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Research Article

Validation of ‘Saltol’ QTL under sodicity

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ABSTRACT

Development of rice varieties with sodicity tolerance would help to boost the rice productivity in salt affected areas of Tamil Nadu. A major QTL ‘Saltol’ has been reported to be responsible for conferring salt tolerance at seedling stage in rice. The present study was conducted to validate the ‘Saltol’ QTL under sodicity and to identify the reliable SSR marker tightly linked to this QTL. The results confirmed that ‘Saltol’ QTL worked under sodicity. The SSR markers *viz*., RM 3412, RM 10748 and SKC 2a especially in a combined manner is suggested to be used for discriminating sodicity tolerance and sensitivity. Possibilities of role of newer QTLs of Pokkali origin in conferring sodicity tolerance at seedling stage was also indicated

Key words
Saltol, Sodicity, QTL and SSR marker

Introduction

In Tamil Nadu 3.55 lakh ha is affected by sodicity and 1.32 lakh ha is affected by salinity (Mandal *et al.,* 2010). The salt stress reduces the potential yield of rice by 40 to 100 per cent depending upon the intensity of the stress. Development of salt tolerant high yielding rice varieties would help to enhance the rice production in salt affected soils. However, the progress in breeding rice for salt tolerance is hindered by the complexity of the inheritance pattern of salt tolerance and difficulties in screening for salt tolerance. Due to linkage drag relatively less success has been met with the simultaneous improvement of rice grain quality and salt tolerance. On the other hand, with the recent advancements in molecular biology a major QTL, ‘Saltol’ in the rice variety Pokkali has been identified to account for 39.2, 43.9 and 43.2 per cent of phenotypic variation for three important salt tolerance associated traits *viz.,* Na+, K+ and Na/K absorption ratio respectively (Naresh Babu *et al* ., 2014). This QTL has been saturated with more than 20 microsatellite markers spanning through 10.7 to 12.2 Mb on the short arm of chromosome number 1. Thomson *et al* (2007) 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. 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 introgress ‘Saltol’ QTL into high yielding but salt sensitive rice varieties (Vu *et al.*, 2012). Markers validation on independent population of different genetic background is essential in determining the effectiveness and reliability of the markers to predict phenotyping (Lin, *et al*.. 1998). 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. Keeping this point in view, the present study was conducted to validate the ‘Saltol’ QTL under sodicity and to identify the most reliable SSR markers linked to ‘Saltol’ QTL to be used in Marker Assisted Breeding programme.

Materials and Methods

Plant Material: The Near Isogenic Lines of ADT 43 (salt sensitive) were developed by initial single cross hybridization between ADT 43 and FL 478 followed by three backcrosses which were later advanced to three selfed generations. From a pool of 250 NILs, a total number of eighty five NILs was picked at random and used for the present study.

Screening for salt tolerance during seedling stage : The field selected for phenotyping was sodic and alkaline in nature. The ESP of the soil was 35 and the pH was 9.5. During Kharif, 2014, the raised beds were formed with 15 cm height and the pre germinated seeds of eighty five NILs were sown in three rows (1m length) in each of three replications adopting Randomised Block Design In each row 30 shallow holes were made at equal distances and two seeds were sown per 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 SES system (Gregario *et al.,* 1997) (Table 1)

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

Six polymorphic SSR markers tightly linked to ‘Saltol’ markers (Table 2) located in 11.2 to 11.7 Mb of ‘Saltol’ QTL were used for the present study. FL 478 halotype was used as the reference halotype.

Results and Discussion

In phenotypic scoring, none of the entries was found to be highly tolerant under sodicity. The ‘Saltol’ QTL donor FL 478 obtained a score of ‘3’ (tolerant) and ADT 43 got the score of ‘9’(Highly sensitive) under sodicity. Out of eighty five lines, eighteen were tolerant and 34 were moderately tolerant and rest of them were either sensitive or highly sensitive. These results indicated the role of ‘Saltol’ QTL in conferring sodicity tolerance.

When the phenotypic scoring and marker data were compared (Table 3) it was found that 33.8, 25.8, 11.3, 17.7, 9.6 and 3.0 per cent of the tolerant / moderately tolerant lines were showing FL 478 halotype for the markers RM 3412, 10748, RM 8094, SKC 2a, SKC 1 and SKC10 respectively indicating the relatively more reliability of RM 3412, RM 10748 and SKC2a compared to other markers. Sabouri *et al* (2013) have reported about the significance of RM 3412 in determining salt tolerance while Mohammedi – Nejad *et al* (2010) have also reported that RM 3412 and RM 10745 are very important for Marker Assisted Selection. Krishnendu Chattobadhyay *et al* (2014) have highlighted the significance of RM 10772. Thomson *et al* (2010) reported that the presence of different ‘Pokkali’ alleles in the ‘Saltol’ region between 11.0 Mb and 12.2 Mb and suggested that the ‘Saltol’ is controlled by the same gene that controls the SKC 1 QTL located at 11.46 Mb which was first detected in Nona Bokra (Ren *et al.*, 2005).

Sixteen out of eighteen tolerant lines (tolerance score 3) had a combination of two or three markers especially RM 3412, RM 10748 and SKC 2a that were specific to FL 478 in ‘Saltol’ region. Hence, it is suggested that instead of depending on a single marker data it is suggested to go in for a combination of more than one marker for discriminating salt tolerance and sensitivity. Naresh Babu *et. al.*, (2014) have also reported similar findings.

On the other hand, some of the salt sensitive entries also had FL 478 halotype of the studied markers in ‘Saltol’ region which may be due to specific epistatic interaction with background genome. Interestingly, there were few lines which were not having FL 478 specific markers but were tolerant indicating the role of some other QTLs from the origin of Pokkali were also involved in conferring salt tolerance during seedling stage as suggested by Krishnendu Chattobahdya *et al.* (2014) who emphasized the need for genome wide mapping to identify additional QTLs even from Pokkali.

The results of the present study clearly indicates the role of ‘Saltol’ QTL in conferring sodicity tolerance at seedling stage and feasibility of Marker Assisted in selection in salinity 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.

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Table 1. Modified SES for screening for salt tolerance

Observation	Phenotypic scores	Salt 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.	**RM 3412**	11.56	210	180
2.	**RM8094**	11.20	130	150
3.	**SKC 1b**	11.40	220	240
4.	**SKC 2a**	11.40	110	120
5.	**SKC10**	11.24	210	180
6.	**RM 10748**	11.70	100	90

Table 3. Comparison of phenotypic scoring data and marker data

Tolerance level	Number of FL 478 halotype					
	RM 3412	RM 10748	RM 8094	SKC 2a	SKC1	SKC10
Highly tolerant	0	0	0	0	0	0
Tolerant	5	4	2	3	2	2
Moderately tolerant	16	12	5	8	3	0
Sensitive	2	4	2	1	1	1
Highly sensitive	3	1	2	3	2	1