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# Mapping osmotic adjustment in an advanced back-cross inbred population of rice

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**Abstract** Osmotic adjustment is one of several characters putatively associated with drought tolerance in rice. Indica cultivars are known to have a greater capacity for osmotic adjustment than japonica cultivars. We developed an advanced back-cross population using an indica donor, IR62266-42-6-2, to introgress osmotic adjustment into an elite japonica cultivar, IR60080-46A. One hundred and fifty $BC_3F_3$ families were genotyped using microsatellites and RFLP markers, and a few candidate genes. We evaluated osmotic adjustment in these lines under greenhouse conditions using the re-hydration technique. Using the composite interval mapping technique, we detected 14 QTLs located on chromosomes 1, 2, 3, 4, 5, 7, 8 and 10 that together explained 58% of the phenotypic variability. Most, but not all, of the alleles with positive effects came from the donor parent. On chromosome 8, two QTLs were associated in repulsion. The QTL locations were in good agreement with previous studies on this trait on rice and in other cereals. Some $BC_3F_3$ lines carried the favorable alleles at the two markers flanking up to four QTLs. Intercrossing these lines followed by marker-aided selection in their progenies will be necessary to recover lines with levels of osmotic adjustment equal to the donor parent. The advanced back-cross strategy appeared to be an appropriate method to accelerate the process of introgressing interesting traits into elite material.

**Keywords** ABC QTL approach · Back-cross inbred lines (BILs) · Drought tolerance · Osmotic adjustment

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## Introduction

Rice is the world's main food crop. It is cultivated and consumed extensively in Asian countries where production conditions are highly diverse (IRRI 1997). Rice is found in a broad spectrum of growing environments ranging from upland to lowland, aerobic to deep water, and temperate to equatorial conditions. In each ecosystem, specific traits are needed for adaptation to local constraints such as drought, cold, submergence or salinity. About 45% of the total rice area is currently planted without irrigation. Drought is the major factor limiting rice production in these rainfed environments. Varieties with better drought tolerance and technologies to improve water-use efficiency are needed. Breeding varieties for stable production under moisture stress is difficult because selection becomes either impossible or inefficient in those years when no representative drought-period occurs (Blum 1988). The efficiency of selection for drought tolerance could be improved considerably through identification of secondary traits that could be used as selection tools. An ideal secondary trait should be easy to measure, highly heritable, genetically correlated with grain yield under stress, and should show genetic variation in the target species. Many secondary traits have been proposed that may be associated with drought tolerance (Ludlow and Muchow 1990). Physio-morphological traits important under moisture stress in rice were reviewed by Nguyen et al. (1997), who concluded that the most-promising traits were root morphology and osmotic adjustment. Osmotic adjustment (OA) is a cellular adaptation by which, under water deficit, plants are able

to actively accumulate solutes. This results in a lower osmotic potential and the maintenance of high turgor. The identity of the accumulated solutes, which is species dependent, is still unclear in rice (Nguyen et al. 1997). While the positive effect of a secondary trait, such as root depth on yield under stress, has been confirmed by field results in upland rice (Lafitte and Courtois 2002), it has been more difficult to prove the interest of OA, notably because of confounding effects of variation for other traits in the studied material (Jongdee et al. 2002). Near-isogenic lines differing only in OA would be the best material to make this demonstration.

Differences in root morphology and OA in rice generally follow the genetic structure of the *Oryza sativa* species. For instance, japonica cultivars under cultivation in the upland environments of South-East Asia are characterized by a constitutive deep-root system (Courtois et al. 1996; Lafitte et al. 2001) and limited OA (Lilley and Ludlow 1996), while lowland indica genotypes have a shallow root system and good OA. Although improvement in the depth of the root system in indica genotypes and enhancement of OA in japonica ones could boost the drought tolerance of each cultivar group, these traits are complex to measure and screening methods are destructive. Therefore, these traits are not routinely used in breeding programs. With the advent of DNA markers, the outlook for improving such complex traits improved significantly. It is now possible to map the genes controlling the traits under study (Quantitative Trait Loci or QTLs), find markers tightly linked to the detected QTLs, and assess the molecular-marker allele variation at the QTLs. These markers can then be used instead of, or in addition to, phenotypic measurements to select superior genotypes throughout breeding generations.

For root depth, QTL mapping has been completed in a doubled-haploid population (Yadav et al. 1997), followed by marker-assisted backcrosses to retrieve plants carrying favorable alleles at the markers flanking the QTLs in the desired genetic background. Phenotypic evaluation of these introgression lines (ILs) as $BC_3F_3$ families, showed that three of the four QTLs manipulated were expressed in the recipient background with the expected effects (Shen et al. 2000). The results highlighted the importance of locating QTLs with the smallest possible confidence interval, detecting QTLs linked in repulsion phase, and the need to consider epistasis. The ILs developed are now under field evaluation at different locations of South and South-East Asia to determine the enhancement of drought tolerance achieved through the introgression of QTLs for root depth. Field evaluations will also allow assessment of linkage drag associated with introgression of these segments.

For OA, two mapping studies have been conducted, both in indica by japonica populations. Lilley et al. (1996) utilized a set of 52 RILs derived from the cross CO39 × Moroberekan, and Zhang et al. (2001) used 154 doubled-haploid lines derived from CT9993 × IR 62266. Though QTLs explaining a significant amount of phenotypic variation were mapped in both studies, exploitation of these results for improvement of OA in rice was not attempted in those populations, due to the lack of agronomic interest of the first one and the wide variation of the second one in terms of phenology and plant type.

One weakness of the approach followed for marker-aided root-depth improvement by Yadav et al. (1997) and Shen et al. (2000) was that QTL mapping and QTL introgression were separated into two successive steps, which delayed the production of useful breeding material. The advanced backcross QTL analysis method proposed by Tanksley and Nelson (1996) allows the simultaneous discovery and transfer of interesting QTLs from an unadapted donor to an elite variety, and produces faster results. The products of the scheme are lines containing different chromosomal segments from the donor but collectively encompassing the whole donor genome. QTL mapping is performed on a $BC_2$ or $BC_3$ generation whose genetic background is the desired one. Moreover, the products are ILs (inbred lines) and several QTLs can easily be pyramided by crossing complementary lines. As suggested by Price and Courtois (1998), such material would allow identification of the particular drought scenarios under which OA contributes to drought tolerance. The value of the ABC-QTL strategy has so far been mostly demonstrated with interspecific introgressions in tomato (Tanksley et al. 1996; Fulton et al. 1997; Bernacchi et al. 1998; Fulton et al. 2000). In rice, it has been used to identify and exploit the beneficial alleles of *Oryza rufipogon* (Moncada et al. 2001) and *Oryza glumaepatula* (Brondani et al. 2002).

This study was undertaken with the objective of introgressing high osmotic adjustment from an indica cultivar into an elite japonica variety with a deep root system. To reach this goal, the first step, reported here, was to develop an advanced back-cross $BC_3F_3$ population, map QTLs for OA in this population and evaluate the possibility of further progress. The choice to advance to the $BC_3$ generation was made to reduce linkage drag and epistatic effects, and to decrease the time necessary to convert introgressed lines into near-isogenic lines (NILs).

## Materials and methods

### Development of the random back-cross inbred population

The mapping population was developed from a cross between IR62266-42-6-2 (abbreviated as IR62266) and IR60080-46A (abbreviated as IR60080). IR62266 is an indica lowland genotype with high OA capacity that was used in the mapping study of Zhang et al. (2001). IR60080 is an improved tropical japonica genotype with a well-developed root system (Courtois et al. 1996) but a poor OA as shown by a preliminary study conducted at IRRI (S. Robin et al., unpublished results). The $F_1$ was backcrossed (BC) to the recurrent parent IR60080. One hundred and fifty $BC_1$ plants were grown in a greenhouse and each of them was individually backcrossed to the recurrent parent up to the $BC_3F_1$. No selection for high OA could be practiced during the population development since OA evaluation is destructive and cannot be conducted on an individual plant basis. The one hundred and fifty $BC_3F_1$ plants obtained were used to produce the $BC_3F_3$ population through single-seed descent. Each $BC_3F_2$ plant was harvested individually. The resulting population,

**Table 1** Sequences of the primers used for amplification of water-stress-related candidate genes

| Designation | Accession number | Primer sequences (F and R) | Gene description |
|---|---|---|---|
| WC | AB016623 | TCGTGGCGACGTTCCTGTTC<br>AGCGGCTAGCTTCATCGATC | Rice water-channel gene-3 |
| S451 | S45168 | CTTCTAGGCGTGACAAT<br>TGATTCCCAGTGGTCAA | Salt and drought stress-responsive gene from rice roots |
| WSI | D26536 | GCCAAGGACACGGCGTCCGA<br>GTGACACCGAACACGTCGAA | Rice cDNA encoding for the LEA WSI18 protein induced by water stress |
| DREB2 | NP180035 | GACCAAGTTCAGGGAGACG<br>AGATGCGGGACTTCTTGTTG | Rice cDNA encoding for a Drought Responsive Element B-like protein |
| BADH1 | BAA21098 | GGATGTCGCTGCATGCTTTG<br>GCAGATGACCGGTCCAAAGA | Rice betaine aldehyde dehydrogenase |
| BADH2 | BAA21098 | GGCCAAGTACCTCAAGGCGA<br>TGTCCCCAGCTGCTTCATCC | Rice betaine aldehyde dehydrogenase |

composed of 150 individual $BC_3F_3$ families (also called random Back-cross Inbred Lines; BILs), was used for genetic and phenotypic characterization.

## DNA analysis and genotyping

Leaf tissue from 20 plants per family was used for DNA extraction using the modified CTAB method (Saghai-Maroof et al. 1984). Fifty nine microsatellite markers published by Akagi et al. (1996) and Temnykh et al. (2000), with a known position in the genome, were used for mapping. They were chosen to cover the whole genome, but a higher density was used in areas where QTLs had been identified in other studies (e.g. chromosome 8 in the study from Lilley et al. 1996). The non-radioactive PCR conditions were similar to those of Chen et al. (1997). Depending upon the base-pair difference between the alleles observed in the parents, PCR products were run either on 2% agarose gels with revelation under UV light or 4% polyacrylamide gels with silver staining, following the protocol described by Panaud et al. (1996). The same DNA was also used for generating restriction fragment length polymorphism (RFLP) data for the population. Digestion, electrophoresis and Southern blotting were performed according to the protocol of Kurata et al. (1994). Southern hybridization and RFLP band detection were done following the protocols of Amersham International. A total of 102 RFLP markers were used.

A list of 35 possible candidate genes possibly involved in the pathways for solute accumulation was established through queries of GenBank rice sequences based on key-words. Primers were designed for all of them based on a 50–60% GC content using the Williamstone on-line primer design program (Willamstone Enterprise Inc, Waltham, US). Only six primer pairs showed polymorphism between the two parents. The candidate genes that were mapped on the population, and their GenBank accession numbers and functions are listed in Table 1.

## Evaluation of the population for osmotic adjustment

One hundred and forty two $BC_3F_3$ families were phenotyped for OA under greenhouse conditions. Because of space constraints, the evaluation was conducted in three successive and independent experiments. Each experiment was conducted with three replications of the 142 genotypes and the two parents. The experiment was conducted following an alpha (0, 1) lattice design (Patterson and Williams 1976) laid out with 12 incomplete blocks per replication, each block consisting of 12 entries. The 12 incomplete blocks were grouped together to form a complete replication of the 144 genotypes. This resolvable balanced design allows for a better control of the variability than a randomized complete block design (RCBD), and can always be analyzed as an ordinary RCBD if the blocking has not been successful in removing variation. It however imposes constraints on the number of entries tested (a multiple of 12 was chosen) and on randomization [every pair of treatments never appear together (0), or are together in only one experimental block (1)]. This is the reason why a sample of 144 lines, composed of 142 randomly chosen lines of the 150 initial lines of the population and the two parents, was used in each replication. The alpha lattice randomization was obtained using the AlphaGen program (Scottish Agricultural Statistics Service, Edinburgh, UK).

The re-hydration method described by Blum and Sullivan (1986) was used to evaluate OA. In each experiment, the experimental unit was a 16-l plastic pot containing three plants from the same family, each of the three plants constituting a sampling unit. The plants were cultivated in a medium composed of peat soil, garden soil, perlite and vermiculite mixed in a 10:5:1:1 proportion. Plants were kept well-watered until 30 days after sowing. The plants, then at the tillering stage, were sampled to measure the initial osmotic potential (control). Water was then withheld. The large pot volume and high moisture retention-capacity of the soil medium favored the gradual induction of moisture stress that is necessary for full expression of OA (Babu et al. 1999). The declining water status in the plants was observed visually, daily during the stress period in the pre-dawn hours (5.30 to 6.30 am). Upon observation of leaf rolling symptoms on a plant, it was tagged and sampled to measure the relative water content (RWC). Five 3-cm-long portions from the midsection of the penultimate fully expanded leaf blade were put in a pre-weighed glass vial to obtain the fresh weight (FW). The leaf tissue in the glass vial was re-hydrated for 4 hours in the dark and weighed to get the turgid weight (TW). The re-hydrated leaf samples were dried in a microwave oven and their dry weight (DW) measured. RWC was calculated for the samples collected on the same morning using the formula $[(FW - DW)/(TW - DW)] \times 100$. If RWC reached 70%, the wilted pot was re-watered in the evening. If the RWC was higher, the pot was left to dry further, and RWC measurement was repeated on subsequent days until 70% RWC was reached. In most cases, however, wilting symptoms were a good predictor of RWC. After plants were re-watered, leaf samples were collected in the morning of the next day on the same plant but on a different tiller that was used for measuring RWC, wrapped in aluminum foil and stored at –80 °C for at least 24 h. The osmotic potential was measured with a Wescor vapor pressure osmometer. OA was calculated as the difference in osmotic potential between leaves at full turgor, collected before and after moisture stress following Blum and Sullivan (1986).

### Linkage analysis

The $BC_3F_2$ marker data were obtained from the analysis of the bulk of 20 $BC_3F_3$ plants. The relative order of the molecular markers was known from previous mapping studies (results in the Gramene database; http://www.gramene.org). A map was built with Mapmaker (Lander et al. 1987) imposing the established order. Removing the constraint did not change the marker grouping but locally changed the marker order in a few cases, with 12 inversions between two markers and seven permutations involving three markers, and reshuffled segments of chromosomes 1, 4 and 6. The Haldane mapping function, which assumes the absence of interference, was chosen to compute genetic distances because of the requirement of the QTL-analysis software PlabQTL. Segregation ratios of individual marker loci were compared with the expected ratios for $BC_3F_2$ using a chi-square-test.

### Statistical analysis

Five families had poor development with very limited biomass for all plants in all three experiments and failed to show leaf-rolling symptoms in the time frame set for the experiment. They were removed from the analyses, which were conducted on the remaining 139 entries. Twenty additional pots spread over experiments and replications showed abnormal growth, or death, of at least two of the three plants. They were considered as missing data. Analyses of variance were carried out separately for the three experiments. The adjusted means were computed and used in further QTL analyses. The three experiments were then pooled together to analyze the interaction between genotype and experiment, with experiment, replications within experiment, line, and experiment × line taken as fixed effects. The experiment × line interaction effect was tested against the model-error term. All analyses were run under SAS.

### QTL analysis

Because this was an advanced back-cross population, the OA distribution was strongly skewed toward the recurrent parent. The OA data were therefore transformed into ranks using the SAS rank procedure. Both raw data and ranks were analyzed. For each marker, an ANOVA comparing the phenotypic mean of the three marker classes was first performed using a SAS program. The test of Kruskal and Wallis included in MapQTL (Van Oijen and Malipaard 1999) was used to perform the same comparisons on ranks. In addition to the single-marker analyses, we used PlabQTL version 1.1 (Utz and Melchinger 1999) to perform composite interval mapping with forward stepwise regression to choose the co-factors. We used the software default values of the F to enter and F to drop in the stepwise regression. The number and nature of co-factors to be used were chosen by the software. A threshold of 3.0 for each individual test corresponded to a 5% risk at the genome level using the permutation method from Churchill and Doerge (1994) with 1,000 runs, and this threshold was adopted for QTL detection. To assess how much the results were affected by the number and nature of the chosen co-factors, we used the cross-validation option of PlabQTL that permits us to sample the data set. The bootstrapping was done on 4/5 of the population, the remaining 1/5 of the lines being used to validate the estimates of additive effect and phenotypic variance explained by the QTLs. For the cross-validation purpose, the CIM analysis with the choice of co-factors was repeated 1,000 times. A QTL was reported in case it was detected in more than 250 of the 1,000 runs. The LOD, $r^2$, additive effects of a QTL were computed from the medians of the values across all runs where it was detected. The QTL × Environment interactions were analyzed using PlabQTL with the three experiments taken as environments.

## Results

### OA of the $BC_3F_3$ progenies

The population and the parents showed significant variation for OA in all three experiments (Table 2; Fig. 1). A large difference was observed between the parents, IR62266 (mean OA of 1.36, 0.92, 0.93 Mpa in the three experiments respectively) and IR60080 (mean OA of 0.83, 0.61 and 0.43 MPa). The population means were 0.87, 0.64 and 0.51 Mpa for the three experiments

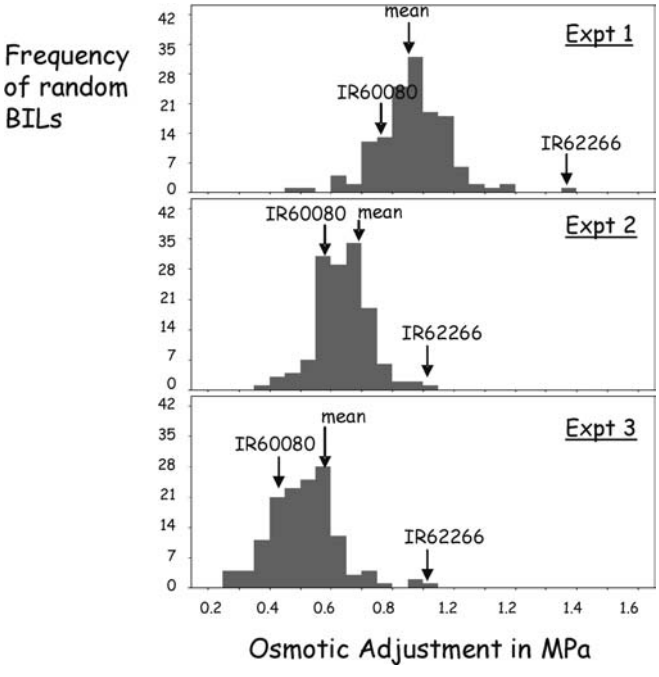


**Fig. 1** Frequency distribution of osmotic adjustment in the three experiments

**Table 2** Mean (MPa) and variance of the $BC_3F_3$ families and parents of the population

| Exp. | IR60080-46A | IR62266 | Mean of the $BC_3F_3$ population | S.D. of the $BC_3F_3$ population | Range of the $BC_3F_3$ population | $h^2$ |
|---|---|---|---|---|---|---|
| Exp. 1 | 0.84 ± 0.051 | 1.36 ± 0.063 | 0.87 | 0.104 | 0.526–1.168 | 0.45 |
| Exp. 2 | 0.61 ± 0.036 | 0.92 ± 0.032 | 0.64 | 0.087 | 0.400–0.894 | 0.43 |
| Exp. 3 | 0.43 ± 0.041 | 0.93 ± 0.033 | 0.51 | 0.105 | 0.263–0.888 | 0.26 |
| Average of the three experiments | 0.64 ± 0.044 | 1.08 ± 0.050 | 0.68 | 0.072 | 0.496–0.866 | 0.56 |

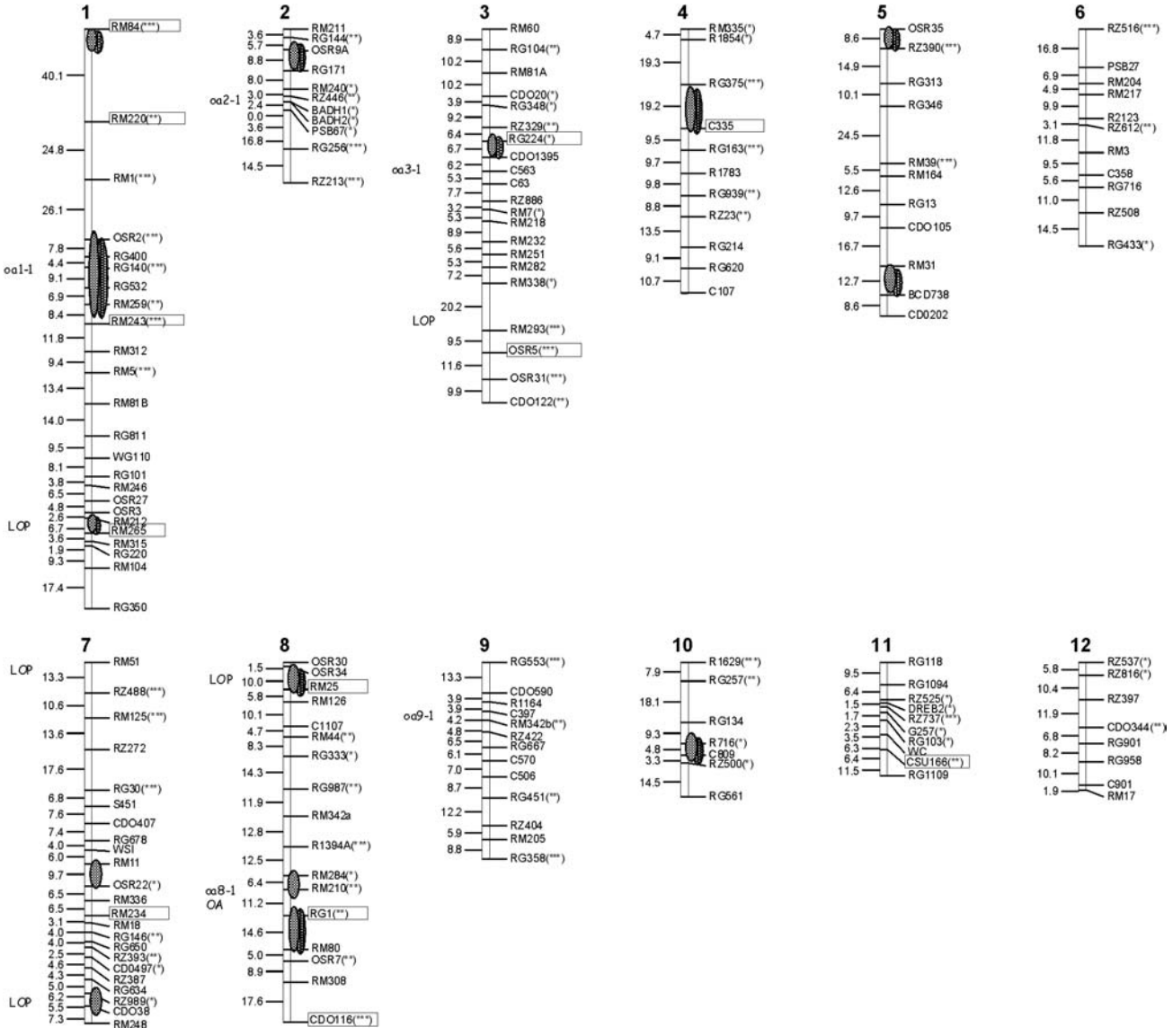


**Fig. 2** Genetic map of the IR62266 × IR60080 population with the position of the main QTLs. The (*, **, ***) marks following the markers indicate segregation distortions at $P < 0.05$, 0.01 and 0.001 respectively. *Boxed markers* are the QTLs for OA located through single-marker analysis. *Shaded blocks* are the QTLs revealed through CIM for raw data (*light grey*) and ranks (*dark grey*). QTLs detected by Liley et al. (1996) and Zhang et al. (1999) are indicated in a *bold letter* on the left side of the chromosomes

respectively. The phenotypic segregation was expectedly skewed towards the recurrent parent (IR60080) with a low OA. Though none of the lines surpassed the OA value of the donor parent (IR62266), a significant increase of OA over the population grand mean was observed in 14 lines based on their mean across the three experiments. Analysis of variance showed significant differences between experiments, but the line × experiment interaction was not significant (Table 3). The line-means of the experiments were positively correlated ($r_{12} = 0.37$; $r_{13} = 0.29$; $r_{23} = 0.25$, all significant at $P < 0.01$). The heritability of the trait was relatively low, notably in the third experiment, but was in the order of magnitude generally observed for this kind of trait (Zhang et al. 2001).

## Linkage map and marker segregation

A total of 102 RFLPs, 59 microsatellites and 6 candidate genes were mapped on 12 linkage groups corresponding to the 12 rice chromosomes (Fig. 2). The total map length measured 1,370 cM with an average inter-marker distance of 7.7 cM. The proportion of plants homozygous for the recurrent parent allele among the $BC_3F_2$ progenies ranged from 98.6% to 60.0% with an average of 89.7% (Fig. 3), while the proportion of plants homozygous for the donor allele ranged between 0.0 and 19.7% with an average of 5.2%. Heterozygosity varied from 0.0 to 28.6% of the loci with a mean of 5.1%. No line carried only recurrent parent alleles.

**Table 3** Variance decomposition in the combined analysis of the three experiments

| Source of variation | *df*[a] | Mean square | *F* value | Probability |
|---|---|---|---|---|
| Experiment | 2 | 1,162.12 | 758.56 | 0.0001*** |
| Replication within experiment | 6 | 10.05 | 6.56 | 0.0001*** |
| Family | 138 | 3.34 | 2.18 | 0.0001*** |
| Family*Experiment | 270 | 1.79 | 1.17 | 0.0529 ns[b] |
| Error | 814 | 1.53 | | |
| Total | 1,230 | | | |

[a] *df*, degree of freedom
[b] ns, non-significant at the 0.05 level; ***, significant at the 0.005 level

**Table 4** Markers correlated with OA through single-marker analysis using an analysis of variance for the raw data and a Kruskall and Wallis test for the ranks

| Markers | Chr. | Level of significance of the tests[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Experiment 1 | | Experiment 2 | | Experiment 3 | | Overall | |
| | | Raw | Ranks | Raw | Ranks | Raw | Ranks | Raw | Ranks |
| RM84 | 1 | *** | * | | | * | * | *** | ** |
| RM220 | 1 | | | * | | **** | * | | |
| RM243 | 1 | | | | * | **** | * | | |
| RG811 | 1 | | | | | *** | * | | |
| RM265 | 1 | | | | | | *** | | ** |
| RG224 | 3 | * | | **** | *** | | | ** | |
| OSR5 | 3 | | | **** | * | | | | |
| C335 | 4 | * | | *** | ** | * | | | |
| RM234 | 7 | *** | ** | | | * | ** | **** | *** |
| RM25 | 8 | | | *** | | | ** | | |
| RG1 | 8 | | | ** | * | *** | * | ** | * |
| CDO116 | 8 | **** | *** | | | | | *** | ** |
| CSU116 | 11 | **** | *** | | | | | ** | |

*, **, ***, ****: significant at the 0.05, 0.01, 0.005, 0.001 levels respectively
[a] The significance level in all experiments is given for markers at least significant at $P < 0.005$ in one of the experiments or in the mean

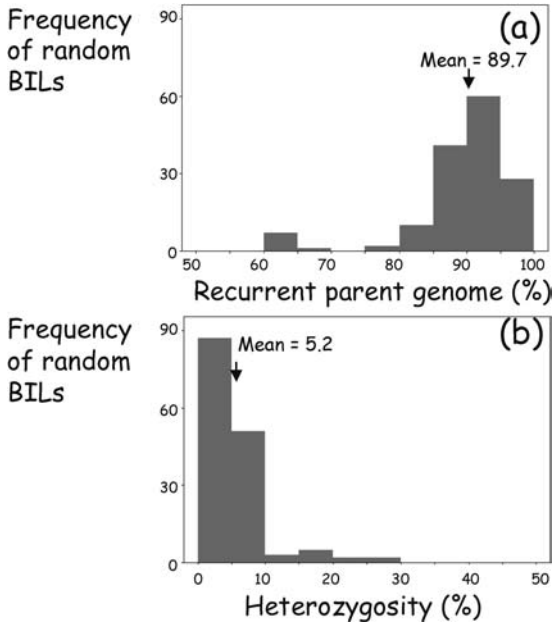


**Fig. 3a, b** Proportion of alleles from the recurrent parent in 150 BILs. **a** Recurrent alleles at the homozygous stage, and **b** at the heterozygous stage

Segregation distortions were observed for 67, 49 and 25 loci among the 161 at a probability of 0.05, 0.01 and 0.001 respectively. The distortions were not in a unique direction but were equally due to an excess of the three genotype classes. The cumulative segregation ratio of 89.7:5.1:5.2, however, was not significantly different from the expected Mendelian frequency of 90.6:6.3:3.1 for a $BC_3F_2$.

## QTL analysis

Single-marker analysis of raw data revealed that 12 markers were significantly correlated with OA ($P < 0.005$) in at least one of the experiments or on the overall mean (Table 4; Fig. 2). Four of them were on chromosome 1, three on chromosome 8, two on chromosome 3, and one each on chromosomes 4, 7 and 11. Among these, six were detected in experiment 1, six in experiment 2, seven in experiment 3 and six for the mean of the three experiments, with significance levels ranging from 0.05 to 0.001. Among the markers, RM84 and RM220 on chromosome 1, RG224 on chromosome 3, RM234 on chromosome 7 and RG1 on chromosome 8 were linked to OA in more than one experiment, and C335 on chromosome 4 was reported in all the three experiments. The Kruskal and Wallis test on the ranks, with the same threshold of $P < 0.005$, was less sensitive. It detected four

**Table 5** Variance decomposition in the QTL × Environment analysis

| Source | *df*[a] | Mean square | *F* value |
|---|---|---|---|
| Experiment | 2 | | |
| Genotypes | 135 | 1.36 | 2.03** |
| QTL | 14 | 7.63 | 5.79** |
| Residual | 121 | 0.64 | 0.97 |
| G × E | 270 | 0.67 | |
| QTL × E | 16 | 0.80 | 1.22 ns[b] |
| Res. × E | 254 | 0.65 | |

[a] *df*, degree of freedom; G, genotype; E, Environment
[b] ns, non-significant at the 0.05 level; ** significant at the 0.01 level

markers significantly linked to OA that had already been identified through the ANOVA and only one additional marker RM265, on chromosome 1, that had not been identified earlier.

PlabQTL was then employed to run composite interval mapping (CIM) to detect QTLs and to assess the QTL × experiment interaction. The QTL × experiment interaction was not significant (Table 5). CIM was therefore run both for raw data and for ranks with the mean of the three experiments. The CIM results are presented in Table 6 and Fig. 2. The QTLs appearing in the table are those detected at least 25% of the times in the bootstrap experiment. A total of 14 genomic regions linked with OA were found on chromosomes 1, 2, 3, 4, 5, 7, 8 and 10, with the phenotypic variation explained by the individual QTL ranging from 14% to 25%. Several QTLs were detected on chromosomes 1, 5, 7 and 8. When combined in a multiple regression, the QTLs explained 58% of the phenotypic variation. Raw data and ranks gave slightly different results, with the raw data allowing the detection of more QTLs, but the QTL positions were identical. Most alleles contributing to an OA increase came from the indica donor IR62666 but, for some of the QTLs on chromosomes 7, 8, and 10, the favorable allele was from the japonica parent. The two QTLs on the long arm of chromosome 8, notably, were linked in repulsion. Seven out of these 14 regions also possessed markers detected by single-marker analysis in one or more experiments.

For the 14 lines with OA significantly greater than the population mean, we used graphical genotyping to visualize the QTLs with favorable alleles. Superior lines had the favorable allele at the homozygous stage for both flanking markers at 0 to 4 QTLs, and had the favorable allele at one flanking marker at either the heterozygous or homozygous stage at 1 to 8 QTLs. Most lines had the favorable allele for QTLs on chromosomes 7, 8 and 10, where the favorable allele was coming from the recurrent parent.

## Discussion

Development of NILs differing for OA QTLs has been advocated as a tool to determine the real impact of OA on rice productivity under moisture stress (Price and Courtois 1998; Zhang et al. 1999). In this study, a population of random BILs was used to map QTLs for OA. Allard (1999) commented on the efficiency of transferring multigenic characters through back-cross breeding. Even in the best case, provided with a donor parent of extreme expression for the trait to be introgressed with good phenotypic evaluation and careful selection, there are chances of losing a few minor genes, thereby reducing trait recovery. In agreement with this prediction, in our study, the population exhibited a mean OA higher than the recurrent parent but consistently lower than the donor parent in all three experiments. This was expected since each line would have recovered only a fraction of the QTLs carried by the donor parent.

The ILs with a very limited frequency of the donor-parent allele posed a challenge in constructing the linkage map but, except for a few segments, the marker order did not differ from the established one. The amount of

**Table 6** QTLs for osmotic adjustment detected using composite interval mapping

| Chr. | Interval | Interval (cM from the top of the short arm) | Most-likely position (cM from the top of the short arm) | LOD | $R^{2a}$ (%) | Add. (MPa) | % of times QTL detected in cross validation (1,000 runs; raw data) | % of times QTL detected in cross validation (1,000 runs; ranks) |
|---|---|---|---|---|---|---|---|---|
| 1 | RM84-RM220 | 0–10 | 0 | 5.7 | 22 | 0.29 | 54 | 42 |
| 1 | OSR2-RM259 | 90–120 | 105 | 4.8 | 18 | 0.40 | 60 | 35 |
| 1 | RM265-RM315 | 215–220 | 215 | 4.4 | 14 | 0.39 | 32 | 40 |
| 2 | OSR9A-RG171 | 10–20 | 15 | 4.7 | 18 | 0.53 | 37 | 38 |
| 3 | RG224-CDO1395 | 50–55 | 50 | 5.1 | 19 | 0.37 | 40 | 28 |
| 4 | RG375-C335 | 35–45 | 40 | 4.5 | 17 | 0.40 | 48 | 43 |
| 5 | OSR35-RZ390 | 0–5 | 0 | 4.0 | 16 | 0.37 | 25 | 46 |
| 5 | RM31-BCD738 | 100–120 | 120 | 4.4 | 19 | 0.54 | 42 | 36 |
| 7 | RM11-OSR22 | 85–90 | 85 | 4.4 | 17 | 0.47 | 25 | 20 |
| 7 | RZ989-CD038 | 140–145 | 140 | 5.0 | 20 | –0.77 | 52 | 21 |
| 8 | RM34-RM25 | 10–25 | 10 | 4.4 | 17 | 0.42 | 31 | 53 |
| 8 | RM284-RM210 | 90–100 | 95 | 5.2 | 19 | 0.51 | 49 | 21 |
| 8 | RG1-RM80 | 110–125 | 120 | 5.7 | 22 | –0.46 | 72 | 62 |
| 10 | C809-R716 | 30–40 | 40 | 6.8 | 25 | –0.43 | 82 | 83 |

[a] $R^2$ multiple regression including all detected QTLs in the model = 58.2%; adjusted $R^2$ = 47.2%

segregation distortion observed in individual loci was not extensive and, because the skewness did not favor any specific genotype, could easily be due to sampling effect. Larger magnitudes of segregation distortion were observed in other BIL populations because they involved interspecific crosses, and because selection was practiced in favor of the recurrent parent (Fulton et al. 1997; Lin et al. 1998; Moncada et al. 2001).

Twelve markers were linked with OA as revealed by single-marker analysis in one or more experiments; of these, six were in the vicinity of QTLs detected by CIM. The similarity of the results between single-marker analysis and CIM was expected because of the limited number of QTLs introgressed per line. Fourteen QTLs for OA were detected by CIM. Lilley et al. (1996), using a 52-line population, mapped one QTL for OA on chromosome 8 and four QTLs for lethal osmotic-potential on chromosomes 1, 3, 7 and 8. The small size of their population explains why they recovered only one QTL for OA with a big effect. This QTL is located in the area where we found two QTLs in repulsion. Zhang et al. (2001) detected five QTLs on chromosomes 1, 2, 3, 8 and 9 in the CT9993 × IR62266 population. The QTLs on chromosomes 1 and 8 were clearly co-localized with QTLs detected in this study while the QTLs on chromosomes 2 and 3 mapped close by.

The region surrounding RG1 on chromosome 8, that was detected in all three studies, needs further analysis. According to our CIM results, two QTLs in repulsion are located in this segment. The QTL between RM284 and RM210 might correspond to that identified by the other teams since the donor allele increased OA, while the QTL between RG1 and RM80 had the opposite effect. Comparative mapping between cereals confirms the importance of this region. The segment consistently detected across rice populations is syntenic to a segment carrying a QTL for OA in both wheat (Lilley et al. 1996) and barley (Teulat et al. 1998). In addition to QTLs for OA, a QTL for root depth was also found in the same area in several populations (Zhang et al. 2001). Fine-mapping is underway to physically map this region (H. Nguyen, personal communication); this should allow separation of these QTLs and confirmation of their individual effects.

Although the candidate-gene approach seems a rather attractive way to identify better markers, it showed some limitations in this study. Only six of the candidate genes were found to be polymorphic, and of these, only one was closely linked to a QTL. This is not unexpected, because the list of genes possibly involved in OA pathways is extensive, and it is difficult to test all of them without high-throughput technology. At present, we have too little understanding of the mechanisms to screen only the most relevant ones. Another limitation is the need for polymorphism within the cross of interest. Lastly, co-segregation is not a proof of causality: genes are often found in clusters and, because the confidence interval of a QTL classically covers 15 to 20 cM, it is not possible to determine which gene is the causal one. Additional evidence is therefore needed before drawing conclusions on what gene lies beneath the QTL.

In order to further characterize the useful introgressions in the japonica background, the 14 lines with significantly higher OA than the population mean were analysed for their genomic constitution with the allele pattern at the 14 QTLs. Eleven of these lines comprised more than 80% of the recurrent genome. The $BC_3F_3$ lines carried the favorable donor alleles at the homozygous or heterozygous stage at both markers flanking the QTLs at up to four segments, with three of the positive alleles coming from the recurrent parent and therefore present in most of the lines. The linkage between the two QTLs in repulsion is not strong enough that it cannot be broken, as shown by the existence of some recombinants homozygous with the favorable alleles at both segments.

Marker-aided selection for the next steps of the BC scheme will eventually deliver a set of NILs introgressed with QTLs for OA in a japonica background. Intercrossing complementary lines will allow recovering NILs with a OA capacity closer to that of the donor parent. In addition to their interest as potential varieties, these NILs will be useful to test physiological hypotheses concerning the value of OA to enhance rice productivity under water-deficit. It is unlikely that OA could help to improve yield under stress in all situations, but such material would allow clarification of which drought scenarios could be better tolerated by rice varieties with enhanced OA.

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