Field Crops Research 123 (2011) 130–138

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Physiological characterization of introgression lines derived from an *indica* rice cultivar, IR64, adapted to drought and water-saving irrigation

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ARTICLE INFO

Article history:
Received 10 March 2011
Received in revised form 8 May 2011
Accepted 8 May 2011

Keywords:
Drought adaptation
Oryza sativa
Root system architecture
Yield potential

ABSTRACT

Water scarcity threatens sustainable rice production in many irrigated areas around the world. To cope with the scarcity, aerobic rice culture has been proposed as a promising water-saving technology. The objective was to elucidate the physiological attributes behind the performance of rice introgression lines in water-saving culture. We evaluated yield potential and physiological adaptation traits to water deficit of BC_3-derived lines with the genetic background of an elite *indica* cultivar, IR64, in the field and in pot experiments. One line, YTH183, had 26% higher yield than IR64 under non-stress conditions (895 vs. 712 $g\,m^{-2}$ on average). This was attributed to enlarged sink capacity due to large grain size, which contributed to more efficient use of assimilates and hence a higher harvest index. YTH183 also showed better dehydration avoidance under intermittent soil drying, due to the adaptive response of deep rooting to water deficiency. The grain yield of YTH183 exceeded that of IR64 by 92–102% under moderate water deficit caused by limited irrigation in aerobic rice culture (143 vs. 72 $g\,m^{-2}$). Two introgressed segments on chromosomes 5 and 6 might, at least in part, confer the higher yield potential and greater dehydration avoidance in YTH183 simultaneously. Advanced backcross breeding combined with molecular genetics and physiological characterization of introgressed segments would be effective for developing new rice cultivars with high yield potential and drought adaptation traits.

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1. Introduction

Declining water availability threatens the sustainability of irrigated rice (*Oryza sativa* L.) production in many countries (Peng et al., 2009). Among the new water-saving technologies being developed, aerobic rice culture can greatly reduce irrigation water requirements (Tuong et al., 2005) with being intensively studied in China, Brazil, India, Australia, and parts of southeast Asia and Africa (Bouman et al., 2007; Humphreys et al., 2010). It aims at maximizing crop water productivity by growing plants without flooding or puddling, in aerobic soil (Kato et al., 2009). Aerobic rice culture was reported to use less than 50% of the water required by conventional flooded rice, but with a significant yield penalty (Peng et al., 2006). Accordingly, its success relies entirely on the development of new suitable cultivars. However, breeding for this purpose confronts completely different challenges from the breeding of rainfed rice, in breaking the yield potential while achieving acceptable adaptation to the moderate water deficit of aerobic soils (Atlin et al., 2006). Because rice cultivars for aerobic culture must perform well in both well-watered and water-limited conditions, it is likely that multiple mechanisms will be necessary to confer a consistent yield advantage (Guan et al., 2010).

It is well-known that there is a wide range of genetic variation in yield potential and drought adaptation in rice. In particular, improvement in root system architecture towards deeper root system would be most effective for better adaptation to occasional or intermittent soil water deficit between irrigation events in aerobic rice culture (Gowda et al., 2011). On the other hand, rice yield potential is often constrained by the limited sink capacity either due to lower spikelet density (spikelet number per unit area) or smaller grain size (Akita, 1999). There is an opportunity for further increasing yield potential by enhancing sink capacity in modern rice cultivars (Ohsumi et al., 2011). By pyramiding the alleles conferring these characteristics into one genome proportionately, it might be possible to develop superior rice cultivars with high yield

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doi:10.1016/j.fcr.2011.05.009

potential and irrigation-use efficiency in water-saving culture (Luo, 2010; Serraj et al., 2011).

Advances in quantitative genetics such as quantitative trait locus (QTL) analysis have fundamentally changed conventional rice breeding in the last decade, and made it possible to tailor genotypes via marker-assisted selection (Ashikari et al., 2005). Molecular genetics has been already adopted in breeding for disease and pest resistance (Fukuoka et al., 2009). It could also provide a powerful tool for raising yield potential (Adachi et al., 2010) and resistance to abiotic stress (Septiningsih et al., 2009) when combined with physiological annotation of favourable alleles. Furthermore, Tanksley and Nelson (1996) suggested the combination of backcross breeding with molecular genetics. The advanced backcross QTL analysis has enabled applications of molecular genetics in plant breeding programs (Collins et al., 2008). Such analysis detects QTLs in elite genetic backgrounds through the use of promising backcross lines while maintaining genetic variability at the whole-genome scale. Advanced backcross breeding may prove efficient for developing new rice genotypes adapted to water-saving agriculture, because it pools the valuable alleles from unadapted germplasms as a list of genotypes which are ready to be isolated. By using these lines, crop scientists can safely avoid the problems of genetic sterility and variation in phenology often encountered in molecular breeding studies.

The objective of this study was to elucidate the physiological attributes underlying the performance of lines with the genetic background of an *indica* rice cultivar, IR64, in advanced backcross breeding for water-saving agriculture. We targeted aerobic rice culture as a promising water-saving technology, and evaluated the genotypes with an emphasis on yield potential and adaptation to water deficit.

2. Materials and methods

2.1. Plant materials

Introgression rice (*O. sativa* L.) lines with the IR64 genetic background (BC_3-derived lines from "New Plant Type" donors; see Fujita et al., 2009 for details) were used for this study. IR64 (recurrent parent) is a semi-dwarf, lowland-adapted *indica* cultivar that is widely grown in the tropics. It has good grain quality, but has medium yield potential and is drought susceptible. The genetic background of these introgression lines is uniform compared with recombinant inbred lines, and the introgression lines have been genotyped (Fujita et al., 2010a). Thus, these introgression lines make a useful resource for both physiological and molecular genetic studies (Farooq et al., 2010). We selected eight introgression lines (YTH146, 183, 243, 254, 272, 288, 302 and 304) on the basis of their performance in preliminary observations at the International Rice Research Institute (IRRI), The Philippines (N. Kobayashi, unpublished data). Several check cultivars were also evaluated as references.

2.2. Experiment 1: Evaluation of yield potential

Field experiments were conducted at the experimental farm of the University of Tokyo, Japan (35°43′N, 139°32′E), during the summer (May–September) of 2009 and 2010; at the experimental farm of Kyoto University, Osaka, Japan (34°51′N, 135°37′E), during the summer of 2010; and at the experimental station of IRRI, Los Baños, The Philippines (14°11′N, 121°15′E), during the dry season (January–May) of 2010 (Table 1). Weather data were obtained from the Tokyo and Osaka meteorological stations (20 km from the sites), or were recorded at meteorological stations located within 2 km of the field in Los Baños. The soils are a Typic Melanudand (clay loam) at the University of Tokyo, a Typic Fluvaquent (clay loam) at Kyoto University, and a Typic Tropaqualf (heavy clay) at IRRI's lowland farm. Fields were puddled and kept flooded after transplanting to evaluate the yield potential of the rice genotypes. A non-puddled, saturated trial with direct-sowing was also set up in Tokyo in 2009 (daily flush irrigation to maintain soil water potential above −10 kPa at 20 cm depth).

Several check cultivars and the IR64 introgression lines were arranged in a randomized complete block design in each trial, with three (Tokyo and Osaka) or four (Los Baños) replicates per genotype (Table 2). IR68522-10-2-2 is a tropical *japonica* lowland-adapted line, one of the first-generation New Plant Type series (Fujita et al., 2009). NSIC Rc158 is a second-generation New Plant Type cultivar (Peng et al., 2008) recently bred for high yield potential in the tropics. PSBRc80 is a check cultivar, well adapted to water-saving culture in lowlands (Bueno et al., 2010). IR55423-01 has one of the highest yield potentials among upland-adapted cultivars in the tropics (Atlin et al., 2006). Takanari is an *indica* lowland-adapted cultivar with the highest yield potential in central Japan (Takai et al., 2006).

Each plot consisted of eight 4-m rows (25- or 30-cm spacing), except in Tokyo in 2009 (six 4-m rows, 20-cm spacing). One 4-leaf seedling in Tokyo and Osaka or three seedlings in Los Baños were transplanted into each hill (20–25 hills m^{-2}): between late May and early June in Osaka and Tokyo, and in mid-January in Los Baños. In the saturated trial, four or five seeds were directly sown on each hill at the same density as for transplanted rice. We applied 32–43 kg ha^{-1} of P and 32–83 kg ha^{-1} of K basally; and 95 kg ha^{-1} of N in three splits in Los Baños, 120 kg ha^{-1} of N in three splits in Osaka, and 160 kg ha^{-1} of N in five splits in Tokyo. Diseases and pests were controlled by the standard practices in the study areas.

At physiological maturity, 1.0 m^2 of rice plants (avoiding border rows) was harvested at ground level to determine the grain yield and yield components. Grain and straw were dried in an oven at 80 °C for 3 days and grain yield and filled grain weight (mg per grain) were calculated on a 14% moisture content basis. Sink capacity [also defined as 'yield capacity' by Yoshida (1972)] was calculated as total spikelet number m^{-2} × single-grain weight according to Mae et al. (2006). Sink capacity represents the potential capacity of vessels that receive carbohydrates during grain filling (Yoshida, 1972). Grain filling ratio was calculated as dry weight of filled grains/sink capacity. At the beginning of anthesis, 8–12 plants were sampled, and the post-anthesis biomass accumulation was also calculated. The apparent translocation of dry matter from non-grain parts was calculated as the dry weight of filled grains minus the post-anthesis biomass accumulation. For calculating the apparent translocation, we have not taken leaf and tiller senescence into account. At full anthesis stage, the SPAD value (a measure of chlorophyll content) and the stomatal conductance of IR64 and YTH183 were determined. The SPAD values of the flag leaf of 20–30 stems per plot were measured with a SPAD-502 chlorophyll meter (Minolta Co., Ltd., Osaka, Japan). The stomatal conductance of the abaxial side of the flag leaf of seven stems per plot was measured with an SC-1 leaf porometer (Decagon Devices Inc., Pullman, WA, USA) in the morning (10:00–12:00) under sunny conditions. Data in each trial were analysed by using the generalized linear model procedure (SAS Institute, 2003), and genotype means were compared by least significant difference at $P = 0.05$. The data of IR64 and YTH183 were also compared by paired *t*-test.

2.3. Experiment 2: Yield response to diverse irrigation intensities

Field experiments were conducted at the IRRI upland farm during the 2009 dry season (Table 1) to evaluate the agronomic performance of the IR64 introgression lines in aerobic culture in

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