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Genetic analysis and validation of quantitative trait loci associated with reproductive-growth traits and grain yield under drought stress in a doubled haploid line population of rice (*Oryza sativa* L.)

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ABSTRACT

Drought is a major constraint for rice production and yield stability in rainfed ecosystems, especially when it occurs during the reproductive stage. Combined genetic and physiological analysis of reproductive-growth traits and their effects on yield and yield components under drought stress is important for dissecting the biological bases of drought resistance and for rice yield improvement in water-limited environments. A subset of a doubled haploid (DH) line population of CT9993-5-10-1-M/IR62266-42-6-2 was evaluated for variation in plant water status, phenology, reproductive-growth traits, yield and yield components under reproductive-stage drought stress and irrigated (non-stress) conditions in the field. Since this DH line population was previously used in extensive quantitative trait loci (QTLs) mapping of various drought resistance component traits, we aimed at identifying QTLs for specific reproductive-growth and yield traits and also to validate the consensus QTLs identified earlier in these DH lines using meta-analysis. DH lines showed significant variation for plant water status, reproductive-growth traits, yield and yield components under drought stress. Total dry matter, number of panicles per plant, harvest index, panicle harvest index, panicle fertility, pollen fertility, spikelet fertility and hundred grain weight had significant positive correlations with grain yield under drought stress. A total of 46 QTLs were identified for the various traits under stress and non-stress conditions with phenotypic effect ranging from 9.5 to 35.6% in this study. QTLs for panicle exsertion, peduncle length and pollen fertility, identified for the first time in this study, could be useful in marker-assisted breeding (MAB) for drought resistance in rice. A total of 97 QTLs linked to plant growth, phenology, reproductive-growth traits, yield and its components under non-stress and drought stress, identified in this study as well as from earlier published information, were subjected to meta-analysis. Meta-analysis identified 23 MQTLs linked to plant phenology and production traits under stress conditions. Among them, four MQTLs viz., 1.3 for plant height, 3.1 for days to flowering, 8.1 for days to flowering or delay in flowering and 9.1 for days to flowering are true QTLs. Consensus QTLs for reproductive-growth traits and grain yield under drought stress have been identified on chromosomes 1 and 9 using meta-QTL analysis in these DH lines. These MQTLs associated with reproductive-growth, grain yield and its component traits under drought stress could be useful targets for drought resistance improvement in rice through MAB and/or map-based positional analysis of candidate genes.

1. Introduction

Rice (*Oryza sativa* L.) is the most important food crop for nearly half of the world's population. About 30.9% of the area planted to rice globally is under rainfed conditions (IRRI, 2009). Drought stress is the primary factor leading to low and instable rice yields worldwide, especially under rainfed conditions (Pandey et al., 2007). The phenological development of the crop at which drought occurs is considered to be an important factor affecting grain yield (Fukai and Cooper, 1995) and the reproductive development stage is highly sensitive to drought stress in rice (Saini and Westgate, 2000). Grain yield reduction up to a maximum of 70% of the control has been observed under water-deficit during the reproductive stage (Lilley and Fukai, 1994). Many complex phenological events between panicle initiation and grain filling are affected by drought stress at this

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stage (Garrity and O'Toole, 1994), leading to severe yield reduction (Saini, 1997). Some of the physiological processes affected by reproductive stage drought stress include days to flowering (Ouk et al., 2006), peduncle elongation, panicle exsertion (Ji et al., 2005) and pollen fertility (Saini and Westgate, 2000). These changes in reproductive growth under drought stress often lead to reduced grain yield by increasing spikelet sterility (Hsiao, 1982). Thus improvement in the performance of these traits under drought would help in increasing grain yield under water-limited environments. However, these traits are not selected for in breeding due to difficulty in phenotypic measurement of the traits, especially under drought stress in the field (Nguyen and Sutton, 2009).

Although plant breeders have made progress through conventional breeding in developing drought resistant lines of some selected crops, the approach is, in fact, highly time-consuming and labor- and cost-intensive and slow in attaining progress. Alternatively, quantitative trait loci (QTL) mapping followed by marker assisted breeding (MAB) could be an efficient approach for identifying genomic regions linked to complex reproductive growth traits and crop performance in stressful environments and pyramiding the desirable alleles to improve drought resistance in crops (Ashraf, 2010). Over the past 15 years, numerous QTLs for drought resistance traits have been identified using many populations in rice (Kamoshita et al., 2008; Courtois et al., 2009; Khowaja et al., 2009). These studies provided information on vital QTLs linked to grain yield and its component traits under drought stress conditions (Serraj et al., 2009, 2011). QTLs for many drought resistance traits viz., primary traits such as root thickness, root penetration index and root pulling force (Zhang et al., 2001; Gowda et al., 2011), secondary traits such as leaf rolling and leaf drying (Babu et al., 2003), phenology (Kumar et al., 2007), and integrative trait such as grain yield (Venuprasad et al., 2002, 2009; Lafitte et al., 2004; Zou et al., 2005; Bernier et al., 2007) have also been reported. QTLs for flag leaf and panicle characteristics under reproductive stage drought stress have also been mapped (Bernier et al., 2007; Liu et al., 2008, 2010). Kamoshita et al. (2008) summarized the current status of QTLs for drought resistance and evaluated the degree to which they affect rice production under drought. However, QTLs linked to reproductive-growth traits correlated with yield and its components under drought stress have not been fully studied in rice.

Among the many populations used in QTL mapping of drought resistance in rice, the doubled haploid (DH) line population derived from the cross CT9993-5-10-1-M/IR62266-42-6-2 is unique in the sense that it has been extensively tested for various drought resistance traits including yield and its components by many researchers across the globe under natural and managed drought stress conditions in the field (Kamoshita et al., 2008). QTLs linked to root- and shoot-related drought resistance traits (Zhang et al., 2001; Nguyen et al., 2004; Kumar et al., 2007), leaf epicuticular wax (Srinivasan et al., 2008), various physio-morphological traits and yield under reproductive stage drought stress (Babu et al., 2003; Lanceras et al., 2004) have been mapped in this DH line population. It is obvious that grain yield and its component traits under drought have been a major focus of QTL analysis in rice. Due to limitations in conducting appropriate drought stress field experiments and variations in QTL analysis, the accurate prediction of stable QTLs associated with yield and its relative traits is difficult. Further, to enable an easier shift from QTLs to the underlying candidate genes, progress is needed in combining the large body of QTL information (Courtois et al., 2009). There are several ways to combine data from different studies to detect consensus QTLs and narrow QTL confidence interval. One way is to perform meta-QTL analysis using the method proposed by Goffinet and Gerber (2000) and improved by Veyrieras et al. (2007). By definition, a meta-analysis is the combined analysis of multiple experiments and it can be used to provide a thorough analysis of one mapping population that has been used in multiple experiments (Laperche et al., 2007). This statistical approach enables QTL results from independent studies to be combined into single result using only published QTL data. Software packages based on this methodology are available such as BioMercator (Arcade et al., 2004) and Meta-QTL (Veyrieras et al., 2007). Meta-QTL analysis is a useful tool to identify the number of 'true' QTLs underlying the observed QTLs and refine the position and confidence interval of the 'true' QTLs (Arcade et al., 2004). It also provides relatively small targets for the identification of positional candidate genes (Khowaja et al., 2009). Although several QTLs under reproductive stage drought stress were reported earlier in this DH line population, a comprehensive meta-analysis of QTL co-location has not been conducted to assess the consistency of QTLs obtained from this population under drought stress. Since the DH population used in this study has been used earlier by several researchers for mapping QTLs under different water regimes, it is desirable to validate the QTLs, especially those associated with reproductive growth processes, yield and its components under drought by collecting additional information specifically on reproductive-growth traits. Such a study will identify candidate QTLs for use in MAB for drought resistance in rice. Therefore the present study was conducted with the specific objectives viz., (i) to identify QTLs linked to reproductive-growth traits related to yield and yield components under reproductive stage drought and (ii) to find out consensus QTLs for drought resistance traits in this DH line population using meta-analysis.

2. Materials and methods

2.1. Experimental population

A subset of 135 DH lines developed from the cross between CT9993-5-10-1-M (abbreviated as CT9993), an upland japonica ecotype from Colombia and IR62266-42-6-2 (abbreviated as IR62266), a lowland indica from Philippines was used in this study. This DH population is unique in that the parents and the DH lines differ considerably for root- and shoot-related drought resistance traits. CT9993 has deep and thick roots and IR62266 has capacity for high osmotic adjustment (Babu et al., 2001; Zhang et al., 2001; Kamoshita et al., 2002). The DH population also has wide variation for drought resistance and has been extensively used in QTL mapping for various drought resistance traits and yield by imposing drought stress at different developmental stages (Kamoshita et al., 2002; Babu et al., 2003; Lanceras et al., 2004; Kanbar et al., 2006; Srinivasan et al., 2008). In this study, phenotypic data for various traits were collected from all the 135 DH lines and used for phenotypic analysis, and QTL analysis was done using a subset of 88 DH lines for which the genotypic data was already available.

2.2. Field experiment

The field experiment using the DH population was conducted in the upland fields of the International Rice Research Institute, Los Banos, Philippines (14°11′N, 121°15′E; 23 m altitude) during the dry season 2007. Two trials with different water regimes were conducted in adjacent fields, one of which was subjected to severe reproductive stage drought stress and the other was irrigated twice weekly throughout the growing season and was considered as irrigated (non-stress) control. Both the trials were laid in a similar alpha lattice design with three replications per treatment. Randomization and field layout were prepared using the CROPSTAT software. Each replication in alpha lattice design had 10 blocks with 14 plots per block. Sowing was taken up during January 2007. Seeds were dry-direct-seeded in plots at the rate of 1.5 g seeds per linear