OPENING MINDS:
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DEVELOPMENT

Mapping and Functional Validation of a Quantitative Trait Loci (QTL) For Salt Tolerance in a Sri Lankan Rice Cultivar

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1 INTRODUCTION

Soil salinity is identified as the second most widespread problem next to drought in rice growing areas worldwide (Waziri *et al.,* 2016). With the increase of world population and reduction of agricultural lands due to industrialization, there is an urgent requirement to utilize salinity affected land to fulfil food requirement in near future (Turan *et al.,* 2012, Ray and Islam, 2008). When considering the Sri Lankan context, Sirisena *et al.,* (2010) have shown that rice production is limited due to soil salinity, both in coastal and inland rice growing areas.

Rice is identified as a salt-responsive crop (Moradi and Ismail, 2007) affected by salinity at varying degrees during different growth stages. It is highly susceptible to salinity at seedling and reproductive stages while more tolerant at germination stage (Gupta and Huang, 2014, Moradi and Ismail, 2007, Zeng and Shannon, 2000).

Salinity stress tolerance in plants is a complex trait. It is determined by the cumulative effect of different mechanisms governed by many genes or quantitative trait loci (Horie *et al*., 2012, Turan *et al.*, 2012). By identifying the QTLs or genes accounting for salinity, they can be utilized to generate improved plants by breeding techniques (Turan *et al*., 2012, Zeng and Shannon, 2000).

A previous study conducted at International Rice Reseach Institute (IRRI) in 2014 identified many promising QTLs leading to salinity tolerance by phenotypic assessment of bi-parental mapping population of At354 (salinity tolerant) and Bg 352 (salinity susceptible) cross. However, usually phenotype and genotype associations vary in different environments and therefore, it is necessary to validate the effects of QTLs in other environments. In view of this we conducted a phenotypic assessment with selected 94 Recombinant Inbred Lines (RILs) of At354 and Bg352 mapping population which had previously genotyped with SNP markers, in Sri Lankan environment in 2017. The present study explains the results obtained from the mapping of Chromosome 3 in Sri Lankan environment in comparison to the QTL hotspot identified in IRRI.

2 METHODOLOGY

2.1 Experimental site and plant material

Previously identified 94 extreme RILs (47 RILs of extremely salinity tolerant and another 47 RILs of extremely salinity susceptible) along with At354 and Bg352 parental lines were grown in a hydroponics system according to the protocol described by Gregorio *et al*., (1997) at the plant house which had 100%

sunlight penetration, Faculty of Agriculture and Plantation Management, Makandura. Experimental setup was established according to the randomized complete block design. Two blocks were used with four individual plants from each line per block representing four replicates per RIL.

2.2 Phenotypic assessment under salinity stress

Initially, the salinity of the nutrient solution was adjusted to 6 dSm^{-1} of electrical conductivity (EC) by adding appropriate amount of analytical grade NaCl. After 2 days, salinity was increased up to 12 dSm^{-1} (100 mM) of EC and the condition was maintained for 21 days for screening. The pH of the nutrient solution was monitored at 5.0 every other day while continuing the higher EC level. The seedlings were assessed using salt responsive morpho-physiological indices viz., salinity survival index (SSI), shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW).

2.3 QTL Analysis

QTL analysis was carried out by composite interval mapping (CIM) approach with Windows QTL cartographer v2.5_011 software using the SNP marker genotpes which were obtained from our previous study, Gimhani *et al*., (2016). Permutation test (500 times) was carried out for each trait to establish a LOD (Logarithm of Odds) threshold value at 0.05 significance level. It was assumed that one million bases on a rice chromosome are equivalent to approximately 3.92 cM, for the estimation of genetic distances between markers for QTL mapping. The physical position of SNP markers of Nipponbare genome in Mb was multiplied by a factor of 4 to approximately estimate the marker distances in centi Morgan (cM).

3 RESULTS AND DISCUSSION

Five salinity responsive morpho-physiological traits were analyzed to detect QTLs on chromosome 3. Present findings revealed one putative QTL for a prominant growth related parameter; shoot dry weight (SDW) exceeding the experimental-wise LOD threshold (Figure 1).

The QTL identified for SDW was localized within the flanking region of 34.1-34.7 Mb (136.5-138.7 cM) with its peak positioning at 34.5 Mb (Table 1). According to the QTL mapping study conducted by Gimhani *et al*., 2016 a promising QTL hotspot has been identified within approximately same flanking region of 34.9-35.8 Mb responsible for five traits viz., SL (*qSL3*), Shoot fresh weight (*qSFW*3), SDW (*qSDW3*), Shoot Na^+ concentration (*qSNC3*) and shoot Na^+/K^+ ratio (*qSNK3*). Interestingly, the QTL identified in the current study for SDW is with close proximity to this QTL hotspot. This result indicated that the QTL *qSDW3*, would be a promising QTL as is validated in two environments, IRRI and Sri Lanka in years 2014 and 2017 respectively. Slight deviation in QTL locations could be raised due to different handling status in two locations and differences in experimental settings at IRRI and Sri Lanka.

Present QTL had a LOD score of 7.66 indicating tight linkage of the QTL with the respective trait. It explained phenotypic variation of 53.5% which is comparatively a higher value while showing a negative additive effect indicating the contribution of the Bg352 allele for increasing the shoot dry weight in favour of salinity tolerance. But it was noted that the salinity tolerant allele donor of SDW in Gimhani *et al.,* 2016 was At354.

The QTL positions were compared with the physical location of markers linked

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with QTLs based on Nipponbare genome and accordingly, the flanking SNP markers of the QTL detected in this study were namely 3501392 and 3522453. There could be slight variations in the physical position of flanking markers in bp wise, since an *indica* mapping population was used in the study.

According to past studies, Sabouri and Sabouri (2008) have identified a QTL for Na^+/K^+ ratio flanking 22.4-36.1 Mb on chromosome 3. In addition Champoux *et al.,* (1995) have identified a QTL associated with drought tolerance within 22.7-35.8 Mb region. The QTL identified in the current study was also embedded within the given regions. Therefore, the QTL for SDW detected on chromosome 3 could be a prominent one and this region might contain genes responsible for regulating salinity stress.

Table 1: Description of the QTL detected for SDW under salinity stress in Sri Lanka.

Feature	Value / name
LOD score	7.66
Chromosome	3
Peak position	137.9 cM (34.5 Mb)
Flanking region	136.5 – 138.7cM
	34.1-34.7 Mb
Flanking SNP markers	3501392 - 3522453
R^2	53.5%
Additive effect	- 0.3053
Salt tolerant allele donor	Bg 352

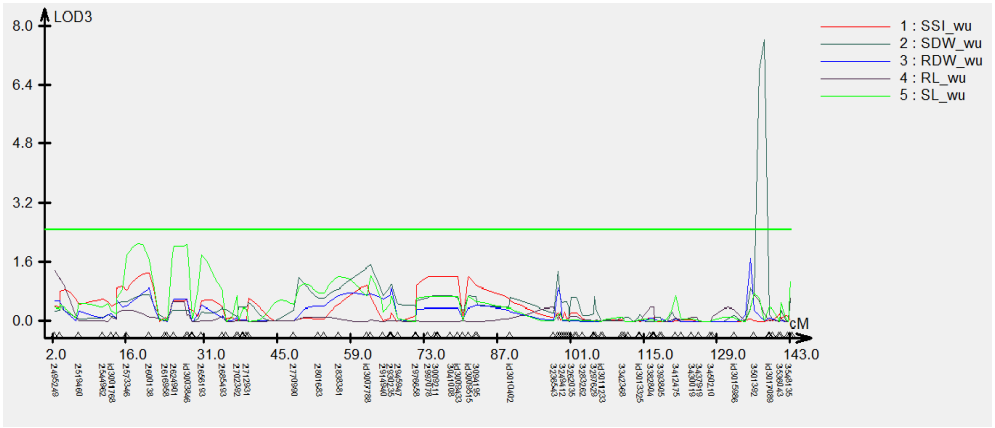

Figure 1: QTL map of chromosome 3 drawn by QTL cartographer v2.5_011 software.

4 CONCLUSIONS

It can be concluded that qtl identified for sdw in the present study validates the previously identified qtl for sdw by gimhani *et al*., 2016. In addition, it is a promising qtl, since qtls for different traits are also being previously reported in the similar region. Therefore, current region which is responsible for different salinity responsive traits could be used for introgression as a unit into elite rice varieties to improve salt tolerant lines followed by breeding

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