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Study of simple sequence repeat (SSR) polymorphism for *Saltol QTL* targeted at chr.1 in elite CMS IR 58025B of paddy and FL478

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Abstract
Marker-assisted selection is an unequivocal translational research tool for crop improvement in the genomics era. IR 58025 B (25B) is an elite cytoplasmic male sterile line which widely accepted all over world for development hybrid paddy. Hybrid rice offers a wide opportunity to improve rice productivity in India. Hybrid rice has the potential to increase yields by 15% to 20% over those of conventionally bred varieties (Virmani, 1994). Most popular CMS source (IR 58025B) for rice hybrids sensitive to salinity. Here, we studied Single sequence repeat polymorphism in between a highly salt tolerant line FL478 and widely adopted salt sensitive CMS source IR 58025B for Saltol Qtl located at chromosome No.1. Saltol Qtl linked 50 markers served for targeted Qtl with both lines, these 50 SSR markers present in between RM 10710 to RM 10838 which is the peak marker for Saltol QTL present on carrier chromosome (Chr 1) There were ten foreground markers found polymorphic between rice cytoplasmic male sterile maintainer 58025B and Saltol Qtl donor FL478 at targeted *Saltol QTL* region on chromosome. These identified polymorphic markers are helpful to for introgression of Saltol Qtl in CMS source of paddy. Saltol Qtl introgressed CMS lines can play a pivotal role in success and sustenance of hybrid rice technology.

Keywords: polymorphism, hybrid paddy, Saltol Qtl

Introduction
Soil salinity is the most widespread soil toxicity problem in rice growing countries. It is one of the major obstacles to increase crop production worldwide. Soils are classified as saline when the electrical conductivity (EC) is 4 dS/m or more which is equivalent to approximately 40 mM NaCl and generates an osmotic pressure of approximately 0.2 MPa. This definition of salinity derives from the EC that significantly reduces the yield of most crops (Pirasteh-Anosheh *et al.,* 2016). Salinity is one of the abiotic stresses limiting rice production globally. In India, total salt affected area is reported to be ~8.1 million ha. In the recent years, salt affected areas are growing at the rate of 10 per cent every year thus making the soil unsuitable for cultivation and thereby rendering crop productivity to decline (Maji *et al.,* 2010). Salinity contribute to the loss of arable lands due to salt accumulation as a result of excessive use of irrigation water with poor or improper drainage, a fact that is likely to be aggravated by sea level rise in coastal areas caused by climate change (Platten *et al.,* 2013). Management of salinity is energy intensive agricultural practice hence developing salinity tolerant crop plants is a best strategy to combat salinity. In order to develop salinity tolerant lines of Improved White Ponni (IWP), *Saltol* QTL on chromosome 1 was transferred from FL478 to IWP using Marker Assisted Backcrossing.

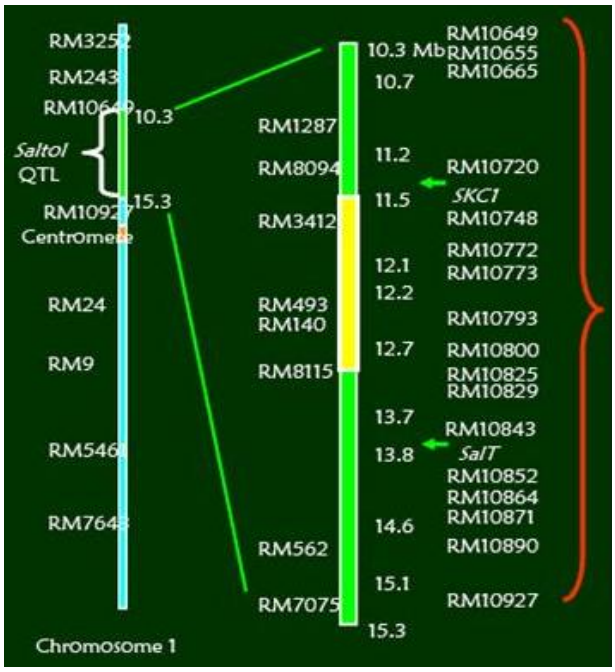
Many other salt-tolerant varieties of rice e.g. CSR 10, SCR 11, CSR 13, CSR 27 for inland situations and CST 7-1, CSR 4 and CSR 6 for coastal areas have been developed and released (Dagar, 2005), But all salinity tolerant varieties are less yielding and no efforts have been made towards develop salinity tolerant hybrids although hybrid rice has proven to be an effective and economical way to increase rice production output. This can provide 10-20% yield advantage along with sustainable stress tolerance. Major constraint in development of Salinity tolerant hybrid is unavailability of Salinity tolerant cytoplasmic Male Sterile Lines and restorers.

Materials and Methods
Plant materials used in the study include: the widely adopted, high productive and good combining elite maintainer line developed by IRRI "CMS 58025B", a salt sensitive CMS line as the recurrent parent and FL478 (IR 66946-3R-178-1-1), a Saltol QTL carrying RIL in the

background of IR29 as donor parent. FL478 has seedling-stage salt tolerance upto 18 dS.m-1 (Thomson *et al*., 2010).

Genotyping

Foreground selection was carried out using markers present in between RM 10710 to RM 10838 which is the peak marker for *Saltol QTL*. Additionally, the markers RM35, RM1287, RM8094, RM10720, RM10748 and RM493 present on carrier chromosome (Chr. 1) flanking the *Saltol QTL* were also used for parental polymorphism Polymorphic DNA markers were identified between the parental lines at targeted Saltol QTL region of chromosome 1.

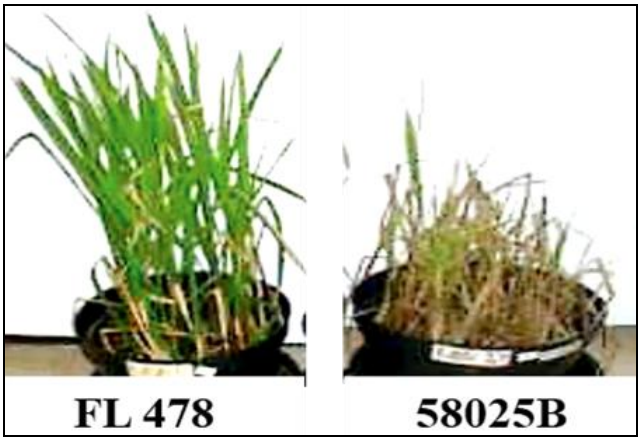


Major Saltol QTL located on chromosome 1 and the flanking markers of QTL (Glenn (1997) mapped in IR29/Pokkali RIL population).

Molecular Analyses: Genomic DNA was isolated from young leaves of the test lines when they were about 21 days old using the standard Cetyl Trimethyl Ammonium Bromide protocol. Polymerase chain reaction (PCR) based amplification of the target genomic fragments by the primer pairs for each selected marker was performed in a 10 μl reaction mix constituted by adding 25–30 ng genomic DNA, 5 pmol each of the two primers, 0.05mM each of the four dNTPs, and PCR buffer (10x) containing 10mM Tris (pH 8.4), 50mM KCl, and 1.8mM MgCl2. To this mix, 0.5U of Taq DNA polymerase was added, and the volume made up to 10 μl using nuclease free water. The PCR was run for 35 cycles comprising of denaturation for one minute at 94°C, followed by annealing for one minute at 55°C, and primer elongation for two minutes at 72°C, sandwiched between an initial denaturation for five minutes at 94°C and the final extension for seven minutes at 72°C. The amplified products were electrophoresed in 3.5% agarose gel, and the products were visualized using a gel documentation system. The marker segregation data was graphically compiled in each generation using Graphical GenoTypes (GGT) version 2.0 software.

Phenotyping

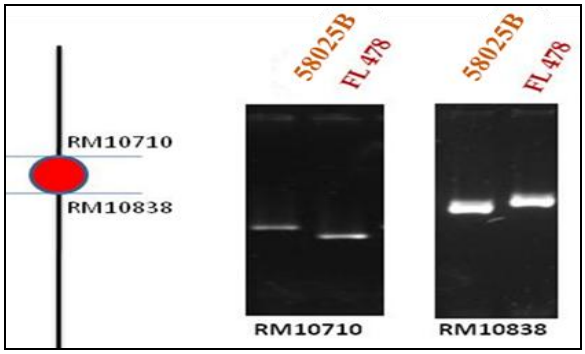
Evaluation of Salinity Tolerance: FL478, as the donor parent for *Saltol* FL478 is a breeding line with very high level of seedling stage salt tolerance; it can endure salt solutions with electrical conductivity (EC) of up to 18 dSm−1 for more than a fortnight. Both the parents were first evaluated for tolerance to 100mM NaCl solution (EC of 11.6 dSm−1) at seedling stage to validate their salt tolerance levels before initiating the crossing programme. In a laboratory experiment, rice seeds were soaked in water for 12 hrs, then placed in special trays and seeds were grown in distilled water. Seedlings were grown in a plastic trays. The 21 days old healthy and homogeneus seedling of both the parent varieties of rice (FL478 and 58025B) were selected and moved into two plastics pots containing soil medium, and were grown in the glasshouse and irrigated with 100 mM of NaCl, up to 4 weeks. The growth response was then observed by measuring the total of leaf area per plant, height, and mortaility. Salt tolerance was scored using the standard evaluation system (SES) for rice developed by the International Rice Research Institute, Manila, Philippines. In the pre-screening, the recurrent parent, 58025B, was found highly sensitive to salt stress and recorded a score of nine, while the donor parent, FL478, was tolerant and recorded a score of one.



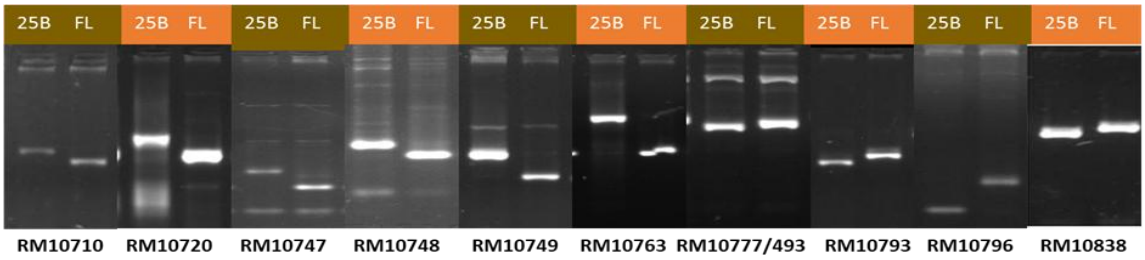
Donor parent FL 478 and recipient parent 58025B grown in the Salinity stress condition created by irrigation of pots with 100 mM NaCl solution

Results

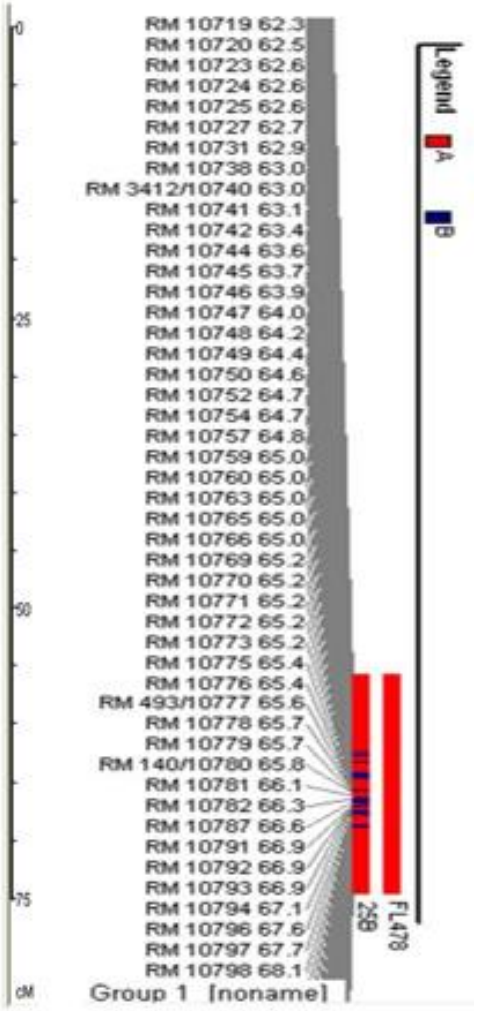
Parental SSR Polymorphism Screening: In this study, 50 SSR markers associated with the *SalTol QTL* region were checked with two parent's in order to find out polymorphic primers to further use for screening the Saltol loci of the crossing populations. The molecular analysis of both the parents was carried out using markers present in between RM 10710 to RM 10838 which is the peak marker for *Saltol QTL*. Additionally, the markers RM35, RM1287, RM8094, RM10720, RM10748 and RM493 present on carrier chromosome (Chr 1) flanking the *Saltol QTL* were also used for parental polymorphism Polymorphic DNA markers were identified between the parental lines at targeted *Saltol* QTL region of chromosome 1. There were ten foreground markers found polymorphic between rice cytoplasmic male sterile maintainer 58025B and *Saltol Qtl* donor FL478 at targeted *Saltol QTL* region on chromosome-1. These study is useful for introgression of saltol Qtl in 58025B background to develop salinity tolerant maintainer lines which can be useful to develop salinity tolerant maintainer lines.



PCR analysis of DNA with flanking primers from 58025B and FL478 plants



PCR analysis of DNA polymorphic markers linked to *Saltol QTL* between 58025B and FL478 parents



Graphical representation of mapping polymorphic markers at targeted Saltol QTL region on chromosome 1 of 58025B and FL478

Table 1: List of polymorphic markers linked to *Saltol QTL* between 58025B and FL478 parents

Sl. No	Primer	Position (CM)	Alleles in (BP)
1	RM 10710	61.754	173
2	RM 10720	62.463	205
3	RM 10747	64.032	96
4	RM 10748	64.165	96
5	RM 10749	64.377	288
6	RM 10763	65.041	238
7	RM 493/10777	65.611	178
8	RM 10793	66.861	124
9	RM 10796	67.555	77
10	RM 10838	70.962	147

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