Available online at www.sciencedirect.com

SciVerse ScienceDirect

Procedia Environmental Sciences 8 (2011) 389 – 395

Procedia Environmental Sciences

ICESB 2011: 25-26 November 2011, Maldives

Identification of QTLs for Morph-Physiological Traits Related to Salinity Tolerance at Seedling Stage in *Indica* Rice

Muhammad Arshad Javed*, Fahrul Zaman Huyop, Alina Wagiran & Faeza Mohd Salleh

Faculty of Biosciences and Bioengineering, University Technology Malaysia, 81310 UTM Johor Bahru, Johor Malyasia.

Abstract

This study was carried out to identify the QTLs, associated with salinity tolerance at seedling stage, in *indica* rice using. $F_{2:3}$ populations. QTL mapping was performed using microsatellite markers. Twelve linkage groups, covering a total size of 1648.9 cM, with an average interval distance 15.2 cM, were made. A total of 22 QTLs were identified, 9 and 13 for four morphological and six physiological traits, respectively. Correlations between salt injury score (SIS) and physiological traits reflected that damage of leaves was attributed to accumulation of Na^+ in shoot by transport of Na^+ from root to shoot in external high concentration. Microsatellite locus, RM 80 on chromosome 8, was found to be associated with SIS, Na^+, K^+ and Na:K in shoot. The correlated traits were found to be associated with the same locus or very close on the same chromosomal region. The QTLs detected for Na^+, K^+ and Na:K in the shoots and the roots did not share the same map locations, suggesting that the genes controlling the transport of Na^+ and K^+ between the shoots and the roots may be different.

Keywords: Quantitative trait loci, microsatellite marker, salt injury score (SIS)

1. Introduction

Rice is considered very sensitive to salinity and response varies with the growth stages. Germination, active tillering and maturity stages are considered to be less susceptible to salinity than seedling stage, early reproductive stage, pollinations and insemination stage (Fageria, 1985; Khatun and Flowers, 1995).

* Corresponding author. Tel.: + (6) 07-5532713; fax: +(6)07-5531112.
E-mail address: majaved@fbb.utm.my.

doi:10.1016/j.proenv.2011.10.061

A significant progress was observed in germplasm improvement (Gregorio et al., 1997) and genetic studies of salinity tolerance in rice (Gregorio and Senadhira, 1993; Garcia et al., 1995; Lee et al., 1996). It is reported that traits related to salinity tolerance were highly influenced by environmental variations (Asch et al., 1997; Boshani et al., 2003). Thus, the most efficient and precise way to identify the genetic background of such a complex trait is to use the molecular marker techniques. Present studies were designed to identify the quantitative trait loci associated with salinity tolerance in indica rice at seedling stage.

2. Materials and methods

2.1. Plant materials

$F_{2:3}$ populations were derived from a cross between Pokkali, an indica rice cultivar possessing high salinity tolerance, and Shaheen Basmati, an indica fine rice variety susceptible to salinity. One hundred and ninety F_3 lines were developed, each line originating from a different F_2 plant.

2.2. Trait evaluations

The phenotyping of parental cultivars and F_3 lines (10 seedlings of each line) was conducted in phytotron. The photoperiod was set for 14 hours and light intensity was 45 µE $m^{-2}s^{-1}$. The salinity stress was continued until the sensitive parental cultivar, Shaheen Basmati, exhibited the significant differences from the salt tolerant cultivar, Pokkali. When seedlings were about five-leaf stage, a salinity of an electrical conductivity (EC) 10 dS/m was imposed (Afza et al., 1999). The culture solution was changed every five days and medium pH was adjusted to 5.8 on an alternate day. After two weeks, salinity stress was stopped and SIS (salt injury score) was assessed as suggested by Gregorio and Senadhira (1995) and described in Table 1. The morphological traits, seedling height and fresh shoot weight, was recorded also. The shoot and root parts were separated; roots were washed in tap water thoroughly. In order to measure the sodium and potassium ionic concentrations, the shoots and roots were kept in forced draft oven at 70 ºC for 72 hours to dry the samples properly. After measuring the dry shoot and root weights, oven-dried samples were used to determine the physiological parameters, sodium and potassium contents, by using "Inductively Coupled Plasma Spectrometer" (ICPS-1000 III Shimadzu) and the concentration of each element was calculated according to its dry weight.

2.3. SSR molecular linkage map and QTL analyses

One hundred and ninty plants of F2 population were employed in the construction of SSR molecular linkage map. DNA extraction was carried out as described by Ikeda et al. (2001). PCR was performed in 50µl reaction containing 5 µl of extracted DNA, 0.2 µM of each primer, 100 µM of each dNTP (dATP, dCTP, dGTP and dTTP), 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 % Tritionx-100, and 1 unit *Taq* DNA polymerase (TOYOBO, Japan). Amplifications were carried out in a Thermal Cycler MP (TAKARA) and PTC100 Programmable Thermal Controller (MJ Research Inc., USA) as follows:

94 ºC for 5 minutes followed by 35 cycles of 94 ºC for 1 minute, 55 ºC for 1 minute, 72 ºC for two minutes and ending with 5 minutes at 72 ºC for the final extension.

In this study 107 primer pairs, which were designed to detect 108 microsatellites loci, were used to amplify the nuclear microsatellite regions. The amplified products, containing microsatellite regions, were electrophoreses in 4 % polyacrylamide denaturing gel with 0.5X TBE buffer. The visualization of microsatellite banding patterns was carried out by non-radioactive silver staining method as prescribed by

Panaud et al. (1996). The molecular linkage map was constructed by multipoint analysis (Lander and Green, 1987), using the program MapMaker v. 2.0 (Lander et al., 1987), based on the genotype data of F_2 population. Map distances between the microsatellite loci were presented in centiMorgan (cM), using the Kosambi function (Kosambi, 1944). Marker order of microsatellites was followed after Temnykh et al. (2001).

Statistical analyses were performed using qGene (Nelson, 1997). QTL mapping was conducted on the basis of F_2 data by regression of trait performance on marker genotype using standard analysis of variance (ANOVA) procedures. A QTL was assumed to be associated with a marker locus at a significance of $P< 0.01$.

3. Results

Using 304 microsatellite markers, a polymorphism survey was conducted to assess the polymorphism between the parental cultivars, Pokkali and Shaheen Basmati. Good amplified band patterns were observed for 157 (51.64%) microsatellite markers. To map the segregation of F_2 population, 107 pairs of microsatellite markers were employed to amplify 108 microsatellite loci, distributed throughout the entire genome. These were used as framework markers for map construction. The total map size and the average distance interval between markers were1648.9 cM and 15.2 cM, respectively.

Correlations studies revealed that FSW and DSW are negative correlations with NS. SIS exhibited maximum correlation with NS (r = 0.822). A correlation between NR and KR is positive and highly significant (r = 0.741), whereas NS and KS showed a negative correlation (r = 0.401). NS showed a high significant correlation with NKS (r = 0.978) while correlation between NR and NKR was non-significant (r = -0.133). KS with NKS and KR with NKR showed negative correlations (r = -0.533 and r = -0.633, respectively).

QTL analyses were carried out by qGene program. In total, 22 QTLs were identified by single marker analysis (Fig. 1). One QTL for seedling height was detected on chromosome 7. The phenotypic variance was observed 5.51%. The probability of association of QTL with microsatellite locus was 0.0053. The Pokkali alleles at this locus showed increased effect for this trait. Three QTLs were detected for fresh shoot weight, two on chromosome 7 and one on chromosome 11. The phenotypic variance was ranged from 5.27 to 5.91 %. Pokkali showed increased effects at these microsatellite loci. The association probabilities of QTLs with microsatellite loci RM18 and RM336 (chromosome 7), and RM 287 (chromosome 11) were 0.0035, 0.0065 and 0.0072, respectively. For dry shoot weight, three QTLs were detected, two on chromosome 1 and one on chromosome 7. The phenotypic variance was ranged from 4.89 to 5.41 %. Pokkali alleles at all the detected QTLs resulted in increased dry shoot weight. The association probabilities of QTLs with microsatellite loci RM431 and RM246 (chromosome 1), and RM 481 (chromosome 7) were 0.0055, 0.0095 and 0.0092, respectively.

For sodium contents in shoot, two QTLs were identified on chromosomes 6 and 8. Both QTLs for this trait showed increased effects from Shaheen Basmati. This trait showed phenotypic variance ranged from 7.82-8.31 %. The association probabilities of QTLs with microsatellite loci RM541 and RM80 were 0.0007 and 0.0004, respectively. Two QTLs significantly influenced the potassium contents in shoot, on chromosomes 3 and 8. The phenotypic variance for this trait ranged from 5.47 to 7.82 %. The increased effects for potassium contents in shoot were observed by Shaheen Basmati allele on chromosome 3 and by Pokkali allele on chromosome 8. These QTLs showed high association probabilities (0.0058 and 0.0006, respectively) with detected microstatellite loci RM545 and RM80 on chromosomes 3 and 8. For this trait, two QTLs were identified on chromosomes 6 and 8. Both QTLs for this trait showed increased effects from Shaheen Basmati. This trait showed phenotypic variance ranged from 5.68 to 8.79. The association probabilities of QTLs with microsatellite loci RM541 and RM80 were 0.0055 and 0.0002,

respectively.

For sodium contents in shoot, five QTLs were detected (two on chromosome 1 and one each on chromosomes 3, 4, and 6). The phenotypic variance was ranged from 5.63 to 8.91 %. Three Pokkali and two Shaheen Basmati alleles showed increased sodium contents in root. The association probabilities of QTLs with six microsatellite loci (RM212, RM428, RM135, RM241 and RM541) ranged from 0.0090 to 0.0005. One QTL for potassium contents in root was detected on chromosome 9. The phenotypic variance was observed 10.29 %. The probability of association of QTL with microsatellite locus, RM242, was 0.0001. The increased effect for this trait was observed by Shaheen basmati allele. The detected QTL for sodium potassium ratio in root were two on chromosome 9. The phenotypic variance was ranged from 5.37 to 10.55 %. The probabilities of association of QTL with microsatellite loci, RM242 and RM201, were 0.0001and 0.0097, respectively. The increased effects for this trait were shown by Shaheen Basmati and Pokkali alleles on microsatellite loci RM242 and RM201, respectively.

For salt injury score, three QTLs were identified on chromosomes 3, 6 and 8. The phenotypic variance was ranged from 4.97 to 9.34 %. The probability of association of QTL with microsatellite loci, RM7, RM541 and RM80 were 0.0085, 0.0006 and 0.0001, respectively. Pokkali allele at locus RM7 showed increased, whereas Shaheen Basmati allele at RM541 and RM80 gave increased effects.

4. Discussion

Among the abiotic stresses, the effects of salinity are more severe on rice production, particularly in highly productive irrigated lands. Recently, not sufficient but limited progress has been reported for the genetic characterization of this very complex quantitative trait (Flowers et al., 2000; Koyama et al., 2001; Prasad et al., 2000; Lin et al., 2004; Yao et al., 2005). It is hard to compare the results due to the differences of plant materials, method of phenotying and genotyping employed in these studies.

QTLs for traits correlated were often mapped in the same chromosomal regions (Vedboom et al. 1994; Lin et al., 2004). A similar trend was observed in present study. A highly significant correlation was observed between salt injury score and sodium contents in shoot. A microsatellite locus, RM80 on chromosome 8 was detected for these two traits. Similarly, sodium contents in shoot and sodium potassium ratio in shoot were found highly correlated traits and same QTL was detected on chromosome 8 for these two traits as well. Potassium contents and, sodium potassium contents ratios in root were significantly correlated. A QTL region on chromosome 9 was detected for these two traits. These results supported the fact that the trait correlation may be attributed to the effects of pleiotropy or to the very close linkage of genes.

The SIS is a criterion that measured salt tolerance of the plant. However, SIS is complex physiological trait related to ion concentration or quantity and to osmosis. Salinity affects almost all processes of the plant, because of the osmotic effects by high ionic concentrations, and because of competitive interference with nutrient uptake and of toxic effects within the plant tissue (Yeo and Flowers 1989). In this study, SIS was correlated with sodium contents in shoot (NS) but not with sodium contents in root (NR) under salt stress. It was suggested that sodium contents in shoot were increased by transport of Na^+ ions from the root to the shoot (leaf) in external high concentration (10 dS/m, NaCl), and the build up of salt in the leaves, which subsequently led to the fact that the leaves were ultimately damaged, because re-translocation from shoot to root is trivial than that from root to shoot (Yeo and Flowers 1989). This suggestion supports the notion that excess Na^+ ions were the primary cause of salt sensitivity in non-halophytes (Greenway and Munns, 1980). On the other hand, the potassium contents in both shoot and root were not correlated with SIS. These results suggested that the high potassium contents did not directly damage leaves. These findings were similar to that of Lin et al. (2004).

Several studies revealed that the processes of sodium and potassium uptake in rice were considered to

be independent under saline circumstances (Garcia et al. 1997; Yadav et al. 1997). Koyama et al. (2001) also supported the previous findings that the uptake of these ions may be independent due to the major pathways of sodium and potassium uptake in rice occur in parallel and not directly in competition. However, there was a negative correlation (r = -0.401) between NS and KS, suggesting that a competition between Na^+ ions and K^+ ions occurred in terms of uptake in the shoots. However, sodium contents and potassium contents in root exhibited high positive correlation (r = 0.741). Lin et al. (2004) reported the similar findings.

The QTLs for salt tolerance in shoots has been well documented (Flowers et al., 2000; Koyama et al., 2001; Lin et al., 2004; Yao et al., 2005). However the relationship of root physiological traits has not been reported except Lin et al. (2004). In present study, the salt injury score (SIS) was negatively correlated with sodium and potassium contents in root but positively with sodium contents and potassium contents ratio in shoot. Moreover, salt tolerant seedlings showed low and high sodium contents in shoot and root, respectively, and vice versa. This reflected that roots may possess some characteristic to reduce the uptake of sodium contents and onward transmission to xylem in salt tolerant cultivars. Guo et al. (2001) have analyzed the expression of PKS2 to PKS8 in shoots and in roots, and their regulation by salt stress; they also found that some were expressed at a higher level in the root than in the shoot. This observation supported our suggestion.

Except Flowers et al. (2000) and Koyama et al. (2001), others QTL analyses were conducted using population derived from *indica* and *japonica* crosses. Koyama et al (2001) used *indica* multiple cross population and were unable to construct linkage map for several linkage groups including chromosome 8. However, important QTLs were detected on chromosome 8 in preset studies. Thus, a comparison could not be made. McCouch et al. (2002) developed 2240 new SSR markers for rice. As saturated map would be an essential part of QTL analysis and by using these advancements a much more saturated map can be constructed in future. Moreover, the identification of QTLs for salinity tolerance at several growth stages, particularly the sensitive ones, is the need of the time to improve the tolerance to salinity in rice by pyramiding these QTLs to produce salt tolerant cultivars.

References

[1] Afza R, FJ Zapata-Arias, F Zwiletitsch, G Berthold and G Gregorio 1999. Modification of rapid screening method of rice mutants for NaCl toleranceusing liquid nutrient solution. Mut. Breed. 44: 25-28.

[2] Asch F, M Dingkuhn, and K Dorffling 1997a. Effects of transpiration on sodium and potassium distribution in salt stressed irrigated rice. J. Exp. Bot. 48 supl. p. 39.

[3] Boshani O, V Valpuesta and MA Botella 2003. Developing salt tolerant plants in a new century: a molecular biology approach. Pl Cell Tissue Org Cult 73: 101115.

[4] Fageria N. 1985. Salt tolerance of rice cultivars. Pl Soil 88: 237-243.

[5] Flowers TJ, ML Koyama, SA Flowers, C Sudhakar, KP Singh and AR Yeo 2000. QTL: their placement in engineering tolerance of rice to salinity. J Exp Bot 51(342): 99-106.

[6] Garcia A, CA Rizzo, J Ud-Din, SL Bartos, D Senadhira, TJ Flowers and AR Yeo 1997. Sodium and potassium transport to the xylem are inherited independently in rice, and the mechanism of sodium:potassium selectivity differs between rice and wheat. Plant Cell Environ. 20: 1167-1174.

[7] Greenway H and R Munns 1980. Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol 31:149–190

[8] Gregorio GB and D Senadhira 1993. Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.). Theor. Appl.Genet. 86: 333-338.

[9] Gregorio GB and D Senadhira. 1995. Rapid screening for salinity tolerance in rice. *In* Proceeding for the 2nd Asian Crop Science, R. Ishii & T. Horie (eds.), Fukui, Japan, 21-23 August 1995, pp. 758-759.

[10] Gregorio GB, D Senadhira and RD Mendoza 1997. Screening rice for salinity tolerance. IRRI Discussion Paper Series No. 22. The International Rice Research Institute. Los Banos, Philippines. pp 30.

[11] Guo Y, U Halfter, M Ishitani and JK Zhu 2001. Molecular characterization of functional domains in the protein kinase SOS2 that is required for plant salt tolerance. Plant Cell 13:1383–1400

[12] Ikeda N, NS Bautista, T Yamada, O Kamijima and T Ishii 2001. Ultra-simple DNA extraction method for marker assisted selection using microsatellite markers in rice. Pl Mol Bio Rept. 19: 27-32.

[13] Khatun S and TJ Flowers 1995a. Effects of salinity on seed set in rice. Plant Cell Environ. 18: 61-67.

[14] Kosambi DD. 1944. The estimate of map distances from recombination values. Ann Eug 12: 172-175.

[15] Koyama ML, A Levesly, RMD Koebner, TJ Flowers and AR Yeo 2001. Quantitative trait loci for component physiological traits determining salt tolerance in rice. Pl Physiol 25: 406-442.

[16] Lander ES and P Green 1987. Construction of multi locus genetic linkage maps in humans. Proc. Natl Acad Sci USA 84: 2363-2367.

[17] Lander ES, P Green, J Abranamson, A Barlow, MJ Daley, SE Lincoln and L Newburg 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

[18] Lee Kyu-Seong, D Senadhira and KS Lee 1996. Salinity tolerance in japonica rice (*Oryza sativa* L.). SABRAO Journal 28: 11–17.

[19] Lin HX, MZ Zhu, m Yano, JP Gao, ZW Liang, WA Su, H Hu, ZH Ren and DY Chao 2004. QTLs for Na+ and K+ uptake of shoot and root controlling rice salt tolerance. Theo Appl Genet 108: 253-260.

[20] McCouch SR, L Teytelman, Y Xu, KB Lobos, K Clare, M Walton, B Fu, R Maghirang, Z Li, Y Xing, Q zhang, I Kono, M Yano, RF Jellstrom, G DeClerck, D Schneider, S Cartinhour, D ware and L Stein 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9: 199-204-7.

[21] Nelson JC 1997. QGENE: Software for marker-based genomic analysis and breeding. Mol Breed 3: 239-245.

[22] Panaud O, X Chen and SP McCouch. 1996. Development of microsatellite markers and characterization of simple sequence lingth polymorphism (SSLP) in rice (*Oryza sativa* L.). Mol Gen Genet 252: 597-607.

[23] Prasad SR, PG Bangali, S Hittalmani and HE Shashidhar 2000. Molecular mapping of quantitative trait loci associated with seedling tolerance to salt stress in rice. Curr Sci 78(2): 162-164.

[24] Temnykh S, G DeClerck, A Lukashowa, L Pivovich, SCartinhour and S McCouch 2001. Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon association and genetic marker potential. Genome Res. 11: 1441-1452.

[25] Veldboom LR, M Lee and WL Woodman 1994. Molecular markerfacilitated studies in an elite maize population. I. Linkage analysis and determination of QTLs for morphological traits. Theor Appl Genet 88: 7-16

[26] Yadav R, B Courtois, N Huang and G McLaren 1997. Mapping genes controlling root morphology and root distribution in a doubled haploid population of rice. Theor Appl Genet 94: 619–632

[27] Yao MZ, JF Wang, HY Chen, HQ Zhai and HS Zhang 2005. Inheritance and QTL mapping of salt tolerance in rice. Rice Sci 12(1): 25-32.

[28] Yeo AR and TJ Flowers 1989. Selection for physiological characters—examples from breeding for salt tolerance. In: Jones HG, Flowers TJ, Jones MB (eds) Plants under stress. Cambridge University Press, Cambridge, pp 217–234

Table. 1. Salt injury score (SIS)

[29]

Score	*Seedling growth conditions*
1	Normal growth, no leaf damage
3	Near normal growth, only the lowest leaf is dry
5	Growth is severely retarded, two basal leaves are dry
7	Complete cessation of growth, only apical leaf survive
9	Dead seedling

Table 2. Correlations among morphological and physiological traits observed in F3 population derived from a cross between a salt tolerant indica rice cultivar, Pokkali, and an indica fine rice cultivar, Shaheen basmati, in saline condition

Trait	SH	FSW	DSW	NS	KS	NKS	NR	KR	NKR
FSW	0.597^{**}								
DSW	0.616^{**}	0.959^{**}							
NS	-0.396^{**}	-0.731^{**}	-0.631^{**}						
KS	0.210^{**}	0.254^{**}	0.159^{*}	-0.401^{**}					
NKS	-0.396^{**}	-0.709^{**}	-0.601^{**}	0.978^{**}	-0.533^{**}				
NR	0.267^{**}	0.599^{**}	0.570^{**}	-0.530^{**}	0.116^{ns}	-0.498^{**}			
KR	0.188^{*}	0.501^{**}	0.441^{**}	-0.586^{**}	-0.003^{ns}	-0.538^{**}	0.741^{**}		
NKR	-0.097^{ns}	-0.267^{**}	-0.195^{*}	0.491^{**}	-0.055^{ns}	0.465^{**}	-0.133^{ns}	-0.633^{**}	
SIS	-0.281^{**}	-0.632^{**}	-0.517^{**}	0.822^{**}	-0.517^{**}	0.817^{**}	-0.468^{**}	-0.417^{**}	0.271^{**}

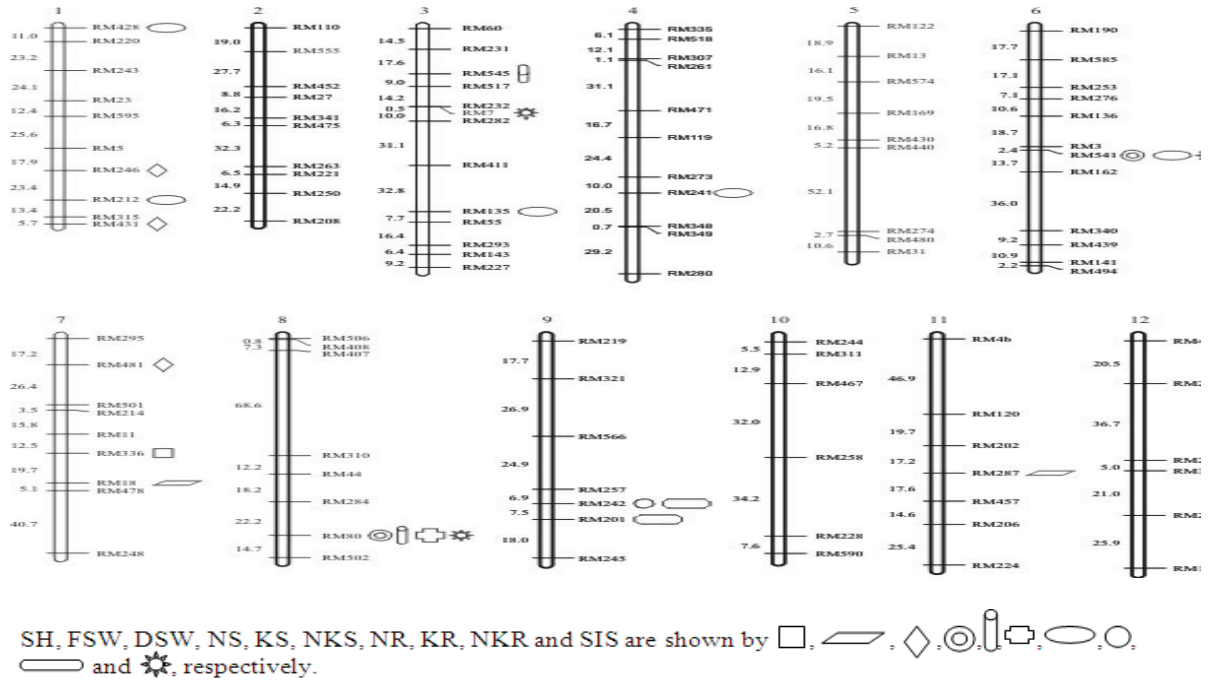

Fig.1. Genetic linkage map showing the location of QTLs for 4 morphological and 6 physiological traits under salt stress (10 dS/m NaCl) in $F_{2:3}$ populations derived from a cross between salt tolerant *indica* cultivar, Pokkali, and *indica* fine rice cultivar, Shaheen Basmati. The marker order and relative distances (in Kosambi mapping units) are after Temnykh et al. (2001).