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Redesigning rice cultivar (*Oryza Sativa* L.) Uma as saline tolerant cultivar through marker-assisted precision breeding

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ABSTRACT

Rice is the only crop adaptable for cultivation in the marshy saline coastal agro ecosystem. Salinity is the second major abiotic stress that limit rice production. The widely cultivated rice variety Uma in Kerala is the best yielder, but only having moderate tolerance to salinity due to partial introgression of Saltol QTL from Pokkali being the male parent of Uma. In this study , complete introgression of Saltol QTL was effected through a Marker Assisted Precision Breeding using the donor parent FL-478 and confirmed through genotyping and phenotyping. The gene sequence of polymorphic foreground marker SKC-10, the key marker for salinity tolerance, was introgressed into Uma, to tailor it into a saline tolerant variety. The background genome recovery of Saltol introgressed inbred lines of Uma was assessed using genome wide polymorphic SSR markers between the parents covering all the 12 chromosomes. The aim of the present study was to improve the salinity tolerance level of the most popular elite rice cultivar Uma of Kerala by applying precision molecular breeding technique.

Keywords: *Marker assisted precision breeding, rice variety uma, salinity tolerance, Saltol QTL, SSR markers*

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food crop in India which feeds more than half of the world population and is the most adaptable crop suitable for cultivation in the marshy saline coastal agro ecosystem. The impact of global warming induced sea level rise is likely to result in extension of saline intrusion which inflicts osmotic effect, ion toxicity and nutritional imbalance in rice. It is important to increase rice production by at least 25% by 2030 to keep pace with predicted population growth. Rice is severely affected by salinity during seedling and reproductive stages. Salinity stress at early seedling stage manifest on leaves and root and at reproductive stage, salinity causes an increase in sterile florets result in high percentage of chaff and as a result the grain yield is reduced drastically. The conventional breeding efforts for salinity tolerance in rice had limited success. The technique of marker assisted back cross breeding has been promoted as a way to take the beneficial QTLs and incorporate them into promising genotypes using fore ground markers at the target locus and background markers across the rest of the genome. A major QTL located on chromosome 1 was identified for salt tolerance using F8 recombinant inbred lines of an IR 29/Pokkali cross is responsible for low Na^+ high K^+uptake Gregorio et al., (1997). The aim of the present study was to improve the salinity tolerance level of the most popular elite rice cultivar Uma of Kerala by applying precision molecular breeding technique.

2. MATERIAL AND METHODS

2.1. Rice Genotypes

The recurrent parent cultivar Uma is the high yielding and widely cultivated rice variety of Kerala which was developed from the cross Pavizham (MO.16) x Pokkali (VTL.1). Though Pokkali is the male parent, the salinity tolerance of Pokkali has not been transferred completely into Uma. It can withstand moderate salinity but will be affected seriously when salinity level increases. The donor parent cultivar FL-478 is one of the highly tolerant recombinant inbred lines (RIL)developed at IRRI through Marker Assisted Selection from a cross between the susceptible rice variety IR29 and Pokkali and thus this line has been promoted as an improved donor of *Saltol* QTL for breeding programs, as it has a high level of seedling stage salinity tolerance and is photoperiod insensitive, semi tall, non lodging and flowers earlier than the original pokkali landrace.

2.2. Parental polymorphism assay and SSR marker selection

Primer combination which have the capability to resolve different alleles of the gene, among the accessions under study are considered as polymorphic markers Joshi et al., (1999) . A total of six SSR markers tightly linked to *Saltol* QTL (telomeric end

and centromeric end) were evaluated for their suitability in foreground screening (Table- 1). The markers flanking on either side of *Saltol* QTL were used for recombinant selection. In this study seven SSR markers were used to select polymorphic flanking markers (Table -2). A total of 600 genome wide SSR markers distributed over the 12 chromosome were evaluated for polymorphism between Uma and FL-478 suitable for background selection.

Table-1:Foreground markers screened for polymorphism between Uma and FL-478.

Sl no	SSR Primers	Chr.no	Position (Mb)	Forward primer	Reverse primer
1	AP3206	1	11.2	TTCTCATCGCACCATCTCTG	GGAGGAGGAGAGGAAGAAG
2	SKC10	1	11.2	ATAGGGGATATTGGCTGCAC	CAACCAAGCGTGACTAAAAAGA
3	RM8094	1	11.2	AAGTTTGTACACATCGTATACA	CGCGACCAGTACTACTACTA
4	RM10713	1	11.2	ATGAACCCGGCGAACTGAAAGG	CTGGCTCCCTCAAGGTGATTGC
5	RM10745	1	11.7	TGACGAATTGACACACCGAGTACG	CTGGCTCCCTCAAGGTGATTGC
6	RM3412b	1	11.5	TCATGATGGATCTCTGAGGTG	GGGAGGATGCACTAATCTTTC

Table- 2: Recombinant markers screened for polymorphism between Uma and FL-478.

Sl no	SSR Primers	Chr.no	Position (Mb)	Forward primer	Reverse primer
1	RM1287	1	10.8	GGAAGCATCATGCAATAGCC	GGCCGTAGTTTTGCTACTGC
2	RM10696	1	10.9	CCTTCGACTCCATGAAACAAACG	TCTCTTTGCCCTAACCCTATGTCC
3	RM10701	1	11.0	GAGACACGGCACAATATACAACG	TTCTATCTCCGACCTCTTCTCAAGG
4	RM10793	1	12.6	GACTTGCCAACTCCTTCAATTCG	TCGTCGAGTAGCTTCCCTCTCTACC
5	RM10711	1	12.6	GCTTCGATCGATGAGAAAGTAGAGG	GAATCTCCCATCCTTCCCTTCC
6	RM493	1	12.3	GTACGTAAACGCGGAAGGTGACG	CGACGTACGAGATGCCGATCC
7	RM 10772	1	12.2	GCACACCATGCAAATCAATGC	CAGAAACCTCATCTCCACCTTCC

2.3. Genomic DNA Isolation

One month old leaves of the healthy plants were collected and immediately genomic DNA isolated to avoid degradation. The isolation process was done by following CTAB method (IRRI). The isolated genomic DNA was purified using RNase and PCI. Extracted DNA was checked through Agarose Gel.

2.4. PCR amplification

PCR amplification was carried out in a 20 µl reaction volume in which 1 X Taq buffer, 2.5 µM MgCl2, 400 µM dNTPs 1 unit of Taq DNA polymerase and 0.4 µM of forward and reverce primer were contained. The PCR program involved initial denaturation at 94°C for 5 min followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing (between 55 and 76°C) and 2 min extension at 72 °C on a thermal cycler (Bio- Rad) and silver stained similar to Benbouza et al.,(2006). DNA banding pattern was documented using imaging system Gel Dic XR (Bio-Rad).

2.5. Molecular marker data analysis

The molecular weights of the different alleles were measured using Alpha Ease Fc 5.0 software. The marker data was analyzed using the software Graphical Geno- typer (GGT 2.0) Berloo Van (2008). The homozygous recipient allele, homozygous dominant allele and heterozygous allele were scored as “A”, “B” and “H”. The percent markers homozygous for recipient parent (%A) and the percent recipient alleles including heterozygous plants (%R) were calculated.

3. RESULT AND DISCUSSION

The rice variety Uma is the high yielding and widely cultivated cultivar of Kerala. This variety has moderate level of salinity tolerance which might have been inherited from Pokkali. Hence a marker assisted precision breeding approach was done for the complete introgression of *Saltol* QTL from FL-478 into elite rice variety Uma.

3.1. Foreground and Recombinant selection

From the six fore ground SSR markers reported as present within the *Saltol* QTL and seven recombinant markers reported as present on either side (centromeric and telomeric ends) of *Saltol* QTL, tested in the polymorphism assay, only the key foreground marker SKC-10 (Figure-1) responsible for Na^+ /K^+ homoeostasis in the seedling stage and one recombinant marker RM 10793 (Figure-2) in the centromeric region (12.5Mb) were observed as polymorphic between recurrent and donor parents. All the other markers were found to be monomorphic with the recurrent parent Uma which might be due to the presence of partial *Saltol* QTL inherited into Uma from its male parent Pokkali. For evaluating the introgression of target *Saltol* QTL selection the only foreground marker (SKC-10) and recombinant marker (RM-10793) were used for screening heterozygous plants in the BC_1F_1 and BC_2F_1 (Figure-3 & Figure-4) progenies respectively. After two generations of screening the promising progenies of Uma *Saltol*BC_2F_2 (Figure-5 & Figure-6) with target locus in homozygous state were selected to develop the next generation . Marker assisted breeding using FL-478 as the donor parent is used to introgress *Saltol* QTL into many Vietnam varieties such as Q5DB ,AS996 etc Huyen et al.,(2012). Report of single feature polymorphisms in the *Saltol* region suggested that FL-478 contained a <1 Mb DNA fragment from Pokkali at 10.6-11.5 Mb on chromosome 1 Kim et al.,(2009).

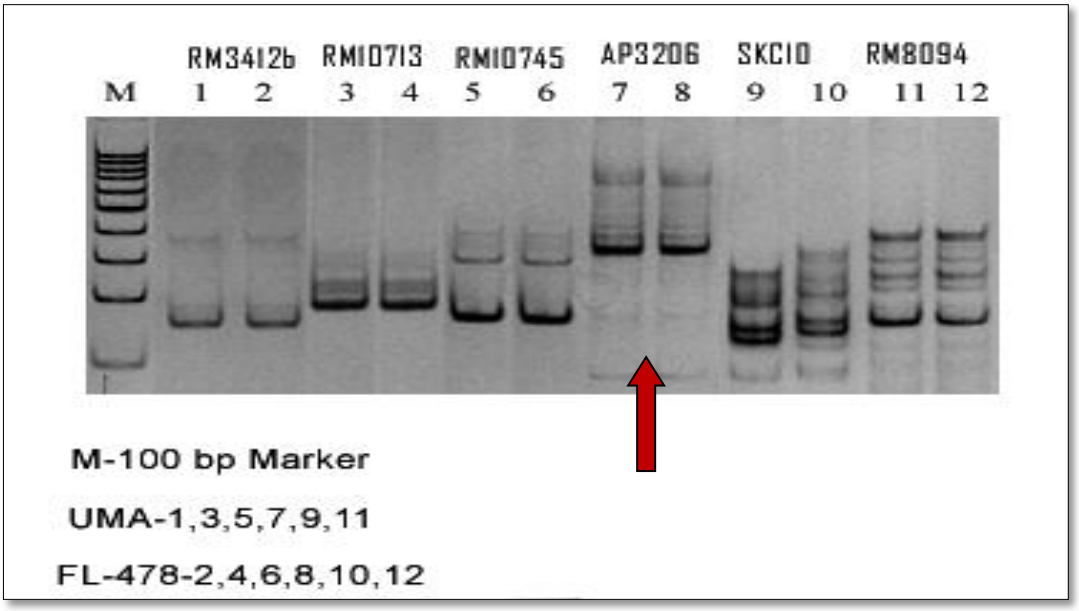

Figure-1: Parental polymorphic screening with *Saltol* specific six foreground markers. Arrow indicate polymorphic marker between recurrent and donor parent.

Figure-2:.Parental polymorphic screening with *Saltol* specific seven recombinant markers. Arrow indicate polymorphic marker between recurrent and donor parent.

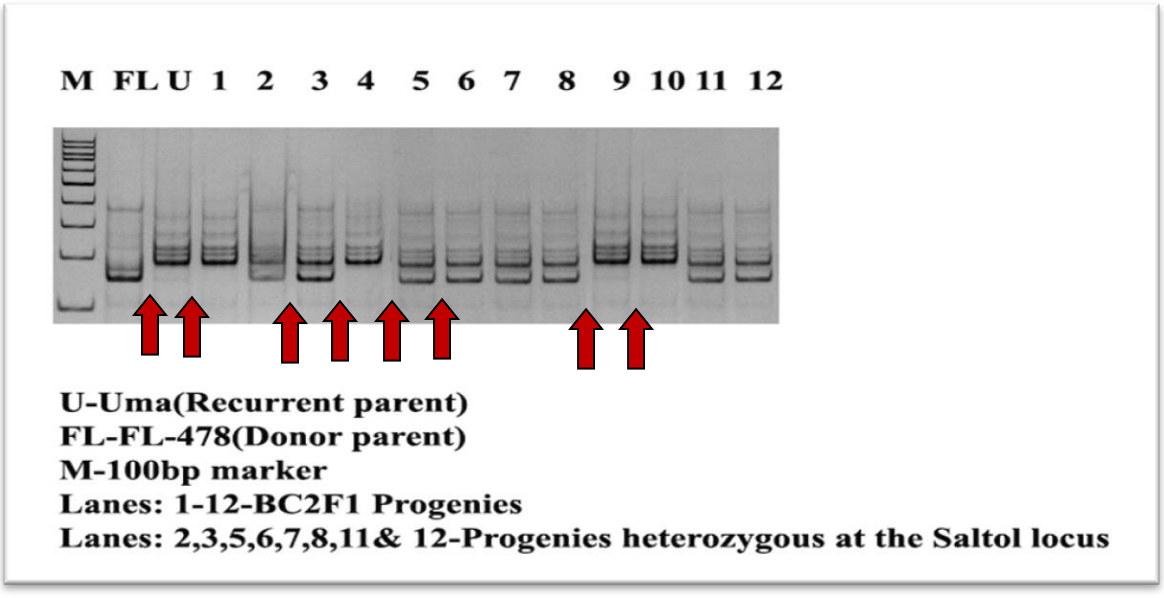

Figure-3:. Screening of BC_2F_1 progeny with foreground marker SKC 10. Arrows indicate heterozygous progenies at *Saltol* locus.

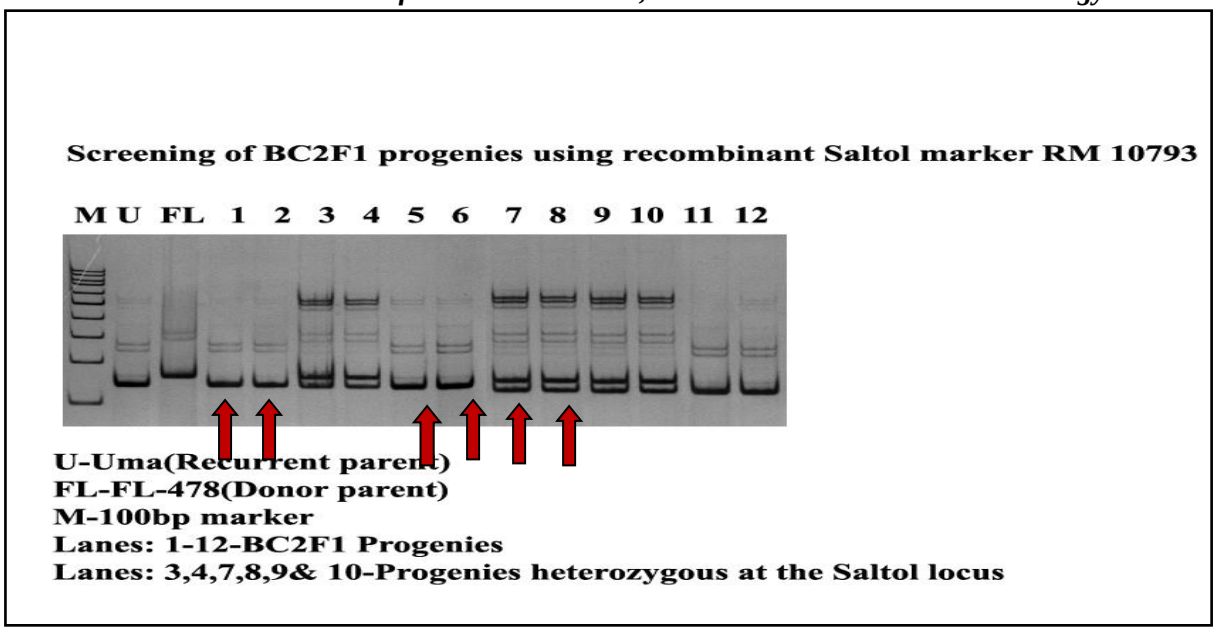

Figure-4:.Screening of BC_2F_1 progeny with recombinant marker RM 10793. Arrows indicate heterozygous progenies at *Saltol* locus.

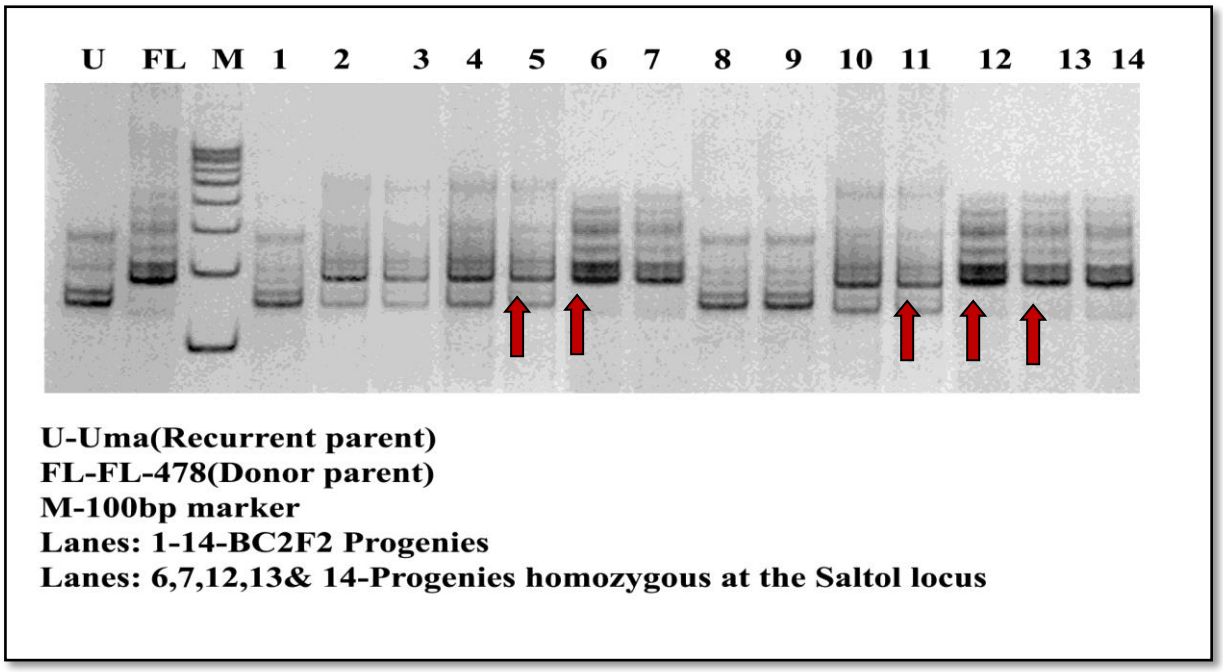

Figure- 5:.Screening of BC_2F_2 progeny with foreground marker SKC 10. Arrows indicate homozygous progenies at *Saltol* locus.

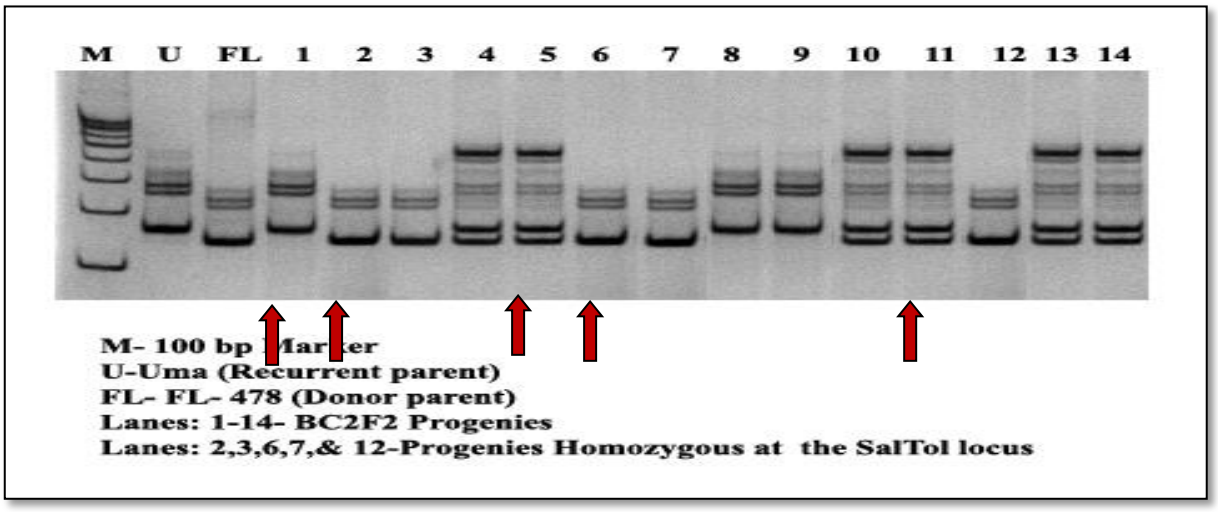

Figure-6:.Screening of BC2F2 progeny with recombinant marker RM 10793.Arrows indicate homozygous progenies at *Saltol* locus.

3.2. Background genome recovery

For effective background genome recovery in the backcross progenies, the thumb rule is to use at least four equidistant polymorphic SSR markers per chromosome. Microsatellite markers unlinked to *Saltol* QTL covering all the chromosomes that were polymorphic between the two parents were used for the genomic recovery of the recurrent parent. Among the 600 SSR back ground markers covering the entire 12 rice chromosomes assayed for the polymorphism between the two parents, seventy-one markers were found to be polymorphic between recurrent and donor parent ranging from 4-11 markers spanned on each chromosome.

Background analysis of the phenotypically and genotypically selected *Saltol* introgressed BC_1F_1plants using the 71 SSR markers indicated an average Recurrent Parent Genome recovery of range71.5% to 74.2% and were then backcrossed again to generate BC_2F_1generation.Background genome recovery of recurrent parent in the BC_1F_1 generation 76.5% – 85.3% was achieved by a single backcross with the recurrent parent Jyothi Rohini and Shylaraj (2017).In this study, 20 plants that were phenotypically selected in the BC_2F_1 generation were subjected to genotypic screening with *Saltol* linked SKC10 marker and could identify seven *Saltol* introgressed plants. Background analysis of these seven plants, using seventy one markers indicated Recurrent Parent Recovery from 86% to 90% in BC_2F_1 generation. All the seven plants were selfed to generate BC_2F_2families and the best two plants having RPG recovery 96.6% and92.0% were selected. Improved NILs of PusaBasmati 1 achieved very high Recurrent Parent Genome (96% -98%) recovery with only two backcrossing Singh et al. (2011) Percentage recovery of recurrent parent genome in the *Saltol* QTL introgressed BC_2F_2lines is presented in (Figure-7) and Graphical genotype of the best plant BIL-5 with maximum recurrent parent genome recovery of 96.6% is presented in (Figure-8).

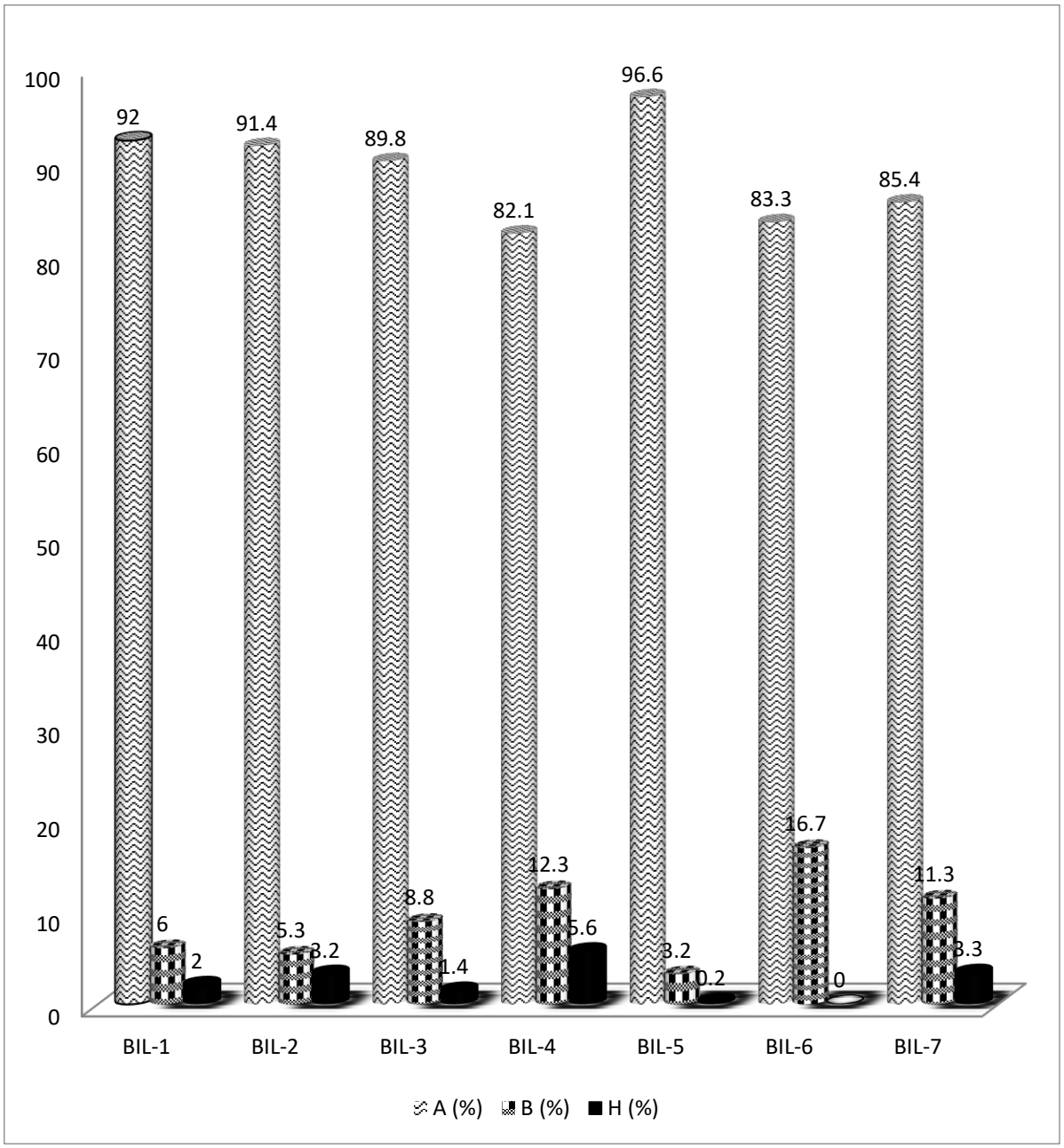

Figure-7:Percentage recovery of recurrent parent genome in the *Saltol*QTL introgressed BC_2F_2 lines
(A)- Recurrent parent genome,(B) – Donor parent genome,(H)- Heterozygous segment

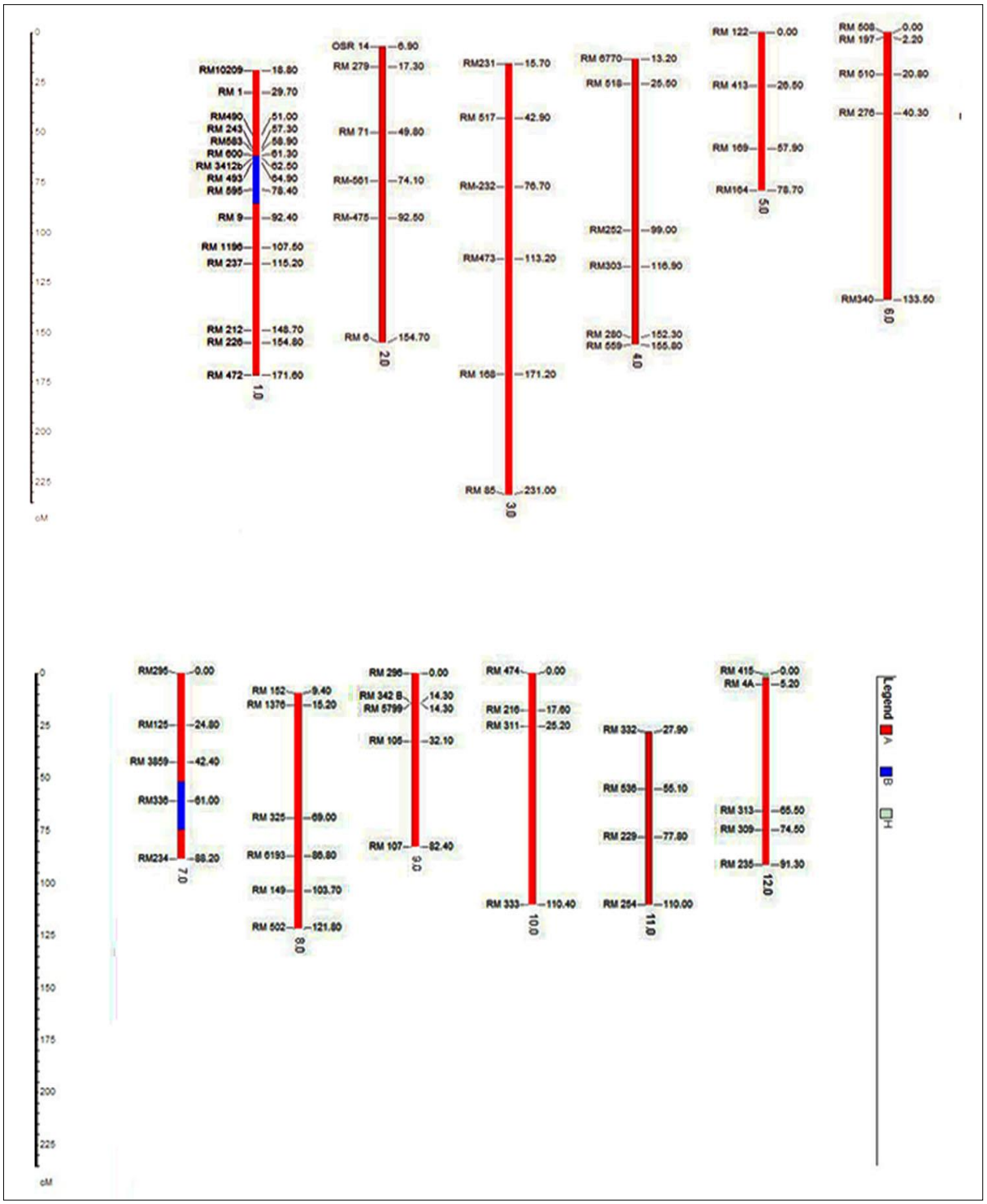

Figure -8. Graphical genotypic representation of BC_2F_2 -BIL -5 plant using GGT2.0. A- Recipient parent (Uma), B- Donor parent (FL-478), H – Heterozygous**.**

4. CONCLUSION

Rice feeds more than half of the world population, but salinity is one of the most important abiotic stresses that can directly affect on plant growth and development. Uma is the most widely cultivated high yielding rice variety in the Vembanad ecosystem but unsuitable for the cultivation in the saline areas. Through the marker assisted precision breeding programme, the variety Uma could be transformed into saline tolerant variety by the complete introgression of *Saltol* QTL locus. The background genome recovery of the seven *Saltol* introgressed lines were assessed with seventy-one polymorphic SSR markers between the parents covering all the 12 chromosome. The phenotypically selected saline tolerant best two lines with *Saltol* QTL locus could recover 96.6% and 92.0% background genotype of Uma. After field evaluation, the most promising lines can be released for commercial cultivation in the saline agro ecosystem of Kerala.

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