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Introgression of Saltol locus into rice variety Sreyas

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Abstract
Rice (*Oryza sativa* L.), one of the most important cereal crops, serves as the staple food for over one-third of the world's population. Susceptibility or tolerance of rice plants to high salinity is a co-ordinated action of multiple stress responsive genes. Introgressing the QTL for salt resistance Saltol into the high yielding variety Sreyas will improve its suitability for cultivation in salinity affected regions. Marker Assisted Backcross Breeding (MABB) can be effectively employed as it helps in eliminating the difficult field level procedure by early screening and identification of tolerant lines carrying the locus. Polymorphism assay of SSR markers was done to select the foreground markers associated with the QTL located on the short arm of chromosome 1 polymorphic between parents and genome wide markers for background selection. Hybridization of the variety Sreyas (MO-22) with the identified salt donor FL478, a source of Saltol QTL induces salt tolerance. F_1 plants were phenotyped *in vitro* and the survived plants under 12 dS m^{-1} (NaCl) were backcrossed with recurrent parent to develop BC_1F_1 generation. More than 200 BC_1F_1 plants were phenotyped *in vitro* and the survived plants under 12 dS m^{-1} (NaCl) were genotyped using polymerase chain reaction and polyacrylamide gel electrophoresis for heterozygosity of the markers linked to Saltol. The plants selected in foreground screening plants were genotyped for background homozygosity with recurrent parent and the BC_1F_1 plants with maximum homozygosity with recurrent parent will be selected and backcrossed with the respective recurrent parents to raise BC_2F_1 generation. The parental population was assayed for foreground polymorphism using 30 SSR markers of which 9 were found to be polymorphic. In case of background polymorphism, of the 200 SSR markers assayed 75 showed polymorphism. F_1 plants phenotyped *in vitro* and genotyped for heterozygosity regarding all the foreground markers. 12 BC_1F_1 plants were selected to be forwarded to next generations to develop the backcross inbred lines.

Keywords: Saltol, salinity tolerance, introgression, rice

Introduction
Rice (*Oryza sativa* L.), one of the most important cereal crops, serves as the staple food for over one-third of the world's population. Salt tolerance is an important constraint for rice which is categorized as a typical glycophyte. However, the productivity of rice is greatly affected due to soil salinity which is the second most widespread stress next to drought in rice growing areas of the world. Susceptibility or tolerance of rice plants to high salinity is a co-ordinated action of multiple stress responsive genes which also interact with other components of stress signal transduction pathways. Introgressing the QTL for salt resistance Saltol (Gregorio *et al.* 2002) [3] into the high yielding variety Sreyas will improve its suitability for cultivation in salinity affected regions. Marker Assisted Backcross Breeding (MABB) can be effectively employed as it helps in eliminating the difficult field level procedure by early screening and identification of tolerant lines carrying the locus. The objective of this research was to introgress QTL Saltol into rice variety Sreyas using identified donor FL478 through MABB.

Materials and Methods
The study was conducted at Rice Research Station, Vytilla during Rabi, 2021-2022. The high yielding variety Sreyas (MO-22) was hybridized with the donor FL478 (Chattopadhyay *et al*, 2014), a source of Saltol QTL to induces salt tolerance. The initial MABC lines for Saltol were developed using FL478 as the donor, due to its high level of tolerance, but without the tallness, photoperiod sensitivity, and late flowering of the original Pokkali landrace. F_1 plants were phenotyped *in vitro* and the survived plants under 12 dS m^{-1} (NaCl) were backcrossed with recurrent parent to develop BC_1F_1 generation. The screening technique used is based on the ability of seedlings to grow in salinized nutrient solution (Gregorio *et al.* 1997, Yoshida *et al*. 1976) [2, 10].

Molecular screening was done by extracting DNA from young leaves of 21 days old rice plant (Doyle and Doyle, 1987) [1]. Genotypic screening included polymorphism assay of SSR markers to select the foreground markers associated with the QTL located on the short arm of chromosome 1 polymorphic between parents. To facilitate background selection genome wide polymorphism assay using SSR markers was done. More than 200 BC_1F_1 plants were phenotyped *in vitro* and the survived plants under 12 dS m^{-1} (NaCl) were genotyped using polymerase chain reaction and polyacrylamide gel electrophoresis for heterozygosity of the markers linked to Saltol. The selected plants will be genotyped for background homozygosity with recurrent parent and the BC_1F_1 plants with maximum homozygosity with recurrent parent will be selected to be backcrossed with the respective recurrent parents to raise BC_2F_1 generation. The breeding scheme is as shown in Fig.1.

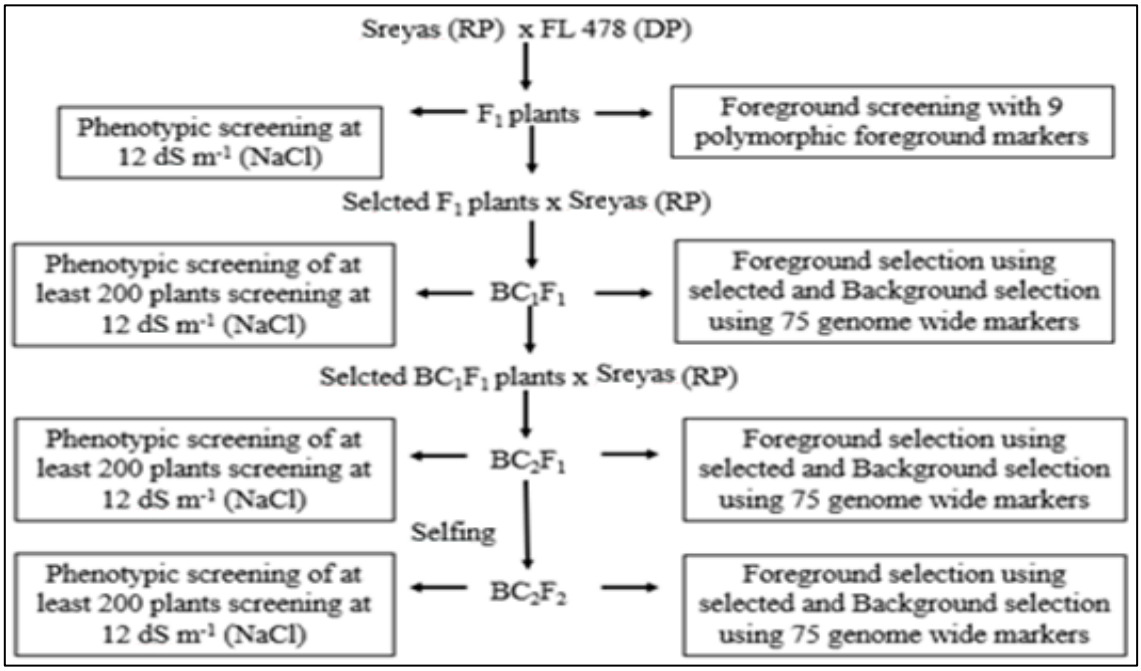

Fig 1: Breeding scheme for marker Assisted Backcross Breeding

Results and Discussion

The parental population was assayed for foreground polymorphism using 30 SSR markers linked to Saltol QTL located on chromosome 1 and genome wide background polymorphism, of the 200 SSR markers. The results obtained were as shown in Table 1.

Table 1: Parental Polymorphism Percentage between Sreyas and FL-478

	Total Markers Screened	Polymorphic Markers	Parental Polymorphism Percentage
Foreground	30	9	30.0
Background	200	75	37.5

Foreground selection revealed the 9 polymorphic markers (Table 2). Thomson *et al.* (2010) [11] stated the best markers within the Saltol QTL region were AP3206, RM8094, and RM3412, the most useful markers flanking the Saltol region were RM1287 and RM10694 (telomeric to Saltol) and RM493 and RM10793 (centromeric to Saltol). Hence the markers polymorphic are highly relevant in foreground selection.

Table 2: Foreground polymorphic markers

Primer Name	Ta (ºC)	Map position
RM1287	58	10.8
RM10701	62	11
RM10711	62	11.2
RM10713	65	11.2
RM8094	55	11.2
RM10720	55	11.3
RM3412	55	11.5
RM493	55	12.3
RM10825	60	13.3

The parents were hybridized and F_1 population was obtained. 150 F_1 plants were phenotyped *in vitro* and 11 plants survived under 12 dS m^{-1} (NaCl) were subjected to genotyping. Salinity stress at early seedling stage manifest on the first leaf, followed by the second and finally on the growing leaf. Salinity suppresses leaf elongation and formation of new leaves (Rajendran *et al*, 2009) [4]. 150 F_1 plants were phenotyped *in vitro* and 11 plants survived under 12 dS m^{-1}

(NaCl) were subjected to genotyping (Fig. 2, 3, 4). All the F_1 plants were found to be heterozygous for all the 9 foreground markers (Fig.5). The plants were backcrossed with the recurrent parent Sreyas.

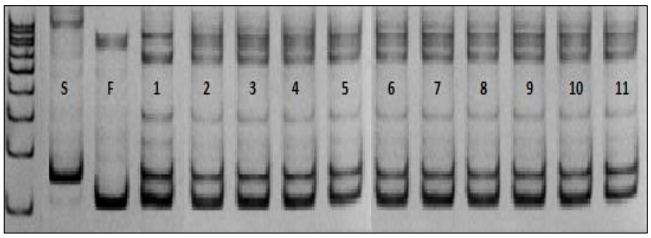

Fig 2: PAGE image of RM1287

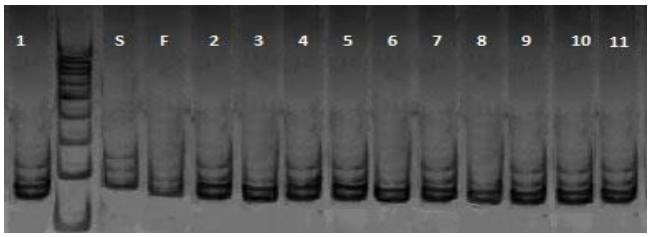

Fig 3: PAGE image of RM10711

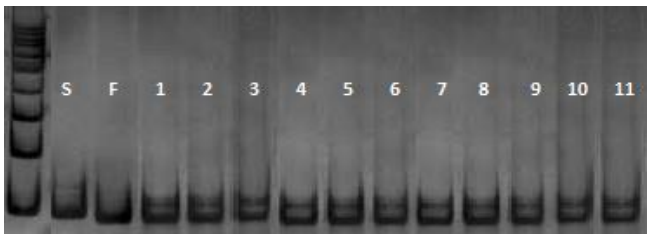

Fig 4: PAGE image of RM10825

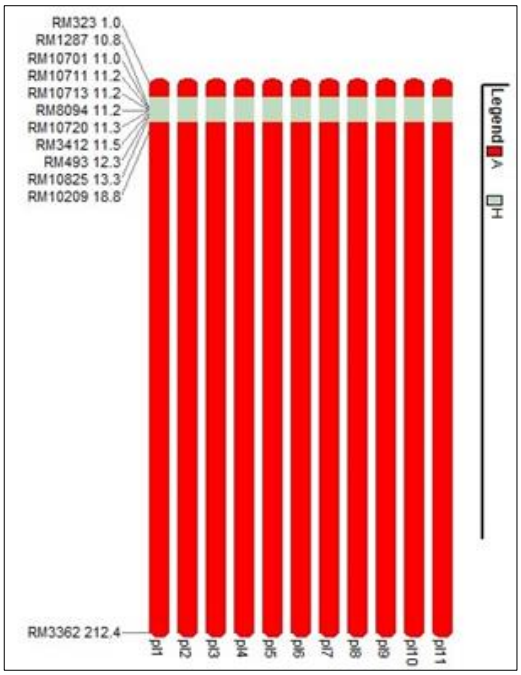

Fig 5: GGT image of F_1 plants heterozygous at Saltol locus

200 BC_1F_1 plants were phenotyped *in vitro* and the 12 plants survived under 12 dS m^{-1} (NaCl) have been selected for foreground and background genotypic screening. Salinity stress (Reddy *et al*., 2017) [5] affects seed germination, seedling growth, leaf size, shoot growth, shoot and root length, shoot dry weight, shoot fresh weight, number of tillers per plant, flowering stage, spikelet number, percent of sterile florets and productivity.

In the life cycle of rice, germination and tillering stages show comparative tolerance while seedling and reproductive stages are susceptible (Sajid *et al*, 2017, Singh and Flowers, 2010) [7, 8]. The Saltol QTL providing seedling stage salinity tolerance was successfully introgressed into an elite variety Jyothi of Kerala through MAS and released for cultivation in Kerala as 'Jyotsna' (Rohini and Shylaraj, 2017) [6].

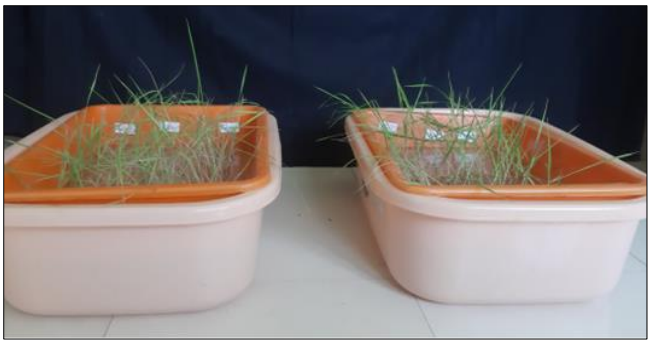

Fig 3: *In vitro* salinity of BC_1F_1 at 12 ds m^{-1} (NaC1) at 10 days of salinization

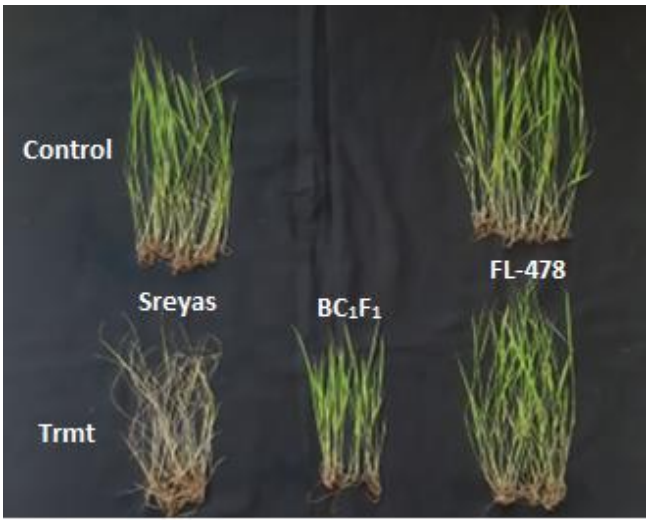

Fig 4: BC_1F_1 plants at 16 days after salinization

Conclusion

The search for salt tolerant lines have led to identification of large variability and numerous accessions. Saltol has been mapped on chromosome 1 in an F_8 Recombinant Inbred Line (RIL) population obtained by a cross between Pokkali (salt tolerant) and IR 29 (salt susceptible) which was successfully used in IRRI breeding programme for salt tolerance (Waziri *et al*, 2016) [9]. The ability of Pokkali, the land race of Kerala was transferred to FL478 in the background of IR-29, a high yielding variety. FL478 is identified as a donor for salt tolerance derived originally from Pokkali. The Saltol locus has been successfully transferred to the backcross progeny of Sreyas and FL478 which showed considerable salinity tolerance at seedling stage with Saltol locus in heterozygous state.

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