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The large-effect drought-resistance QTL *qtl12.1* increases water uptake in upland rice

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ABSTRACT

Drought stress is the most important abiotic factor limiting upland rice yields. Identification of quantitative trait loci (QTL) conferring improved drought resistance may facilitate breeding progress. We previously mapped a QTL with a large effect on grain yield under severe drought stress (*qtl12.1*) in the Vandana/Way Rarem population. In the current paper, we present results from a series of experiments investigating the physiological mechanism(s) by which *qtl12.1* affects grain yield under drought conditions. We performed detailed plant water status measurements on a subset of lines having similar crop growth duration but contrasting genotypes at *qtl12.1* under field (24 genotypes) and greenhouse (14 genotypes) conditions. The Way Rarem-derived allele of *qtl12.1* was confirmed to improve grain yield under drought mainly through a slight improvement (7%) in plant water uptake under water-limited conditions. Such an apparently small increase in water uptake associated with this allele could explain the large effect on yield observed under field conditions. Our results suggest that this improvement of plant water uptake is likely associated with improved root architecture.

1. Introduction

Breeding rice (*Oryza sativa* L.) varieties with improved drought resistance has progressed slowly over the past decade due to a lack of understanding of the traits and genetic mechanisms that confer adaptation to water deficit in the different environments; and to the inability of breeders to select for these traits (Bernier et al., 2008). Recently, direct selection for grain yield under managed drought stress has been found to be effective in improving drought resistance (Venuprasad et al., 2007; Kumar et al., 2008; Venuprasad et al., 2008), but the reliable application of managed stress is not possible for most upland rice breeding programs. This has led to the hypothesis that breeding for drought resistance in rice could be facilitated by mapping quantitative trait loci (QTL) related to secondary component traits assumed to be related to drought resistance (Price and Courtois, 1999), for use in marker-assisted selection (MAS). Pursuing this approach, QTL for osmotic adjustment (Zhang et al., 2001), relative water content (RWC) (Courtois et al., 2000), root-related traits (Zhang et al., 2001; Courtois et al., 2003; Yue et al., 2006), stomatal conductance (Price et al., 1997) and several other traits potentially linked to drought resistance have been mapped. Some attempts have been made to use such QTL in MAS schemes. Unfortunately, most QTL identified have not been repeatable over environments and/or populations (Bernier et al., 2008), or have not consistently affected either the target trait (Shen et al., 2001; Steele et al., 2006) or grain yield under stress (Steele et al., 2007) when introgressed into a susceptible cultivar.

Relatively few experiments have attempted to map quantitative trait loci for grain yield under drought, and, as for secondary traits, most of the reported QTL were not repeatable across multiple trials or locations. The few QTL detected across multiple trials at a single location include one mapped in the CT9993/IR62266 doubled haploid population under lowland field conditions in India (Kumar et al., 2007) and a QTL detected in a rainout shelter pipe experiment in China (Yue et al., 2006). However, the largest and possibly most reliable QTL for grain yield under drought stress in the field reported to date in rice has been *qtl12.1* in the Vandana/Way Rarem F_3-derived population, which explained 51% of the genetic variation for grain yield under drought conditions over 2 years of field evaluation at IRRI, with a very low QTL × year interaction (Bernier et al., 2007). The Way Rarem-derived allele of this QTL has been shown to improve grain

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yield under severe or moderate upland drought conditions in 9 out of 10 trials, but appears to have no effect on yield under lowland conditions (Bernier et al., submitted for publication).

The original QTL mapping experiment where *qtl12.1* was identified (Bernier et al., 2007) focussed on identifying QTL with large effect on grain yield under drought stress, but did not provide an understanding of the physiological mechanisms by which drought resistance was improved. Grain yield under water deficit conditions is determined by three main factors: water uptake by the plant, water-use efficiency (WUE), and harvest index (HI) (Passioura, 1977). Those three components are relatively independent, but obviously interact with each other in a complex manner (Condon et al., 2004). In order to determine which factor was predominant in explaining the effect of *qtl12.1*, and to gain a better understanding of the physiological processes affected by this QTL, we conducted a series of field and greenhouse experiments. Our objectives were: (1) to characterize in more detail the yield components affected by *qtl12.1* under field conditions; (2) to determine if *qtl12.1* improves WUE and/or plant water uptake; (3) to determine if rooting distribution and architecture and/or internal root anatomy are affected by *qtl12.1*.

2. Materials and methods

2.1. Plant material

All lines used in the current trial were selected from a set of 92 unselected lines from the Vandana/Way Rarem F_3-derived recombinant inbred line (RIL) mapping population that had previously been genotyped at 126 loci (Bernier et al., 2007). Vandana is an upland-adapted drought-resistant variety originating from India, while Way Rarem is an upland-adapted drought susceptible variety originating from Indonesia. The first criterion used for selecting the lines was the presence or absence of the Way Rarem allele in the entire *qtl12.1* interval, which spans the genomic region between RM28048 and RM511. The second criterion was to select lines with as narrow a range of flowering dates as possible. This led to selection of lines with an average number of days to 50% flowering under non-stress conditions ranging from 65 to 80 days after seeding (DAS). In the 2006 field trials, a set of 28 lines (14 homozygous for the Way Rarem allele at *qtl12.1* and 14 homozygous for the Vandana allele) and four checks (the upland-adapted cultivars Apo, Vandana and Way Rarem and the irrigated lowland-adapted cultivar PSBRC80) were planted once in each replication. After analysis of the 2006 data, four lines were discarded (two from each set) because their flowering date did not fall within the desired range. Thus, in the 2007 field trials, only the 24 lines used in 2006 were planted, with every check being repeated twice per replication to compensate for the removal of four lines from the experiment. Both trials contained 32 plots per replication and used an 8×4 α-lattice design. A total of 14 lines randomly chosen from within the 24 field-tested lines were used in the greenhouse experiments.

2.2. Field evaluation

Field evaluation was conducted under upland conditions at IRRI, Los Baños, Philippines (14°N 121°E, 21 m above sea level) during the dry seasons of 2006 and 2007 (Table 1). The soil of the IRRI upland farm is a Maahas clay loam. Plots were 4 m long and 6 rows wide, with inter-row spacing of 0.25 m and a seeding rate of 2 g of seed per linear meter, resulting in a density of approximately 390 seeds m^{-2}. Basal fertilizer applications equivalent to 40 kg P and 40 kg K ha^{-1} were applied in the form of single super phosphate and potassium chloride, and 120 kg ha^{-1} of N in the form of ammonium sulphate was applied in three even splits around 21, 42 and 61 DAS. Both well-watered control trials and drought stress trials were conducted. In the 2006 well-watered trial, irrigation was supplied weekly by basin irrigation for the entire season. All other trials were sprinkler-irrigated, with drought trials being irrigated twice weekly during establishment and early vegetative growth, but subsequently drought-stressed. Plots were re-irrigated periodically when soil water tension fell below −50 kPa at 30 cm soil depth. At this soil water potential, most lines exhibited visual stress symptoms such as leaf drying and leaf rolling. Plots were then re-irrigated and a new soil drying cycle was imposed. This type of cyclical stress was repeated until harvest, and is considered to be efficient in screening for drought resistance in populations consisting of genotypes with a broad range of growth duration (Lafitte et al., 2004), ensuring that lines of all durations are stressed during reproductive development.

In both years, several physiological traits were regularly measured from the moment stress was initiated until crop maturity. Relative water content was measured according to Turner (1981) by sampling the last fully expanded leaf of three plants per plot. Leaf collection was always performed between 9:00 a.m. and 10:30 a.m. The leaves were cut and placed into sealed vials and stored on ice. Fresh leaf weight was then recorded as quickly as possible and distilled water was added to the vials, which were then stored at 4 °C in the dark for 24 h. Leaves were then removed from vials, excess water on the leaves was removed using paper towels and the turgid weight was recorded. Leaves were then placed inside paper envelopes and oven-dried at 80 °C for 72 h before recording the dry weights. We measured RWC twice in 2006 and eight times in 2007. Stomatal conductance (g_s) was measured using a Li-1600 steady-state porometer (Li-Cor Biosciences, USA). Measurements were taken on the abaxial (lower) side of the last fully elongated leaf. We measured g_s in 2006 only. Leaf water potential (LWP) was measured in the last fully expanded leaf using the pressure chamber technique (Scholander et al., 1965; Turner, 1981). Leaf rolling and leaf drying scores were

Table 1
Description of all field trials used to contrast lines with and without the Way Rarem allele of *qtl12.1* under upland conditions in the dry season: IRRI 2006 and 2007

	2006		2007	
	Stress	Non-stress	Stress	Non-stress
Planting date	07 January	21 January	12 January	14 January
Number of replications	3	3	2	2
Beginning of drought stress (DAS)	40		43	
Mean minimum temperature at flowering (°C)	24	24	24	23
Mean maximum temperature at flowering (°C)	33	32	32	32
Maximum temperature at flowering (°C)	35	35	35	34
Mean line yield (kg ha^{-1})	502	1844	420	2598
Mean line harvest index	0.14	0.32	0.13	0.38
Mean line biomass accumulation (kg ha^{-1})	3599	4695	4264	8553