Research paper

Genetic analysis of yield and agronomic traits under reproductive-stage drought stress in rice using a high-resolution linkage map

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ABSTRACT

Drought stress at the reproductive stage of rice crop leads to a huge loss in grain yield. Identification and introgression of large effect drought tolerant QTLs are necessary to develop drought-tolerant rice varieties. Compared to the high-density linkage maps, widely spaced markers lead to the identification of QTLs with large confidence intervals which are difficult to incorporate in a breeding program. A previously generated geno-typing-by-sequencing (GBS) based linkage map consisting of 4748 SNP markers was used to map QTLs in Cocodrie × N-22 recombinant inbred line (RIL) population. Twenty-one QTLs were discovered for days to flowering (DTF), plant height (PH), leaf rolling score (LRS), plant dry matter content (DM), spikelet fertility (SF), grain yield (GY), yield index (YI), and harvest index (HI) under drought stress. A major QTL *qPH1.38* was identified in a narrow confidence interval on chromosome 1. The QTLs, *qDTF3.01* and *qPH1.38,* overlapped with the previously identified QTL *qDTY1.1* and *Hd9*, respectively. Another large-effect QTL *qLRS1.37* was identified close to the *sd1* locus on chromosome 1. A grain yield QTL *qGY1.42* located on chromosome 1 contained only 4 candidate genes. There was no overlapping of QTLs for the root traits and the yield attributes. The important candidate genes present within the large effect QTL regions are MYB transcription factors, no apical meristem protein (NAC), potassium channel protein, nuclear matrix protein1, and chlorophyll A-B binding protein. The large effect QTLs (*qDTF3.01*, *qPH1.38*, and *qLRS1.37*) and a novel grain yield QTL *qGYS1.42* can be used to incorporate in elite breeding lines to develop drought-tolerant rice varieties.

1. Introduction

Rice is an important food crop grown all around the world. Due to high water requirement, rice cultivation in the rainfed and water lim-iting environments is severely affected. Irrigated rice covers 55% of the rice-growing areas and produces 75% of the total production while the remaining is contributed by areas exposed to moisture stresses (CGIAR Science Council, 2009). Since rice productivity in irrigated areas has stagnated, efforts should be made to increase rice production in the rainfed ecosystem. Exploitation of natural genetic variation to develop drought-tolerant varieties and effective water management practices are some of the strategies to address this challenge.

Drought tolerance in rice is inherently complex involving multiple mechanisms. Phenotyping for drought tolerance should involve primary traits (plant height, root length, and number of tillers), secondary traits (plant water status, leaf rolling, and leaf death) and integrated traits (spikelet fertility, harvest index, and test weight) (Kamoshita et al., 2008). Identification of quantitative trait loci (QTLs) and genes

controlling the root traits (Uga et al., 2013; Bhattarai and Subudhi, 2018) and various other physiological traits (Zhang et al., 2001; Nguyen et al., 2004) has been done in the past. But due to poor cor-relation of these traits with yield, there has been little progress in im-proving rice productivity in drought-prone areas. Yield improvement is dependent upon several yield component traits. Progress has been made in developing drought tolerant cultivars by direct selection for yield. However, due to low heritability of yield, it is difficult to make im-provement in both yield and drought tolerance simultaneously (Palanog et al., 2014). Plant height, spikelet fertility, plant biomass, and harvest index are some associated traits that influence grain yield in rice (Prince et al., 2015). Therefore, QTLs for yield along with other highly heritable yield component traits would be an effective strategy to enhance yield under drought stress.

Efforts have been made to identify drought-tolerant donors and to develop suitable selection criteria for breeding drought-tolerant vari-eties (Kumar et al., 2014). Several QTLs for drought tolerance have been identified in the past (Bernier et al., 2007; Saikumar et al., 2014;

Palanog et al., 2014; Dixit et al., 2014a, 2014b; Prince et al., 2015; Solis et al., 2018). Most of these QTL studies were based on direct selection for yield. QTLs for yield have been identified on chromosomes 1, 2, 3, 6, and 12. The *qtl12.1* was the first yield QTL for drought tolerance at the reproductive stage (Bernier et al., 2007). This QTL has been used in marker-assisted breeding to develop a drought-tolerant version of "Sabitri", a popular rice variety of Nepal (Dixit et al., 2017b). A large effect reproductive stage drought responsive QTL (*qDTY1.1*) has been identified on chromosome 1 (Ghimire et al., 2012; Venuprasad et al., 2012) with a consistent effect in various genetic backgrounds. A possible linkage between *qDTY1.1* and *sd1* affects the introgression of this yield enhancing QTL to elite varieties (Vikram et al., 2015). Besides this, the drought-tolerant QTLs on chromosome 2 (*qDTY2.1, qDTY2.2,* and *qDTY2.3*) for grain yield were also identified (Palanog et al., 2014). A reduction in flowering duration due to the interaction of *qDTY3.2* with two other QTLs *qDTY1.1* and *qDTY12.1* was observed. Drought tolerant rice varieties could be developed by marker-assisted breeding and QTL pyramiding of drought-tolerant QTLs. An interaction between two or more QTLs was reported to increase grain yield under drought stress (Swamy et al., 2013; Sandhu et al., 2018). Pyramiding drought tolerant QTLs (*qDTY2.2, qDTY3.1,* and *qDTY12.1*) in Malaysian rice variety showed increase in yield (Shamsudin et al., 2016). Identification of stable large effect QTLs across various environments and genetic backgrounds is needed to develop drought-tolerant rice varieties.

QTL based identification and cloning of genes has been helpful for rice improvement (Uga et al., 2013). A limited effort has been made in the past to study drought tolerance in USA rice varieties. In this study, we identified the drought-tolerant QTLs for yield and various yield attributing traits in rice at the reproductive stage drought stress using a recombinant inbred line (RIL) population involving a susceptible southern US rice variety "Cocodrie".

2. Materials and methods

2.1. Development of mapping population

The mapping population comprised of RILs derived from a cross between Cocodrie and N-22. Cocodrie is a high yielding rice variety developed by Louisiana State University Agricultural Center and is sensitive to drought (Linscombe et al., 2000). N-22 is a popular drought tolerant cultivar with low yield potential (Kumar et al., 2014). The F$_1$s derived from a cross between Cocodrie and N-22 were selfed for six generations and the seeds were advanced by single seed descent method to generate F$_7$ RILs for drought phenotyping and genotyping.

2.2. Phenotyping under drought stress

One hundred eighty-one RILs and two parents (Cocodrie and N-22) were phenotyped for drought tolerance. The experiment was conducted inside the greenhouse of Louisiana State University Agricultural Center during summer 2016. Plants were grown in 2-gallon plastic pots filled with silty clay soil. The pots were placed in a plastic covered concrete bench filled with water. Nutrient solution (0.2%) was prepared using Jack's Professional (20-20-20) (J.R. Peters, Inc.) and was applied every week for the first four weeks of plant growth. Slow release fertilizer "Osmocote" (4 g) was applied in every pot after 35 d and 55 d of plant growth. Three plants were grown in each pot. There were three replications and the experiment was conducted in completely randomized design. The two replications were subjected to drought treatment and one set was used as control. The pots were transferred from the irrigated bench to the non-irrigated bench when the plants started to emerge panicle. The plants were left un-irrigated for one week. The degree of drought severity was assessed by observing > 50% spikelet sterility in majority of the RILs and some irreversible damage in highly susceptible RILs. After one week, the plants were replaced in the bench filled with water. Irrigation was continued until the plants attained

physiological maturity. The control bench was continuously irrigated until harvest. Measurements were taken on three plants from each pot in each replication for morphological and yield parameters. Days to flowering (DTF) was determined as the number of days for emergence of the panicle. Plant height (PH) was measured from the base of the culm to the tip of the plant. Leaf rolling score (LRS) was determined following the standard evaluation system (SES) of rice (IRRI, 2002). Dry matter content (DM) was measured as the ratio of dry plant mass to the fresh plant mass and was expressed in percentage. Grain yield per plant (GY) was measured averaging the gain yield from all drought treated plants in each replication. Yield index (YI) was calculated as the ratio of the plant yield at drought and control condition. Harvest index (HI) was calculated as the percentage ratio of grain yield to the biomass yield under drought stress. Root length, dry root mass, and root-shoot ratio were measured under both stress and control environment in the same mapping population in an earlier experiment and the detailed protocol on phenotyping was described in Bhattarai and Subudhi (2018).

2.3. Statistical analysis

Statistical analysis was carried out using SAS 9.3 (SAS Inc., 2011). Frequency distribution graphs were constructed using a pivot table in Microsoft Excel 2010. Mean, standard deviation, and coefficient of variance were computed using PROC MEANS procedure. Analysis of variance (ANOVA) was estimated using PROC MIXED procedure in SAS, considering the line as fixed effect and replication as random effect. Broad-sense heritability (based on family mean basis) was estimated in SAS using the SAS code (Holland et al., 2003). Pearson correlation coefficients were calculated using PROC CORR procedure. The previously generated data on root traits under both stress and non-stress environments in the same mapping population was used for determining correlation with four yield-related traits (spikelet fertility, grain yield under stress, yield index, and harvest index). Principal component analysis (PCA) was done to understand the relationship among the variables. PCA was done using JMP software (SAS Inc., 2013).

2.4. Linkage mapping and QTL analysis

A total of 4748 SNPs previously generated by genotyping by sequencing (GBS) in Cocodrie x N-22 RILs were used for the construction of linkage map and QTL mapping (Bhattarai and Subudhi, 2018). Linkage mapping and QTL analysis were performed using interval mapping (IM) and inclusive composite interval mapping (ICIM) software (Meng et al., 2015). The markers were placed on the linkage map based on the physical map of the reference genome Nipponbare. Kosambi mapping function was used to calculate the genetic distance between the markers (Kosambi, 1944). Both additive and epistatic QTLs were identified. The additive QTLs having LOD scores > 2 were considered as significant and the epistatic QTLs with LOD scores > 4 were considered as significant. The nomenclature of QTLs was done following the procedure described earlier (Bhattarai and Subudhi, 2018). For example, a QTL for days to flowering in chromosome 3 and 1 Mb region is written as *qDTF3.01*. A positive additive effect represented contribution of Cocodrie allele toward increased trait mean and a negative additive effect indicated N-22 allele responsible for increasing the trait mean.

2.5. Gene ontology and annotation

The QTL intervals were determined by the physical position of the SNP markers flanking the respective QTLs. All the genes present in each QTL interval was retrieved from the MSU rice genome annotation project database (http://rice.plantbiology.msu.edu/). Trait-wise gene ontology annotation was done using agriGo gene ontology analysis toolkit (Tian et al., 2017). Singular enrichment analysis (SEA) tool in