Evaluation of rice (*Oryza sativa* L.) near iso-genic lines with root QTLs for plant production and root traits in rainfed target populations of environment

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ABSTRACT

Drought stress is the major constraint in rainfed rice production. Deep and thick roots are thought to contribute in drought resilience. Selection for root traits is hampered by difficulty in phenotypic measurements. Mapping quantitative trait loci (QTLs) for root traits and their use in marker assisted breeding (MAB) will hasten the development of genotypes with improved root system. Consistent QTLs for root traits have been mapped in rice. Developing and testing near iso-genic lines (NILs) with QTLs for root traits in target populations of environment (TPE) will help to verify the agronomic value of the QTLs. NILs were developed by introgression of three root QTLs from CT9993, an upland *japonica* into IR20, a lowland *indica* cultivar through MAB. Considerable variation in drought response and grain yield under rainfed condition in TPE was observed among the NILs. Five out of 41 NILs gave higher yields under rain-fed and irrigated conditions as compared to IR20. Two NILs *viz.*, 212 and 297, with three and two root QTLs, respectively had thicker and longer nodal roots and higher total and deep nodal root weights than IR20. In addition, NIL 297 had more nodal root volume and surface area, while NIL 212 had more number of nodal roots compared to IR20. Further evaluation of the NILs with farmers for yield and grain quality may help in identification of high-yielding, resilient rice suitable for drought-prone rainfed ecosystems. The NILs may also be useful to study the genes underlying the QTLs and their functions for drought resilience improvement in rice.

1. Introduction

Rice is one of the most important crops for world's food security (Lobell et al., 2011). Drought stress is the major abiotic stress limiting rice production (Luo, 2010), especially in rainfed ecosystems. Rainfed lowland and upland systems contribute only 21% of the total rice production from more than 38% of the cropped area (Vikram et al., 2011). Developing cultivars combining drought resilience and high yield potential will help to increase rice production in rainfed target population of environments (TPEs). TPE is the set of all environments, farms and future seasons in which an improved variety will be grown (IRRI, 2006). In rainfed TPE, the variation in available soil moisture is a major determinant of crop growth and breeding cultivars with specific adaptation to the prevalent water supply is key for yield increase (Fischer et al., 2003). Drought avoidance is considered more relevant for mitigating agricultural drought and maintaining crop performance (Serraj et al., 2011). Roots are important for maintaining crop yields, especially when plants are grown in soils with insufficient water and nutrients (Bengough et al., 2011). Deep and thick roots have been proposed to contribute in drought resistance (Fukai and Cooper, 1995; Nguyen et al., 1997; Liu et al., 2008; Li et al., 2011) and were shown to have positive correlations with yield under drought in rice (Babu et al., 2003; Ling et al., 2002; Cairns et al., 2009). Rice has remarkable level of genetic variation in root morphology (Babu et al., 2001; Lafitte et al., 2001) and can be harnessed for improving rice adaptation to drought (Gowda et al., 2011). However, genetic improvement of root system through conventional breeding is impractical due to difficulty in phenotyping root traits (Pennisi, 2008). Mapping quantitative trait loci (QTLs) for root traits and their use in marker assisted breeding (MAB) is an alternate approach in selecting for root traits that are difficult to phenotype (Coudert et al., 2010).

There has been considerable progress in mapping QTLs for root traits and several of them are consistent across genetic backgrounds and environments in rice (Kamoshita et al., 2008; Courtois et al., 2009; Khowaja et al., 2009). Introgression of these root QTLs into elite varieties through MAB may have impact on the sustainability of rice production in drought-prone rainfed environments (Venuprasad et al., 2011). Attempts have been made to improve yield under drought with the introgression of root QTLs into elite lines through MAB (Shen et al., 2001) and develop a few

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introgression lines with improved root phenotype (Steele et al., 2006) and yield under rainfed environments (Steele et al., 2007). However, it is not clear whether the higher yields of the introgression lines were primarily due to improved root phenotype, since certain other lines without the root QTLs also gave higher yields under rainfed conditions (Steele et al., 2007). Small phenotypic effect (some <10%) and large intervals (3.7–7.3 Mb) of the root QTLs used in MAB are more likely to confer linkage drag of unwanted effects (Shen et al., 2001; Steele et al., 2006, 2007). Further, low heritability of certain root traits is also a common breeding concern (Gowda et al., 2011). Identifying large effect QTLs with narrow intervals are critical for improving the efficacy of MAB (Bernier et al., 2008).

In most rainfed lowland rice growing areas of Asia, the soil density increases under drought leading to soil compaction (Ingram et al., 1994) and root elongation is limited by both water deficit and mechanical impedance (Bengough et al., 2011). Rice plants with thicker roots are able to better penetrate hardpans and access soil moisture at depth (Yu et al., 1995). A large effect QTL (37.6%) for basal root thickness was detected on chromosome 4 in CT9993/IR62266 doubled haploid (DH) rice lines (Zhang et al., 2001). The same QTL was also linked to root pulling force in these DH lines (Nguyen et al., 2004). High root pulling force is associated with ability of the rice plant to develop deep and large diameter roots with greater penetration ability (Lafitte et al., 2001; Clark et al., 2008). Root pulling force had positive correlations with plant water status and yield stability under drought in rice (Kumar et al., 2004). The QTLs for basal root thickness and root pulling force on chromosome 4 co-located with QTLs for grain yield under drought stress in these rice DH lines (Babu et al., 2003). QTLs for putative traits overlapping with QTLs for yield under drought stress are good candidates for MAB for drought resistance (O'Toole, 2004). The co-location of QTLs for putative traits with QTLs for yield provides clues on the casual relationships (Hochholdinger and Tuberosa, 2009) and assist breeders in identifying the best QTL alleles for a successful MAB program (Landi et al., 2010). A QTL for penetrated root thickness was mapped on chromosome 9 in CT9993/IR62266 DH lines (Zhang et al., 2001). These QTLs on chromosomes 4 and 9 were found to be meta-QTLs (Courtois et al., 2009) and are thought to improve rice yields under drought through increased water uptake by roots (Kamoshita et al., 2008). Developing and testing NILs for these QTLs will help to verify their agronomic value and also to understand the mechanism of drought resistance in rice.

In the present study, efforts were made to develop NILs for root trait QTLs using IR20 as recurrent parent. IR20 is drought sensitive (Kanbar and Shashidhar, 2011) and has shallow and fine root system (Ekanayake et al., 1985). Developing IR20 NILs with root QTLs and evaluating them for drought response in TPE is likely to provide the proof of the concept that improved roots are key components of drought resilience in rice. Thus, the present study was conducted with the objectives: (i) to develop IR20 NILs by introgressing QTLs for root traits from CT9993 using MAB and (ii) to understand the agronomic value of the QTLs by evaluating the NILs for drought resistance and root traits under rainfed condition in TPE.

2. Materials and methods

2.1. Parental lines

CT9993-5-10-1-M (abbreviated CT9993), an upland-adapted tropical *japonica* ecotype from Columbia was used as a donor. CT9993 is drought resistant and known for its deep and thick root system, hardpan penetration ability and root proliferation to depth (Babu et al., 2001; Kamoshita et al., 2004; Siopongco et al., 2009),

but low yielding. IR20, a lowland indica cultivar from Philippines was used as a recurrent parent. IR20 is drought sensitive (Kanbar and Shashidhar, 2011) and has shallow and thin roots (Ekanayake et al., 1985). IR20 is grown on considerable area under semi-dry condition in south Tamil Nadu, India representing the rainfed TPE and is preferred by farmers and consumers for its high yield potential and superior grain quality.

2.2. Root QTLs for introgression

QTLs for root traits have been mapped in CT9993/IR62266 rice DH lines (Zhang et al., 2001; Nguyen et al., 2004). Three QTLs on chromosomes 4 and 9 linked to basal root thickness, root pulling force and penetrated root thickness were chosen for development of NILs using MAB strategy. The selected QTLs had relatively small confidence interval (5.2–9.7 cM; 1.1–3.1 Mb) and large phenotypic effect, 16.3–37.6% (Table 1). The flanking SSR markers, RM252–RM348 and RM348–RM280 for basal root thickness and root pulling force QTLs on chromosome 4 and RM257–RM242 for penetrated root thickness QTL on chromosome 9 were used in foreground selection of backcross progenies (Table 1). Introgression of non target region from the donor parent was prevented through background selection of the genome of the selected backcross progenies using SSR markers from rice linkage map (Temnykh et al., 2001)

2.3. Development of IR20 NILs

IR20 (female) was crossed with CT9993 (male) by hand emasculation and artificial pollination as described by Chaisang et al. (1967). The F_1 hybrids were fixed morphologically based on plant height, tiller number and leaf size at maximum tillering and days to heading. The hybridity was also verified using SSR markers flanking the root QTLs. Back crossing was done using true F_1 hybrids as male and IR20 as recurrent female. In the BC_1F_1 generation, individual plants that were heterozygous at the loci flanking the root QTLs were identified through foreground selection. Subsequent back cross was made between the selected BC_1F_1 hybrids carrying the root QTLs and recurrent parent to get BC_2F_1 progenies. Similar strategy was followed to get BC_3F_1 and BC_4F_1 progenies (Fig. 1). Two backcross progenies, BC_4F_1-(2) and BC_4F_1-(16), with three and two root QTLs, respectively were selected. These two BC_4F_1 progenies were generation advanced by selfing and evaluated for drought response, plant production and root traits under rainfed condition in TPE.

2.4. Genotyping

Leaf samples of F_{1s}, backcross progenies and parents were collected from field-grown seedlings and freeze dried. DNA was extracted from the leaf tissues using cetyl trimethyl ammonium borate buffer (Gawel and Jarret, 1991). The quantity and quality of DNA was assessed in 0.8% agarose gel and concentration was adjusted to 50 ng/μl by comparing DNA standards. Polymerase chain reactions (PCR) were performed in a volume of 15 μl in thermal cycler (DNA Engine®, Peltier Thermal Cycler, BIO-RAD, USA or Eppendorf Master Cycler Gradient, Eppendorf, Germany). The reaction mixture contained each primer of 1 μM (Sigma Aldrich, USA), 100 μM deoxy nucleotide, 1× Taq buffer, 0. 02 U Taq polymerase (Bangalore Genei, India) and 50 ng of template DNA. After 5 min at 94 °C, the PCR involved 36 cycles of amplification, each cycle comprising 1 min at 94 °C, 1 min at 57 °C, 1 min at 72 °C and with a final extension step of 5 min at 72 °C. Foreground and background selection for target root QTL regions were made with primer amplicons separated on 3% Metaphor agarose gels (Bio-Whittaker