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Drought-tolerant QTL *qVDT11* leads to stable tiller formation under drought stress conditions in rice

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ABSTRACT

Drought is an important limiting factor for rice production, but the genetic mechanisms of drought tolerance is poorly understood. Here, we screened 218 rice varieties to identify 32 drought-tolerant varieties. The variety Samgang exhibited strong drought tolerance and stable yield in rain-fed conditions and was selected for further study. To identify QTLs for drought tolerance, we examined visual drought tolerance (VDT) and relative water content (RWC) phenotypes in a doubled haploid (DH) population of 101 individuals derived from a cross between Samgang and Nagdong (a drought-sensitive variety). Three QTLs from Samgang were identified for VDT and explained 41.8% of the phenotypic variance. In particular, *qVDT11* contributed 20.3% of the phenotypic variance for RWC. To determine QTL effects on drought tolerance in rain-fed paddy conditions, seven DH lines were selected according to the number of QTLs they contained. Of the drought-tolerance-associated QTLs, *qVDT2* and *qVDT6* did not affect tiller formation, but *qVDT11* increased tiller number. Tiller formation was most stable when *qVDT2* and *qVDT11* were combined. DH lines with both of these drought-tolerance-associated QTLs exhibited the most stable tiller formation. Together, these results suggest that *qVDT11* is important for drought tolerance and stable tiller formation in rain-fed paddy fields.

1. Introduction

Rice, wheat, and maize are important cereal crops and staple foods worldwide. In irrigation systems without drought stress, rice production is two- to three-fold higher than production in rain-fed systems [1,2]. During the past 50 years, rice varieties sensitive to drought stress have been developed for use within irrigation systems [1,3]. However, 20% of rice fields in Asia are estimated to be drought-prone [4]. Drought disturbs panicle initiation, floret initiation, flowering and grain filling, in addition to reducing rice yield [5–7]. Recent drought conditions caused by global climate change seriously threaten commercial rice production in irrigated fields as well as rain-fed fields [8]. Moreover, the predicted climate-change scenarios warn of the possibility that drought intensity, duration, and frequency will increase in agroecosystems and decrease crop yield [9]. Thus, the development of drought-tolerant varieties is an important global strategy to maintain sustainable rice production worldwide.

Marker-assisted breeding is an efficient strategy to improve drought resistance and ensure stable production of drought-prone rice varieties. However, drought resistance is considered a complex trait. Rice has three mechanisms for drought resistance regulated by multiple QTLs: drought avoidance, drought tolerance, and drought recovery [10]. Drought-resistance QTLs also interact with environmental conditions and recipient genetic backgrounds [11]. Therefore, effective phenotype evaluation criteria for target environments must be applied to QTL analyses. Several traits related to drought characteristics, such as root architecture, leaf water content, and grain yield, are combined into an evaluation index that also accounts for photosynthesis parameters (e.g., stomatal conductance and electron transport capacity) [12,13].

Root architecture, especially root length and thickness, is related to drought resistance [14,15]. Many QTLs for root architectural traits have been identified with different phenotype evaluation systems. In a wax-petrolatum-layer test system, Zhang et al. found

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QTLs underlying seven traits related to drought resistance, including root penetration [16]. Additionally, several QTLs for root traits have been reported using a PVC pipe culture system [17,18]. Deep rooting is determined primarily through a combination of root growth angle and maximum root length [19,20]. The basket method was developed specifically to investigate the ratio of deep rooting; five major QTLs for this trait from Kinandang Patong (a deep-rooting variety) have been identified [21–23]. The *DRO1* gene that induces a downward direction to the root growth angle in rice has been cloned and shown to improve drought avoidance significantly [24].

Several major drought-yield QTLs with high heritability have been found through direct selection under drought-stress field conditions. The QTL *qDTY6.1*, identified in the drought-tolerant aerobic-adapted rice varieties Apo and Vandana, explains 40–66% of the genetic variance in grain yield during drought stress [25]. Four QTLs with major effects on yield under drought conditions have been identified in the stress-tolerant variety AdaySel, and near-isogenic lines (NILs) harboring two of these QTLs ($qDTY_{2.2}$ and $qDTY_{4.1}$) improved yield under drought conditions in irrigated fields [26–28]. Recently, Kumar et al. described the development of drought-tolerant rice varieties with high yields in drought-prone rain-fed fields; they used a marker-assisted backcross breeding approach and identified QTLs with large effects on grain yield under drought [29,30]. However, little is known about drought-resistance QTLs in rice. In this work, we aimed to aid the development of new rice varieties for stable production through screening drought-tolerant varieties and investigating QTLs associated with drought tolerance.

2. Materials and methods

2.1. Plant materials

A total of 218 rice (*Oryza sativa*) germplasms were evaluated for drought tolerance in this study (Table S1). The mapping population was developed from a cross between Samgang and Nagdong. Samgang is a drought-tolerant Tongil-type lowland variety. Nagdong is a drought-sensitive temperate *japonica* variety. Both were developed in the National Institute of Crop Science, RDA, Korea. The 101 doubled haploid (DH) lines were obtained via anther culture using F_1 plants [31]. Each DH line was harvested individually and used for the identification of drought-tolerance related QTLs.

2.2. Agronomic trait experiments under rain-fed and irrigated paddy field conditions

The agronomic performance of Samgang, Nagdong, and selected SN-DH lines was assessed under rain-fed and irrigated paddy field conditions at the Department of Southern Area Crop Science (35°49′N, 128°74′E), National Institute of Crop Science, Miryang, Korea in 2014 and 2015. The soil type is of the Deogpyeong series: fine, mixed family of typic Epiaqualfs. A randomized complete block design was applied with three replications for each condition. A single 30-day-old seedling was transplanted on each hill with 30 cm (line) × 15 cm (row) spacing between hills. For the drought treatment (defined as "rain-fed paddy field conditions"), water supply was withheld from 7 days post-transplantation until harvest. Grain yield was recorded as the average grain weight per 100 plants of each plot. Grain weight per plot was adjusted to a moisture content of 13% and calculated on a per area basis (0.1 ha). Yield loss was calculated using the following formula: Yield loss (%) = Grain yield in rain-fed conditions/Grain yield in irrigated conditions × 100.

2.3. Evaluation of drought phenotypes in the greenhouse

To screen varieties and evaluate the DH population, pre-germinated seeds were sown in a seed box (60 × 30 cm). Three-week-old seedlings were transplanted with 15 cm (line) × 10 cm (row) spacing to the rice nursery bed (9 × 1.5 m, depth 30 cm) filled with Deogpyeong series soil in the greenhouse. Seedlings were grown for 7 additional days to acclimate them; water was then withheld for six weeks. Then, the varieties were evaluated for drought-tolerance phenotypes based on degree of leaf drying 7 days post-watering resumption: sensitive (>70% of all leaves fully dried), intermediate (20–70% of all leaves dried), and tolerant (<20% of leaf tips dried). The visual drought tolerance (VDT) phenotype of the DH population was examined using a 1 to 9 scale according to the standard evaluation system for rice [32].

Relative water content (RWC) of each leaf was calculated on the fifth week of observing visual drought phenotypes. The flag leaf on the main culm was excised about 12 cm below the tip, and the fresh weight (FW) of the detached leaf tip was measured. Leaf tips were put in water for 12 h to measure the turgid weight (TW). Finally, leaf tips were oven-dried for 2 d at 60 °C for measuring the dry weight (DW). RWC was then calculated using the following formula: RWC(%) = (FW-DW)/(TW-DW) × 100. Ten independent leaf tips from each line comprised one sample and three replicate samples were analyzed.

2.4. Genotyping, linkage map construction, and QTL analyses

Genomic DNA was extracted from leaf tissue of 14-day-old seedlings using the cetyltrimethyl ammonium bromide method [33]. Simple sequence repeat (SSR) primers developed by McCouch et al. were used for mapping [34]. Depending upon the sequence size difference (in bp) between the parental alleles, PCR products were electrophoresed on 8% non-denaturing polyacrylamide gels with silver staining on or 3% agarose gels and visualized under UV light [35].

A genetic linkage map was constructed with 185 SSR and sequence tagged site (STS) polymorphic markers from the parental varieties in IciMapping ver. 4.0 (Chinese Academy of Agricultural Sciences, China), with the following parameters: grouping by 3.0 of logarithm of odds (LOD) threshold, ordering algorithm by input, and rippling by sum of adjacent recombination fractions (SARF) [36]. The Kosambi mapping function was used to calculate map distances [37]. The linear order of DNA markers on each rice chromosome (n = 12) was determined in accordance with a physical map (http://www.gremene.org).

QTL analysis was performed for the adjusted phenotypic mean values of VDT and RWC using the inclusive composite interval mapping of additive (ICIM-ADD) module in QTL IciMapping. The walking step for QTL scanning was set to 0.1 cm. The threshold LOD score for declaring the significance of putative QTLs was determined for drought-tolerance traits on the basis of 1000 permutations at $P < 0.05$ [38]. The symbol of an additive effect for each QTL was used to confirm the donor for the proper allele [39]. The negative value of additive effects for VDT-related QTLs indicated that Samgang alleles increase the RWC trait value and vice versa. The percentage of phenotypic variation explained by individual QTLs was estimated with the coefficient of determination (R^2). The QTL nomenclature followed McCouch and CGSNL [40,41].

2.5. Effect of drought-tolerance-associated QTL combinations in the DH population

The seven DH lines were divided into groups based on the peak marker of each QTL. Mean VDT values were calculated and