Pak. J. Bot., 40(6): 2369-2381, 2008.

GENETIC MAPPING OF QTLs, CONTROLLING SHOOT FRESH AND DRY WEIGHT UNDER SALT STRESS IN RICE (*ORYZA SATIVA* L.) CROSS BETWEEN Co39 × MOROBEREKAN

TANVEER UL HAQ[1*], JAVAID AKHTAR[1], JOHN GORHAM[2], KATHERINE A. STEELE[2] AND MUHAMMAD KHALID[3]

[1]Centre for Advance Studies in Applied Genetics and Saline Agriculture (CAGSA), University of Agriculture, Faisalabad, 38040, Pakistan
[2]CAZS–Natural Resources, University of Wales, Bangor, Thoday Building, Deiniol Road, Bangor, Gwynedd, Wales, LL57 2UW, U.K.
[3]Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad, 38040, Pakistan

Abstract

A mapping population of 120 recombinant inbred lines (RILs) derived from the cross between Co39 (lowland, Indica) and Moroberekan (upland, Japonica) rice (*Oryza sativa* L.) cultivars was used to map QTLs associated with shoot growth traits under salinity on all chromosomes of rice. The dilution of salt concentration in shoot tissues by higher vegetative growth is an established mechanism of salt tolerance in plants. The fresh weight of shoots was recorded after 42 days of growth at 100 mol m^{-3} NaCl + 5.0 mol m^{-3} $CaCl_2$ in nutrient solution in a flood bench system. We also determined shoot dry weight and shoot fresh/dry weight ratio traits from the above data. A total of 7 QTLs for 3 shoot growth traits were detected on chromosomes–1, 2 and 6 in this study. Out of 4 QTLs for shoot fresh/dry weight ratio, 2 were located on chromosome–1 and one on each of chromosome–2 and 6, respectively. These QTLs explained 5.52–26.13% of the total phenotypic variation for shoot fresh/dry weight ratio trait. From 2 QTLs for shoot fresh weight one was found on each of chromosome–1 and 2 and the phenotypic variation explained by these QTLs was 9.89–13.17%. Whereas a single QTL for shoot dry weight was positioned on chromosome–2, in this study and explained 10.07% of the total phenotypic variation. Detection of QTLs for shoot growth traits at different chromosomes indicated that this character is controlled by multiple loci.

Introduction

Rice is an important staple food and cash crop feeding more than three billion people in the world (Ma *et al*., 2007). Soil salinity is a major constraint for crop production in arid and semi arid regions of the world (Ashraf, 1994; Afzali *et al*., 2006; Ashraf *et al*., 2008) and about 6.67 M ha (40%) of cultivated land is affected to various degrees of soil salinity in Pakistan (Khan, 1998). On a global basis, around one third of the total irrigated land (Epstein *et al*., 1980) and about 30% of the world's rice growing land is affected by salinity (Prasad *et al*., 2000). In Pakistan one million hectares of rice growing area is salt–affected (Qureshi *et al*., 1991). Of the 130 million hectares of land where rice is grown, about 30% contain levels of salt too high to allow normal rice yield (Mishra, 2004). Rice yields can be reduced by up to 50% when grown under moderate (6 dS m^{-1}) salinity levels (Zeng *et al*., 2002). Soil salinity is a serious constraint to rice cultivation under irrigated agriculture in countries like Pakistan and elsewhere due to poor quality of water (Abdullah & Ahmad 1982). Salt tolerance is a complex mixture of different morpho–physiological processes which are controlled by many genes across the rice genome and manipulation of any of these genes may contribute to improved salt tolerance. Consequently, increased tolerance to salt in rice would help promote global rice production and solve future food shortage problems.

*Corresponding author: Tanveer ul Haq; tanveerulhaqro@yahoo.com

To obtain better yield from saline soils and saline irrigation waters on a sustained basis, it is imperative that along with improved agronomic practices the genetic resources should be exploited with the help of modern plant molecular techniques, such as QTL mapping, marker assisted breeding, map based cloning and gene transformation to develop high yielding salt tolerant rice varieties. Marker-assisted selection (MAS) is helpful in identifying qualitatively and quantitatively inherited desirable traits and biotic and abiotic stress resistance (Ali *et al*., 2008). DNA markers have great scope in the construction of linkage maps for a range of crop species. Linkage maps can be utilized for the identification of QTLs/genes controlling simple or quantitative traits (Collard *et al*., 2005). DNA markers which are tightly linked to important genes may be used as molecular tools for marker–assisted selection in plant breeding (Ribaut & Hoisington, 1998).

The genetics of salt tolerance in rice has been unraveled for morphological and physiological traits, such as shoot fresh weight, shoot dry weight, shoot length, root length, and shoot Na^+/K^+ ratio in saline field (Akbar *et al*., 1986; Jones, 1986; Yeo & flowers, 1986; Flowers & Yeo, 1995; Ashraf, 2004; Masood *et al*., 2004). All these analysis revealed that genetic variation of salt tolerance was associated with multiple genes. Analysis of QTLs for salt tolerance using molecular markers has also been conducted in water culture in greenhouse (Flowers *et al*., 2000; Prasad *et al*., 2000; Tuan *et al*., 2000; Koyama *et al*., 2001; Masood *et al*., 2004) and by cultivation in saline paddy fields (Takehisa *et al*., 2004). Takehisa *et al*., (2004) identified QTLs associated with shoot fresh weight by using backcross inbred lines population BC1F1 (Nipponbare/Kasalath/Nipponbare) in saline and non-saline field. A total of 6 QTLs associated with shoot fresh weight were detected on chromosomes–1, 3, 5, 6, 7 and 12 in the saline group. In the control, 13 QTLs were identified on chromosomes–1, 2, 3, 6, 7, 9 and 12 for the four cropping seasons. Prasad *et al*., (2000) examined the seedling vigor of a double haploid population derived from rice cultivars IR64 and Azucena and detected QTLs for salt tolerance on chromosome–6. In RI lines mapping population from the cross between Tesanai 2 (moderately salt tolerant) and CB (salt sensitive) rice varieties, Masood *et al*., (2004) identified 14 QTLs for dry shoot weight on chromosome-1 (3 QTLs), 3 (3 QTLs), 6 (1 QTL), 8 (8 QTLs), 9 (1 QTL), 10 (1 QTL) and 11 (2 QTLs), in a hydroponics experiment at an EC level of 12 dS m^{-1}. These QTLs together explained 97.9% of the phenotypic variation in dry shoot weight. Tuan *et al*., (2000) reported QTLs for some traits as indicators of salt tolerance on a genome including chromosome–6. Koyama *et al*., (2001) evaluated the shoot dry weight as an indicator of salt tolerance and then distinguished QTLs on chromosome–6 using a different mapping population.

This study used the Co39/Moroberekan RIL population to map QTLs for shoot fresh weight, shoot dry weight and shoot fresh/dry weight ratio under salt stress on all chromosomes of rice. Additional markers were added to the region on chromosome–1 and the improved map was used to investigate whether QTLs for shoot fresh and dry weight can be identified in this population for their potential use in plant breeding programs to develop resilient salt tolerant local rice varieties.

Materials and Methods

Plant material: The F9 recombinant inbred line (RIL) mapping population employed in this study was developed at the International Rice Research Institute (IRRI) from the cross Co39/Moroberekan. The maternal parent variety Co39 is a salt tolerant lowland indica cultivar with medium height having originated from India. Moroberekan is salt sensitive, tropical upland japonica variety of long stature having its origin in West Africa. This cross was made in IRRI in 1988 and the population was derived from 15 F_1 seeds

that produced about 300 F_2 seed. Single seed descent and a Rapid Generation Advance greenhouse (Vergara *et al*., 1982) were used to develop the mapping population. The population is skewed towards the Co39 alleles (80%). The effect of this distribution on linkage analysis has been discussed previously (Wang *et al*., 1994; Manly *et al*., 1994; Champoux *et al*., 1995).

Assessment of phenotypic response under salt stress: The parents, Co39 and Moroberekan, and 120 F_9 recombinant inbred lines (RILs) were evaluated for salt tolerance in a flood bench system at Pen–y–Ffridd Research Station, Bangor, UK. Supplementary 400 Watt high pressure sodium vapour lamps were used to maintain a minimum photon flux density of photosynthetically active radiation (400–700 nm) of 350 µmol m^{-2} s^{-1} during the 16 hour photoperiod. The minimum temperature was maintained at 25°C during the photoperiod and 20°C during the dark period.

The rice seeds were sown in seed plug (P–84) trays (Plantpak, Malvern, England) having John Innes compost No. 1 (Corwen, Clwyd, U.K.) as growth medium and watered every day until transplantation. Twenty–three days after sowing two seedlings of each line were transplanted into 2 L plastic pots lined with porceline cloth and filled with John Innes No. 1 compost with two uniform, healthy seedlings per pot. Twenty–four pots were placed into each flood bench tank on upturned, empty p–576 plug trays to improve drainage in a randomized layout with three replications. The tanks were arranged on an iron bench frame and 200 L reservoirs were placed on the concrete glasshouse floor underneath the tanks. Submersible electric pumps were placed in the reservoirs to pump nutrient solution or nutrient plus salt solution into the tanks. Nutrient solution at a concentration of 1g dm^{-3} Phostrogen plant food (Phostrogen, Corwen, Wales, U.K.) plus 0.5 ml dm^{-3} micro–nutrients (Hoagland & Arnon, 1950) plus 0.1 ml dm^{-3} Sodium silicate were supplied to the plants with the irrigation solution. Salt stress was started 28 days after sowing in daily increments of 25 mol m^{-3} and 1.25 mol m^{-3} for NaCl and $CaCl_2$ respectively and stress reached 100 mol m^{-3} NaCl + 5.0 mol m^{-3} $CaCl_2$ 32 days after sowing. A Na^+ to Ca^{2+} ratio of 20:1 was developed with $CaCl_2$. Salinity level was maintained in the solution, this was by recording the electrical conductivity (EC) with portable water proof conductivity meter (Hanna Instruments, U.K.) in each reservoir twice a week by adding water, up to 200 L. Tanks were flooded with the solution once a day for at least 15 minutes.

Data collection at final harvesting: The plants were harvested after 42 days of full salt stress in flood bench system. Plant shoots were cut off from the surface of the soil/compost and weighed immediately and stored in labeled paper bags. The fresh samples were dried in an oven at 70°C for one week and shoot oven dry weights were recorded for all replications of RILs. The data were statistically analyzed by using the software package SPSS for Windows release 11.5.1 (Anon., 2002).

Genotyping

Extraction and quantification of genomic DNA: Six–week old seedlings that were not transplanted from plug trays, 3 plants for each RIL and parent line, were used for DNA extraction. DNA was extracted from 100 mg of the fresh leaf tissue from three plants using DNeasy plant mini kits (Qiagen, UK) according to the manufacturer's instructions. DNA quantification was done using a PicoGreen dsDNA quantification kit (Molecular Probes Leiden, The Netherlands) following the manufacturer's protocol. The

fluorescence intensity was measured using a FluoStar Galaxy florescence micro plate reader (BMG Lab–technologies Inc, Durham NC). DNA samples were diluted to a final concentration of 5–6 ng/µl for use in PCR.

Microsatellites markers: Microsatellites were selected from the database (www.gramene.com) on the basis of their map locations on rice chromosome–1. A total of 56 microsatellite markers were used to test polymorphism in the parents. The 29 SSR (52%) that were polymorphic in the parents were used to genotype 120 RILs. All PCR amplifications were performed in 16 µl reaction volume containing 8.0 µl of Ready Mix (Abgene, UK) with 3.0 mM $MgCl_2$, 2.0 µl each of forward primer (2.0 µM), reverse primer (20 µM) and optional dye labeled primer (20 µM) and 5–10 ng of genomic DNA template per sample. Genomic DNA was amplified by using PCR program: 94ºC for 5 min., 35 cycles of 94ºC for 1 min., 55ºC for 1 min., 72ºC for 2 min., followed by final extension at 72ºC for 10 min. PCR products were separated using 2.5% metaphor super fine resolution (SFR) agarose (Anachem, Lutin, U.K) gel. The RILs having Moroberekan allele were scored as 'A' whereas those showing Co39 allele were scored as 'B' at each marker locus. A gel photograph showing segregation of marker K061 (Chromosome 1) in a part of RIL population is illustrated in Fig. 1. For markers where the PCR products differed by less than 10 bp, the dye labeled primer (Proligo, U.K) was included in the PCR reaction and PCR products were run on a CEQ 8000 capillary sequencer (Beckman Coulter, High Wycombe, U.K) to determine the size of the amplified fragments. A primer consisting of the M13 sequence labeled with a florescent dye was added in each PCR so that PCR products could be detected on CEQ 8000. Three different Beckman Coulter dyes, D2 (Black), D3 (Green) or D4 (Blue) were used to label primers (Proligo, U.K).

RFLP markers: The genotypic data for 127 RFLP markers on all chromosomes were obtained from Brigitte Courtois (CIRAD, Montpellier, France). The probe sequences for these markers were searched on 'www.gramene.org' and these sequences were run for blast search to identify the similarity on genome database of rice, to determine their position and order on the map. The cMap option of the "Gramene" website (www.gramene.org) was also used to order these markers on the map where probe sequences were not available.

Linkage maps construction: Linkage maps of 12 chromosomes were created based on genotypic data of 120 RILs from the Co39/Moroberekan, with 29 SSR primers and 127 RFLPs by using Kosambi mapping function (Kosambi, 1944) of MapMaker/Exp. Version 3.0, computer software (Lander *et al*., 1987).

QTL analysis: To identify the genetic loci involved in the variation in shoot fresh weight, shoot dry weight and shoot fresh/dry weight ratio, QTL analysis was performed by composite interval mapping (CIM) using the computer program, Windows QTL Cartographer version 2.5 (Wang *et al*., 2007). The mapping was run with the default setting for model 6 (5 background markers and window size of 10 cM). The percentage of the total phenotypic variation explained by QTLs identified for each trait was estimated as the R^2–value. The data were permuted 1000 times to determine the threshold LRS values for the declaration of putative QTLs (Churchill & Doerge, 1994).

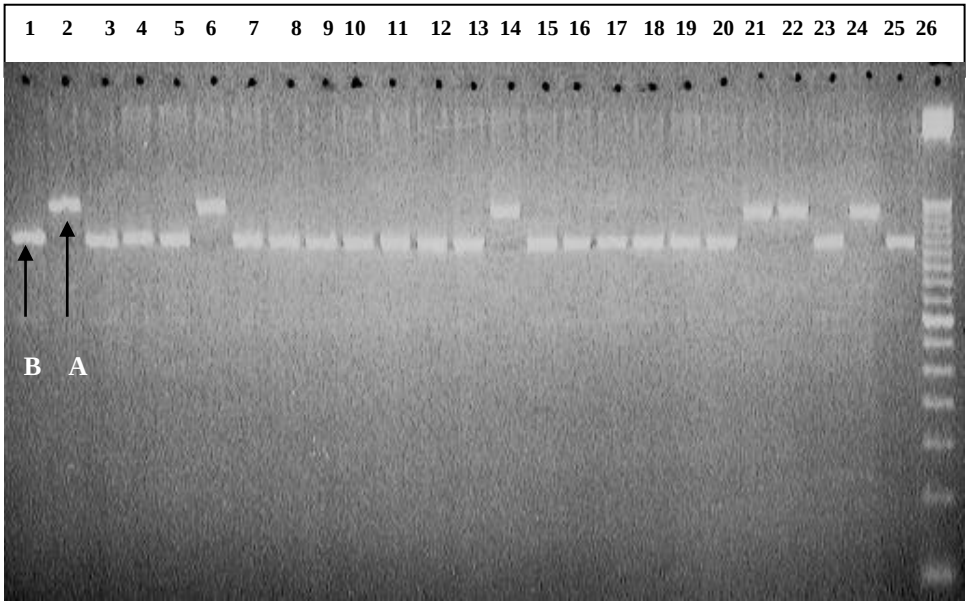

Fig. 1. Amplification of Co39, Moroberekan and RILs DNA with primer K061 (Chrom.1) showing polymorphism. The first two lanes are with parental DNA (Co39 and Moroberekan) followed by DNA from RI lines (part of a segregating population). The lane 26 is 50 bp DNA size marker. The band of size as Co39 incase of progeny was scored as B and the band of size as Moroberekan was scored as A. Black arrows indicate polymorphic bands.

Results

Variation in shoot fresh weight, shoot dry weight and shoot fresh/dry weight ratio: The data were checked for frequency distribution of the traits of shoot fresh weight, shoot dry weight as well as shoot fresh/dry weight ratio, using the software package SPSS for Windows release 11.5.1 (Anon., 2002). The frequency distribution for shoot fresh weight, shoot dry weight and shoot fresh/dry weight ratio of RILs showed wide variation (Fig. 2). For shoot fresh weight, shoot dry weight and shoot fresh/dry weight ratio transgressive segregation was observed towards high shoot weight parent Co39. The mean values for the parents as well as RILs are given in Table 1.

Correlation among three growth traits: Shoot fresh weight, shoot dry weight and shoot fresh/dry weight ratio were significantly and positively correlated with each other (Table 3). For shoot fresh weight the correlation was highly significant with shoot dry weight ($r = 0.91^{***}$) and shoot fresh/dry weight ratio ($r = 0.52^{**}$), whereas significant correlation was observed between shoot dry weight and shoot fresh/dry weight ratio ($r = 0.18^{*}$) traits under salinity in this study (Table 3).

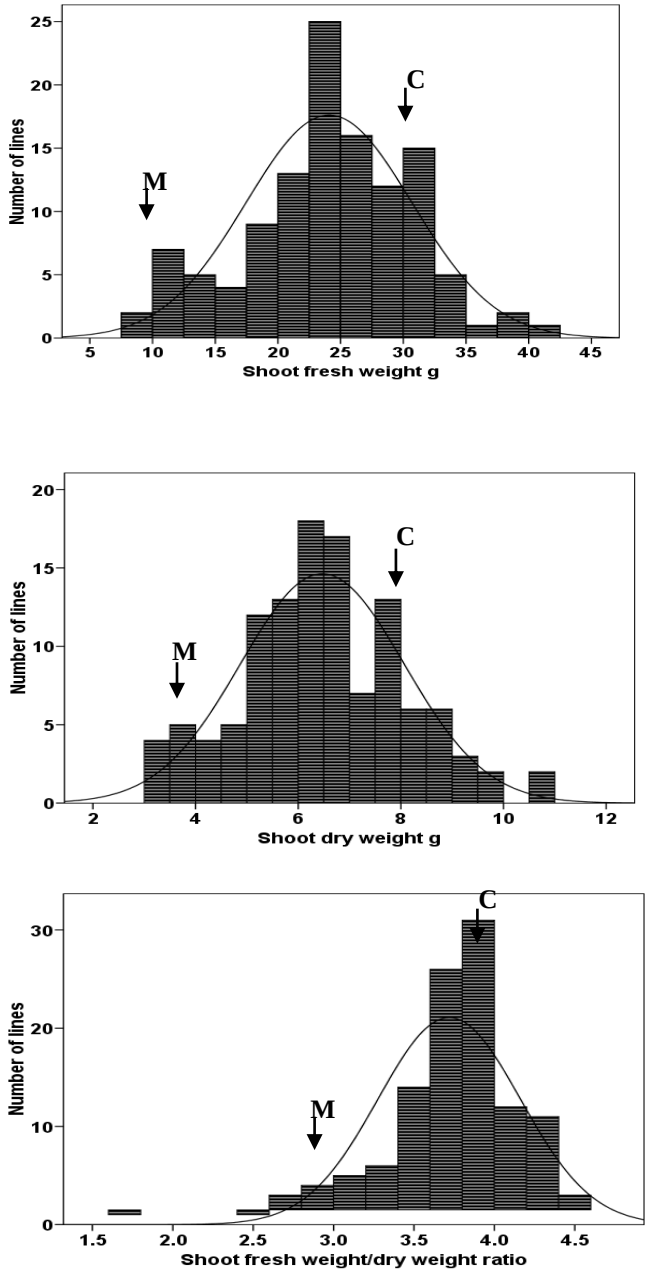

Fig. 2. Frequency distribution of shoot fresh weight, shoot dry weight and shoot fresh/dry weight ratio after 42 days at 100 mol m^{-3} NaCl + 5 mol m^{-3} $CaCl_2$ salt stress in Co39/Moroberekan RIL population (n=120). Arrows are indicating mean values for parents (C = Co39, M = Moroberekan). Normal curve is also shown over the bars.

Table 1. The Means ± S.E of phenotypic traits analyzed for parents Co39 and Moroberekan along with RIL population n = 3.

Traits	Means ± S.E			
	Name	Co39	Moroberekan	RILs
Shoot fresh weight (g)	SFW	29.52 ± 1.16	11.04 ± 1.78	24.07 ± 0.61
Shoot dry weight (g)	SDW	7.78 ± 0.08	3.81 ± 0.12	6.47 ± 0.15
Shoot fresh /Dry weight ratio	FW/DW	3.80 ± 0.18	2.89 ± 0.42	3.72 ± 0.04

QTL mapping

QTLs for growth traits on chromosome–1: Total of three QTLs for shoot fresh weight (1 QTL) and shoot fresh/dry weight ratio (2 QTL) were identified on chromosome–1 (Table 2 & Fig. 3). A QTL for shoot fresh weight coincided with shoot fresh/dry weight ratio–QTL, near the start of chromosome–1 (Fig. 3). The positions of the shoot fresh weight (qSFW1) and shoot fresh/dry weight ratio–QTLs (qSF/DW1–a & qSF/DW1–b) were 44.10, 45.0 and 89.30 cM on our genetic map of chromosome–1 and these QTLs explained 13.17, 26.13 and 5.52% of the phenotypic variation for respective growth traits, under salt stress (Table 2). The Moroberekan allele decreased shoot fresh weight as well as shoot fresh/dry weight ratio at all three loci. No QTL was detected for shoot dry weight trait under salt stress on chromosome–1 of rice in this study.

QTLs for growth traits on chromosome–2: One QTL each for shoot fresh weight, shoot dry weight and shoot fresh/dry weight ratio was detected on chromosome–2, after 42 days, salt stress of 100 mol m^{-3} NaCl + 5 mol m^{-3} $CaCl_2$, respectively (Table 2 & Fig. 3). All three QTLs have different locations on chromosome–2. The positions of the QTLs for shoot fresh weight (qSFW2), shoot dry weight (qSDW2) and shoot fresh/dry weight ratio (qSF/DW2), were 17.70, 120.10 and 105.20 cM on our genetic map of chromosome–2 (Table 2). The above QTLs explained 9.89, 10.07 and 14.56% of the total phenotypic variation for these traits under salt stress (Table 2). The Co39 allele at all these loci increased above mentioned traits respectively, in this experiment.

QTLs for growth traits on chromosome–6: A QTL for shoot fresh/dry weight ratio was identified on chromosome–6, after 42 days salt stress at 100 mol m^{-3} NaCl + 5.0 mol m^{-3} $CaCl_2$ in flood bench system (Table 2 & Fig. 3). The position of this QTL (qSF/DW6) was 7.0 cM with peak LOD score of 3.83, on the genetic map (Table 3). The phenotypic variation of 9.33% was explained by this QTL (qSF/DW6). The Moroberekan allele reduced shoot fresh/dry weight ratio for this QTL. No QTLs were found for shoot fresh weight and shoot dry weight traits on chromosome–6, in this study.

Discussion

This study found that shoot growth of rice is controlled by multiple genes, located on different chromosomes, under salt stress. We have identified total of seven QTLs for shoot fresh weight (2 QTLs), shoot dry weight (1 QTL) and shoot fresh/dry weight ratio (4 QTLs) traits on chromosome–1, 2 and 6. Three regions carrying QTLs for various growth and physiological traits under salt stress were detected on chromosome–1, 4 and 8 respectively (Gong *et al*., 2001). The dilution of salt concentration in shoot tissues by higher vegetative growth is an established mechanism of salt tolerance in plants and

Table 3. Correlation coefficients for shoot fresh weight (SFW), shoot dry weight (SDW) and shoot fresh/dry weight ratio (SF/DW) after 42 days of 100 mol m^{-3} NaCl + 5.0 mol m^{-3} $CaCl_2$ salt stress in flood bench system.

Traits	SFW	SDW
Shoot fresh weight (SFW)	1.0	
Shoot dry weight (SDW)	0.91***	
Shoot fresh/Dry weight ratio (SF/DW)	0.52**	0.18*

*, **, *** = Correlation is significant at ≤ 0.05, 0.01, and 0.001 levels, respectively (Pearson Correlation, 2 tailed)

appeared to be a reliable measure for salt tolerance (Yeo *et al*., 1990). Vigorous growth at seedling stage is desirable because of high sensitivity of rice to salts at this growth stage. Therefore high concentration of different ions can be explained partly in terms of low vigor. Koyama *et al*., (2001) emphasize the difference between ion concentration (amount per unit dry weight, and hence related to vigor expressed as total dry weight) and uptake (amount per shoot). This is to some extent, supported by our results. So vigor is an important factor for the comparison of ion concentration in plants and can not be ignored. QTL analyses for rice salt tolerance have been reported by different workers (Bonilla *et al*., 2002; Masood *et al*., 2004; Lin *et al*., 2004; Ren *et al*., 2005; Lee *et al*., 2007).

In this study we have identified three new QTLs differed from those detected by other researchers (Prasad *et al*., 2001; Koyama *et al*., 2001; Lin *et al*., 2004). However it is difficult to compare the chromosomal locations of QTLs because different materials and marker maps were used (Lin *et al*., 2004). Our results are also in line with Akhtar (2000) who identified a QTL for shoot fresh weight under salt stress on chromosome–1.

High concentration of Na^+ is crucial for plant growth in rice and could be responsible for the reduction in shoot fresh weight. An inverse relationship between growth and Na^+ concentration was also observed by Flowers *et al*., (1985). Aslam *et al*., (1993b) had similar results for different rice varieties under salinity.

For shoot fresh weight the correlation was highly significant for shoot dry weight (r = 0.91***) and shoot fresh/dry weight ratio (r = 0.52**), whereas significant correlation was observed between shoot dry weight and shoot fresh/dry weight ratio (r = 0.18*) traits. Veldoom *et al*., (1994) and Xiao *et al*., (1996) demonstrated that correlated traits often have QTLs mapping to the same chromosomal location. The similar trend was observed in this study. For shoot fresh weight, shoot dry weight and shoot fresh/dry weight ratio transgressive segregation was observed towards high shoot weight parent Co39. Genetically, transgression is defined as the appearance of individuals in segregating population that fall beyond the parental phenotypes. In this mapping population, lines having phenotypic values greater than the higher parent and lesser than the lower parent were observed for all the traits measured.

The molecular markers that are nearest to the QTLs (Table 2) may also be useful for markers assisted selection (MAS) in rice breeding program aimed at developing new varieties with a high level of salt tolerance. The near isogenic lines (NIL) of rice differing only in the presence of a single and specific QTL for shoot growth in saline environment should be developed to precisely map genes controlling each trait and to elucidate the biological functions of the QTLs. The knowledge of QTLs for salt tolerance in saline environment will be valuable tool in future plant breeding programs for producing high yielding rice varieties.

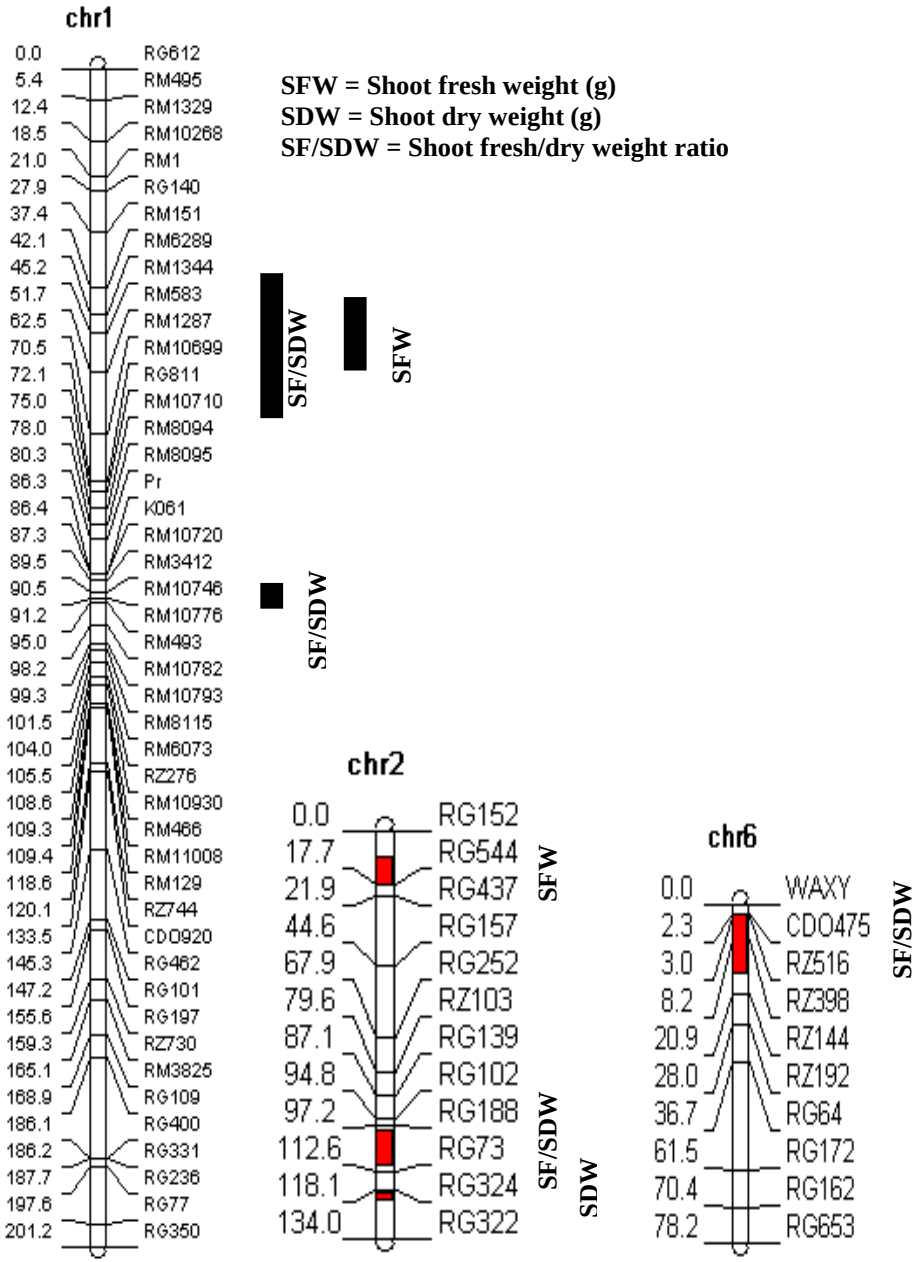

Fig. 3. Map of rice chromosome 1, 2 and 6, showing QTLs (solid bars) associated with shoot fresh weight (SFW), shoot dry weight (SDW) and shoot fresh dry weight ratio (SF/SDW Ratio) under 100 mol m^{-3} NaCl + 5 mol m^{-3} $CaCl_2$ salt stress after 42 days in flood bench system in Co39/Moroberekan RIL population (n=120). Kosambi mapping distances (cM) are to the left and markers are to the right of chromosomes, respectively.

Acknowledgements

This work was supported in part by a split doctoral fellowship to first author from a project funded by EU–Commission, for human resource development to the University of Agriculture, Faisalabad, Pakistan. We thank Dr. Brigitte Curtosis (CIRAD, Montpellier, France) for providing RFLPs data and some of the rice lines for this study.

References

Abdullah, Z. and R. Ahmed. 1982. Salt tolerance of *Solanum tuberosum* L., growing on saline soils amended with gypsum. *Z Acker und Pflanzenbau*, 151: 409-416.

Afzali, S.F., M.A. Hajabbasi, H. Shariatmadari, K. Razmjoo and A. H. Khoshgoftarmanesh. 2006. Comparative adverse effects of peg- or nacl- induced osmotic stress on germination and early seedling growth of a potential medicinal plant *Matricaria chamomilla*. *Pak. J. Bot*., 38(5): 1709-1714.

Akbar, M., G.S. Khush and D. HilleRisLambers. 1985. Genetics of salt tolerance in rice. In: *Proceedings of the Rice Genetics*, International Rice Research Institute (IRRI), Manila, Philippines, pp. 399-409.

Akhtar, N. 2000. *Salt tolerance studies in rice* (*Oryza sativa* L.) Ph.D. Thesis. School of Biological Sciences, University of Wales, Bangor, UK.

Ali, H., N. Iqbal, M. Ahsan ul Haq, T.M. Shah, B.M. Atta and A. Hameed. 2008. Detection of QTLs for blight resistance in chickpea genotypes with DNA based markers. *Pak. J. Bot*., 40(4): 1721-1728.

Anonymous. 2002. *Statistical Package for Social Sciences* (SPSS for Windows). Released 11.5.1. SPSS Inc. Chicago.

Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora*, 199: 361-376.

Ashraf, M., H.R. Athar, P.J.C. Harris and T.R. Kwon. 2008. Some prospective strategies for improving crop salt tolerance. *Adv. Agron.*, 97: 45-110.

Ashraf, M.1994. Breeding for salinity tolerance in plants. *Crit. Rev. Plant Sci*., 13: 17-42.

Aslam, M., R.H. Qureshi and N. Ahmad. 1993b. Mechanism of salinity tolerance in rice In: *Towards the rational use of high salinity tolerant plants*. (Eds.): H. Lieth and A.A. Masoum. Vol. 2: 135-138. Kluwer Acad. Publ. Dordreacht.

Bonilla, P., J. Dvorak, D. Mackill, K. Deal and G. Gregorio. 2002. RFLP and SSLP mapping of salinity tolerance genes in chromosome–1 of rice (*Oryza sativa* L.) using recombinant inbred lines. *The Philippine Agric. Scientist*, 85(1): 68-76.

Champoux, M.C., G. Wang, S. Sarkarung, D.J. Mackill, J.C. O'Toole, N. Huang and S.R. McCouch. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. *Theor. Appl. Genet*., 90: 969-981.

Churchill, G.A. and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. *Genetics*, 138: 963-971.

Collard, B.C.Y., M.Z.Z. Jahufer, J.B. Brouwer and E.C.K Pang. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker–assisted selection for crop improvement: The basic concepts. *Euphytica*, 142: 169-196.

Epstein, E., J.D. Norlyn, D.W. Rusk, R.W. Kingsbury, D.B. Kelly, G.A. Cunningham and R.A. Wrona. 1980. Saline culture of crops; a genetic approach. *Science*, 210: 399-404.

Flowers, T.J. and A.R. Yeo. 1995. Breeding for salinity resistance in crop plants: where next? *Aust. J. Plant Physiol*., 22: 875-884.

Flowers, T.J., D.R. Lachno, S.A. Flowers and A.R. Yeo. 1985. Some effects of sodium chloride on cells of rice cultured *In vitro*. *Plant Sci*., 39: 205-211.

Flowers, T.J., M.L. Koyama, S.A. Flowers, C. Sudhakar, K.P. Singh and A.R. Yeo. 2000. QTL: their place in engineering tolerance of rice to salinity. *J. Exp. Bot*., 51: 99-106.

Gong, J.M., X.W. Zheng, B.X. Du, Q. Qian, S.Y. Chen, L.H. Zhu and P. He. 2001. Comparative study of QTLs for agronomic traits of rice (*Oryza sativa* L.) between salt stress and non–stress environment. *Sci. China Ser. C–Life Sci*., 44: 73-82.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plant without soil. *California Agri. Exp. Sta. Cir*., 347: 39.

Jones, M.P. 1986. Genetic analysis of salt tolerance in mangrove swamp rice. In: *Proceedings of the Rice Genetics*, International Rice Research Institute (IRRI), Manila, Philippines, pp. 411-422.

Khan, G.S. 1998. Soil salinity/sodicity status in Pakistan. *Soil Survey of Pakistan*, Lahore. pp. 59.

Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Ann. Eugen*., 12: 172-175.

Koyama, M.L., A. Levesley, R.M.D. Koebner, T.J. Flowers and A.R. Yeo. 2001. Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiol*., 125: 406-422.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. 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.

Lee, S.Y., J.H. Ahn, Y.S. Cha, D.W. Yun, M.C. Lee, J.C. Ko, K.S. Lee and M.Y. Eun. 2007. Mapping QTLs related to salinity tolerance of rice at the young seedling stage. *Plant Breeding*, 126: 43-46.

Lin, H.X., M.Z. Zhu, M. Yano, J.P. Gao, Z.W. Liang, W.A. Su, X.H. Hu, Z.H. Ren and D.Y. Chao. 2004. QTLs for Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance. *Theor. Appl. Genet*., 108: 253-260.

Ma, H., K. Chong and X.W. Deng. 2007. Rice research: past, present and future. *J. Integrative Plant Biol*., 49(6): 729-730.

Manly, K.F. 1994. Establishing genetic linkage using recombinant inbred lines with an abnormal segregation ratio. *Genetics*, 136: 1433-1434.

Masood, M.S., Y. Seiji, Z.K. Shinwari and R. Anwar. 2004. Mapping quantitative trait loci (QTLs) for salt tolerance in rice (*Oryza sativa* L.) using RFLPs. *Pak. J. Bot*., 36(4): 825-834.

Mishra, B. 2004. Present status issues and future strategies for increasing quality rice productionand export. In: *National Symposium on strategies for enhancing export of quality rice held atNBPGR, New Delhi*, pp. 1-16.

Prasad, S.R., P.G. Bagali, S. Hittalmani and H.E. Shashidhar. 2000. Molecular mapping of quantitative trait loci associated with seedling tolerance to salt stress in rice (*Oryza sativa* L.). *Curr. Sci*., 78: 162-164.

Qureshi, R.H., M. Aslam, G. Mustafa and J. Akhtar. 1991. Some aspects of physiology of salt tolerance in wheat (*Triticum aestivum* L.). *Pak. J. Agric. Sci*., 28: 199-209.

Ren, Z.H., J.P. Gao, L.G. Li, X.L. Cai, W. Huang, D.Y. Chao, M.Z. Zhu, Z.Y. Wang, S. Luan and H.X. Lin. 2005. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nat. Genet*., 37: 1141-1146.

Ribaut, J.M. and D. Hoisington. 1998. Marker–assisted selection: New tools and strategies. *Trends Plant Sci*., 3: 236-239.

Takehisa, H., T. Shimodate, Y. Fukuta, T. Ueda, M. Yano, T. Yamaya, T. Kameya and T. Sato. 2004. Identification of quantitative trait loci for plant growth of rice in paddy field flooded with salt water. *Field Crops Res*., 89: 85-95.

Tuan, V.D., Y. Fukuta and M. Yano. 2000. Mapping quantitative trait loci for salinity tolerance in rice (*Oryza sativa* L.). *Omonerice*, 8: 27-35.

Veldboom, L.R., M. Lee and W.L. Woodman. 1994. Molecular–marker–facilitated studies in an elite maize population. 1. Linkage analysis and determination of QTL for morphological traits. *Theor. Appl. Genet*., 88: 7-16.

Vergarba, S., G. Patena and F.S.S. Lopez. 1982. Rapid generation advance of rice at the International Rice Research Institute. *IRRI Res. Pap. Ser*., 4: 1-10.

Wang, G.L., D.J. Mackill, M. Bonman, S.R. McCouch, M.C. Champoux and R.J. Nelson. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics*, 136: 1421-1434.

Wang, S., C.J. Basten and Z.B. Zeng. 2007. *Windows QTL Cartographer 2.5*. Department of Statistics, North Carolina State University, Raleigh, NC.

Xiao, J., J. Li, L. Yuan and S.D. Tanksley. 1996. Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a specific rice cross. *Theor. Appl. Genet*., 92:230-244.

Yeo, A.R. and T.R. Flowers. 1986. Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. *Aust. J. Plant Physiol*., 13: 161-173.

Yeo, A.R., M.E. Yeo, S.A. Flowers and T.J. Flowers. 1990. Screening of rice (*Oryza sativa* L.) genotypes for physiological character contributing to salinity resistance, and their relationship to overall performance. *Theor. Appl. Genet*., 79: 377-384.

Zeng, L., M.C. Shannon and C.M. Grieve. 2002. Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters. *Euphytica*, 127:235-245.

(Received for publication 5 March 2008)