Quantitative Trait Loci for Component Physiological Traits Determining Salt Tolerance in Rice[1]

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Rice (*Oryza sativa*) is sensitive to salinity, which affects one-fifth of irrigated land worldwide. Reducing sodium and chloride uptake into rice while maintaining potassium uptake are characteristics that would aid growth under saline conditions. We describe genetic determinants of the net quantity of ions transported to the shoot, clearly distinguishing between quantitative trait loci (QTL) for the quantity of ions in a shoot and for those that affect the concentration of an ion in the shoot. The latter coincide with QTL for vegetative growth (vigor) and their interpretation is therefore ambiguous. We distinguished those QTL that are independent of vigor and thus directly indicate quantitative variation in the underlying mechanisms of ion uptake. These QTL independently govern sodium uptake, potassium uptake, and sodium:potassium selectivity. The QTL for sodium and potassium uptake are on different linkage groups (chromosomes). This is consistent with the independent inheritance of sodium and potassium uptake in the mapping population and with the mechanistically different uptake pathways for sodium and potassium in rice under saline conditions (apoplastic leakage and membrane transport, respectively). We report the chromosomal location of ion transport and selectivity traits that are compatible with agronomic needs and we indicate markers to assist selection in a breeding program. Based upon knowledge of the underlying mechanisms of ion uptake in rice, we argue that QTL for sodium transport are likely to act through the control of root development, whereas QTL for potassium uptake are likely to act through the structure or regulation of membrane-sited transport components.

It is now recognized that tolerance of salinity by higher plants, in common with other environmental stresses, is genetically and physiologically complex, and that salt affects numerous plant processes at all levels of organization. At the very least, ion transport, selectivity, excretion, nutrition, and compartmentation are involved, together with growth, water use, and water use efficiency. However, some single-gene effects have been identified, particularly via genomic sequence comparisons with yeast, which have begun to demonstrate commonality in some aspects of the responses to salinity stress of yeast and plants. This approach has been exploited notably in the use of yeast *NHX1* to identify *AtNHX1*, which when overexpressed in Arabidopsis, markedly improves tolerance to salt stress (Apse et al., 1999); *OsNHX1,* a rice (*Oryza sativa*) cDNA homolog, showed increased expression under salt stress (Fukuda et al., 1999). In a similar manner, transformation of tomato with yeast *HAL1* (halotolerance) is reported to improve its level of salt tolerance (Gisbert et al., 2000), while Zhang et al. (1999) were able to demonstrate that allelic variation in one copy of a small family of H^+ ATPase genes was correlated with a quantitative trait locus (QTL) for salt tolerance in rice.

However, most of the processes found, empirically, to be important in plant resistance or tolerance of salinity exhibit quantitative inheritance; that is they show continuous variation and a high degree of environmental sensitivity. Although many component traits in salinity tolerance have now been extensively described (e.g. compartmentation in halophytes, minimizing sodium uptake, maximizing selectivity of potassium over sodium, and the ability to synthesize compatible solutes) and in some cases the underlying mechanism is at least partially understood (e.g. sodium/potassium selectivity in wheat: Gorham et al., 1997; bypass flow in rice: Garcia et al., 1997; and compatible solute synthesis in *Mesembryanthemum*: Bohnert and Shen 1999), the application of this knowledge to the improvement of cereal crops such as rice remains hampered because of the quantitative nature of the genes involved (which are difficult to handle in a breeding program).

Investigations of plant response to environmental stress are now frequently revealing relatively small numbers of major QTL (for review, see Yano and

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Sasaki, 1997) despite the certainty that large numbers of genes must contribute to the overall phenotypes: recent data on drought responses in rice are particularly pertinent (Champoux et al., 1995; Price and Tomos 1997; Price et al., 1997; Yadav et al., 1997). The prospects of changing a phenotype through genetic manipulation or through conventional breeding are much greater if one or a few defined regions of chromosome are of crucial importance than if generating a desired phenotype depends upon changes in a large number of genes, each with small effect, scattered all over the genome. The identification of QTL has, therefore, practical importance to attempts to enhance stress tolerance.

It is now possible to begin to dissect a complex physiological trait such as salt tolerance in rice using improved methods of identifying and measuring the physiological components (e.g. Yeo et al., 1990), improved mapping techniques, and software (Jansen and Stam, 1994; Kearsey and Hyne, 1994; Kearsey and Farquhar, 1998), together with one of the densest plant genetic maps available (Nagamura et al., 1997). The study reported in this paper sought to identify and map major QTL associated with the salinity tolerance traits of low sodium uptake and regulation of Na:K ratio. Markers closely associated with major QTL for salt tolerance might then be used for breeding programs in rice using marker-assisted selection.

RESULTS

Construction of Genetic Linkage Maps

Of the 85 amplified fragment length polymorphism (AFLP) primer combinations tested against parental DNA, 33 produced at least three clear and scorable polymorphic bands, giving rise to 221 mappable AFLPs. A Chi-square test ($P \leq 0.005$) was performed on each marker to verify the expected 1:1 segregation ratio, resulting in 199 AFLP markers being retained for mapping. Microsatellite and restriction fragment-length polymorphism (RFLP) markers were used to anchor the AFLP linkage map. Twenty-nine of the 84 microsatellites screened discriminated between the parents and were used to genotype the population of recombinant inbred lines (RIL). Two microsatellites showed distorted segregation ratios ($P \leq 0.005$).

From a parental screen of 107 RFLP probes, 28 loci were polymorphic. Fourteen of these were used in the full population screen to target chromosomal regions requiring anchoring. Two loci exhibited segregation distortion ($P \leq 0.005$). Using Joinmap 2.0 (Stam, 1993), nine linkage groups were constructed representing chromosomes 1 to 6 and 9 to 11 of rice, with a minimum LOD score of 3.0 and a maximum recombination fraction of 0.49. The LOD score is defined as the base-10 logarithm of the ratio of the maximum likelihood values assuming linkage versus no linkage. No polymorphisms were found on chromosomes 7, 8, and 12 for microsatellites or RFLPs.

Trait Performances

The dry mass and the amounts of sodium, potassium, and chloride ions were measured in three replicate samples of the mapping population. These data were used to calculate nine separate phenotypic parameters. Table I gives the mean value of each trait measurement for the mapping population and the properties of the trait distributions. The percentage coefficient of variation is also given for the traits of Na^+ uptake, K^+ uptake, Na^+:K^+ ratio, dry mass production, and concentrations of Na^+ and K^+ ions across the three replicate treatments together with F values. These calculations were not made for the traits involving Cl^- ions as, in subsequent marker

Table I. *The ANOVA of the nine traits over three replicate treatments using shoot dry mass (g)*

Rows indicate the information for each trait. Column 1 contains the trait names and the units measured, column 2 has the mean of all the values measured for that trait across the population, column 3 shows the percentage coefficient of variation between the three replicate treatments, column 4 contains the F values for the coefficients of variation, and columns 5 and 6 give the properties of the trait distribution.

Trait	Mean	Coefficient of Variation	F Ratio ($P < 0.0001$)	Kurtosis	Skewness
		%			
Dry mass (g)	0.43	13	13.22	0.17	0.07
Na^+ (mmol g^{-1})	0.95	23	15.01	3.13	1.52
K^+ (mmol g^{-1})	0.55	14	7.16	0.61	0.43
Na^+ (mmol)	0.37	25	12.16	4.53	1.50
K^+ (mmol)	0.22	14	12.47	2.87	1.08
Na^+:K^+	1.74	22	10.99	1.20	0.90
Na^+:Cl^-	1.43	nc[a]	nc	2.25	1.31
Cl^- (mmol g^{-1})	0.68	nc	nc	2.25	1.30
Cl^- (mmol)	0.27	nc	nc	0.04	0.58

[a] nc, Not calculated.

regression analysis, no significant QTL were found for these traits.

QTL Analysis

Single Marker ANOVAs

ANOVA was initially used to identify markers showing a significant association with all nine traits listed in Table I (using Genstat [Numerical Algorithm Group Ltd, Oxford] with $P \leq 0.005$). Twenty-five AFLP markers distributed across chromosomes 1, 4, 6, and 9 were identified in this way. The results are summarized in Table II. Fourteen of these markers were associated with QTL for dry mass (vigor) and mapped to chromosome 6. These dry mass markers coincided with all nine of the markers for Cl^- ion concentration (mmol g^{-1} dry mass shoot tissue), with five out of seven markers for Na^+ concentration, with six of the nine markers for K^+ concentration, and with two out of five markers for total K^+ uptake (mmol) into the rice shoot.

It is important in terms of the interpretation of the data to emphasize at this point the difference between the concentration of an ion in shoot tissue (a quantity per unit dry mass) and the quantity per se of an ion in a shoot. Because the latter accumulated during the course of the experiment it can be equated with the ion uptake occurring over the period (20 d after salt stress was first applied). Single unique markers for Na^+ concentration were located on chromosomes 4 and 6; for K^+ concentration, one marker was found on chromosome 4 and two on chromosome 1. The Na^+ concentration marker on chromosome four also showed significant association with the trait controlling Na:K ratio.

Of the remaining six markers, two were associated with a QTL controlling the Na^+:K^+ ratio (chromosomes 1 and 4), one with total Na^+ uptake (mmol; chromosome 1), and three with total K^+ uptake (mmol) in the shoot (chromosomes 4 and 9). As the markers for these three traits were independent of dry mass (vigor), it is believed that they are related specifically to the uptake of these ions at the root level. No markers were found relating to the traits of Cl^- uptake or Na^+:Cl^- ratio.

In light of these results further analysis was focused on chromosomes 1, 4, 6, and 9 and the traits of Na^+ uptake, K^+ uptake Na^+:K^+ ratio, dry mass production, and concentrations of ions. Figure 1, A and B show the maps for each of these chromosomes with the full set of markers. The positions of those AFLP markers given in italic in Table II are not shown on the chromosome figures (Fig. 1, A and B). This was because the genotype data for these markers was

Table II. *Single-marker ANOVA results; traits and associated markers at $P \leq 0.005$*

Each row indicates with an asterisk the traits with which a particular AFLP marker was significantly associated. AFLP markers in italics were not used for subsequent map construction because the segregation data for these markers had skewed segregation ratios.

Marker No.	Chromosome	Traits Associated with Markers						
		Dry mass	Na^+	K^+	Cl^-	Na^+:K^+	Na^+	K^+
		g		*mmol g^{-1}*			*mmol*	
E12M35-8	6	*	*	*	*			
E12M57-3	*6*	*						
E12M55-2	6	*						*
E12M86-4	6	*	*	*	*			
E12M71-4	*6*	*	*					
E12M61-1	*6*	*						
E12M74-1	*6*	*		*	*			
E12M74-8	*6*	*		*				
E12M80-2	6	*			*			*
E12M81-5	6	*	*	*	*			
E12M41-5	*6*	*			*			
E12M51-2	*6*	*			*			
E15M51-3	*6*	*			*			
E15M53-3	6	*	*	*	*			
E12M79-6	4		*					
E15M48-2	6		*			*		
E12M65-3	*1*			*				
E12M74-2	*1*			*				
E12M79-4	4			*				
E12M57-1	1					*		
E15M41-4	*4*					*		
E12M65-1	4							*
E12M37-1	1						*	
E12M73-2	*4*							*
E12M48-2	9							*

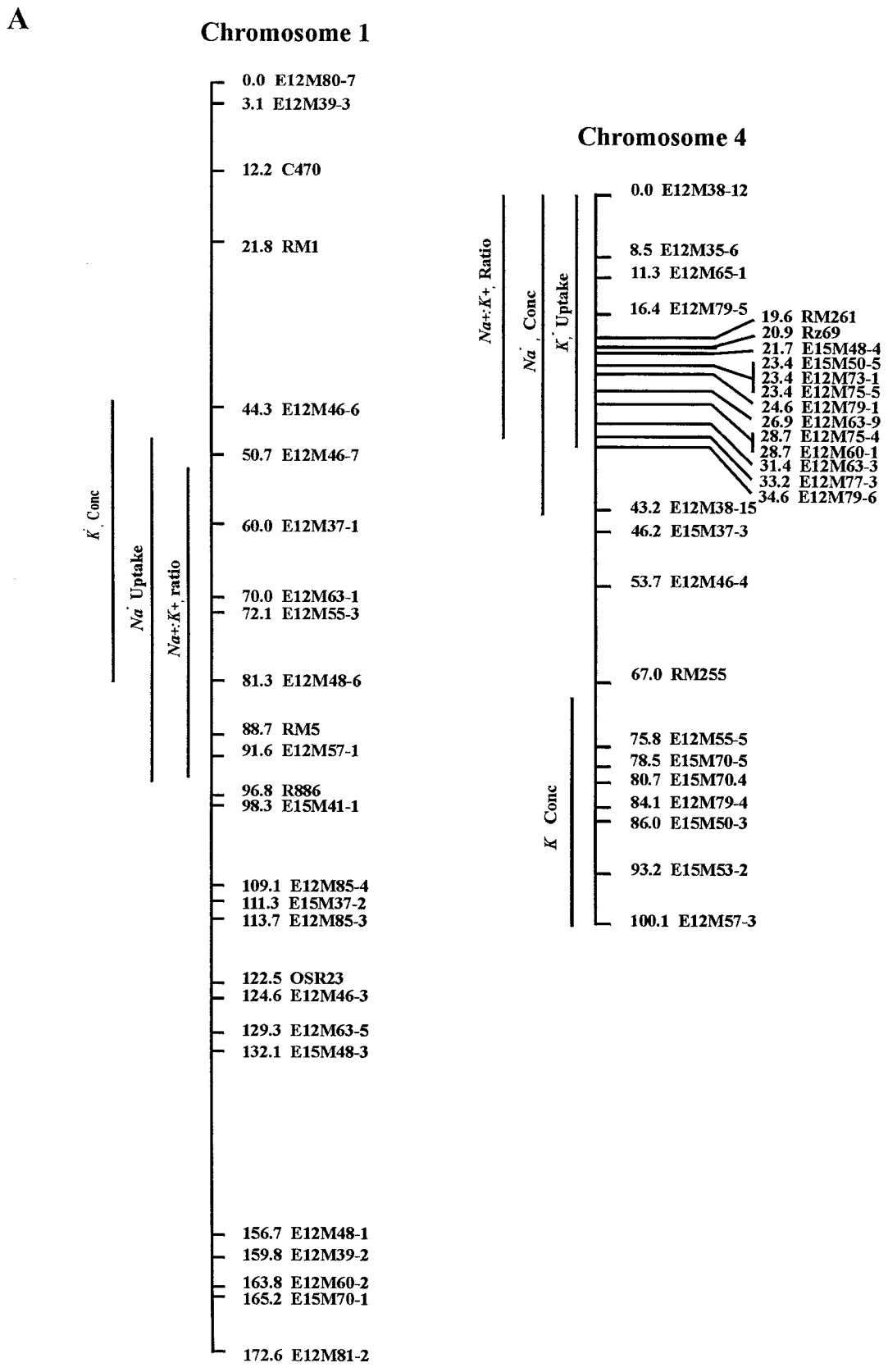


Figure 1. (Figure continued on next page.)

B

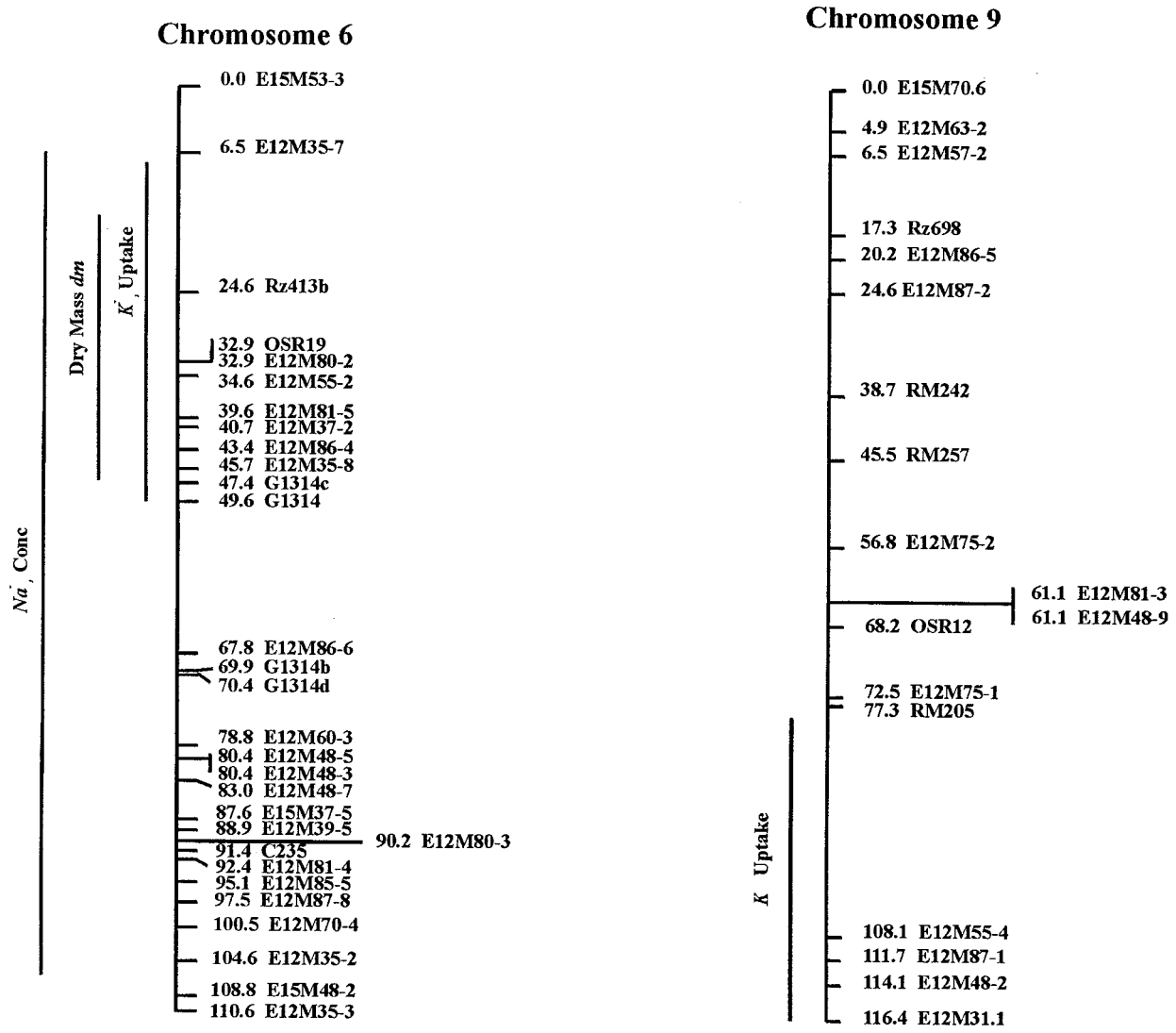


Figure 1. (Figure continued from previous page.) Chromosomal maps. All molecular markers are shown with centimorgan distances from the top of each chromosome before the marker name. The 95% confidence intervals (CIs) of QTL positions are indicated. AFLP markers are named by the selective primer pair combination used following the nomenclature given in Zabeau and Vos (1993), with E denoting the *Eco*Rl primer and M denoting the *Mse*l primer; the hyphenated figure is the band number on the gel, e.g. E15M53-3. Markers named Rz-, R-, G-, or C- are RFLP markers and microsatellite markers are denoted as Rm- or OSR-. A, Chromosomes 1 and 4. B, Chromosomes 6 and 9.

skewed, which could have interfered with marker order in the linkage groups; they were therefore omitted from the chromosome maps.

Marker Regression

Marker regression analysis was performed using values for each trait in turn with marker values for each of chromosomes 1, 4, 6, and 9. A subset of markers from the chromosomal maps (Table III) were used so as to provide equal spacing between markers of between 10 to 20 cM for this analysis (Davarsi et al., 1993). QTL positions that were significant for each trait are shown in Table IV and the 95% CIs of each QTL can be found in Figure 1, A and B. The proportion of phenotypic variation explained for any of the traits varied from 6.4% to 19.6%. Figure 2, A through K

Table III. *Summary of nos. and positions of marker loci used for regression analysis*

The complete set of markers used for the construction of the chromosomes can be found in Figure 1.

Chromosome	Chromosome Length (Kosambi cM)	No. of Markers	Average Distance between Markers (Kosambi cM)
1	172.6	16	14.4
4	100.1	12	8.3
6	110.6	12	9.2
9	116.4	12	9.7

shows the marker regression graphs for each "trait x chromosome" combination.

One significant QTL was located for sodium uptake (Na^+) on chromosome 1 at 74 cM and explained 8.9% of the total variation for this trait (Fig. 2A). Of the three QTL for potassium uptake, K^+_1 on chromosome 4 explained 6.8% of the trait variation (Fig. 2F), K^+_2 on chromosome 6 accounted for 7.6% (Fig. 2I), whereas K^+_3 on chromosome 9 explained 19.6% (Fig. 2K). Together they explained nearly 34% of the variation for potassium uptake.

Two QTL were located associated with Na^+:K^+ ion ratio. Na^+:$K_1{}^+$ on chromosome 1 explained 9.1% (Fig. 2B) and is located in a similar position as the QTL for Na^+ uptake at 74 cM. Na^+:$K_2{}^+$ on chromosome 4 is located at 14 cM (Fig. 2E) and explained 9.6% of the trait variation. Together they explain 18.7% of the variation of this trait.

A significant QTL for dry mass (*dm*) occurred on chromosome 6 at 34 cM (Fig. 2H), explaining 9.7% of the variation for this trait. A QTL for Na^+ ion concentration ($Na_2{}^+$) was found on the other arm of chromosome 6 at 106 cM (Fig. 2J), explaining 6.4% of the variation for this trait; a further 6.7% was explained by another QTL on chromosome 4 ($Na_1{}^+$) located at 24 cM (Fig. 2D); together they explained 13.1% of the variation. Two significant QTL for K^+ concentration, K^+_1 and K^+_2, were found on chromosomes 1 and 4 (Fig. 2, C and G) and explained 10.6% and 8.8% of the variation for this trait, respectively.

DISCUSSION

Genetic Map

Chromosomes 7, 8, and 12 were not represented on our genetic map due to a lack of detectable genetic polymorphism between the parents of the mapping population. This is most likely due to the fact that these parents are *indica* genotypes. This does not mean that there were no QTL for traits relating to salt tolerance located on these chromosomes, but merely that they cannot be detected because there are no discernible allelic differences. Because rice breeding programs mainly use the same group for crosses, such as *japonica/japonica* and *indica/indica*, the QTL identified between closely related varieties are far more interesting and useful to rice breeders (Yano and Sasaki, 1997) than intergroup crosses. To date, only one QTL analysis of a *japonica/japonica* cross has been reported (Redona and Mackill, 1996).

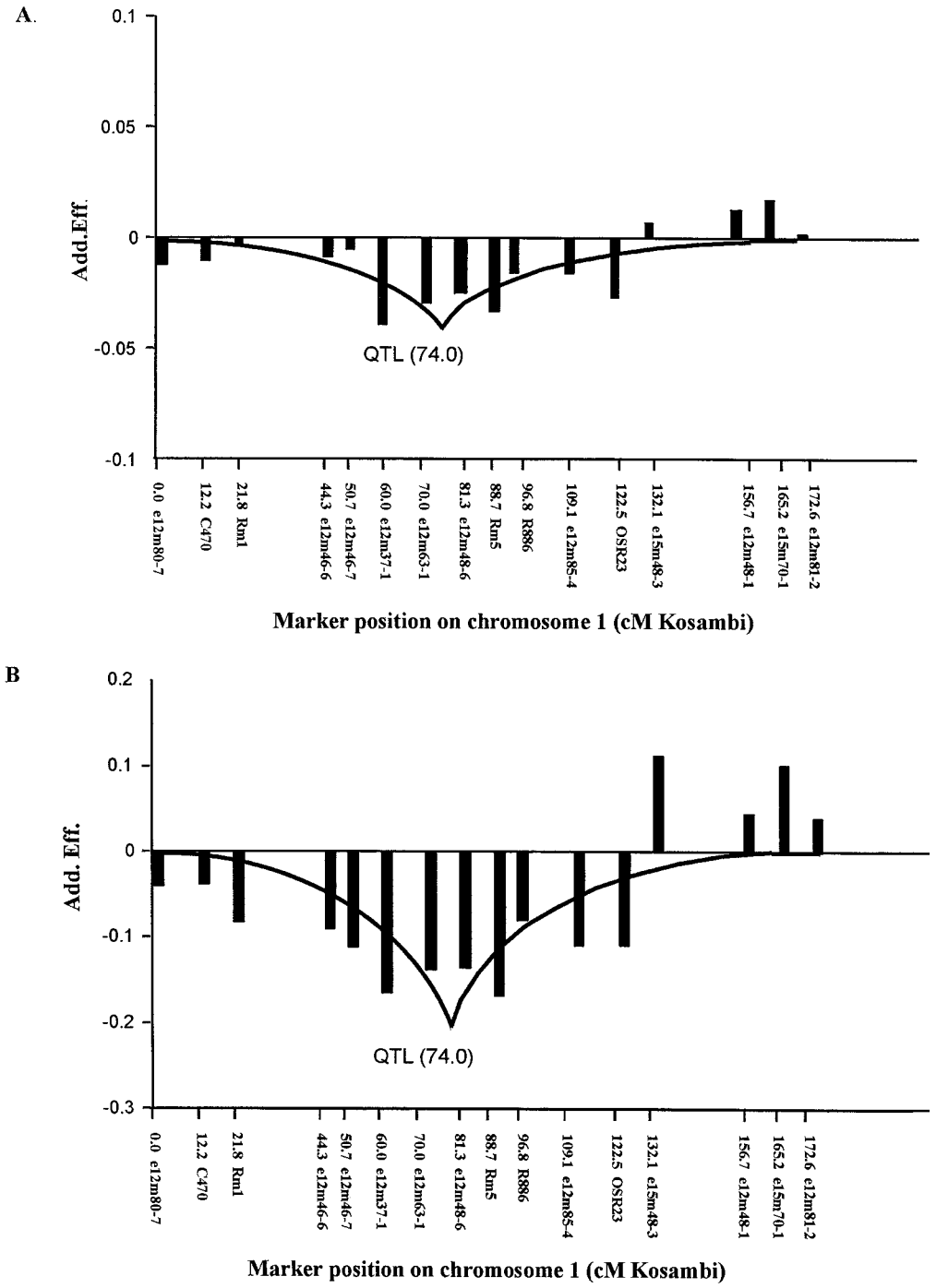
Interpretation of QTL Analysis

Complex physiological traits have on recent occasions been described by a small number of major QTL (Kearsey and Farquhar, 1998). Problems arise in finding useful QTL for a particular trait when there are numerous QTL associated with it, as the smaller their individual contribution, the more difficult they are to detect. Although the sensitivity of the analysis

Table IV. *Properties of located QTL*

Results on estimated QTL position, together with their additive effects and the percentage of the genetic variance explained by each QTL for a particular trait.

Chromosome	Trait QTL	Position cM	Additive Effect	% Variance Explained
1	Na^+ uptake	74	−0.04	8.9
1	K^+_1 concentration	56	0.05	10.6
1	Na^+:K^+_1 ratio	74	−0.22	9.1
4	K^+_1 uptake	10	0.02	6.8
4	K^+_2 concentration	90	0.05	8.8
4	Na^+_1 concentration	24	−0.013	6.7
4	Na^+:K^+_2 ratio	14	−0.22	9.6
6	Dry mass	34	−0.04	9.7
6	K^+_2 uptake	30	0.02	7.6
6	Na^+_2 concentration	106	−0.12	6.4
9	K^+_3 uptake	96	−0.03	19.6



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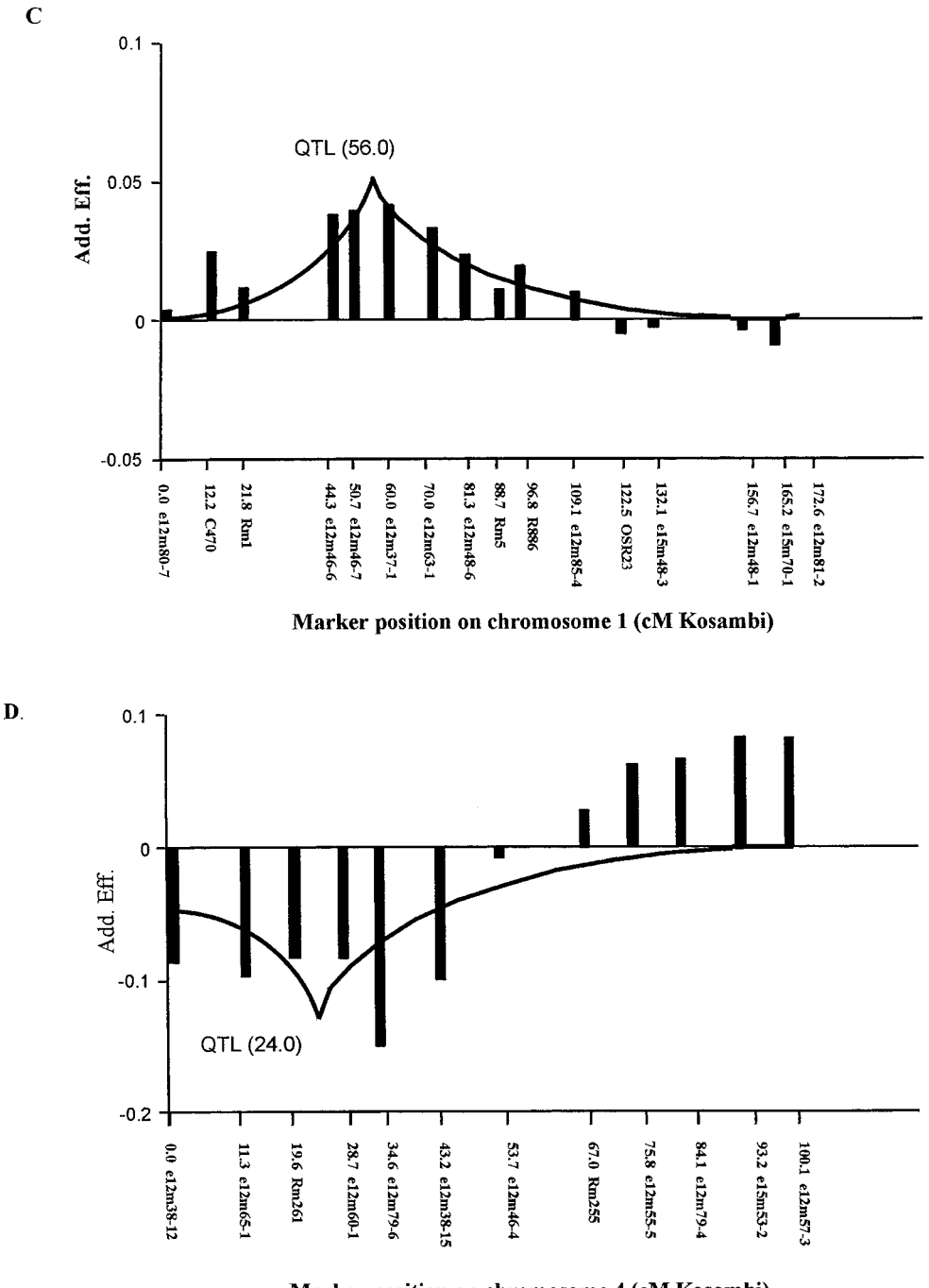
may fail to detect QTL with small effects, giving rise to the biased view that there are only a few QTL with large effect, the fact that major QTL for complex processes can be detected is promising for plant breeding. Single-marker analysis is generally a good choice when the goal is simply detection of a QTL linked to a marker. However, estimation of its position and its effects requires further complex analysis such as marker regression (Kearsey and Hyne, 1994) or interval analysis (Haley and Knott, 1992).

The QTL associated with Na^+ uptake found on chromosome 1 coincides with the estimated location of a QTL affecting Na^+:K^+ ratio (Na^+:K_2^+; see Fig. 1A). Both of these QTL explain a similar amount of variation for each trait. However, the single-marker ANOVA analysis shows two significantly different



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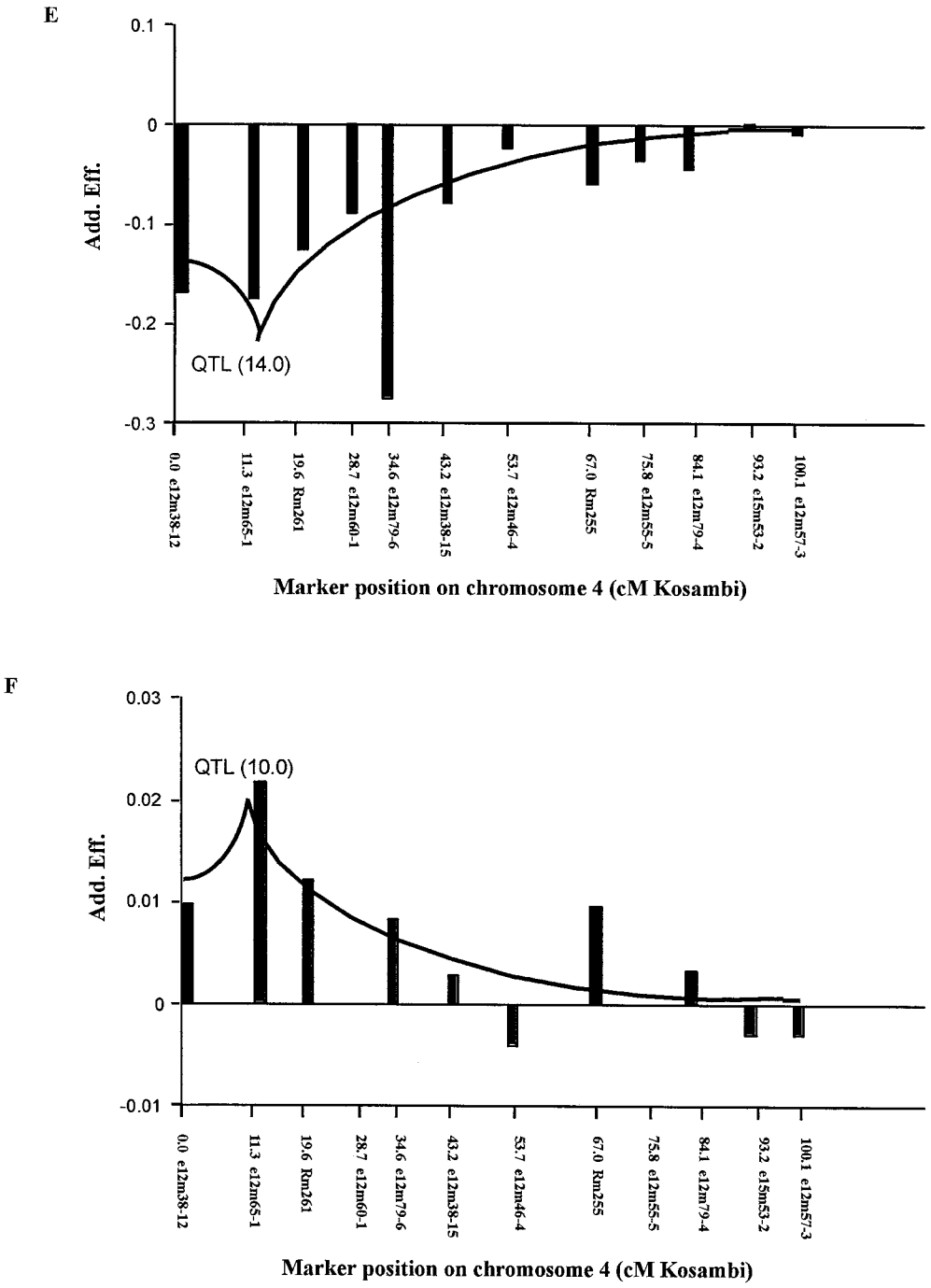
markers for each trait when the full marker data set for this chromosome is analyzed (Table II). The question then arises whether there is one QTL affecting both traits or whether there are QTL affecting two separate traits, but located adjacent to each other. This highlights one of the problems of QTL analysis. It is not yet possible to discern whether significant effects at several linked markers are due to a common QTL or due to several linked QTL. Although specific tests for the presence of linked QTL in adjacent intervals using sets of three overlapping markers have been suggested (Haley and Knott, 1992; Martinez and Curnow, 1993), these tests have their problems (Whittaker et al., 1996). The QTL K^+_1 is also found on chromosome 1 with its CI overlapping those of the QTL associated with Na^+ uptake and Na^+:K^+ ratio (Fig. 1A). However, the ANOVA and marker regression analysis identified a significantly different marker that was at some distance (56 cM) from the Na^+ uptake and Na^+:K^+ ratio QTL and



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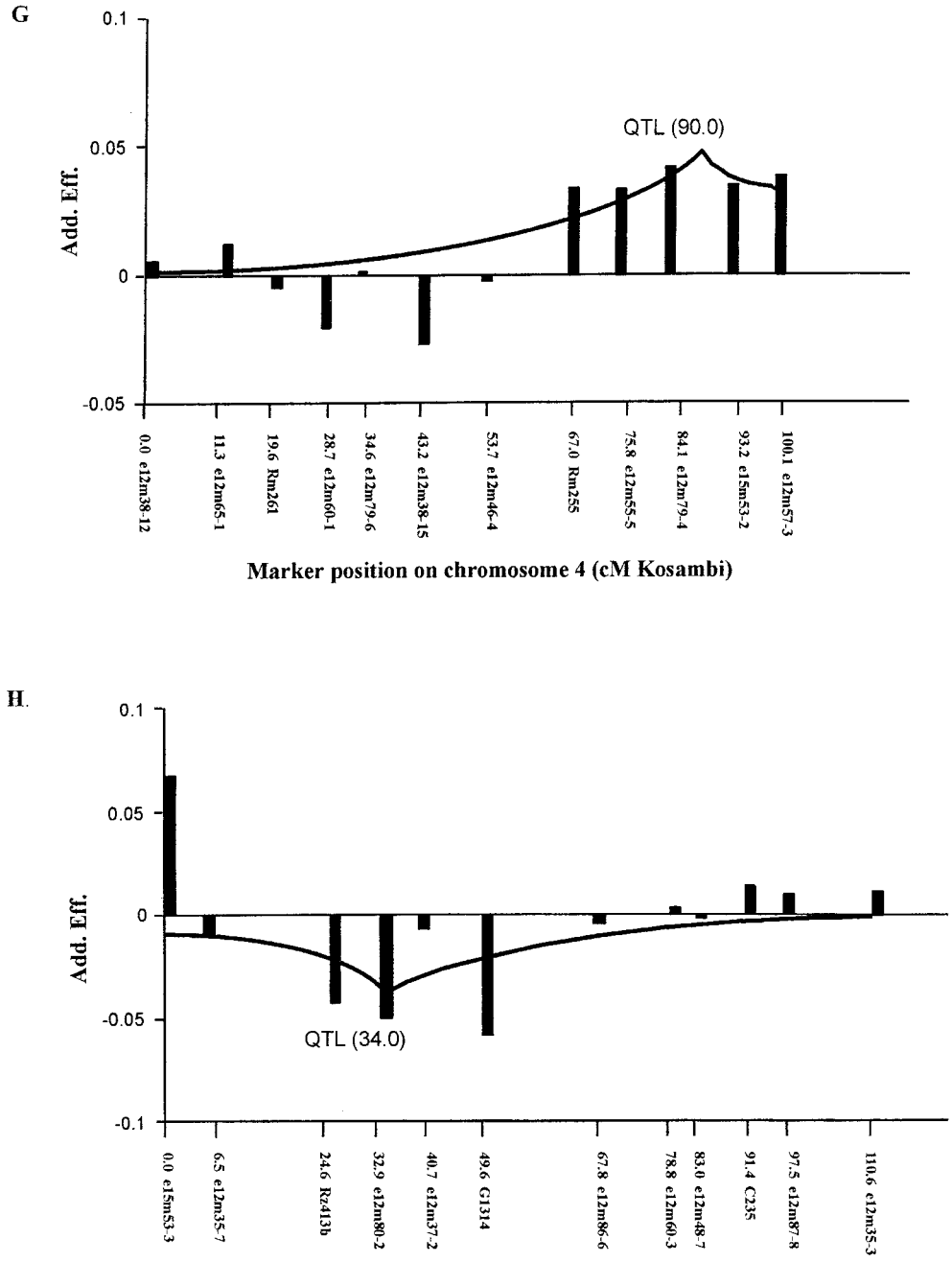
therefore K^+_1 appears to be a separate QTL. The regression graph in Figure 2C indicates one clear-cut QTL for this trait.

Chromosome 4 harbors four QTL. Three of these, associated with the traits of Na^+ concentration, K^+ uptake, and Na^+:K^+ ratio, appear to have overlapping CIs in one region (Fig. 1A), but their estimated positions are different: at 10 cM for K^+ uptake, 14 cM for Na^+:K^+ ratio, and 24 cM for Na^+ concentration QTL, respectively (Table IV). It could be that there are three QTL here, one associated with K^+ uptake and one with Na^+ concentration of equal effect; both of them affect the QTL controlling these ions (i.e. $Na^+:K_2^+$). This situation bears certain similarities to that on chromosome 1 where there is a QTL controlling ion ratio and two other QTL, only this time one for Na^+ uptake and the other for K^+ concentration. All these QTL are close together in one region of around 45 cM. It seems likely that these chromosomal areas appear to be involved in the monitoring and



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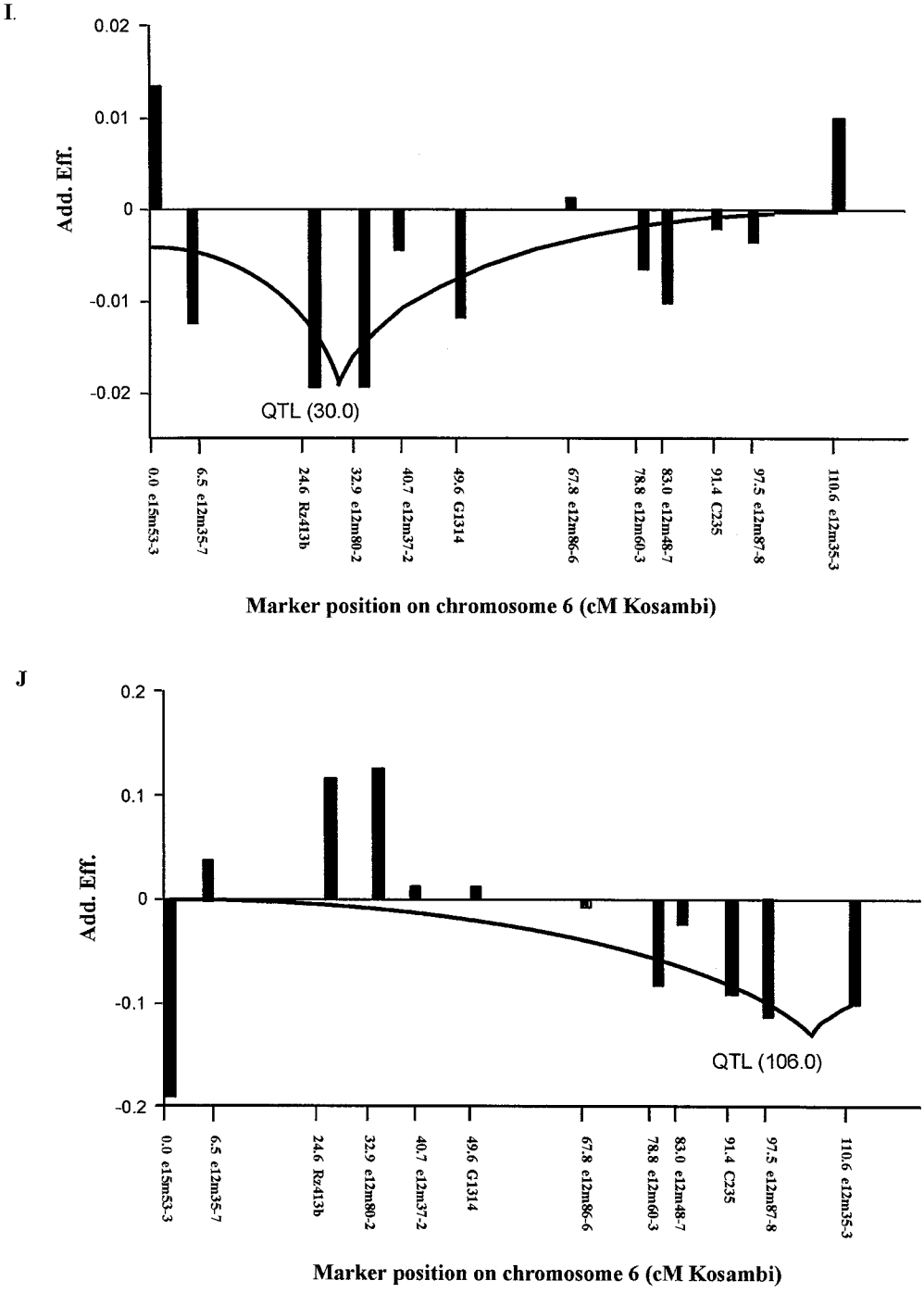
regulation of the levels of Na^+ and K^+ ions. At the opposite end of chromosome 4, a QTL for K^+ concentration was found. It was not associated with the QTL for plant vigor and explains 8.8% of the variation in this trait. The regression graph in Figure 2G shows one significant chromosomal area with a CI of approximately 30 cM.

One QTL associated with plant vigor (dry mass) was found on chromosome 6, with a discrete CI of approximately 15 cM and a position of 34 cM (Fig. 1B). The QTL associated with K^+ uptake, K^+_2, has been located at 30 cM, just distal to the plant vigor QTL, but with an overlapping interval. As it is in a similar region as the QTL for plant vigor, there is a possibility that this trait is affected by the growth of the plant and does not govern a mechanism for K^+ uptake per se (see also below). Supporting this finding, Prasad et al. (2000) also found a QTL on chromosome 6 associated with seedling tolerance to salt stress and dry mass. The CI of the QTL for Na^+



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concentration appears to span most of the length of the whole chromosome (Fig. 1B), but the estimated position of this QTL is at the opposite end of chromosome 6 from the K^+ and plant vigor QTL, at 106 cM. The reasons for this become clearer if one refers to its regression graph (Fig. 2J). Although no markers appeared to be significantly associated with Na^+ concentration in the K^+ and plant vigor QTL region of the chromosome, there is an indication of a region approaching significance, but in dispersion. It is possible that there is another QTL here, close to the plant vigor QTL, but of a relatively weak effect. This would result in the location of the stronger effect QTL at one end of the chromosome, but has caused the estimate of the QTL location to have a large CI (Hyne and Kearsey, 1995). This represents another drawback of any many QTL analysis techniques in that they cannot provide useful models for chromosomes that

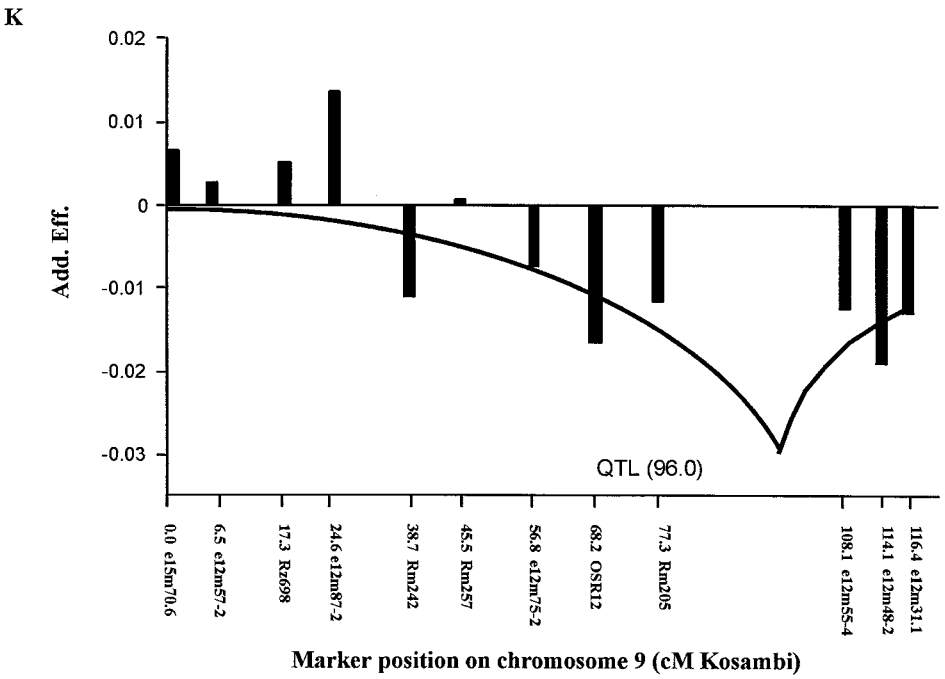

Figure 2. (Figure continued from previous page.) Marker regression histograms for traits associated with salt tolerance. The *x* axis represents the length of the chromosome together with the location of molecular markers in centimorgans. Each vertical bar at a marker position represents the level of additive effect (Add.Eff.) of the marker position on the trait scores. Because the significance of the effect is highest at the location of the QTL and reduces with centimorgan distance either side of it, a graph can be superimposed on the histogram indicating the estimated QTL position along the chromosome. A, Na^+ uptake, chromosome 1; B, Na^+:K^+ ratio, chromosome 1; C, K^+ concentration, chromosome 1; D, Na^+ concentration, chromosome 4; E, Na^+:K^+ ratio, chromosome 4; F, K^+ uptake, chromosome 4; G, K^+ concentration, chromosome 4; H, dry mass, chromosome 6; I, K^+ concentration, chromosome 6; J, Na^+ concentration, chromosome 6; K, K^+ uptake, chromosome 9.

may have more than one QTL (Goffinet and Mangin, 1998). The relatively large size of some CIs makes it difficult to distinguish two QTL on a chromosome unless they are far apart. Sometimes the ANOVA tests may indicate that there are markers showing significance in two different regions of the chromosome. However, when there are two markers on one chromosome in dispersion, one QTL tends to reduce the effects of the other so making the detection of either more difficult. In the case of two QTL being in association, their individual effects could combine to give the appearance of a false or ghost QTL somewhere between them. One can only conclude from inspection of the results that the model of just one QTL cannot explain the data in such cases.

The QTL for K^+ uptake (K^+_3) with largest effect was found on chromosome 9 at 19.6 cM, within a CI of 40 cM. This QTL explains 19.6% of the variation for this trait alone. No other traits were found to be associated with any of the markers of this chromosome.

Epistatic interactions between markers were not investigated because our population was small (118 RILs) and thus interactions would be difficult to detect (Yano and Sasaki, 1997). Epistatic effects and pleiotropy can play a large part in the interaction and function of QTL, the presence of one very small effect QTL may have a massive effect on regulatory pathways. In this way unlinked QTL can alter QTL detection as segregation at such loci contributes to the overall phenotypic variance. Reducing or removing the effects of a major QTL (such as plant vigor) in some cases can reduce the residual variance for another marker under consideration sufficiently to enable detection of additional QTL (Lin et al., 1995).

It is now possible to compare cereal chromosomal regions for particular traits due to genomic synteny within the Poaceae (Devos and Gale, 1997). As the genetic basis of salt tolerance is physiologically and genetically complex in cereal genomes studied for these traits, meaningful comparisons are difficult to find at present. For example the *Kna1* gene associated with salt tolerance in wheat (controlling Na^+/K^+ discrimination) has been mapped to chromosome arm 4DL (Dubcovsky et al., 1996) and this region is probably equivalent to the tip of chromosome 3S in rice. However, no QTL associations for Na^+/K^+ ratio were detected in this region. This may be due to, for example, different mechanisms operating in wheat compared with rice, or the presence of the same allele(s) for this QTL in both the parents of our mapping cross. In barley, QTL have been found associated with salt tolerance involving multiple loci expressed at different developmental stages of the plant (Ellis et al., 1997; Mano and Takeda, 1997).

These are scattered throughout the barley genome and thus difficult to compare with rice.

In rice there have been other reports of QTL associated with salt tolerance. Zhang et al. (1995), using a salt tolerant mutant line, have detected a QTL involved in salt tolerance on chromosome 7. Gong et al. (1999) have reported a major QTL for salt tolerance in rice on chromosome 1, but it is unclear how this relates to the positions of the QTL reported here on chromosome 1. Prasad et al. (2000) have also mapped a QTL on chromosome 6 related to salt tolerance and dry mass, which may be related to the QTL found in this study for dry mass.

An Important Distinction: Ion Quantity and Concentration

One of the major confounding effects in interpreting ion uptake data under saline conditions is that of plant vigor. An external concentration of 50 mM NaCl may not, in itself, be damaging to rice (Yeo et al., 1991); it is the increase in internal concentration with time that leads to damage and this feeds back positively, once damage reduces growth (Munns, 1993). As long as the rate of new growth is sufficient to allow the concentration of salt in the leaves to remain tolerable by the plant, then damage is minimal. Once the concentration of Na^+ and Cl^- in the leaf causes a growth reduction, then there is less material into which additional salt can be distributed. The NaCl concentration in the leaf then rises faster, growth decreases even more, the concentration rises further, and so a catastrophic event is precipitated. It is the long-term build up of salt in the leaves that ultimately leads to damage (Munns and Termaat, 1986; Yeo et al., 1991).

In our analysis we have emphasized QTL associated with the quantity of ions in the shoot (rather then their concentration), as the quantity is determined simply by the quantity of ions transported from the root to the shoot; retranslocation from shoot to root is trivial in relation to that from root to shoot (Yeo and Flowers, 1982). Ion concentration, on the other hand, is confounded with dry mass, so that QTL related to concentration might really be determined by mass, a parameter that in turn reflects plant vigor.

In this study the QTL analysis is entirely consistent with the known physiological and anatomical basis of sodium and potassium uptake in rice and with studies of the heritability of sodium and potassium transport. The mechanism (Yeo et al., 1987; Yadav et al., 1997) and the heritability (Yeo et al., 1988; Garcia et al., 1997) indicate independence of the processes of sodium and potassium uptake in rice in saline conditions. Sodium uptake occurs primarily via bypass-flow leakage along an apoplastic continuity into the xylem. Potassium is able to follow this pathway, but the quantitative contribution is directly proportional to the outside concentration. Although apoplastic uptake is substantial for sodium at an external concentration of 50 mM, it is trivial for potassium at 1 mM. Relative to the uptake of K^+ by membrane-based processes the apoplastic leakage of K^+ is essentially invisible.

The uptake of potassium is likely to be due to selective channel(s) and/or transport protein(s) (Sussman, 1994; Rubio et al., 1995) according to the external and internal activities and membrane potential. Although a number of potassium carriers and channels may allow the passage of sodium (e.g. Roberts and Tester, 1995; Amtmann et al., 1997; Maathuis et al., 1997a, 1997b; Wegner and DeBoer, 1997; Maathuis and Amtmann, 1999), these are all likely to be masked by the apoplastic pathway in the case of rice (Garcia et al., 1997). It is quite possible that the QTL for Na^+:K^+ ratio, which was independent of the QTL for sodium or potassium uptake per se, reflects selectivity by membrane-based transport systems.

Because the major pathways of uptake of sodium and potassium in rice are in parallel and not directly in competition, the uptake of the two ions would be expected to be independent. This was found in the heritability studies reported earlier (Garcia et al., 1997) and in the results described here in which the major QTL for sodium and potassium were located on different chromosomes. Gregorio and Senadhira (1993) also observed in rice that two groups of genes were involved in the sodium and potassium uptake; one group was envisaged to control sodium exclusion and the other to control potassium absorption.

The genes governing the transport of sodium and potassium are predictably different. At any typical soil concentration, the transport of potassium will be a membrane-determined process mediated by one or more of a range of carriers and channels (Maathuis and Amtmann, 1999) and the contribution of the apoplastic pathway will be small. For sodium at saline concentrations, the uptake is largely apoplastic and in rice this masks the entry of sodium via the range of possible carrier/channel pathways (for review, see Davenport et al., 1997; Roberts and Tester, 1997; Amtmann and Sanders, 1999; Maathuis and Amtmann, 1999). Although potassium uptake is expected to be controlled by genes related to the structure or regulation of carriers and channels, the transport of sodium in rice in saline conditions is expected to be controlled by genes affecting root developmental anatomy and architecture. The apoplastic pathway of uptake is presumed to involve leakage around and through the rhizodermal and endodermal barriers (Yeo et al., 1988, 1999; Yadav et al., 1996). The pathway may be partially blocked by colloidal silica (Yeo et al., 1999). The development and integrity of the rhizodermis and endodermis, lateral root development, and the repair of the disruption they cause to these barriers are likely to be important factors in the apoplastic leakage to the xylem. It is

therefore likely that the QTL for sodium transport relate to genes governing root development rather than membrane transport processes.

MATERIALS AND METHODS

Mapping Population

A mapping population of rice (*Oryza sativa* L. sub. *indica*) segregating for the traits of interest was identified from an initial screening of potential parents. Five pairs of elite *indica* breeding lines were crossed (at the International Rice Research Institute [IRRI], Manila, Philippines, by the late Dr. D Senadhira), the choice of parents being based on extensive screening of genotypes for a range of physiological traits (Yeo et al., 1990). The mapping population here (designated IR55178) was chosen because it demonstrated good heritability of the traits of sodium and potassium transport (narrow-sense, 45%: Garcia et al., 1997) and there was an adequate level of genetic (RFLP) polymorphism between the parents (30%). The parents of IR55178 (IR4630- and IR15324-) show extreme phenotypes for sodium transport and for tissue tolerance (the concentration of sodium in the tissue that can be accommodated for the same degree of damage) and differ also for potassium and for chloride transport to the shoot (Yeo et al., 1990; Garcia et al., 1997). The pedigrees of the parents are traceable to the beginning of the crossing program at IRRI in the 1960s (International Rice Research Institute, 1985). Both parents are modern, elite breeding lines of good agronomic character. This does limit the molecular polymorphism, but the results are more relevant agronomically than when the parents are the genetic extremes generally used in crosses made for experimental purposes. The mapping population would be regarded as semi-dwarf by agronomists, though there was appreciable variation in vigor among the lines.

This study was based on a population of 118 RILs from the cross advanced by single seed descent to F_6 in greenhouses at the University of Sussex and at La Mayora, an Institute of the Consejo Superior de Investigaciones Cientificas in southern Spain. Plants were bagged to prevent cross pollination. Heritability studies were conducted by regression of F_4 on F_3 means (Nyquist, 1991) using a random subsample of 44 lines (Garcia et al., 1997). Each of the RILs was bulked at F_6 at the IRRI and used for phenotyping and mapping.

Phenotyping

Seeds of each RIL were heated at 44°C for 5 d to break any possible dormancy, soaked for 24 h in aerated water, and sown directly onto nylon mesh supported on floating Perspex grids. The grids were floated on large (1 m^2) interconnected tanks containing culture solution (total volume 0.5–1.0 m^3) that was recirculated. The plants were grown in a greenhouse where the conditions were as described in detail by Yeo et al. (1990). The culture solution was that of Yoshida et al. (1972), but with sodium salts replaced with potassium and the phosphate concentration reduced (because phosphate toxicity had often been observed at high transpirational demand); for theoretical and modeled concentrations see Yeo et al. (1999). Three completely randomized blocks containing all the lines were grown at the same time. The culture solution was salinized at 10 d after planting, by slowly adding 5 M NaCl to the header tank of a recirculating pump so that the concentration rose gradually to 50 mM over a period of about 24 h and was maintained for 12 d, after which the concentration was increased to 100 mM for a period of 8 d. The shoots were then harvested.

Shoots were dried, weighed, and extracted in 100 mM acetic acid for 2 h at 90°C. Sodium and potassium were determined in the extract by atomic absorption spectroscopy (Unicam 919, Unicam, Cambridge, UK) and chloride with an ion-specific electrode. Results were calculated as the concentration of various ions in the shoot on a dry mass basis and as the quantity of ions in the shoot (the product of concentration and dry mass).

Construction of the Genetic Map

DNA was extracted from 2-week-old leaves as described by Dellaporta et al. (1983). A genetic map was constructed with AFLP markers and anchored with microsatellite and RFLP markers. AFLP analysis was carried out following the method of Vos et al. (1995), using *Eco*RI and *Mse*I restriction enzymes and corresponding primers as described by Zabeau and Vos (1993). Five additional bases were added in total to the core primer sequences during selective amplification, two on the core *Eco*RI primer (5′-GTA GAC TGC GTA CCA ATT C-3′), where primer E12 had additional bases AC, and E15 had CA; and three on the core *Mse*I primer (5′-GTA GAG TCC TGA GTA A-3′), where e.g. M35 had additional bases ACA (see Zabeau and Vos, 1993 for full details of M-selective primers). *Eco*RI primers were radioactively labeled with ^{33}P for detection purposes. PCR products were separated on a 6% (w/v) denaturing polyacrylamide gels, after which the gels were dried onto filter paper and exposed to film. A total of 85 primer combinations were tested on the parents of the cross. AFLP markers were identified by their primer pair combination, following the nomenclature given by Zabeau and Vos (1993), with the band number as suffix. The polymorphic bands were numbered serially in descending order of M_r. Only clear unambiguous bands were scored. Markers were scored for presence or absence of the corresponding bands among the segregating RIL population according to the genotype of the parent (IR4630 or IR15324).

AFLP markers were anchored using microsatellite analysis. Eighty-four published microsatellite markers were evaluated for polymorphism between the parents of the cross (Wu and Tanksley, 1993; Agaki et al., 1996 [*Oryza* simple sequence repeat coded]; Panaud, et al., 1996 [rice microsatellite coded]; Chen et al., 1997). Polymorphic markers were scored in the segregating RIL population as above. The amplification profile was as used by Panaud et al. (1996). Forward primers were radioactively labeled with ^{33}P for detection of amplified fragments. PCR products

were separated on 6% (w/v) denaturing polyacrylamide gels, after which the gels were dried and exposed to film.

RFLP analysis was applied to those chromosomal regions where the microsatellite analysis failed to detect any polymorphisms. Restriction digestion, gel electrophoresis, Southern transfer, and DNA/DNA hybridization followed standard techniques (Sambrook et al., 1989). Four restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, and *Hin*dIII) were used. The probes coded as RZ were provided by Cornell University, whereas those coded R, C, or G were provided by the Rice Genome Project, Tsukuba, Japan.

Genetic maps were constructed using Joinmap 2.0 (Stam, 1993), with a minimum LOD score of 3.0 and a maximum recombination fraction of 0.49. This is commonly used as a likelihood ratio statistic (Ott, 1985) to perform a test for marker, QTL linkage. Map units (centimorgans) were derived using the Kosambi mapping function (Kosambi, 1944).

QTL Analysis

Associations between genetic markers and traits were detected by single-marker ANOVA for each trait using GENSTAT ($P < 0.005$). QTL analysis was subsequently focused on those chromosomes found harboring markers associated with target QTL. A subset of loci were chosen for each chromosome to provide an even coverage and also because marker spacing narrower than 10 to 20 cM does not increase mapping power, regardless of the population size and gene effect (Davarsi et al., 1993). The subsets were spaced approximately 9 to 14 cM apart (Table III).

QTL analysis was performed using the marker regression approach of Kearsey and Hyne (1994) using the software package QTL Café at the web site http:/web.bham.ac.uk/g.g.seaton/. Using marker regression, ANOVAs of the phenotypic data based on the genotype at each marker position test for the presence of one or more QTL. By performing 1,000 simulations, the probabilities associated with the F values of the items in the ANOVA, as well as the CIs of the estimated positions and gene effects, are obtained. Models are accepted when the residuals are no longer significant. Studies have been carried out to compare the efficiency of this method against the interval mapping approach of Haley and Knott (1992), giving rise to essentially similar results using marker intervals of up to 20 cM (Davarsi et al., 1993; Bohuon et al., 1998). The output of the marker regression analysis (Fig. 2) is essentially a histogram showing increasing significance at marker positions associated with QTL.

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