Molecular mapping of quantitative trait loci associated with seedling tolerance to salt stress in rice (*Oryza sativa* L.)

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Quantitative trait loci (QTL) controlling various rice seedling traits conferring salt tolerance were mapped on the molecular map of rice, generated by using a doubled haploid population derived from the cross between IR64 and Azucena. Seven QTL were identified [threshold LOD (log of odds ratio) ≥ 2.00] for seedling traits under salt stress, i.e. two for seed germination (%), one for seedling root length (cm), three for seedling dry matter (mg) and one for seedling vigour. Among the seven QTL four were located on chromosome 6. A QTL for root length on chromosome 6 was flanked by restriction fragment length polymorphism (RFLP) markers RG162–RG653, exhibited a very high phenotypic variance of 18.9% and a peak LOD score of 2.852. Homozygous genotypes containing quantitative genes tolerant to salt stress were identified. The markers tightly linked to salt tolerance can be used in marker-assisted selection after testing their stability and reliability.

RICE breeders have used genetic variability to produce cultivars that have high yield potential, resistance to disease and insect damage and tolerance for cold, drought and other abiotic stresses. One-third of the world's irrigated rice area is affected by excess salt accumulation due to irrational management and defective irrigation practices[1]. In spite of soil reclamation and improved drainage systems, breeding varieties for tolerance to salinity stress is considered as an exeunt in the long run. Very little work has been done until recently to identify and breed cultivars adapted to salinity. Lack of screening techniques is one of the limitations in identifying rice genotypes for salt tolerance which is difficult, expensive and time consuming. Hence, dissection of such a complex trait by means of quantitative trait loci (QTL) mapping and identifying chromosomal regions associated with DNA markers would be a useful tool for large-scale screening of genotypes. The present study was envisaged with the objective of identifying homozygous lines tolerant to excess salt stress at seedling stage and mapping of salt tolerant QTL in rice.

In this study we used a mapping population consisting of seventy-six doubled haploid (DH) lines developed from the cross between IR64, an *indica* variety and Azucena, a *japonica* variety[2]. The molecular map for this DH population was earlier generated by Huang *et al.*[3]. The parents of the DH population IR64 and Azucena were evaluated and stress was induced with various concentrations of sodium chloride (NaCl) solution. The optimum concentration of NaCl was estimated to be 0.5% with pH of 8.5 when there was considerable tolerance in IR64 and susceptibility in Azucena. Phenotyping of rice seedlings was carried out *in vitro* in petri plates with filter paper moistened with 0.5% NaCl. Distilled water was used as medium in the control experiment. Fifteen seeds each of the DH lines, parents, resistant check Pokkali, IR64 and susceptible check Azucena were grown in 0.5% NaCl solution in two replications. The petri plates were covered with paper and proper care was taken to avoid loss of water by evaporation. Though there could be slight increase in the concentration of NaCl its overall concentration was maintained around 0.5% which was optimum as suggested by Zapata *et al.*[4]. Seedlings were allowed to grow for 14 days. NaCl solution was added at regular intervals to all the treatments and distilled water added to the control. Observations were recorded for five randomly selected plants on seedling characters such as seed germination (GEM) (%), seedling shoot length (cm), seedling root length (SRTL) (cm), seedling dry matter (SDM) (mg) and seedling vigour (SV).

Salt tolerance index for each character and for each DH line was calculated as the ratio of mean of saline solution indicator to the mean of the control solution indicator. Analysis of variance was carried out on the mean values of five randomly selected seedlings from each of the two replications and the control[5]. The DH lines exhibited considerable amount of variation for all the five characters[5]. Considering seedling vigour, DH line P389 was found to be the most salt tolerant followed by P232, P332 and P22 plants. QTL mapping was done by using MAPMAKER/QTL (refs 6 and 7) (v.1.1 b) with a threshold LOD ≥ 2.00. Salt stress tolerance QTL associated with four seedling traits is presented in Table 1.

The nomenclature for QTL here is as per McCouch *et al.*[7]. Major part of the variability for seed germination was explained by the QTL qSGEM-7 flanked by CD059–RG477 on chromosome 7, which exhibited phenotypic variance of 19.5% and peak LOD of 2.828. The length of this QTL is 26.8 cM and identification of any tightly linked markers in this region will serve as a candidate gene for fine-mapping and further use in marker-assisted selection (MAS). Major loci for seedling root length (qSRTL-6) were bracketed by RG162–RG653 spread over 38.4 cM on chromosome 6 (Figure 1). Two QTL were identified for dry matter, with qSDM-5 flanked by RZ70–RZ225 and qSDM-6 flanked by CD0544–*Amy2A*. QTL for seedling vigour (qSV-6)

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Table 1. QTL identified for salt tolerance traits at 0.5% NaCl in IR64 × Azucena DH population by interval mapping (threshold LOD > 2.00)

Traits	Chromosome number	QTL[a]	Flanking markers	Variation (%)	Peak LOD
Seed germination (%)	6	qSGEM-6	RZ398–RG213	16.30	2.735
	7	qSGEM-7	CDO59–RG477	19.50	2.828
Seedling root length (cm)	6	qSRTL-6	RG162–RG653	18.90	2.852
Seedling dry matter (mg)	5	qSDM-5	RZ70–RZ225	17.90	2.882
	6	qSDM-6	CDO544–*Amy2A*	16.70	2.471
	10	qSDM-10	RZ625–RZ500	13.50	2.000
Seedling vigour	6	qSV-6	CDO544–*Amy2A*	15.80	2.402

QTL[a], QTL nomenclature as suggested by McCouch *et al.*

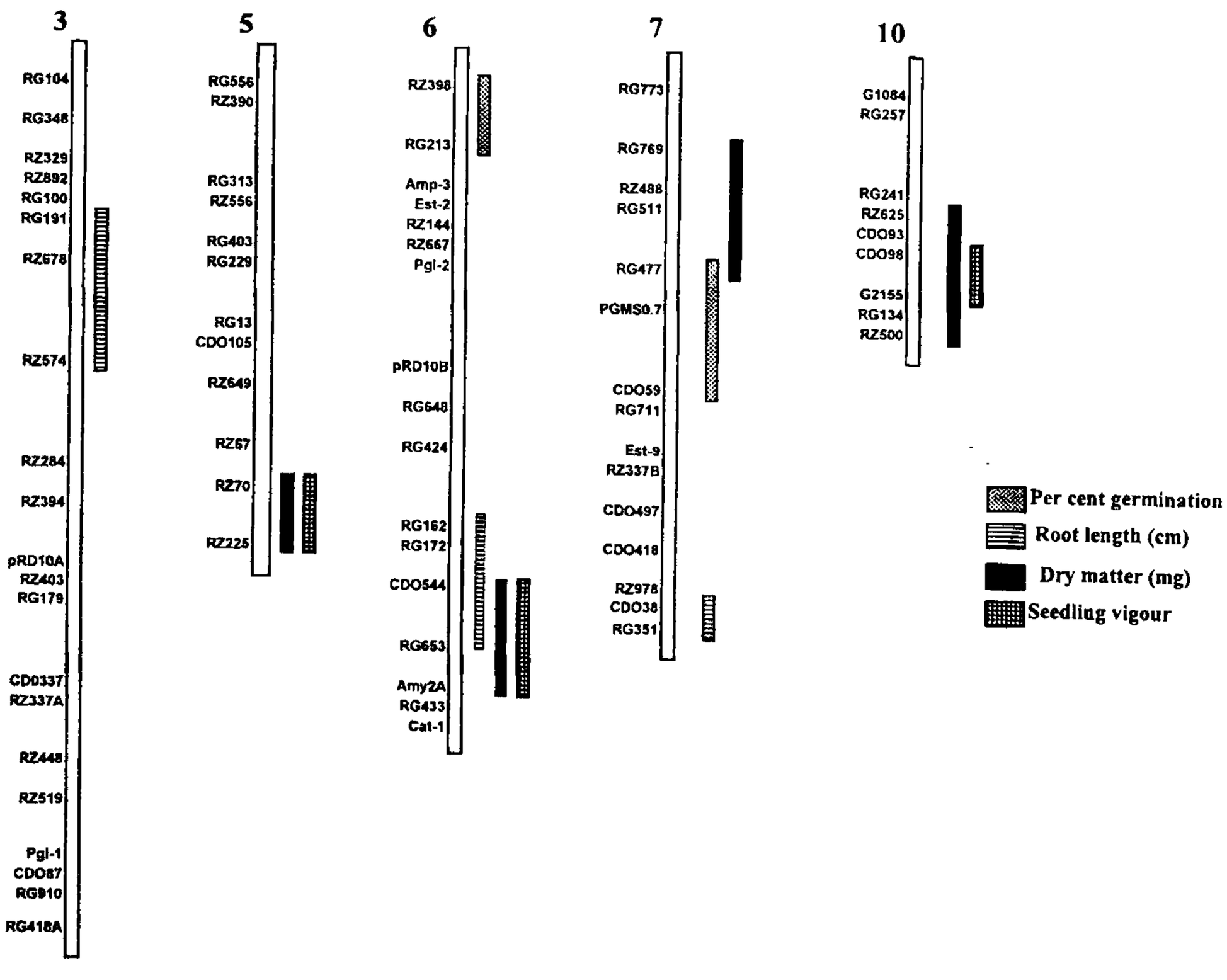

Figure 1. Rice chromosome map showing the position of QTL associated with seedling tolerance to salt stress for various seedling characters at 0.5 per cent NaCl stress (LOD > 1.50).

overlapped with qSDM-6 and qSRTL-6. The pleiotropic effect of QTL was hypothesized. This multiple effect of QTL on the same chromosomal region could be due to the fact that seedling vigour is derived from the product of per cent germination and seedling dry matter. We hypothesize that QTL for associated traits will be localized at the same region on the chromosome which is in agreement with the results of Bagali[9]. Zhang *et al.*[10] tagged a salt-tolerant gene to a single copy DNA probe, RG4, which was located on chromosome 7, using F_2 mapping population derived from a cross between a salt-tolerant rice mutant M-20 and its sensitive original

Acc.77–170. The QTL identified in this study did not overlap with this major salt-tolerant gene[10], indicating elite or novel loci for salt tolerance. In anticipation of its significant contribution and pleiotropic nature, the QTL on chromosome 6 may contain a new major gene for salt stress tolerance at seedling stage in rice. This needs to be confirmed by conducting field trials in saline soils for two or three seasons to test if the QTL are stable across seasons and growth phases of the crop. Chromosome substitution at this loci can be done after fine-mapping and back-crossing to desired lines.

Rice breeders are resorting to molecular marker technology for developing salt-tolerant varieties, as traditional breeding practices, many a times, turned out to be difficult exercise in tackling complex traits. QTL mapping is the first step in applying marker technology 'o the molecular breeding programme. QTL identified by this technique, after fine-mapping, could be used for indirect selection of salt-tolerant traits to be used in MAS.

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Somatic embryogenesis and plantlet regeneration in Amrapali and Chausa cultivars of mango (*Mangifera indica* L.)

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Somatic embryogenesis has been obtained from nucellus of two monoembryonic Indian mango cultivars 'Amrapali' and 'Chausa'. Among the four auxins (IAA, IBA, NAA & 2,4-D) tested, only 2,4-D stimulated callus initiation and induction of proembryogenic callus in cultured bisected ovules containing nucellus minus zygotic embryos. The proembryogenic calli produced up to 130 somatic embryos when transferred to 2,4-D-free medium. The presence of 2,4-D in the medium inhibited progression of development of somatic embryos. The best medium for the production, development and maturation of somatic embryos was the modified M4E medium which contained full-strength B5 macrosalts, MS microsalts, MS iron-EDTA and MS organics along with 400 mg/l L-glutamine, 6% (w/v) sucrose and 0.8% (w/v) agar. The mature somatic embryos gave rise to plantlets in liquid medium containing half-strength B5 macrosalts and 1.0 mg/l GA_3. The *in vitro* raised plantlets of Amrapali cultivar have been successfully transplanted in earthen pots containing garden soil, but those of Chausa failed to survive in the garden soil but have been established in pots containing sand and soil (3:1) mixture.

MANGO is a prized summer fruit crop of India with over one thousand recognized varieties consumed as fresh fruit or variously processed. Mango pickles constitute an important ingredient of the poor man's daily meal. The wild trees are a source of timber. India is a major producer of monoembryonic mango cultivars, many of which are esteemed for the high quality fruit and considered far superior to the polyembryonic counterparts[1]. Cultured tissues of mango are recalcitrant to plantlet regeneration. Any biotechnological approach, aimed at improvement of plant quality and yield, requires an efficient plant regeneration system[1]. Somatic embryogenesis has been reported in several mango cultivars (most of which are polyembryonic); yet reports on the development of plantlets from somatic embryos are limited to a few cultivars[1,2]. As the responses are cultivar-dependent, the methods may not be applicable to other varieties[2]. Serious efforts have not been made to utilize the advantages of tissue culture for enhancing the availability of planting material of Indian monoembryonic

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