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Research Article

Development of sodicity tolerant rice varieties through marker assisted backcross breeding

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Abstract
The 'Saltol' QTL, responsible for salt tolerance at seedling stage of rice was successfully introgressed into ADT 43, a salt sensitive but a popular commercial rice variety of favorable irrigated rice ecosystem of Tamil Nadu, through Marker Assisted Backcross Breeding (MABB) technique. The newly developed 'Saltol' lines were confirmed to be salt tolerant, high yielding and with good cooking quality traits. The importance of RM 3412, RM 8094 and SKC 2a was envisaged in diagnosing the sodiicty tolerance. Role of other QTLs like qSES and qCHL on chromosome 3, in conferring sodicity tolerance at seeding stage was also indicated.

Keywords: Rice, Saltol, QTL, introgression, MABB

Introduction

In India, about 6.73 m.ha area is salt affected and in Tamil Nadu, the major rice producing State of India, a total area of 4.97 lakh ha is affected either by salinity or sodicity (Mandal *et al*., 2010). In Tamil Nadu the area affected by sodicity is almost 75 per cent more than the area affected by salinity. Hence more attention is required for increasing agricultural productivity under sodality. Rice is the main crop in sodic soils of Tamil Nadu due to its ability to tolerate inundated water condition. Rice productivity in salt-affected areas is very low, <1.5t/ha, but can reasonably be raised by atleast 2t/ha (Ponnamperuma,1994), thereby providing food for more than 10 million of the poorest people living off these lands. High sodicity causes even 70 per cent yield reduction (Tiwari *et al*., 2016). Though, by way of application of gypsum the sodic soil could be reclaimed, the adoption rate of this technique is poor as it requires copious quantity of good quality water, good drainage facilities, more horizontal level of adoption, more cost and labour. Hence, growing salt tolerant rice varieties would be a sustainable way to increase rice yield under sodicity. In Tamil Nadu, four salt tolerant rice varieties have been released for commercial cultivation. However, the area under these varieties is relatively low because of the less preference in the market. Hence, farmers are pushed to grow salt sensitive fine grain rice varieties at the expense of grain yield. Under these circumstances, there is a dire need to develop salt tolerant rice varieties coupled with good grain quality to increase the overall rice production of Tamil Nadu. However, the success of development of sodicity tolerant rice varieties coupled with fine grain quality through conventional breeding approaches is relatively low due to the complex inheritance pattern of sodicity tolerance, difficulties in screening and linkage drag as reported by Jairia *et al*. (2009). On the other hand, with the recent advancements in molecular biology, a major QTL, the 'Saltol' QTL which explains about 64.3-80.2 % of the variability in shoot Na+/K+ ratio at seeding stage has been identified in the rice variety Pokkali, (Krishnendu Chattopadhyay *et al*., 2014) has been identified to be handy tool for the breeders to selectively introgress the salt tolerance in high yielding salt sensitive rice varieties. Pokkali is the most widely used salt tolerant donor in salinity rice breeding. This major QTL is located in the region of 10.5 to 12.5 Mb of short arm of chromosome 1 of Pokkali and flanked by 21 SSR markers (Niones, 2004). Thomson *et al*. (2010) identified the tightly linked SSR markers to selectively transfer this QTL into the desired genetic background by which the difficulties in screening for salt tolerance and linkage drag could be overcome. Since SSR markers have been found to be linked to some of the specific traits of interest and used as the tools of biotechnology it is possible to transfer valuable genes of salt tolerance in rice without linkage drag (Mackill *et al*., 2006).

A newly developed line, FL 478 derived from IR29/Pokkali is being used as a novel source of salinity tolerance at seedling stage in Marker Assisted Breeding programme to selectively introgress 'Saltol' QTL into high yielding but salt sensitive rice varieties (Vu *et al.*, 2012). Single feature polymorphism in the 'Saltol' region showed that FL 478 contained a 0.9 Mb fragment from a Pokkali accession at 10.6 to 11.5 Mb on chromosome 1 flanked by IR 29 allele (Kim *et al*.,

2009). This QTL has been introgressed from FL 478 into the background of different popular varieties (Lang *et al*., 2010, Hung *et al*., 2012, Vu *et al*., 2012) through Marker Assisted Back Cross Breeding. Lang *et al*. (2008) applied MAS for salt tolerance in rice variety OMCS 2000 which is widely cultivated in North Vietnam.

Rice is sensitive to sodicity at seedling stage as well as reproductive stage and these two are controlled by independent genes. In Tamil Nadu, sodicity injury is more experienced during seedling stage rather than reproductive stage as the latter stage usually coincides with monsoon period of main cropping season. Hence, rice varieties with sodicity tolerance at seedling stage are required to be developed.

So far made reports on 'Saltol' QTL are limited to salinity only and the effect of this QTL in conferring sodicity tolerance has not been studied much. However, the important sodicity tolerance parameter, Na/K homeostatis, is governed by this QTL. Keeping these points in view, the present study was conducted with an objective of selective introgression of 'Saltol' into a high yielding salt sensitive fine grain rice variety ADT 43 through Marker Assisted Backcross Breeding.

Materials and Methods

The variety ADT 43 (recurrent parent) released by Tamil Nadu Rice Research Institute, Aduthurai, was used as a female parent and FL 478 (developed by International Rice Research Institute, Philippines) was used as a male parent. The true hybrids were fixed based on genotyping with RM 10793 (**Fig 1**). The hybrids which showed heterozygosity for RM 10793 were used for further backcrossing.

The stabilized backcross progenies of BC_3F_4 and BC_4F_3 were developed. During the development of BC_nF_1 the recurrent parent was used as female parent and the respective 'Saltol' positive (heterozygous for foreground marker) BC_nF_1 plants with least donor alleles for the background markers were used as male plants in addition to the consideration of similarity of BC_nF_1 plant traits as that of the recurring parent. A total number of 149 BC_1F_1 seeds were produced and a total number of randomly selected ten plants were used for developing BC_2F_1. From 53 BC_2F_1 plants, thirteen 'Saltol' positive plants with 44 – 90 per cent homozygosity for the background markers were used for developing BC_3F_1 and further BC_4F_1 generations. By repeated selfings of selected BC_3F_1 and BC_4F_1, the BC_3F_4 and BC_4F_3 plants (NILs) were developed for further studies. A total number of 42 'Saltol' positive (homozygous for 'Saltol' markes) NILs with atleast 80 per cent background genome recovery were selected and studied for their seedling stage tolerance and *per se* grain yield was also recorded to compare the yielding potential of the NILs

The Exchangeable Sodium Per cent of the experiment plot was 35 and the pH was 9.5 and hence the soil was sodic and alkaline in nature. During Kharif, 2014, the raised beds were formed with 15 cm height and the pre-germinated seeds of 42 NILs were sown in three rows (1m length) in each of five replications adopting Randomised Block Design. The probable error due to soil heterogeneity was minimized because of randomization of entries and with five replications. In each row 30 shallow holes were made at equal distances and two seeds were sown per one hole. At two leaved stage one seedling was pulled out from each hill and only one seedling was maintained per hill. The seeds of salt tolerant check FL 478 and salt sensitive check ADT 43 were raised in between every five NILs. After 21 days the seedlings were scored for their tolerance to sodicity based on Modified Standard Evaluation System (Gregario *et al*., 1997) (**Table 1**). The mean of five replications was considered for the analysis.

The tolerant and moderately tolerant lines were later transplanted to the main field (ESP = 35, pH : 9.5) for yield evaluation at a spacing of 15 x 10 cm in six rows in three replications during 2013-2014 and 2014-2015. The grain yield of the entries was recorded at maturity the average performance during two years was considered for analysis. The per cent increase in grain yield over the tolerant local check variety TRY 2 and the 'Saltol' donor FL 478 was calculated. Cooking quality analysis was done as detailed by Juliano and Perez (1984).
Hi The Na and K were estimated in shoot and roots of 21 days old seedlings using the standard procedure described by Jackson (1973). The Na /K was derived from the values of estimated Na and K values

The total genomic DNA was isolated using Modified CTAB Mini Prep procedure (Doyle and Doyle, 1990) and was subjected to Polymerase Chain Reaction (Eppendorf Master cycler AG Germany). The amplified Polymerase Chain Reaction products were resolved in agarose (1.5%) gel electrophoresis and photographed using gel documentation system (Alpha Imager $^{TM.}$ 1200, Alpha InnoTech, CA, USA).

A total number of 25 markers of 'Saltol' QTL region was studied for their polymorphism and six were found to be highly polymorphic between two parents (**Table 2**). The peak marker RM 3412 (**Fig. 2**) along with five other linked markers located in the region of 11.2 to 11.7 Mb of 'Saltol' QTL was used for foreground selection. FL 478

was used as a reference halotype for foreground markers.

For background screening a total number of 300 primers were studied. The primers were selected in such a way that they are distributed throughout the particular chromosome with equal distance between markers. Out of 300 primers, 50 were found to be polymorphic and used for background genotyping. The background genome recovery was expressed as per cent of homozygous background markers. The polymorphic flanking markers above and below 'Saltol' QTL lying at 11.1 and 12.0 respectively were fixed as given in Table 2. To fix the recombinant lines, the polymorphic markers that flank the 'Saltol' QTL were used as detailed in **Table 3**.

Results and Discussion

The variety and FL 478 and 'Saltol' QTL have been reported to confer tolerance for salinity (Electrical Conductivity of more than 4.0) only and very meager reports are available about their reaction to sodicity (High Exchangeable Sodium Per cent) . However, the results of the present study clearly indicated that FL 478 is tolerant not only to salinity (high Electrical Conductivity) and also to sodicity (High Exchangeable Sodium Per cent). Hence, FL 478 could be used as a donor for sodicity tolerance also. Likewise, the SSR markers linked to 'Saltol' QTL could be used not only to discriminate the salinity tolerant lines and also the sodicity tolerant rice genotypes. The true F_1 plants confirmed through genotyping, were found to be moderately tolerant which may be due to the polygenic control as reported by Krishnendu Chattopadhyay *et al*. (2014) while Muthuvijayaragavan and Murugan (2017), Kannan and Ganesh (2016), Gopikannan and Ganesh (2014), Shanthi *et al*. (2011), Geetha *et al*. (2006) and Gregario and Senedhira (1993) have reported the role of both additive and nonadditive effects in conferring sodicity tolerance.

The introgression of specific region of 'Saltol' QTL of Chromosome 1 alone from FL 478 was ensured by effecting the genotypic selection for recombinant markers **(**recurrent parent halotype) RM 580 (10.5Mb) and RM 10793 (12.56Mb) (**Table 4)**. The genotypic selection for flanking markers (FL 478 halotype) RM 10711 (11.1 Mb) and Pect 4 (12.0 Mb) which are above and below the 'Saltol' QTL helped in confirming the introgression of 'Saltol' QTL.In general the results of foreground marker data analysis revealed that all the six polymorphic markers were the diagnostic markers for sodicity tolerance; however more importance of RM 3412, RM 8094 and SKC2a was realized. Islam *et al*. (2012) have reported that entries that had one of the FL 478 type allele for the locus RM 8094 were found to be either tolerant or moderately tolerant and Naresh Babu *et al*. (2014) have highlighted the importance of RM 3412 while Nejad *et al*., 2008 reported that

RM 8094 and RM 10745 consistently discriminated the salt genotypes of rice It was interesting to witness that a very few of the 'Saltol' positive plants were found to highly sensitive to sodicity which may be due to specific epistatic interaction with the background genome requiring further studies. Reversely, few of the 'Saltol' negative plants identified during early generations were found to be sodicity tolerant which may be due to the involvement of other QTLs from Pokkali origin in conferring sodicity tolerance at seedling stage as reported by Krishnendu Chattobahdyay *et al*. (2014) who emphasized the need for genome wide mapping to identify additional QTLs even from Pokkali. Another interesting observation of this study is that all the 'Saltol' lines were positive for RM 6329 present on chromosome 3 (halotype of FL 478) , the peak markers for the qSES 3 and qCHL3 related to Initial SES scoring and Leaf Chlorophyll content respectively as reported by Micheal *et al*. (2010) indicating the role of other QTLs of Pokkali in conferring seedling stage tolerance and also consideration of RM6329 for diagnosing sodicity tolerance and also using the level of chlorophyll content as one of the important criteria while selecting sodicity tolerant rice varieties besides Na/K. The values of shoot Na/K ranged from 0.40 (TR 13 077) to 5.0 (ADT 43). All the superior 'Saltol' lines were found to possess lower shoot and root Na/K when compared to the sensitive recurrent parent and similar type of results have been reported by Nejad *et al. (*2008). Some of the 'Saltol' entries (NILs) *viz*., TR 2013 069, 073, 077, 080 and 083 were found to show even lower values than that of tolerant check FL 478. In case of root Na/K, the values ranged from 0.96 (TR 13 – 077) to 5.81 (ADT 43) and the 'Saltol' entries *viz.*, TR 2013-083 and 077 showed lesser values than that of tolerant check for root Na / K also. These results clearly depicted the role of 'Saltol' QTL in maintaining Na/K homeostatis under sodicity. Ren *et al.* (2005) reported that eight QTLs were found to be responsible for the variation in their K and Na content, among which SKC 1 in 'Saltol' region on Chromosome 1 was distinguished as a major QTL for the K and Na shoot content. In the present study also, the SKC 'QTL' linked markers 'SKC1a, SKC 2a and SKC 10 were used as foreground markers; however, other markers RM 3412, RM 3412b, RM8094 and RM 10748 were also diagnostic for low Na/K. Micheal *et al*. (2010) have reported that the marker RM 8094 is the peak marker of the QTLs qSNC1 (Shoot Na^+ content) and qRKC 1(Root K^+ content of rice).

When the grain yield performance (**Table 5**) of the entries was assessed over two years of replicated yield trials, it was found that the 'Saltol' entry TR 13 083 registered 13.3 and 8.0 per cent increased yield over the local tolerant check variety, TRY2 and the donor FL 478 respectively. Another entry TR 13-069 also has recorded 10.7 and 5.4 per cent increased yield over the local check and the donor respectively. The results clearly confirmed the effectiveness of 'Saltol' QTL in conferring seedling stage sodicity tolerance in rice which in turn helped in better establishment of plants in sodic soils leading to better grain yield. Further the results of this present experiment strongly indicate the possibilities of development of sodicity tolerant fine grain rice varieties by selectively introgressing the 'Saltol' QTL in the desired background genome using Marker Assisted Backcross Breeding. Huang *et al*. (2012) have also reported about successful introgression of Saltol in BT 7 using SSR markers RM 493 and 3412b were efficient foreground selection through Marker Asssited Backcross Breeding.

When the cooking quality parameters (**Table 6**) were evaluated, for grain L/B except TR 13 077, all the test entries were of medium slender type which was the targeted grain type. All test entries have shown better LER than ADT 43 which is a desirable improvement. While considering the breadth of cooked rice TR 13 083,070 and 071 have been found to be superior over the recurrent parent. With respect to Alkali Spreading values the entries TR 13 071,067,070, 083 and 069 have been found to be better than the recurrent parent and TR 13 076 and 069 are matching the quality of the recurrent parent. Except the entry TR 13-077 all the entries have shown soft gel consistency. These results confirmed the possibilities of selective introgression of 'Saltol' QTL in the background genome of salt sensitive fine grain rice varieties through Marker Assisted Backcross Breeding without affecting the grain qualities of the recurrent parent. In some cases, the grain quality was enhanced than its recurrent parent which may be due to various types of interactions.

To conclude, through the results of the present study, the role of 'Saltol' QTL in conferring sodicity tolerance at seedling stage and feasibility of Marker Assisted in selection in sodicity breeding to introgress 'Saltol' QTL into salt sensitive rice varieties. Use of FL 478 specific SSR markers *viz*. RM 3412, RM 10748 and SKC 2a in a combined manner than singly, would yield better results in Marker Assisted Breeding programme aimed at sodicity tolerance. With critical observations, it was understood that instead of completely relying on the background genome recovery for selecting the plants for backcrossing more emphasis might be given to select the plants that have desirable morphological similarities as that of the recurrent parent while developing back cross progenies for getting fruitful results. It is also suggested that more studies on other QTLs like qSES and qCHL on chromosome 3 are required.

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Table 1. Modified SES for screening for salt tolerance

Observation	Phenotypic scores	Sodicity tolerance level
Normal growth, no leaf symptom	1	Highly tolerant
Nearly normal growth but leaf tips or few leaves whitish rolled	3	Tolerant
Growth severely retarded, most leaves rolled and only a few are elongating	5	Moderately tolerant
Complete cessation of growth , most leaves dry , some plants dying	7	sensitive
Almost all plants dead or dying	9	Highly sensitive

Table 2. Details of 'Saltol' linked SSR markers

S.No.	Polymorphic markers	Position (Mb)	Product size (Mb)	
			ADT 43	FL478
1.	RM8094	11.20	130	150
2.	SKC10	11.24	210	180
3.	SKC 1b	11.40	220	240
4.	SKC 2a	11.40	110	120
5.	RM3412b	11.45	140	120
6.	RM 3412	11.56	210	180

Table 3. Details of flanking markers

Flanking marker (above 'Saltol')	Position (Mb)	Product size (bp)		flanking marker (below 'Saltol')	Position (Mb)	Product size (bp)	
		ADT 43	FL 478			ADT43	FL478
10711	11.1	280	260	PECT4	12.0	90	100

Table 4. Details of recombinant markers

Upper recombinant marker	Position (Mb)	Product size(bp)		Lower Recombinant marker	Position(Mb)	Product size (bp)	
		ADT 43	FL 478			ADT 43	FL478
580	10.5	200	290	RM10793	12.56	150	130
1	5.05	180	160	RM10793	12.56	150	130

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Table 5. Grain yield performance of 'saltol' over two years

S.No	Entries	Grain yield (Kg/ha)			% increase over		Shoot Na/K	Root Na/K	SES score
		2013-2014	2014-2015	Mean	TRY 2	FL 478			
1	TR13-067-Saltol	3583	4885	4234	--	--	0.50	2.69	5
2	TR13-070-Saltol	5000	4595	4798	5.4	10.7	1.12	2.70	3
3	TR13-069- Saltol	4833	4990	4912	8.0	13.3	**0.56**	**2.81**	**3**
4	TR13-083- Saltol	5167	5160	5164	13.5	19.2	**0.40**	**0.88**	**3**
5	TR13-073- Saltol	2833	4600	3717	---	---	0.38	1.86	5
6	TR13-071- Saltol	4500	4350	4425	---	---	0.75	1.18	5
7	TR13-080 - Saltol	3750	4745	4248	---	2.1	0.40	5.75	5
8	TR13-079 - Saltol	3667	4750	4209	--	--	0.88	1.36	5
9	TR13-077- Saltol	4500	3625	4063	--	--	0.40	0.96	3
10	TR13-081- Saltol	3337	4250	3794	--	--	0.97	1.20	5
11	ADT 43 – Sensitive check	3083	3380	3232	--	---	5.0	5.81	9
12	FL 478 – 'Saltol'donor	4500	4600	4550	--	--	0.73	0.98	3
13	TRY 2 – local check	4167	4500	4334	--	--	1.26	1.82	5

Table 6: Evaluation of cooking quality traits

SI. No.	Entries	Un cooked rice Length (cm)	Cooked rice length (cm)	Linear Elongation Ratio (LER)	Un cooked rice Breadth (cm)	Cooked rice Breadth (cm)	Breadth wise elongation ratio (BER)	L/B ratio of un cooked rice	Alkali Spread Value (ASP)	Alkali digestion	Volume expansion (ml)	Gel consistency (cm)	Texture of cooked rice
1	TR 13-069-Saltol	6.0	10.4	1.7	2.1	2.7	1.3	2.9	intermediate	Low to intermediate	1:4.3	7.3	soft
2	TR 13-070 - Saltol	6.2	8.6	1.4	2.0	2.2	1.1	3.1	low	Low	1:4.1	6.5	soft
3	TR13-067-Saltol	6.1	9.0	1.5	1.9	2.5	1.3	3.2	low	Low	1:4.8	7.2	soft
4	TR13-071 - Saltol	6.1	8.4	1.4	2.0	2.3	1.2	3.05	low	Low	1:4.1	8.6	soft
5	TR 13-077 - Saltol	6.2	8.5	1.4	2.0	2.6	1.3	3.1	Low to intermediate	Intermediate	1:3.1	3.8	Flaky and hard
6	TR13-076 - Saltol	6.2	9.5	1.5	1.9	2.5	1.3	3.3	high	High	1:4.5	6.9	soft
7	TR13-083	5.8	8.5	1.5	2.1	2.2	1.0	2.3	low	Low	1:3.8	6.7	soft
8	TR 13-079	5.6	8.2	1.5	2.3	2.5	1.1	1.9	intermediate	Intermediate	1:4.7	7.0	soft
9	FL-478	7.4	11.0	1.5	2.2	3.0	1.4	3.4	Low to intermediate	Intermediate	1:4.6	3.8	Flaky and hard
9	ADT 43	6.0	8.9	1.5	1.9	2.5	1.3	3.2	low	Low to intermediate	1:4.6	9.0	soft
10	TRY 2 (local check)	6.9	10.3	1.49	2.2	3.0	1.36	2.54	high		4.1	10.6	soft

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Fig1. Genotyping hybrids with RM 10793

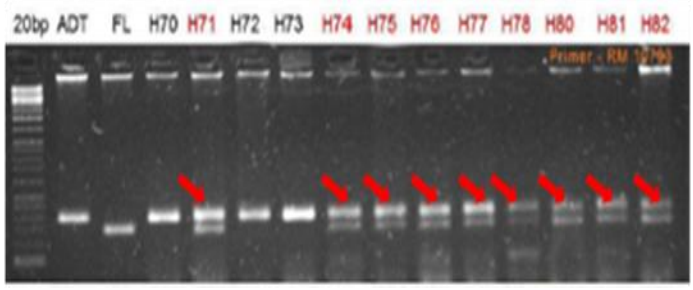

Fig 2. Fixing up of foreground markers