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## Yield response to water deficit in an upland rice mapping population: associations among traits and genetic markers

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**Abstract** A population of recombinant inbred rice lines from a cross between the upland japonica cultivar Azucena and the upland indica cultivar Bala was evaluated in a series of upland field experiments. Water stress was imposed during the reproductive stage by managed irrigation during the dry season, while control treatments were maintained in aerobic, well-irrigated conditions. Water deficit resulted in a yield reduction of 17 to 50%. The genetic correlation between stress and control yields was quite high when stress was mild, and the heritability of yield was similar in stress and control treatments across both years of this study. Genetic correlations between secondary traits such as leaf rolling and drying and yield under stress varied from high (leaf drying) to insignificant (leaf rolling). Lines with superior yield tended to have fewer panicles and larger grain size than the high-yielding parent, Bala, even though the panicle number was positively correlated with yield and the thousand-grain weight was not associated with yield for the population as a whole. Analysis of quantitative trait loci (QTLs) for yield and yield components allowed the identification of 31 regions associated with growth or yield components. Superior alleles came from either parent. Several of the regions identified had also been reported for root mass at depth or maximum root length in this population in other studies made under controlled environments, and for leaf drying (LD) in field studies. However, the direction of the

effect of QTLs was not consistent, which indicates that there was not necessarily a causal relationship between these secondary traits and performance. We conclude that mapping populations can provide novel insights on the actual relationships between yield components and secondary traits in stress and control environments and can allow identification of significant QTLs for yield components under drought stress.

**Abbreviations** DAS: Days after sowing · GPP: Grains per panicle · QTL: Quantitative trait locus · RWC: Relative water content · SPP: Spikelets per panicle · TGW: Thousand-grain weight · VPD: Vapor pressure deficit

### Introduction

Rice is particularly susceptible to water deficit compared to other crop species, and this sensitivity is especially severe around flowering. A considerable body of research has been undertaken to find genetic variation in traits that are expected to influence the response of rice to water deficit. Cultivars differ dramatically in their vegetative response to water deficit, with some cultivars having extensive leaf drying while others cease growth but their leaves remain green and tightly rolled. Extensive screening of these traits has been undertaken (DeDatta et al. 1988), but scores of leaf responses have not been closely associated with yield under stress unless severe stress occurs near flowering and scoring is done at that time (Garrity and O'Toole 1995; Mitchell et al. 1998). Differences in phenology strongly affect the response to stress near flowering (Garrity and O'Toole 1994), so such screening is more difficult than vegetative-stage screening. Rice cultivars also differ widely in root traits such as root distribution and root thickness (Lafitte et al. 2001). Lines with more extensive root systems tend to have better leaf survival under stress (Ekanayake et al. 1985), and in some, but not all cases, more extensive rooting has been associated with greater grain yield under drought stress (Fukai and Cooper 1995). Because of the difficulty of

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screening for genetic variation in root traits, these characters have not been used in crop improvement programs.

Through the use of mapping populations, many quantitative trait loci (QTLs) have been identified in rice for traits that are putatively associated with performance under drought. QTLs have been reported for root morphology (Price and Courtois 1999), osmotic adjustment (Zhang et al. 2001), leaf membrane stability (Tripathy et al. 2000), and visual symptoms of leaf stress such as rolling and drying (Courtois et al. 2000). In lowland environments, QTLs have been identified for major yield components such as panicle number, spikelets per panicle, and individual grain weight (Courtois et al. 1995). An analysis of grain yield in several upland rice experiments with or without stress imposed at the reproductive stage revealed a number of genomic regions associated with yield components in upland conditions (Lafitte et al. 2002a). These QTLs did not consistently cosegregate with drought avoidance traits such as leaf rolling or root morphology. In that study, the semi-dwarfing gene *sd1* dominated differences in yield components, just as it had in the lowland study mentioned earlier. While little cosegregation of root and leaf QTLs has been found in some other populations as well (Price et al. 2002a), there are recent reports of some overlap between root QTLs and performance (Babu et al. 2003).

Drought screening is complicated by difficulties in field management, variation in phenology, and unexpected rainfall events. For this type of screening to be useful in the context of a breeding program, it must provide additional information that cannot be obtained in an unstressed control plot, and which predicts how the line will perform under natural stress in the target environment. Information on the repeatability of traits measured in stress experiments, the heritabilities of yield and secondary traits, and the genetic correlations among these traits are needed to decide whether or not such nurseries are useful (Fukai and Cooper 1995). Studies with mapping populations can help to provide this information. A difficulty with evaluating yield in many rice mapping populations, however, is that the parental lines are usually chosen to maximize polymorphism, so parents often have very different adaptations. In this case, performance estimates may reflect relative adaptation (pest and disease susceptibility, tolerance of transplanting shock or aerobic soil, etc.) rather than line response to water deficit per se. Mapping populations derived from parents with very different adaptation might, therefore, be unlikely to reveal meaningful associations between performance under stress and secondary traits.

The objectives of these experiments were to:

1. Assess crop response to moderate water deficit beginning around flowering in a mapping population based on upland-adapted parents.
2. Determine the correlation between performance and secondary traits, and their relative heritabilities.

3. Identify genetic regions associated with superior performance in control and stress environments, and compare conclusions drawn from correlation analysis with those from QTL analysis.

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## Materials and methods

### Germplasm

This study used progeny of a cross between the cultivars Bala and Azucena. Bala is an upland-adapted semi-dwarf indica cultivar from Eastern India. It is noted for early maturity and a fairly good level of drought tolerance. It has rather short, thin roots. Azucena is a traditional japonica cultivar from the Philippines. It has comparatively thick, deep roots, and low but stable yields under mild stress in upland conditions. These two cultivars respond quite differently to drought stress (Lafitte and Courtois 2002). F<sub>6</sub> seeds were collected from a total of 205 F<sub>5</sub> plants produced by single-seed descent from an original set of 310 F<sub>2</sub> plants (Price et al. 2000). A total of 176 of these lines were grown in a lowland (flooded) field at IRRI in 1996 (Price et al. 2002c) and their seeds were collected. Flowering dates were recorded in this sowing. The 96 lines used for yield evaluations were selected for early and uniform flowering in order to minimize direct effects of maturity on performance.

### Experiments and analysis

Lines were evaluated in field experiments in the 1999 and 2000 dry seasons, with two replications in each of two water levels in each year. In 1999, seed was sown on raised 3-m long beds (90 cm inter-bed spacing, two rows per bed with 20 cm between rows). All plots received rainfall supplemented by sprinkler irrigation to maintain the soil moisture tension below 20 kPa until 57 days after sowing (DAS). From that time until harvest, the control treatments were furrow-irrigated twice weekly, while the stress plots were irrigated once each week. This treatment resulted in weekly cycles of soil drying in the stress treatments that reached a soil moisture tension above 70 kPa at a depth of 30 cm. The control treatment did not dry to more than 40 kPa at that depth.

In 2000, seed was sown in 3-m plots comprising four rows spaced at 25 cm. Plots received 230 mm rainfall together with sprinkler irrigation 2–3 times per week until 56 DAS. After that date, plots were irrigated using a drip system that applied approximately 25 mm of water three times per week. When panicles were visible on three or more hills in a plot, irrigation to that individual plot was discontinued and no water was applied for 16 days. This sudden withholding of water had an immediate effect on progress toward flowering, and delayed the date of 50% flowering in the remaining hills. A single rainfall event occurred during the time when the plots were being stressed, and this added 19 mm of water. Average potential

evapotranspiration was 5.1 mm/day during this period, and average vapor pressure deficit (VPD) was 0.6 kPa. Soil moisture tension in control plots was maintained at <15 kPa, and stress plots dried to more than 80 kPa at both 15 and 30 cm depths.

Data collected from these experiments included yield, yield components, biomass production, flowering date, and plant height. Flowering date was the day when panicles were visible on half of the hills in the plot. Flowering delay was the difference between 50% flowering in the stress and control plots, or between aerobic plots and a lowland seed increase conducted in 1999. In 1999, the rate of drying of excised leaf segments was used to estimate epidermal conductance (Mitchell et al. 1998). The percent fresh weight of turgid leaves and specific leaf area were also recorded from those samples. Midday leaf and panicle water potentials were recorded for Azucena and Bala on two occasions after flowering to estimate stress severity.

Lines from this population have been screened for their response to water deficit at the vegetative stage in the 1996 and 1998 dry seasons at IRR (Price et al. 2002c). Data from these experiments were used to evaluate correlations between traits measured under a severe early stress and grain yield under reproductive stage stress. In summary, those experiments were also dry-seeded in rows spaced 25 cm apart, with two replications. Sprinkler irrigation was applied three or four times per week until 40 DAS in 1996

or 45 DAS in 1998. At that time, water was withheld and plots were allowed to dry. Data collected included leaf rolling scores, leaf drying scores, leaf chlorophyll content (SPAD meter reading), specific leaf weight, nodal root thickness, canopy temperature, radiation interception, and relative water content (RWC). Root growth has been examined in this population and QTLs for root thickness and root depth have been identified (Price et al. 2002b). Root penetration ability has also been reported (Price et al. 2000).

Data were analyzed with SAS/STAT software, v6.12 of the SAS System for Windows NT, using SAS procedures GLM and CORR (Copyright 1989–1996 SAS Institute, Cary, N.C., USA.). Heritability was estimated from variance components (SAS procedure VARCOMP) when traits were measured across years. Heritability within each water level was calculated as:

$$H = \frac{G}{(G + (G \times E/2) + (E/4))}$$

where  $G$  is the genetic variance,  $E$  is the environmental variance, and  $G \times E$  is the variance for the genotype by environmental interaction.

When data were available only for a single year, repeatability was used as an estimate of heritability, and this was calculated as  $1 - (1/F$  for line effect). The genetic

**Table 1** Performance of parents and 96 recombinant inbred lines ( $\pm$  standard deviation) across two water levels when grown in the field in 2 years. Flowering dates recorded in lowland fields were 70 days

for Bala, 89 days for Azucena, and  $74 \pm 6$  for the lines. *NA* Not available. Significant effects are indicated for water level ( $W$ ), entry ( $E$ ) and the interaction between entry and water level ( $E \times W$ )

Year	Control			Stress			Significant effects
	Azucena	Bala	Lines	Azucena	Bala	Lines	
1999							
Yield (ton/ha)	2.74	3.67	2.58 $\pm$ 0.99	1.26	2.23	1.18 $\pm$ 0.62	W*, E**
Panicles/m <sup>2</sup>	219	614	330 $\pm$ 78	144	494	252 $\pm$ 79	W**, E**
Sterile panicles(%)	27	3	15 $\pm$ 14	12	3	31 $\pm$ 15	W**, E**, E $\times$ W*
Spikelets/panicle	91	81	83 $\pm$ 19	80	59	81 $\pm$ 17	E**
Fertile spikelets(%)	71	60	57 $\pm$ 8.5	58	58	47 $\pm$ 10	W**, E**
Weight of 1,000 grains (g)	25.6	15.1	22.0 $\pm$ 1.2	22.5	15.1	20.6 $\pm$ 1.6	W**, E**, E $\times$ W*
Biomass (ton/ha)	11.7	8.4	7.9 $\pm$ 2.2	6.2	5.2	5.1 $\pm$ 1.3	W**, E*
Leaf water potential midday (MPa)	-1.2	-2.1	NA	-1.3	-1.7	NA	E*
Panicle water potential midday (MPa)	-0.8	-1.1	NA	-1.2	-2.0	NA	W**, E*
2000							
Yield	2.38	2.97	2.84 $\pm$ 0.66	1.86	2.50	2.36 $\pm$ 0.48	W**, E**
Panicles/m <sup>2</sup>	228	465	310 $\pm$ 66	198	339	292 $\pm$ 65	W*
Sterile panicles(%)	12	4	5 $\pm$ 5	8	11	7 $\pm$ 7	NS
Spikelets/panicle	95	82	95 $\pm$ 45	105	82	90 $\pm$ 15	E**
Fertile spikelets(%)	73	71	67 $\pm$ 8	64	68	62 $\pm$ 7	W**, E**
Weight of 1,000 grains (g)	24.5	15.9	21.9 $\pm$ 2.6	25.2	15.8	21.8 $\pm$ 1.5	E**
Biomass (ton/ha)	7.8	6.8	6.5 $\pm$ 1.4	6.2	5.4	5.8 $\pm$ 1.1	W**, E*
Across years							
Plant height (cm)	145	81	111 $\pm$ 14	136	75	101 $\pm$ 14	W**, E*
Flowering date (days)	90	65	68 $\pm$ 3	92	65	69 $\pm$ 3	W**, E**, E $\times$ W*

\* $P < 0.05$ ; \*\* $P < 0.01$

**Table 2** Phenotypic correlations between traits measured under two different water levels, and correlations between grain yield and other traits in either stress or control treatments. Chlorophyll levels were measured for the two water levels in 1996. The RWC and PAR interception was measured under vegetative stage stress conditions in 1998. Correlations were between the line means averaged across

four replications (2 years × 2 replications per year). Correlations were also calculated for subsets of lines having Azucena alleles in the Chromosome 1 region RM212-C86-C949 (group A; 45 lines) or Bala alleles in that region (group B; 31 lines). Values in bold differed in significance between the two groups

Trait	Control with stress	Trait measured in control with yield measured in control			Trait measured in stress with yield measured in stress		
		All lines	Group B	Group A	All lines	Group B	Group A
Grain yield	0.48**						
Flowering date	0.95**	NS	NS	NS	-0.16	<b>-0.43**</b>	NS
Plant height	0.75**	NS	<b>-0.33*</b>	NS	-0.38**	<b>-0.54**</b>	NS
Biomass	0.43**	0.57**	0.50**	0.62**	0.64**	0.59**	0.64**
Panicles/m <sup>2</sup>	0.56**	0.41**	0.45**	0.43**	0.34**	NS	<b>0.46**</b>
Sterile panicles(%)	0.21*	<b>-0.53**</b>	<b>-0.46**</b>	<b>-0.64**</b>	<b>-0.57**</b>	<b>-0.68**</b>	<b>-0.52**</b>
Spikelets/panicle	0.60**	NS	NS	NS	NS	NS	NS
Spikelet fertility(%)	0.57**	0.39**	0.42*	0.31*	0.61**	0.71**	0.55**
Weight of 1,000 grains	0.86**	NS	NS	NS	NS	NS	NS
Harvest index	0.51**	0.73**	0.82**	0.74**	0.81**	0.87**	0.82**
Chlorophyll		NS	NS	NS	0.26*	NS	<b>0.43**</b>
RWC		NS	<b>0.43*</b>	NS	0.32*	<b>0.79**</b>	NS
PAR interception		<b>-0.35*</b>	NS	NS	<b>-0.35*</b>	<b>-0.48*</b>	NS
Leaf rolling		NS	NS	NS	NS	NS	NS
Leaf drying		NS	<b>-0.60**</b>	NS	<b>-0.45**</b>	<b>-0.46*</b>	<b>-0.39*</b>

\* $P < 0.05$ ; \*\* $P < 0.01$

correlation between yield under stress and other traits averaged across the 2 years was estimated as:

$$r_g = \frac{COV_{\text{stress yield, trait}}}{(G_{\text{stress yield}} * G_{\text{trait}})^{0.5}}$$

To compare genetic correlations between water levels in each year, the genetic correlation was estimated as the intraclass correlation coefficient:

$$H = \frac{G}{G + (G \times W)}$$

where  $G \times W$  is the genotype by water regime variance.

The construction of the linkage map with 102 RFLP, 34 AFLP and 6 microsatellite markers on 15 linkage groups and total length of 1,779 cM has been described elsewhere (Price et al. 2000). Composite interval mapping to identify QTLs was conducted using QTL Cartographer software (Basten et al. 2001). A threshold probability level of 0.005 was used in the analysis, and only QTLs with LOD score >3.6 are reported.

## Results

The flowering dates of the RILs were generally similar to Bala, because early-flowering lines were chosen for this study, but plant height was intermediate between the two

parents (Table 1). When midday water potential was measured in 1999, values for panicles just after flowering were significantly lower in the stressed plants than in the controls, and Azucena tended to maintain higher leaf and panicle water potentials than Bala. Azucena yielded less than Bala in all environments. Bala produced more panicles/m<sup>2</sup> and smaller grains than Azucena.

Yields were reduced after water stress by about 55% in 1999 and by about 17% in 2000. The effect of water treatment was significant for yield and for all yield components except for the number of spikelets per panicle (Table 1), though thousand-grain weight (TGW) was significantly affected by water treatment only in 1999. No significant line by water level interaction was observed for yield, panicles/m<sup>2</sup>, spikelets per panicle (SPP), or spikelet fertility in either year. When the results were combined across both years, significant differences were observed among the lines for all the traits measured except the total biomass production in the control treatment. Across both years, the line by water level interaction was significant for yield, the fraction of fully sterile panicles, fraction of fertile spikelets, harvest index and TGW (data not shown). Flowering delay in stress plots compared to control plots was minimal in 2000, and differences were not observed among the lines. In 1999, the average flowering delay between water levels was 4±4.3 days, and differences among the lines were significant, ranging from -6.5 to 31 days. In that environment Azucena had a greater flowering delay (3 days) than Bala (0.5 days). Flowering delay between the lowland field and aerobic fields ranged



**Table 3** Observed heritabilities for different traits and genetic correlations among traits. Data were combined from the 1999 and 2000 seasons unless noted otherwise. For PAR interception and RWC, data were taken from a single environment only. Other data are based on analysis across the 2 years or, for chlorophyll, across

two water levels. The phenotypic correlation is between grain yield (or residual grain yield) measured under stress and the trait measured in the same environment for which the genetic correlation is reported. The residual from the regression of the stress grain yield on the control grain yield is also reported

Trait	Heritability		Genetic correlation between yield under stress and trait		Phenotypic correlation	
	Measured under control conditions	Measured under stress	Measured under control conditions	Measured under stress	Stress yield and trait	Residual of regression and trait
Flowering date	0.94	0.93	NS		NS	NS
Height	0.78	0.66	-0.62**		-0.44**	-0.37**
Yield	0.45	0.45	0.99**		0.51**	
Panicles/m <sup>2</sup>	0.57	0.52	0.76**		0.48**	0.28*
Spikelets/panicle	0.89	0.67	-0.33*		-0.25*	NS
Spikelet fertility (%)	0.59	0.38	0.41*		0.23*	NS
Weight of 1,000 grains	0.84	0.91	NS		NS	NS
Leaf rolling score		0.63		NS	NS	NS
Leaf drying score		0.33		-0.87**	-0.39*	-0.34*
PAR interception		0.24		-0.78**	-0.35*	NS
Chlorophyll		0.81		0.69**	0.31*	0.30*
RWC		0.39		0.63**	NS	NS
Flowering delay		0.17			NS	NS

\* $P < 0.05$ ; \*\* $P < 0.01$

from -8.8 to 0 days for the lines, and Azucena tended to show a greater delay than Bala (data not shown).

Performance measures were generally well correlated across both water levels (Table 2). Neither spikelets per panicle nor TGW were correlated with grain yield—important yield components were the number of panicles, the fraction of those panicles that were completely sterile, and the percentage of fertile spikelets. These same components were highly correlated with yield under both stress and control conditions. Plant height was negatively correlated with grain yield under stress but not in control conditions. Performance under stress was not strongly correlated with scores of leaf rolling (Price et al. 2002c) or canopy temperature (unpublished) that were collected under severe vegetative stage stress. A correlation was observed between yield under stress (mean of the 1999 and 2000 experiments) and light interception early in the stress period in the 1998 trial, and also with leaf chlorophyll content measured in the 1996 trial and LD score. Variation was observed for panicle exertion among these lines and shorter plants had a tendency for poor exertion ( $r = -0.50^{**}$ ).

In order to assess the relationships between secondary traits and yield in more uniform genetic backgrounds, the lines were divided into two groups on the basis of which alleles were present near the *sd1* locus [markers C89-C949-RZ14; (Price et al. 2002c)]. The group with Azucena alleles at that region was on average 20% taller than the group with Bala alleles. It also had significantly cooler leaves, greater flowering delay with stress (1.2 days more delay), more leaf rolling and 20% sterile panicles compared to 15% in the group with Bala alleles. In contrast, the group with Bala alleles in that region had

25% more panicles/m<sup>2</sup>, more rapid water loss from excised leaves, poorer panicle exertion, and 15 to 25% greater harvest index under both stress and control conditions. When the correlation analysis was repeated by group, grain yield under stress was associated with plant height, flowering date, RWC and radiation interception in the set of lines with Bala alleles in that region, but not for the lines with Azucena alleles (Table 2). The only secondary trait showing a significant correlation with grain yield in both sets was leaf drying, and lines with more drying had reduced yield. Chlorophyll content was correlated with stress yield only in the group with Azucena alleles. The different yield components had consistent relationships with yield in both sets of lines, except for panicles/m<sup>2</sup> under stress, which was significant only for lines with Azucena alleles.

The heritability ( $h^2$ ) of grain yield in these experiments was moderate, and the heritability of yield under stress was similar to the yield in control plots (Table 3). Some secondary traits, such as leaf rolling, had fairly high heritability. Flowering date, plant height, spikelets per panicle and TGW were highly heritable. Other traits were measured in only a single season, so the estimate of  $h^2$  is less reliable. Nonetheless, high values indicate high repeatability of the trait and significant genetic variation among lines. Genetic correlations between yield under stress and other traits tended to be greater than phenotypic correlations (Table 3). In particular, leaf drying scores and plant height both had strong negative genetic correlations with yield under stress. The genetic correlation between yield under stress and under the control conditions was very high when based on the average values across both years. When  $r_g$  was estimated separately for each year, it

was near 1 in 2000, when water deficit reduced yield by an average of 35%. The genetic correlation between stress and control yields was only 0.66 in 1999, when the yield loss due to water deficit was 50%.

Because the yields under stress and control conditions were significantly correlated, stress yield was regressed against control yield and phenotypic correlations were computed between the residual from that regression and other traits. For plant height, leaf chlorophyll, and leaf drying, correlations with the residuals were generally similar to those observed for stress yield itself (Table 3). The influence of these traits on yield under stress was independent of the control yield of the line. In contrast, SPP and radiation interception were correlated with yield under stress but not with the residual. The influence of these traits on yield was similar in the stress and control environments.

While some lines yielded more than Bala, the high-yielding parent, the yield difference was not significant. Yield components from the best ten lines under stress and the best ten lines under the control conditions were examined to see how they compared to Bala. Under the control conditions, all high-yielding lines produced fewer panicles/m<sup>2</sup> and larger grains than Bala (Fig. 1). In the stress selections, high yielding lines had more spikelets per panicle and greater TGW, but fewer panicles/m<sup>2</sup> than Bala. This result contrasts with the conclusions that could be drawn from correlation analysis for the entire population, where greater yield was associated with more panicles and was independent of TGW. For panicle number this result is easily explained: Bala produced significantly more panicles/m<sup>2</sup> than any of the RILs.

The QTL analysis identified a number of genomic regions that were associated with maturity, plant height, or yield components (Table 4). An important region was identified on Chromosome 3 for flowering date in several environments, accounting for up to 50% of the variation, and for panicle number and biomass production. Twelve QTLs were identified for flowering delay, but only four of these coincided with QTLs for flowering date. The QTLs for delay were identified from the contrast between flowering dates measured in the lowland field and in an aerobic field under either well-watered or stress conditions. A major QTL for plant height was identified on Chromosome 1 near the *sd1* gene, accounting for 20% of the observed variation in the control conditions and over 40% under drought stress. Of the seven QTLs identified for panicle length, only two coincided with height QTLs, indicating some independence of these two traits. A QTL for average yield and grains per panicle (GPP) in the control was identified on Chromosome 3, accounting for 17% of the variation in yield. Additional QTLs for average yield were detected on chromosomes 8 and 11. No QTLs were found specifically for yield under stress, but ten QTLs were found for spikelet fertility under stress. Both parents contributed positive alleles for this trait. LOD scores for TGW were particularly high, reflecting the high heritability of this yield component, but again, the small-seeded parent, Bala, sometimes contributed the positive

allele. Five QTLs were identified for harvest index; four of these coincided with plant height QTLs and the effect of the allele was as predicted, with taller plants having a lower harvest index. Two QTLs were observed for nodal root thickness in the field (Table 4); these coincided with QTLs for spikelet fertility but thicker roots were associated with lower fertility

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## Discussion

### Effects of water deficit

Rice is particularly sensitive to water deficit in the period from about 10 days before flowering until about 7 days after flowering (O'Toole 1982). In 1999, the onset of differential irrigation was 56 DAS, meaning that water deficit began from -1 to 32 days before flowering in the control plots, and continued throughout grain filling. In contrast to other studies, however, the stress was not continuous or extremely severe—it was relieved at least weekly by rainfall or irrigation. In 2000, stress plots were not irrigated for a 16 day period that began on average 3 days before 50% flowering in the control plots. As in 1999, the stress was generally mild because of moderate VPD and several light rainfalls that occurred during the period of water exclusion. The date of the last irrigation was not correlated with indicators of stress impact such as yield reduction or flowering delay. This differs from results reported elsewhere, where there was a major and fairly precise effect of stress timing on performance (Garrity and O'Toole 1994). Nonetheless, the yield component most affected by water deficit during this period was the spikelet fertility, as has been reported in studies with more severe stress imposed around flowering (Lafitte and Courtois 2002). These results indicate that mild chronic drought stress beginning after the date of maximum tillering appears to be somewhat less sensitive to the date of stress onset. This type of irrigation regime may therefore be suitable for screening breeding populations with varying flowering dates for sensitivity to drought. While further confirmation of the value of dry-season screening to predict grain yield in drought-prone environments is required, early results suggest that performance under managed stress in the dry season at IRRI is well correlated with grain yields observed in an important target region, the short-season plateau uplands of Eastern India, where the cultivar Bala was developed (Atlin et al., submitted).

The genetic correlation between yields in stress and control treatments was very high in 2000, when water deficit reduced yield by less than 20%. This means that even though stress had a significant effect, the drought treatments did not contribute much additional information about line performance. This is not the same conclusion as would be reached based on the phenotypic correlation between stress and control yields, where the yield from the control plots explained less than 25% of the variation observed in the stress plots. While most lines that yielded



Table 4 (continued)

Chr	Marker 1	Marker2	Position (cM)	Flowering date	Flowering delay	Plant height	Panicle length	Panicle no. per m <sup>2</sup>	Fraction sterile panicles	Grains per panicle	Spikelet fertility	Weight per grain	Grain yield	Biomass index	Harvest index	Root thickness	Other traits
8	R902	A12367	8–15									3.42s	–4.70a		5.58		+RD
8	G1010b	C225	22							–5.92s		10.10s					–RD
8	RG598	R662	114	4.89c				–5.50a									+RD
9	A123613	G1085	106–109	5.26c	–4.66c					3.41c							
10	C701	G89d	0		–6.56c						–3.59s						
10	A12372	G1082	55–64		–15.99												
10	C16	C223	141					–4.25c				5.05s					
11	G44	A1245y	60														
11	A123615	C189	73–88						4.39s				5.49c				
11	C189	G1465	102–114	–5.10c		–3.82s			–3.87s		4.12s	4.99c		3.78c			
12	CDO127	RM247	14–25		–9.00					–4.34s							+RD

well under stress also yielded well in the control conditions, a few lines performed better under stress than would have been expected on the basis of their control yields. These are candidates for further study to identify drought-adaptive traits. The genetic correlation between stress and control plots was much weaker in 1999, when stress reduced the yield to 50% of its control value. We conclude that this level of yield reduction is desirable to ensure that stress nurseries provide added value to breeding programs. A 50–80% yield reduction has been targeted as the desired level in maize drought stress nurseries (Bänziger et al. 2000).

The influence of some secondary traits, such as flowering date and RWC, on yield under stress was apparent only when the *sd1* gene was present (i.e., in lines with Bala alleles at the plant height QTL interval on Chromosome 1). In another rice mapping population, a similar dependence of correlations on this structural gene was observed (Lafitte et al. 2002b). This result may reflect epistatic interactions among genes, or it may be a more direct physiological effect. For example, the set of lines with Bala alleles in that region had more panicles/m<sup>2</sup> and greater harvest index. Further yield improvements may depend on improved plant water status only after these structural aspects are improved. The influence of *sd1* on yield was considerably weaker in this population than in the IR64 × Azucena mapping population, where the semi-dwarf parent is adapted to lowland cultivation (Lafitte et al. 2002a).

#### QTLs for drought-related traits

Despite the small population size used in this study, a number of significant QTLs were identified for maturity, plant type, and yield components. Only three QTLs were observed for the more integrative trait, yield. The level of significance observed was similar to that reported for another indica-by-japonica rice population (Babu et al. 2003), and there was some correspondence between the QTLs detected for flowering date and plant height in that study. Some correspondence was also found for published QTLs for flowering date and yield components in an interspecific population (Moncada et al. 2001). In some cases, QTLs for yield components corresponded to locations of QTLs reported for putative drought-adaptive traits (Table 4). However, the directions of the observed effects of these QTLs seldom supported a causal relationship between them. For example, a major QTL for plant height on Chromosome 1 coincided with a QTL for RWC (Price et al. 2002c), with taller plants having lower water content. This supports the observation that LD scores are strongly influenced by plant size, particularly the amount of leaf area present when stress begins (Mitchell et al. 1998). On Chromosome 7, however, a QTL for biomass coincided with a QTL for LD where the Azucena allele increased biomass, but reduced leaf drying. Other studies also report inconsistent relationships between QTLs for leaf traits and root traits (Price et al. 2002a). Nonetheless,



the genetic correlation between stress yield and LD was high. The inconsistent QTL effect may reflect the absence of a biological relationship between these traits, or it may reflect low precision of QTL estimates in a small population (Utz et al. 2000), or it may be due to low heritability of the trait in the comparatively mild stress imposed in these studies. It would be worthwhile to investigate more consistent screening systems that could reliably reveal genetic differences in LD near flowering in plants of more uniform size.

Previously reported root QTLs (Price et al. 2002b) often coincided with QTLs identified for height, flowering date, and tiller number. The *sd1* gene, which was detected here as a major QTL on Chromosome 1, is generally associated with high tiller number and shallow rooting, and the semidwarf parent, Bala, had much greater tiller numbers. In rice, a cohort of new roots is produced with each tiller, and extensive tillering thus results in comparatively more roots in superficial soil layers than for low-tillering types. In five of the six locations where QTLs for spikelet fertility coincided with reported QTLs for root depth, the relationship between the two traits was negative. The QTLs for root traits and panicle number or plant height did not show a consistent relationship. The genetic correlation between panicles/m<sup>2</sup> and yield under stress was high and positive (Table 3); the benefits of high tillering capacity may overwhelm any impact of root effects on performance. Near-isogenic lines are needed to directly test the value of greater rooting depth on rice yield, independent of tillering capacity.

The parents of this population are both adapted to upland cultivation conditions, so we anticipate that the QTLs identified are not specific for aerobic adaptation. Bala always out-yielded Azucena by 0.6–1 ton/ha, largely because of its greater tiller number. QTLs for yield and spikelet sterility did not generally coincide with QTLs for tiller number, indicating that there are additional alleles to improve aerobic rice yield coming from Azucena. The yield QTLs identified on chromosomes 3 and 11 are good candidates for improving the performance of Bala by introgression of Azucena alleles. At the same time, it is clear that greater tiller numbers will be required to raise yield potential, and that this can be achieved without sacrificing stress tolerance.

Quantitative trait loci analysis was not very helpful in identifying other secondary traits that had similar genetic control as yield components measured under stress. Chlorophyll content appeared to be a promising secondary trait because it showed a high genetic correlation with yield under stress; it could be measured early in the season and was not affected by water level, and it had a high heritability. Only two QTLs were identified for leaf chlorophyll content. The Azucena allele had a positive effect at both positions, but also had a negative effect on important yield components. Flowering delay is another trait that has been suggested as an indicator of drought susceptibility (Pantuwan et al. 2002). In this study, a strong QTL was detected on Chromosome 3 for flowering delay between lowland and sprinkler experiments, but this

region was also associated with late maturity, and it was not associated with yield components. In contrast, all but three of the QTLs detected for delay between lowland and drip irrigated experiments cosegregated with QTLs for yield components, but in these regions a greater delay was associated with better performance. Flowering date in the lowland was often later than in the stress treatments. These results do not support this estimate of flowering delay as an indicator of drought susceptibility in this population, which was derived from upland-adapted parents.

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## Conclusions

Correlation analysis has commonly been used to estimate the association of different traits with grain yield, or to establish the potential utility of secondary traits to improve selection efficiency. Alternatives to that approach are: (1) observation of the mean values for different traits in a fraction of the population selected for grain yield, and (2) observations of cosegregation between QTLs for traits and QTLs for yield or yield components. In the present study, the phenotypic and genetic correlations between yield and spikelets per panicle or TGW were low. Nonetheless, these yield components differed significantly from Bala in the group of lines selected for yield, particularly for yield under stress. Thus, the examination of high-yielding lines revealed successful combinations of yield components that were not predicted from the correlation analysis. There was variable cosegregation between the QTLs for secondary traits and for yield components, and when it did occur, the effects of parental alleles for the two traits were not always as expected. These results do not support the use of such QTLs as markers for improving yield under conditions of drought. The QTLs reported here do highlight interesting genetic regions for further investigation, and confirm the opportunity for low-yielding parents to contribute superior alleles for yield components. Yields measured in the stress and control plots had similar heritabilities, indicating that selection for yield per se in managed stress environments may be a promising approach for improving drought tolerance in rice. On the other hand, secondary traits may provide supplemental information for selection where yield cannot be measured due to logistical reasons such as lodging, small plot size, or very large numbers of entries. Leaf drying was the trait most suited for this, because it had a high genetic correlation with yield when stress occurred near flowering and the trait is easy to score. Stress must be rather severe, however, for differences to be apparent.

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