Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP2: 156-162

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National Conference on Conservation Agriculture
(ITM University, Gwalior on 22-23 February, 2018)

Marker-Assisted Introgression of *Saltol QTL* to enhances seedling stage salt tolerance in cytoplasmic male sterile background of rice

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Abstract
Rice is a most salt sensitive cereal crop with a threshold of 3 dSm^1 for most cultivated varieties and it is one of the major obstacles to increase rice production worldwide. 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 sources of rice hybrids also 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 IR58025B for *Saltol QTL* located at chromosome No.1. *Saltol QTL* linked 50 markers served for targeted QTL with both lines and 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 served on F_2 population of IR58025BXFL478 to recover the targeted *Saltol QTL* on chr.-.1 for identification of maintainability, Rf marker RM6100 used to avoid the restorer plants and selected plants utilized to develop homogenous maintainer population.

Keywords: salt tolerance, cytoplasmic male, homogenous maintainer population

Introduction
Climate change and food security are the two burning issues now-a-days. Agricultural production is extremely vulnerable to climate change. It is causing threatening impacts on rice production, which is the most important cereal crop for the food security worldwide. Rice (*Oryza sativa L.*) is an important staple crop that feeds more than one half of the world's population and is the model system for Monocotyledonous plants. However, rice is very sensitive to salinity and is the most salt sensitive cereal crop with a threshold of 3 dSm^1 for most cultivated varieties. The most common damages of salinity are attributed to osmotic imbalance, membrane destabilisation, and failure of photosynthetic machinery. 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) [26]. Management of salinity is energy intensive agricultural practice hence developing salinity tolerant crop plants is a best strategy to combat salinity. Salt tolerance in rice is manifested through morphological, physiological, and metabolic responses that includes stomatal changes, sodium exclusion, tissue tolerance, apoplastic salt compartmentalization, salt sequestration into older tissues, and regulation of the antioxidants [2–5]. Apart from the understanding of physiological and metabolic responses to salt stress, quantitative trait loci (QTLs) and genes governing salt tolerance have also been reported in rice. 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) [6], 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: (a) 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 (b) FL478 (IR 66946-3R-178-1-1), a Saltol QTL carrying RIL in the background of IR29 as donor parent. FL478 can endure salt tolerance upto 18 dS.m-1 at seedling satge (Thomson *et al*., 2010) [22, 33]. 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. 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, IR 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 (Supplementary Figure 1).

Breeding Strategy

IR58025B was crossed as the male parent with FL478, and the hybridity of the F_1 was confirmed using the SSR marker, RM10710 and RM 10838. The confirmed F_1s were selfed and developed F_2 population. All the F_2 plants screened in saline micro tanks along with RP (salt sensitive) and DP (salt tolerance) and survived plants screened from a rice microsatellite marker RM6100, tightly linked to fertility restoration gene located on the long arm of chromosome 10, which can distinguish maintainers from fertility restorers was used for screening of F_2 population to distinguish the fertility restoration and maintainer type of plants (Supplementary Figure 7);. Selected 10 maintainer type of plants screened for foreground selection The parental lines were screened for polymorphism at the target QTL locus using twenty-one Saltol-linked SSR markers, of which ten markers RM10710, RM 10720, RM 10747, RM 10748, RM 10749, RM 10763, RM 493/10777, RM 10793, RM 10796 and RM 10838 were found to be polymorphic (Supplementary Figure 8); All the ten markers were used for foreground selection of 10 F2 population (Supplementary Figure 10 &11); and homozygous plants for Saltol Qtl. forwarded for F3 population. Selected F3 plants selfed and raised F4 gen. The family showing the highest level of salt tolerance was transplanted in the field and evaluated for agronomic characters. Agronomically superior members of the tolerant family can be use for development of A/B pairs.

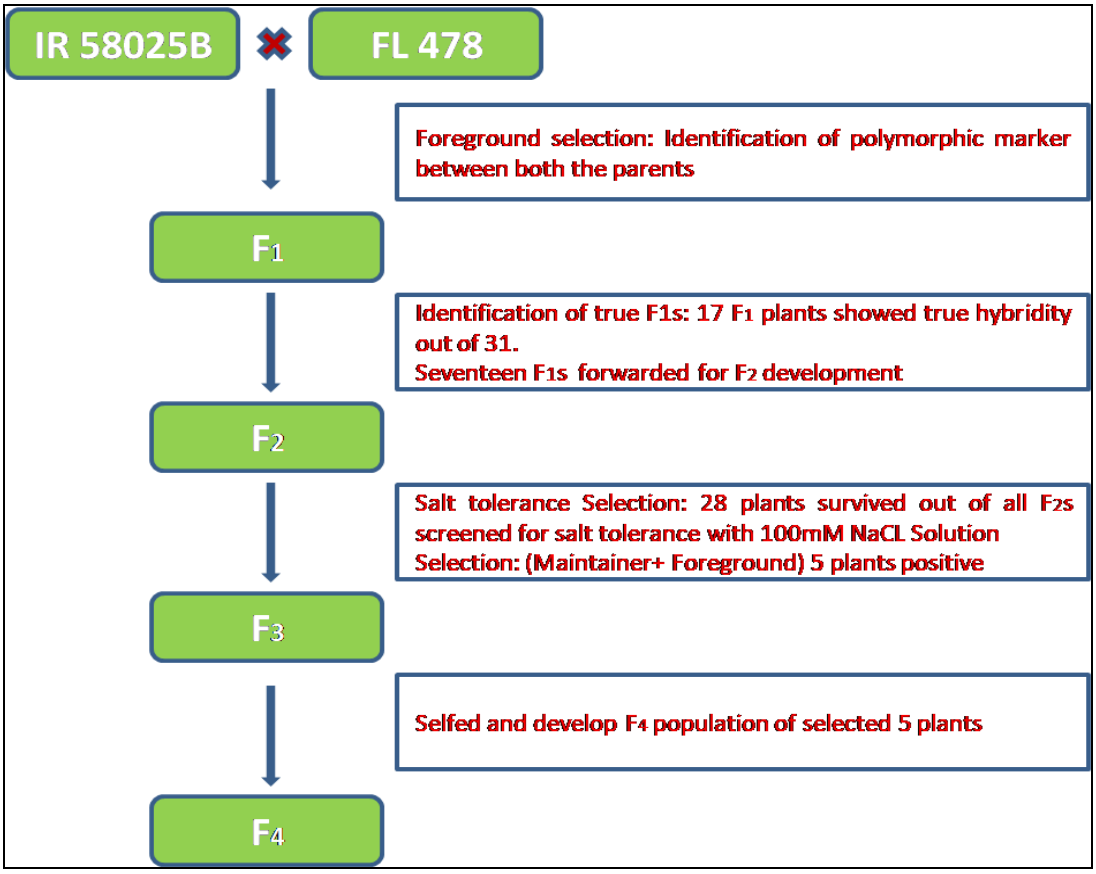


Fig 1: Breeding scheme used in the marker-assisted programme for the transfer of *Saltol* locus in the background of the elite rice maintainer line IR58025B.

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

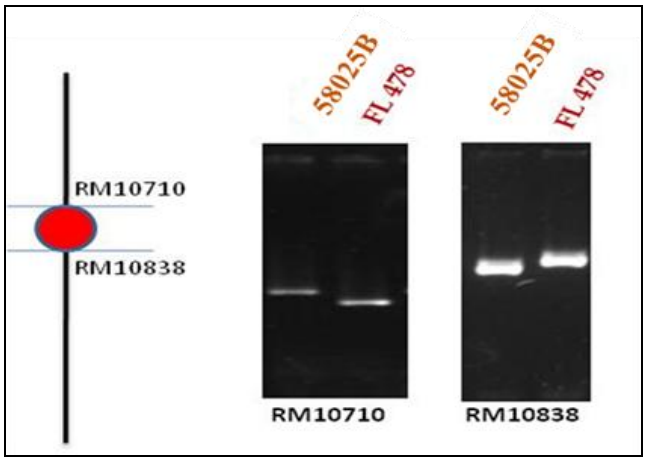
reaction mix constituted by adding 25–30 ng genomic DNA, 5 pmol each of the two primers, 0.05mM each of the four dNTPs, and PCR buffer (10x) containing 10mM Tris (pH 8.4), 50mM KCl, and 1.8mM MgCl2. To this mix, 0.5U of Taq DNA polymerase was added, and the volume made up to 10 μl using nuclease free 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.

Marker-Aided Selection: 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. Ten Saltol-linked SSR markers, RM10710, RM 10720, RM 10747, RM 10748, RM 10749, RM 10763, RM 493/10777, RM 10793, RM 10796 and RM 10838 found polymorphic and used for foreground selection, details of these Saltol-linked SSR markers such as their physical position on chromosome 1 and physical locations within the *Saltol QTL* are given in Supplementary Table 1

A rice microsatellite marker RM6100, tightly linked to fertility restoration gene located on the long arm of chromosome 10, which can distinguish maintainers from fertility restorers, was used for screening of F_2 population to distinguish the fertility restoration and maintainer type of plants.

Result

Identification of True F_1s: The F_1 progenies obtained from the crossbreeding IR58025XFL478 which provided introduction of donor resistance alleles into the genotype of the recurrent parent used for confirmation of hybridity. Thirty one F_1 plants screened with two polymorphic flanking SSR markers RM10710 and RM 10838 for confirmation of hybridity, 18 plants scored as true hybrid plants out of 31 plants.



Fige 2: PCR analysis of DNA with flanking primers from IR58025B and FL478 plants

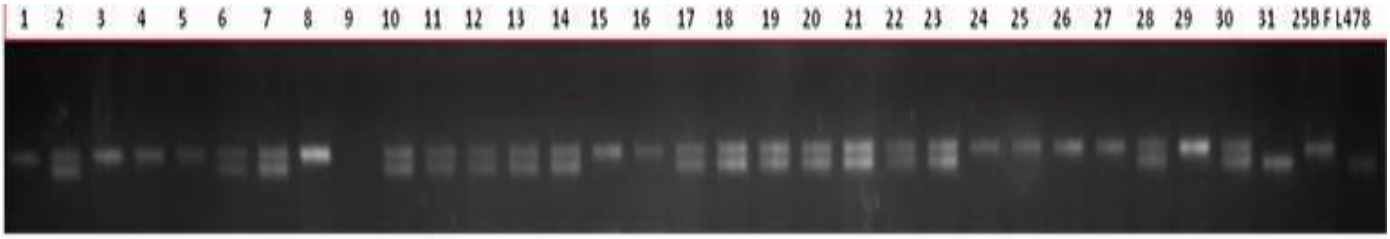


Fig 3: Representative agrose gel picture of identification of true F1 plants. The PCR analysis of IR58025B/FL478 F_1 plants with foreground specific flanking marker RM 10710 (1 to 31 are F1 plants, IR 58025B-RP, FL478-DP)

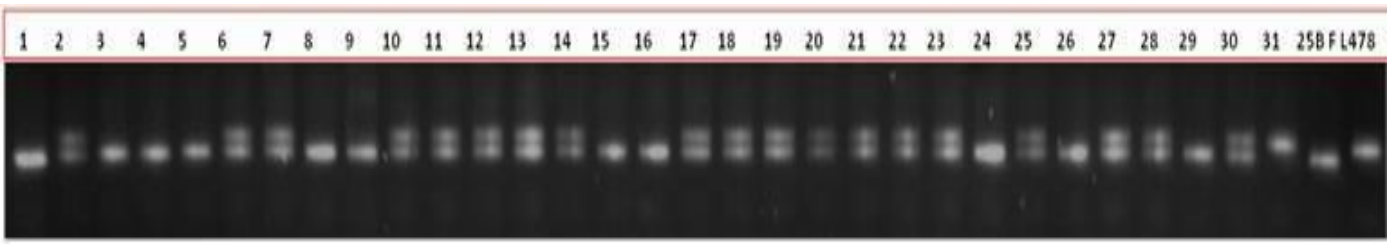


Fig 4: Representative agrose gel picture of identification of true F_1 plants. The PCR analysis of IR58025B/FL478 F_1 plants with foreground specific flanking marker RM 10838 (1 to 31 are F_1 plants, IR 58025B-RP, FL-478-DP)

Phenotyping: Evaluation of Salinity Tolerance: Donor parent 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. All the F_2 plants developed from true F_1s, screened for salt sensitivity in 100mM NaCl solution (EC of 11.6 dSm−1) at seedling stage to discard the plants population segregated for *Saltol QTL*. In this experiment, F_2 seeds were soaked in water for 12 hrs, then placed in trays and seeds were grown in distilled water. Seedlings were grown in plastic trays. The 21 days old healthy seedling of F_2 population (IR58025BX FL478) were moved into two plastics trays 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 mortality. Salt tolerance was

scored using the standard evaluation system (SES) for rice developed by the International Rice Research Institute, Manila, Philippines. In the salinity screening, only survived plants (score of one) selected for Molecular screening.

Fig 5: F2 population grown in the Salinity stress condition created by irrigation of trays with 100 mM NaCl solution (EC of 11.6 dSm−1)

Screening of Maintainer plants: The identification of maintainers and restorers is fundamental for the commercial exploitation of heterosis breeding programme using cytoplasmic male sterility (CMS) system. To differentiate restorers and maintainers in *Saltol QTL* introgressed F_2 population, A rice microsatellite marker RM6100, tightly linked to fertility restoration gene located on the long arm of chromosome 10 used to distinguishing between maintainers and fertility restorers plants of F_2 population. All the 28 F_2 generation survived plants screened with the microsatellite marker RM6100 to distinguish the fertility restoration and maintainer type of plants. 10 plants scored maintainer type bands. Those 10 plants subjected to foreground screening.

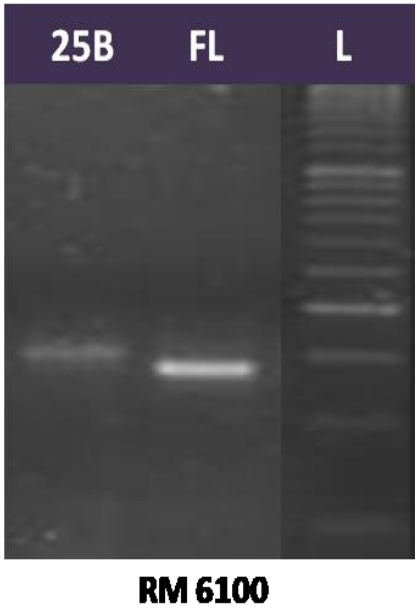


Fig 6: PCR analysis of DNA with polymorphic marker RM 6100 with IR58025B and FL478 plants

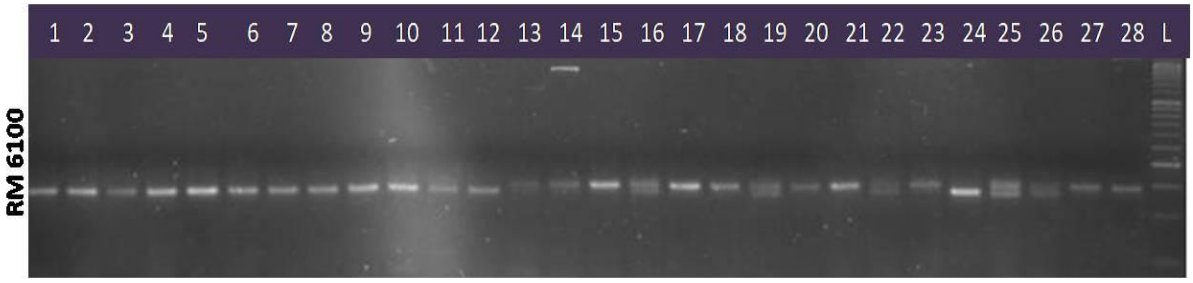


Fig 7: Representative agrose gel picture of identification of maintainer type plants. The PCR analysis of IR58025B/FL478 F_2 plants with fertility restoration marker RM 6100 (1 to 28 are F_2 plants)

Polymorphism between the Parents: 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*. 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 IR58025B and *Saltol Qtl* donor FL478 at targeted *Saltol QTL* region on chromosome-1.

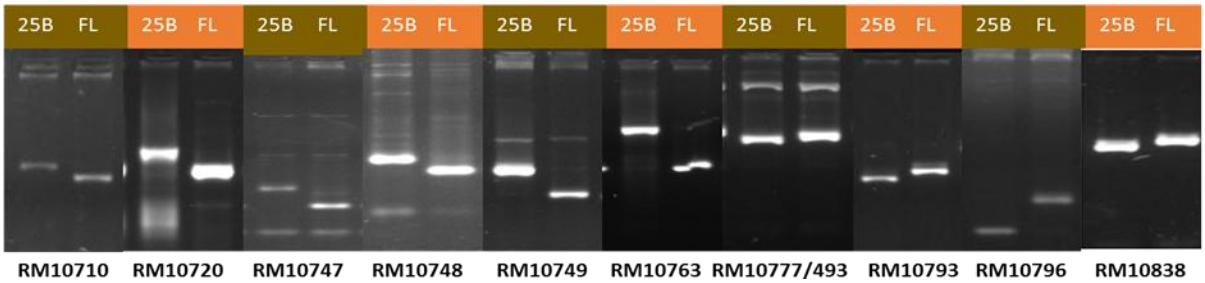


Fig 8: PCR analysis of DNA polymorphic markers linked to *Saltol QTL* between IR58025B and FL478 parents

Table 1: List of polymorphic markers linked to *Saltol QTL* between 25B 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

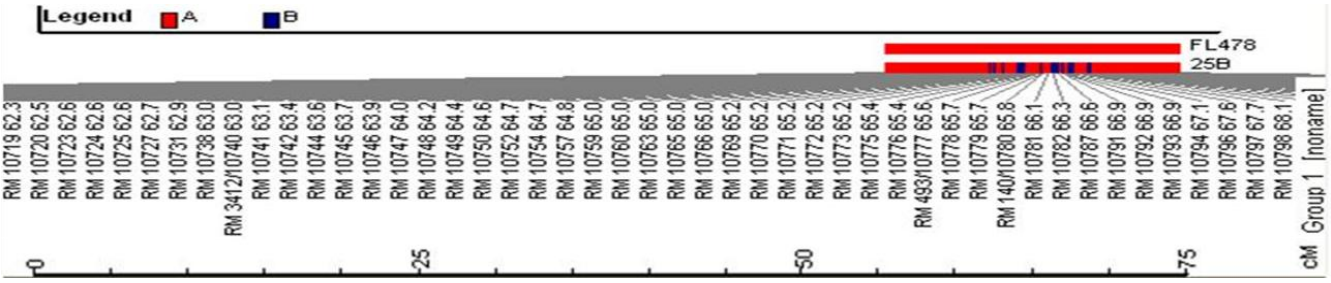


Fig 9: Graphical representation of mapping polymorphic markers at targeted *Saltol QTL* region on chromosome 1 of IR58025B and FL478

Foreground selection: Ten maintainer plants of F_2 population screened for presence of *Saltol* Locus with 10 parental polymorphic DNA markers (RM10710, RM 10720, RM 10747, RM 10748, RM 10749, RM 10763, RM 493/10777, RM 10793, RM 10796 and RM 10838) present in between RM 10710 to RM 10838 which is the peak marker for *Saltol QTL*.

Plant No. 3, 4, 5, 8 and 9 scored homgygous banding pattern similar to the donor parent with all the 10 polymorphic SSR markers. Plant No. 1,2,6,7 and 10 scored hetrogygous banding pattern with one and more SSR markers. Five plants with homogygous banding pattern selected to develop F_3 population. All the plants with heterogygous banding pattern rejected to avoid the segregation in introgressed *Saltol QTL*. Selected 5 plants used to develop homogenous population and forwarded up to F_4 population.

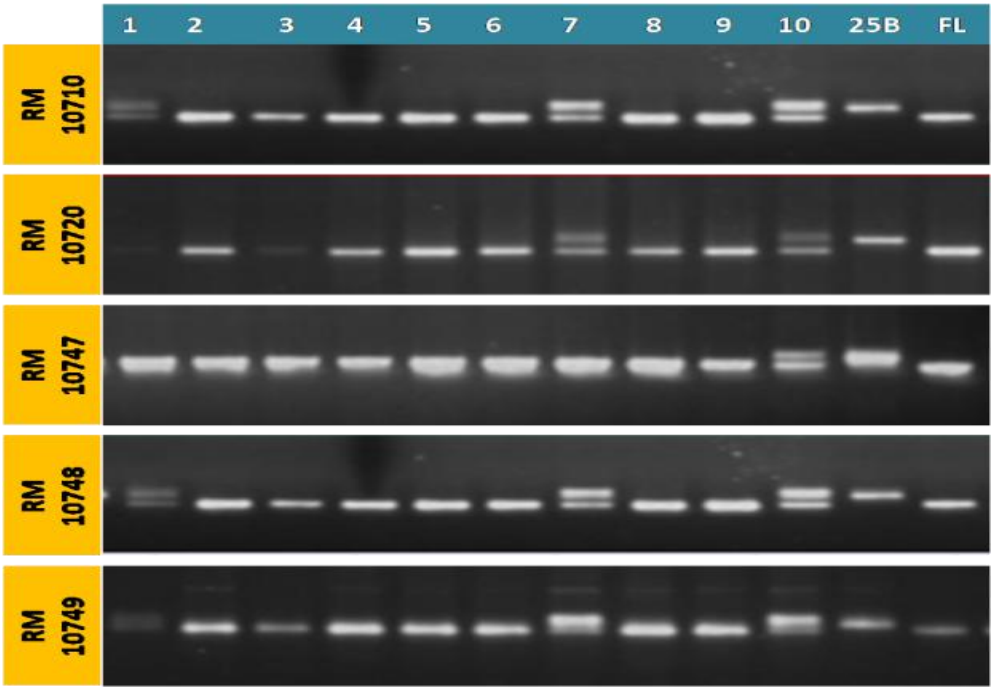


Fig 10: PCR analysis of DNA polymorphic markers (RM 10710, RM10720, RM 10747, RM10748 and RM10749) linked to *Saltol QTL* for F_2 population of IR58025BX FL478

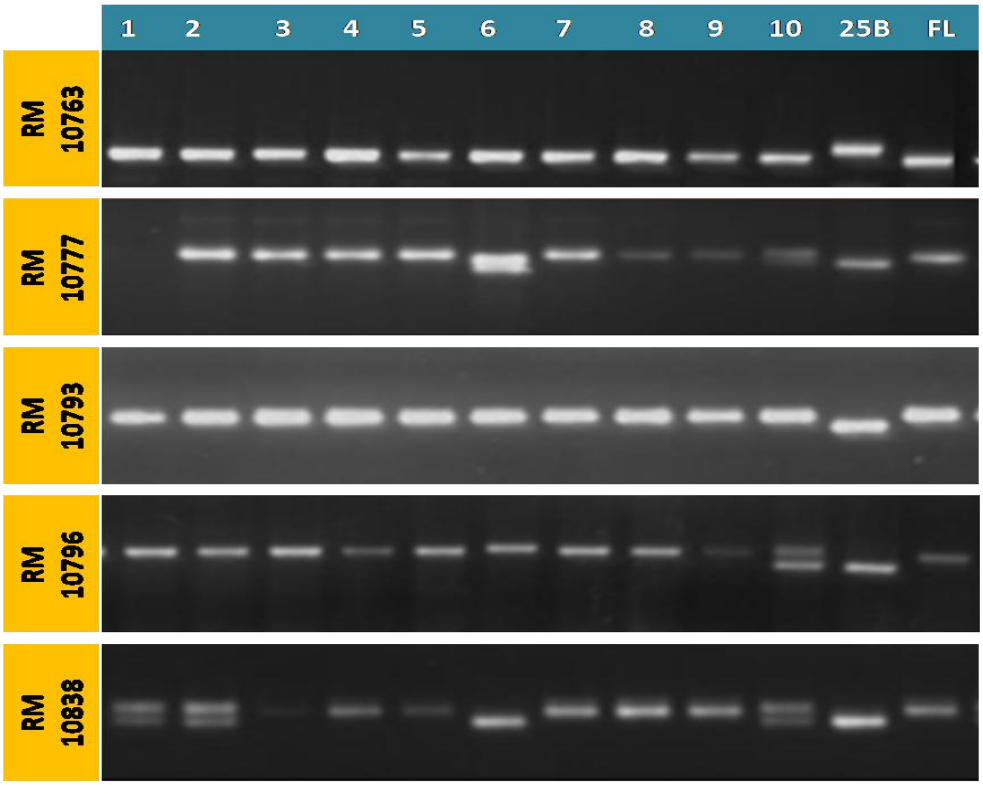


Fig 11: PCR analysis of DNA polymorphic markers (RM 10763, RM10777, RM 10793, RM10796 and RM10838) linked to *Saltol QTL* for F_2 population of IR58025BX FL478

Acknowledgement
The authors wish to acknowledge the IAHS, Bangalore India and N.D University of Agriculture and Technology, Faizabad India for providing necessary facility and support to carry out this Project. Authors are also thankful to Bhagwant University for accepting this research work.

References

1. Ismail AM, Heuer S, Thomson MJ, Wissuwa M. "Genetic and genomic approaches to develop rice germplasm for problem soils," Plant Molecular Biology. 2007; 65(4):547-570
2. Yeo R, Flowers TJ. "Salinity resistance in rice (Oryza sativa L.) and a pyramiding approach to breeding varieties for saline soils," Australian Journal of Plant Physiology. 1986; 13(1):161-174
3. Ashraf M, Akram NA, Mehboob-Ur-Rahman, Foolad MR. Marker-assisted selection in plant breeding for salinity tolerance. Methods Mol. Bio., 2012; 913:305-333. DOI: 10.1007/978-1-61779-986-0_21
4. Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van, Dillen PM *et al*., Rapid and simple method for purification of nucleic acids. J Clin. Microbiol. 1990; 28:495-503.DOI: 0095-1137/90/030495-09$02.00/0
5. Collard BC, Mackill DJ. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. Philo. Trans. B, 2008; 363:557-572. DOI: 10.1098/rstb.2007.2170
6. Dagar JC. Salinity Research in India an Overview. *Bulletin of the National Institute of Ecology.*, New Delhi & Jaipur *(in* Ecology and Environmental Management**:** Issues and Research Needs. 2005; 15:69-80
7. Moradi F, Ismail AM. "Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice," Annals of Botany. 2007; 99(6):1161-1173
8. Fu C, Wu T, Liu W, Wang F, Li J, Zhu X *et al*. Genetic improvement of resistance to blast and bacterial blight of the elite maintainer line Rongfeng B in hybrid rice (*Oryza sativa* L.) by using marker-assisted selection. Af. J Biotechnol. 2012; 11:13104-13114.
9. Gregorio GB. Tagging salinity tolerance genes in rice using amplified fragment length polymorphism (AFLP), Ph.D. Thesis University Philippines Los Banos Laguna Philippines, 1997.
10. Gregorio GB, Senadhira D, Mendoza RD. Screening Rice for Salinity Tolerance, IRRI Discussion Paper Series no 22, Los Baños, International Rice Research Institute, 1997.
11. Gregorio GB, Islam MR, Vergara GV, Thirumeni S. Recent advances in rice science to design salinity and other abiotic stress tolerant rice varieties, SABRAO Journal of Breeding and Genetics. 2013; 45:31-41.
12. Hari Y, Srinivasarao K, Viraktamath BC, Prasad H, Arremsetty S, Laha GS *et al*. Marker-assisted introgression of bacterial blight and blast resistance into IR 58025B, an elite maintainer line of rice. *Plant Breeding*. 2013; 132:586-594. 10.1111/pbr.12056
13. Hospital F. Challenges for effective marker assisted selection in plants. Genetica. 2009; 136:303-310. DOI: 10.1007/s10709-008-9307-1
14. Hu S, Tao H, Qian Q, Guo L. Genetics and molecular breeding for salt-tolerance in rice. Rice Genomics Genetics. 2012; 3:38-39. DOI: 10.5376/rgg.2012.03.0007
15. Walia H, Wilson C, Condamine P. "Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage," Plant Physiology. 2005; 139(2):822-835
16. IRRI, Standard Evaluation System (SES) for Rice, Los Baños, International Rice Research Institute, 5th edition, 2013.
17. IRRI, STAR Version 2.0.1, Biometrics and Breeding Informatics, PBGB Division, International Rice Research Institute, Los Baños, 2014.

18. Vinod KK, Krishnan SG, Babu NN, Nagarajan M, Singh AK. Improving salt tolerance in rice: looking beyond the conventional," in Salt Stress in Plants: Signalling, Omics and Adaptations, P. Ahmad, M. M. Azooz and M. N. V. Prasad, Eds., Springer, New York, 2013, 219-260,
19. Kumar V, Singh A, Mithra SVA, Krishnamurthy SL, Parida SK. Genome-wide association mapping of salinity tolerance in rice (Oryza sativa). DNA Research: An Int. J. Rapid Pub. Reports Genes Genomes. 2015; 22:133-145. DOI: 10.1093/dnares/dsu046
20. Huyen LTN, Cuc LM, Ismail AM, Ham LH. "Introgression the salinity tolerance QTLs Saltol into AS996, the elite rice variety of Vietnam," American Journal of Plant Science. 2012; 3(7):981-987
21. Mardani Z, Rabiei B, Sabouri H, Sabouri A. "Identification of molecular markers linked to salt tolerant genes at germination stage of rice". Plant Breeding. 2014; 133:196-202. DOI: 10.1111/pbr.12136
22. Thomson MJ, Ocampo M, Egdane J. "Characterizing the Saltol quantitative trait locus for salinity tolerance in rice," Rice, 2010; 3(2, 3):148-160
23. Negrao S, Courtois B, Ahmadi N, Abreu I, Saibo N. Recent updates on salinity stress in rice: From physiological to molecular responses. Critical Rev. Plant Sci., 2011; 30:329-377.DOI: 10.1080/07352689.2011.587725
24. Babu NN, Vinod KK, Krishnan SG. "Marker based haplotype diversity of Saltol QTL in relation to seedling stage salinity tolerance in selected genotypes of rice," Indian Journal of Genetics and Plant Breeding. 2014; 74(1):16-25
25. Babu NN, Krishnan SG, Vinod KK. "Marker aided incorporation of Saltol, a major QTL associated with seedling stage salt tolerance, into Oryza sativa 'Pusa basmati 1121'," Frontiers in Plant Science, 2017, 8
26. Platten JD, Egdane JA, Ismail AM. Salinity tolerance, Na+ exclusion and allele mining of HKT1; 5 in Oryza sativa and O. glaberrima: Many sources, many genes, one mechanism. BMC Plant Biol, 2013. DOI: 10.1186/1471-2229-13-32
27. Aliyu R, Adam AM, Muazu S, Alonge SO, Gregario GB. "Tagging and validation of SSR markers to salinity tolerance in rice," in 2010 International Conference on Biology, Environment and Chemistry (IPCBEE), Singapore. 2011; 1:328-332,
28. Mohammadi R, Mendioro MS, Diaz GQ, Gregorio GB, Singh RK. "Genetic analysis of salt tolerance at seedling and reproductive stages in rice (Oryza sativa)," Plant Breeding. 2014; 133(5):548-559
29. Van Berloo R. "Computer note. GGT: software for display of graphical genotypes," Journal of Heredity. 1999; 90(2):328-329
30. Basavaraj SH, Singh VK, Singh A. "Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid," Molecular Breeding. 2010; 26(2):293-305
31. Shanti M, Shenoy V, Devi GL, Kumar VM, Premalatha P, Kumar GN *et al*. Marker-assisted breeding for resistance to bacterial leaf blight in popular cultivar and parental lines of hybrid rice. *J. Plant Pathol.* 2010; 92:495-501.
32. Tanweer FA, Rafii MY, Sijam K, Rahim HA, Ahmed F, Ashkani S *et al*. Identification of suitable segregating SSR markers for blast resistance in rice using inheritance and disease reaction analysis in backcross families. *Austral. Plant Pathol.* 2015a; 44:619-627. 10.1007/s13313-015-0380-5a
33. Thomson MJ, Ocampo M, Egdane J, Rahman MA, Sajise AG. Characterizing the Saltol quantitative trait locus for salinity tolerance in rice. Rice, 2010; 3:148-160. DOI: 10.1007/s12284-010-9053-8
34. Hoang TML, Tran TT, Nguyen TKT. "Improvement of salinity stress tolerance in rice: challenges and opportunities," Agronomy, 2016; 6(4):54
35. Vu TTH, Le DD, Ismail AM, Le HH. "Markerassisted backcrossing (MA-C) for improved salinity tolerance in rice (Oryza sativa L.) to cope with climate change in Vietnam," Australian Journal of Crop Science, 2012; 6:1649-1654
36. Singh VK, Singh VK, Ellur RK, Krishnan SG, Singh AK. "Validation of rapid DNA extraction protocol and their effectiveness in marker assisted selection in crop plants," Indian Journal of Genetics and Plant Breeding. 2015; 75(1):110-113
37. Ho VT, Thomson MJ, Ismail AM. "Development of salt tolerant IR64 near isogenic lines through marker-assisted breeding," Journal of Crop Science and Biotechnology. 2016; 19(5):373-381
38. Ren ZH, Gao JP, Li LG. "A rice quantitative trait locus for salt tolerance encodes a sodium transporter," Nature Genetics. 2005; 37(10):1141-1146
39. Zhou P, Tan Y, He Y, Xu C, Zhang Q. Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. Theor. Appl. Genet. 2003; 106:326-331.