Field Performance and SSR Analysis of Drought QTL Introgression Lines of Rice

Jung-Pil Suh*, Yong-Jae Won, Eok-Keun Ahn, Jeong-Heui Lee, Woon-Goo Ha, Myeong-Ki Kim, Young-Chan Cho, Eung-Gi Jeong, Bo-Kyeong Kim

National Institute of Crop Science, RDA, Suwon 441-857, Republic of Korea

ABSTRACT A set of five Korean rice cultivars and seven drought-tolerant indica lines were screened under irrigated non-stress and drought-stressed conditions in the 2011 and 2012 dry seasons at IRRI, Philippines. The drought-stressed experiment received mild to moderate stress. Under drought stress, 'IR86918-B-439-B' had the highest grain yield among all tested lines and 'Hanareumbyeo' had the highest grain yield among the five Korean rice cultivars. 'IR86918-B-439-B' also had the highest yield under irrigated non-stress conditions. The grain yield of 'Hanareumbyeo' was similar to 'IR86918-B-439-B' under non-stress conditions. SSR marker analysis was performed using 125 SSR markers for detection of polymorphic markers between the Korean rice cultivars and the drought-tolerant indica lines, and for genetic diversity analysis. Twelve polymorphic markers were identified in the region of three major drought QTLs (DTY1.1, DTY2.2, DTY3.1) in two of the Korean rice cultivars and three of the drought-tolerant lines. These polymorphic markers will be useful as foreground genotyping markers for drought-QTL introgression in Korean rice genetic backgrounds.

Keywords Drought tolerance, Genetic diversity, Rice, SSR marker, Yield

INTRODUCTION

Drought is one of the most severe constraints reducing rice grain yield in rainfed environments. Recent predictions of climate change suggest a further increase in water deficit in the coming years (Wassmann *et al.* 2009), leading to an increase in the intensity and frequency of drought (Bates *et al.* 2008). In severe drought years, total rice production losses in drought-prone rice growing countries is very high. In 2004, a severe drought affected more than 2 million ha of total cropped area and over 8 million people in Southeast Asia (Pandey *et al.* 2007). Drought also affects production on millions of hectares in irrigated areas dependent on surface irrigation, where river flows and water impounded in ponds, tanks, and reservoirs may be insufficient to irrigate the crop in dry years (Maclean *et al.* 2002). Despite the importance of drought as a major constraint, little success has been achieved in developing drought-tolerant rice cultivars. Most improved cultivars grown in drought-

prone areas are the varieties originally bred for irrigated conditions and are highly susceptible to drought (Kumar *et al.* 2008). Drought stress during the cropping season directly affects grain yield, which is particularly devastating at the reproductive stage (Venuprasad *et al.* 2009a). As rainfed environments are highly variable, breeding rice for drought-prone rainfed ecosystem requires genotypes that combine high yielding ability with good drought tolerance in addition to tolerance to prevalent biotic stresses. Varieties with high yield potential and improved drought tolerance could reduce risk and help alleviate poverty in drought-prone rice growing areas. Recently drought-tolerant lines such as Apo, Vandana, Way Rarem, N22, and Adaysel have been shown to out-yield susceptible lines such as IR64 and IR72 by several-fold under upland or lowland drought (Venuprasad *et al.* 2007; Venuprasad *et al.* 2009b; Bernier *et al.* 2007; Vikram *et al.* 2011; Swamy *et al.* 2011). Crosses between such tolerant and susceptible lines are useful to identify important QTL underlying variation in

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*Corresponding author Jung-Pil Suh, suhjp@korea.kr, Tel: +82-31-290-6710, Fax: +82-31-290-6730

grain yield under drought stress. QTLs showing large and consistent effects can be utilized through marker-assisted breeding (MAB), a rapid and precise approach to develop high-yielding drought-tolerant rice varieties. To date, several QTLs with large and consistent effects on grain yield under drought stress have been identified at IRRI. Bernier *et al.* (2007) reported DTY12.1, a QTL on chromosome 12 in the Vandana/Way Rarem population, explaining about 51% of the genetic variance for yield under severe upland drought stress over two years. A consistent major-effect QTL for grain yield, DTY1.1, was identified to show an effect in several populations. Vikram *et al.* (2011) detected DTY1.1 consistently over two years in three RIL populations derived from the donor variety N22 crossed to drought-susceptible varieties Swarna, IR64, and MTU1010. Two major-effect QTLs, DTY3.1 and DTY2.1, were identified in an Apo/2*Swarna population explaining 30% and 15% of the phenotypic variance, respectively (Venuprasad *et al.* 2009a). Four major-effect QTLs, DTY2.2, DTY4.1, DTY9.1, and DTY10.1, were identified in Adaysel/IR64-derived populations (Swamy *et al.* 2011; Swamy *et al.* 2013). Drought grain yield QTLs DTY2.2, DTY4.1, DTY9.1 and DTY10.1 have been successfully introgressed into an IR64 background and DTY12.1 has been introgressed into a Vandana background.

The drought tolerant IR64-NILs and Vandana-NILs have been successfully evaluated, their yield advantage under drought has been validated, and lines are being processed for release (Kumar *et al.* 2008; Kumar 2011). The objectives of this study were to evaluate the grain yield of drought tolerant-NILs under irrigated non-stress and drought stress conditions and to detect polymorphic SSR markers between Korean rice cultivars and drought tolerant-NILs to facilitate the introduction of drought tolerance QTLs into Korean rice cultivars.

MATERIALS AND METHODS

Plant materials

Five Korean rice cultivars and seven drought tolerant-NILs were used for drought screening along with controls (Table 1 and 2). IR86918-B-223-B, IR86918-B-305-B, IR86918-B-382-B and IR86918-B-439-B are nearly isogenic to IR64 and have the drought tolerance QTL, DTY1.1 from N22 (Vikram *et al.* 2011). IR77298-14-1-2 is also nearly isogenic to IR64 and has drought tolerance QTL, DTY2.2 from Aday Sel (Swamy *et al.* 2011; Swamy *et al.* 2013). IR81896-B-B-431 is nearly isogenic to Swarna and has the drought tolerance QTL, DTY3.1 from Apo (Venuprasad *et*

Table 1. List of Korean rice varieties for QTL introgression.

Variety	Ecotype	Remarks	Grain characteristics
Jinmibyeo	Japonica	Termperate and tropical adaptable	Non-glutinous
Unkwangbyeo	Japonica	High yielding in Korea	Non-glutinous
Hanareumbyeo	Tong-il	High yielding in Korea	Non-glutinous
Gayabyeo	Tong-il	High compatibility	Non-glutinous
Sangnambatbyeo	Japonica	upland rice in Korea	Glutinous, long-grain

Table 2. List of drought tolerant QTL-NILs developed from International Rice Research Institute.

Variety (GID#)	Genetic background	Ecotype	QTLs	QTL source
IR 86918-B-223-B (#2963514)	IR64	Indica	DTY _{1.1}	N22
IR 86918-B-305-B (#2963523)	IR64	Indica	DTY _{1.1}	N22
IR 86918-B-382-B (#2963599)	IR64	Indica	DTY _{1.1}	N22
IR 86918-B-439-B (#2963506)	IR64	Indica	DTY _{1.1}	N22
IR 77298-14-1-2 (#909971)	IR64	Indica	DTY _{2.2}	Aday sel
IR 81896-B-B-431 (#2155763)	Swarna	Indica	DTY _{3.1}	Apo
IR 84984-83-15-18-B-B (#2719923)	Vandana	Indica	DTY _{12.1}	Way Rarem

al. 2009a). IR84984-83-15-18-B-B is nearly isogenic to Vandana with the drought tolerance QTL, DTY12.1 from Way Rarem (Bernier *et al.* 2007). Seeds of these drought tolerant-NILs were obtained from the Plant Breeding, Genetics and Biotechnology Division of the International Rice Research Institute (IRRI), Los Banos, Philippines. Seeds of the Korean rice cultivars were obtained from the Rice Research Division of the National Institute of Crop Science (NICS), Rural Development Administration (RDA), Republic of Korea. 'Nonglimna1', 'GL33', 'Moroberekan' and 'Ilpumbyeo' were also used to analyze genetic diversity using SSR markers.

Management of drought stress and non-stress trials in tropical region

In the lowland drought stress trials, seeds were sown in the nursery and 21-day-old seedlings were transplanted to the main field of IRRI, Philippines, during the 2011 and 2012 dry seasons. One seedling was transplanted per hill at a spacing of 10 cm between hills in a row. In non-stress trials, after transplanting, ~5 cm of standing water was maintained in the field until drainage before harvest while in stress trials, stress was imposed by draining water from the paddy at 30 DAT (days after transplanting) and withholding irrigation until the soil moisture tension reached -50 kPa at 20 cm depth. Fields were then re-irrigated by flash flooding, and drained again after ~24 h. This cycle was repeated until harvest. Severe leaf rolling was observed during each stress period. All the experiments were laid out in an alpha lattice design and consisted of two replications. Spacing between rows was 0.25 m in all trials. Plots were single-row in the selection experiments but mostly two-row in the evaluation experiments. Length of the plot was 5 m in lowland. Recommended levels of fertilizer were added, and weeds and pests were controlled when necessary. Days to flowering (DTF), plant height (PH), grain yield of rough rice (Yield; kg/10a) were estimated as drought tolerance index. More experimental details can be found in Venuprasad *et al.* (2007 and 2008).

Evaluation of agricultural traits in temperate region

Seven drought tolerant-NILs including five Korean rice

cultivars also were planted in a four-row plot with 35 plants per row by 30×15 cm spacing in a randomized complete block design with three replications and evaluated for agronomic traits in the rice experimental plot of NICS, Cheolwon, Korea, using the standard evaluation method of rice (RDA 2003). For each line, five plants in the middle rows were used to determine heading date (HD), culm length (CL), panicle number per plant (PN), panicle length (PL), number of spikelet per panicle (NSP), percent of ripened grain (PRG), 1,000-grain weight of the brown rice (GW), and grain yield of rough rice (Yield; kg/10a).

SSR marker and genetic diversity analysis

The 16 rice genotypes including drought tolerant-NILs were genotyped using 125 SSR markers distributed evenly on the 12 chromosomes to generate a dendrogram. Seventeen SSR markers flanking the DTY1.1, DTY2.2 and DTY3.1 regions were used for detecting polymorphic markers between recipients and donors. Genomic DNA was extracted from the fresh frozen leaves of the rice cultivars by a modified CTAB method (Rogers and Bendich, 1988). The procedure used in the PCR assay was as described by Suh *et al.* (2009). The PCR products were detected using 4% denaturing polyacrylamide gel electrophoresis with silver staining. The DNA bands generated were analyzed and scored 1 (present) or 0 (absent) for each allele. The similarity matrices obtained using the simple matching coefficient were subjected to unweighed pair-group methods using arithmetic average (UPGMA) clustering and represented in dendrogram form using NTSYS-pc program (Rohlf 1993). Two Korean rice cultivars (Jinmiby eo and Hanareumbyeo) and only three QTL donors were used for parental polymorphism survey.

RESULTS

Drought tolerance of Korean rice cultivars and drought tolerant-NILs under tropical conditions

Seven drought tolerant-NILs including five Korean rice cultivars were screened under irrigated non-stress and drought stress at IRRI, Philippines during the 2011 and

2012 dry seasons. Under drought stress, all drought tolerant-NILs showed higher grain yield than IR64 and the Korean rice cultivars (Table 3). In particular, IR86918-B-439-B had the highest yield followed by IR77298-14-1-2 and IR81896-B-B-431 under drought stress. Among the five Korean cultivars, Hanareumbyeo yielded the highest

followed by Sangnambatbyeo but their yields were lower than those of drought tolerant-NILs (Table 3). Three drought tolerant-NILs, IR86918-B-305-B (DTY 1.1), IR77298-14-1-2 (DTY2.2), IR81896-B-B-431 (DTY3.1) were selected for transferring the drought-QTLs to Korean rice cultivars, Jinmibyeo and Hanareumbyeo (Table 3).

Table 3. Morpho-agronomic traits and yield performance of Korean rice varieties and drought tolerant QTL-NILs under irrigated non-stress and mild-moderate drought stress conditions in 2011-2012 dry season at IRRI.

Variety	Irrigated non-stress ^z			Drought stress		
	DTF (days)	PH (cm)	Yield (kg/10a)	DTF (days)	PH (cm)	Yield (kg/10a)
Jinmibyeo	69	84	386ab	80	68	104a
Unkwangbyeo	63	76	354a	74	70	106a
Gayabyeo	76	85	487c	84	72	157a
Hanareumbyeo	77	81	616e	85	66	242c
Sangnambatbyeo	74	92	366a	78	74	218c
IR 86918-B-223-B	75	149	544d	82	141	352de
IR 86918-B-305-B	75	156	543d	78	133	328d
IR 86918-B-382-B	75	129	563d	81	117	356de
IR 86918-B-439-B	74	137	653e	78	122	380e
IR 77298-14-1-2	83	84	533d	89	71	373e
IR 81896-B-B-431	93	93	629e	99	63	360e
IR 84984-83-15-18-B-B	84	125	573de	87	105	309d
IR 64	75	104	569d	87	73	130a

^zDTF: Days to flowering, PH: Plant height, Yield: Grain yield of rough rice. Means followed by the same letter are not significant at the 5% significance level by the least significant difference test (LSD=0.05).

Table 4. Yield performance and agronomic traits of drought tolerant-NILs under irrigated non-stress condition in Korea.

Variety	^z HD (M.D)	CL (cm)	PL (cm)	PN	NSP	PRG (%)	GW (g)	Yield (kg/10a)
Jinmibyeo	7.30	69	20	12	68	84.5	22.07	616d
Unkwangbyeo	7.30	62	20	11	80	80.0	23.97	699e
Hanareumbyeo	8.10	68	21	10	75	91.9	24.83	707e
Gayabyeo	8.7	75	21	10	85	75.2	23.92	624d
Sangnambatbyeo	8.7	75	24	9	81	80.2	21.01	518bc
IR86918-B-223-B	7.28	116	23	12	51	80.7	18.54	344a
IR86918-B-305-B	8.10	128	24	12	66	81.4	20.20	487b
IR86918-B-382-B	8.4	113	21	12	53	68.1	29.31	461b
IR86918-B-439-B	8.9	106	27	10	72	74.8	26.28	561c
IR77298-14-1-2	8.10	84	26	12	83	63.5	25.88	606d
IR81896-B-B-431	Not flowering							
IR84984-83-15-18-B-B	8.4	103	21	11	90	86.4	21.31	512bc

^zHD: Heading date(M: month, D: day), CL: Culm length, PL: Panicle length, PN: Panicle number per plant, NSP: Number of spikelet per panicle, PRG: Percent of ripened grain, GW: 1,000 grain weight of brown rice, Yield: Grain yield of rough rice. Means followed by the same letter are not significant at the 5% significance level by the least significant difference test (LSD=0.05).

Jinmibyeo and Hanareumbyeo were selected as recipient parents because these rice cultivars are well adapted in temperate and tropical regions.

Yield potential of drought tolerant-NILs under non-stress temperate conditions

Seven drought tolerant-NILs including five Korean rice cultivars were also evaluated for yield potential under irrigated non-stress experimental plot at Cheolwon substation, NICS, Korea in 2012. The agronomic traits and yield performance of IR81896-B-B-431 could not be evaluated because it does not flower in Korean environmental conditions. All Korean rice cultivars except Sangnambatbyeo showed higher yield than those of drought tolerant-NILs

collected from IRRI (Table 4). It is likely that the lower yield potential of the drought tolerant-NILs comparison with the Korean rice cultivars is related to their selection under tropical environmental conditions, and hence their lack of adaptation to temperate conditions. However, IR77298-14-1-2 showed similar yield potential to the Korean rice cultivars. Thus, IR77298-14-1-2 may be the best adapted donor for introducing drought tolerance QTL into temperate *japonica* rice.

Parental polymorphism survey for foreground and background selection markers

The three drought tolerant-NILs, IR86918-B-305-B (DTY1.1), IR77298-14-1-2 (DTY2.2), IR81896-B-B-431

Fig. 1. PCR assay for amplification of identified QTL regions using SSR markers linked with QTLs for drought tolerance on chromosomes 1, 2, 3, and 12.

Table 5. Flanking SSR markers for drought-QTL region and DNA polymorphism between donor and recurrent parents.

Donor parent	QTLs	Polymorphism (%)		Flanking markers
		Jinmibyeo	Hanareumbyeo	
IR86918-B-305-B	(DTY _{1.1})	70	46	RM431, RM11943, RM12023, RM12233, RM12146, RM12091
IR77298-14-1-2	(DTY _{2.2})	63	37	RM109, RM279, RM236
IR81896-B-B-431	(DTY _{3.1})	66	28	RM520, RM416, RM16030

(DTY3.1), were used as the donor parents for drought tolerance, and Jinmibyeo and Hanareumbyeo were used as the recipient parents. The parental polymorphism survey was performed with 17 SSR markers flanking the target QTLs on two of the Korean rice cultivars and three of the drought tolerant-NILs. Twelve polymorphic markers were identified between recipient and donor parents at target QTL regions for drought tolerance (Table 5 and Fig. 1). The SSR markers, RM431, RM11943, RM12023, RM12233, RM12146 and RM12091 on chromosome 1 showed polymorphism between two Korean rice cultivars and IR86918-B-305-B in DTY1.1 region. The SSR markers, RM109, RM279 and RM236 on chromosome 2 showed polymorphism between two of the Korean rice cultivars and IR77298-14-1-2 in DTY2.2 region. The SSR markers, RM520, RM416 and RM16030 on chromosome 3 showed polymorphism between two of the Korean rice cultivars

and IR81896-B-B-431 in DTY3.1 region. SSR markers that showed polymorphism between the two recipient parents and three donor parents around the target drought-QTLs were used as flanking markers to introduce the drought-QTLs of donors (Table 5).

Genetic diversity of drought tolerant lines using SSR markers

Cluster analysis for the 16 rice germplasm was performed based on similarity coefficient matrices calculated from 125 SSR markers to generate a dendrogram (Fig. 2). The sixteen rice cultivars could be separated into two main clusters such as *japonica* and *indica* groups. Four rice cultivars (Sangnambatbyeo, Nonglimna1, GL33, Moroberekan) were sub-grouped in *japonica* cluster. Sangnambatbyeo, Nonglimna1 and Moroberekan are upland *japonica* rice and GL33 is a *japonica* weedy rice.

Fig. 2. Dendrogram of nine Korean rice germplasm and seven donors for drought tolerance clustered by the similarity coefficients of 125 SSR markers.

DISCUSSION

As rainfed environments are highly variable, breeding rice for drought-prone rainfed ecosystems requires genotypes that combine high yielding ability with good drought tolerance in addition to tolerance to prevalent biotic stresses. The molecular breeding program for drought at IRRI has identified several QTLs for grain yield under drought (Bernier *et al.* 2007; Venuprasad *et al.* 2009a; Vikram *et al.* 2011; Dixit *et al.* 2012). The present study evaluated the grain yield of drought tolerant-NILs under irrigated non-stress and drought stress conditions and identified polymorphic SSR markers between Korean rice cultivars and drought tolerant-NILs to introduce the drought QTLs into Korean rice cultivars.

All drought tolerant-NILs showed higher grain yield than those of IR64 and Korean rice cultivars under drought stress in tropical conditions. IR86918-B-439-B yielded highest followed by IR77298-14-1-2 and IR81896-B-B-431 under drought stress. IR86918-B-439-B possessed drought tolerance QTL, DTY1.1 from N22 in IR64 genetic background (Vikram *et al.* 2011). The drought tolerance QTL, DTY1.1 had a major positive effect for grain yield under reproductive-stage drought stress and non-stress conditions. The effect of this QTL is also consistent in the different genetic backgrounds, Swarna and MTU1010 (Vikram *et al.* 2011). IR77298-14-1-2 is nearly isogenic to IR64 but differs in its tolerance to drought and tungro disease. It has a drought tolerance QTL, DTY2.2 from Adaysel in IR64 genetic background (Swamy *et al.* 2011; Swamy *et al.* 2013). The drought tolerance QTL, DTY2.2 has high and consistent additive effect under severe lowland drought stress (Dixit *et al.* 2012). QTLs for root length and thickness adjacent to the qDTY2.2 have been reported (Champoux *et al.* 1995; Kamoshita *et al.* 2002; MacMillan *et al.* 2006). IR77298-14-1-2 is a valuable breeding line because it not only has drought tolerance but also tungro resistance (Venuprasad *et al.* 2007). IR81896-B-B-431 has the drought tolerance QTL, DTY3.1 from Apo in a Swarna genetic background (Venuprasad *et al.* 2009a). DTY3.1 had a large effect on grain yield under severe lowland drought stress. It also explained considerable variance for yield under mild stress in lowland conditions

and aerobic environments (Venuprasad *et al.* 2009a).

Six SSR markers showed polymorphism between two Korean rice cultivars and IR86918-B-305-B in the region of DTY1.1. This region has been identified between RM431 and RM11943 on chromosome 1 in the background of highly popular elite rice varieties showing consistent effects against different genetic backgrounds (Vikram *et al.* 2011). RM431 and RM11943 markers also showed polymorphism between two Korean rice cultivars and IR86918-13-305-B in this study. These markers are very useful as flanking markers for DTY1.1 introduction. Three SSR markers, RM109, RM279, and RM236 showed polymorphism between two of the Korean rice cultivars and IR77298-14-1-2 in the region of DTY2.2. DTY2.2 was identified between RM279 and RM555 having an effect on grain yield under severe stress conditions by CIM analysis (Dixit *et al.* 2012). The SSR marker RM279 was found polymorphic for the two Korean rice cultivars and IR77298-14-1-2. Three SSR markers were also polymorphic between two of the Korean rice cultivars and IR81896-B-B-431 in the region of DTY3.1. The peak of DTY3.1 was located at 10.0cM and flanked by RM520 (9.1cM) and RM416 (10.0cM) (Venuprasad *et al.* 2009a). Moreover, RM520 and RM416 were also found polymorphic between the two Korean rice cultivars and IR81896-B-B-431. These markers will therefore be useful as flanking markers to introduce the DTY3.1 into Korean rice cultivars. The group of polymorphic SSR markers at the target drought QTLs, DTY1.1, DTY2.2 and DTY3.1 can be utilized to introduce those QTLs into Korean rice cultivars as foreground selection markers.

The sixteen rice cultivars could be separated into two main clusters corresponding the *japonica* and *indica* groups following cluster analysis using SSR marker results. The lines IR77298, IR81896 and IR84984 sub-grouped with the Korean Tong-il cultivars. The cultivars Sangnambatbyeo, Nonglimna1, GL33 and Moroberekan sub-grouped in the *japonica* cluster. The cultivars Sangnambatbyeo, Nonglimna1, GL33, and Moroberekan were slightly genetically different from the *japonica* rice cultivars because those lines are upland or *japonica* weedy rice. The four IR86918 lines sub-grouped within the *indica* group. These lines are drought tolerant-NILs derived from a cross IR64 (recipient) and N22 (donor for drought tolerance) by backcrossing

(Kumar 2011; Vikram *et al.* 2011), and as expected from their pedigree, they are genetically very similar. Although lines developed from IR77298 were derived from a cross between IR64 and Adaysel, they were genetically distinct from the IR86918 lines but more closely related to the Tong-il cultivars from Korea. This suggests that the lines derived from IR77298 did not recover as much of the IR64 genetic background as the IR86918 lines. Lines derived from IR81896 and IR84984 also sub-grouped with Korean Tong-il cultivars. These lines are drought tolerant-NILs from crosses between Swarna, Vandana (recipients) and Apo, Way Rarem (donors for drought tolerance). IR77298 and IR84984 lines also demonstrated desirable agronomic traits and yield potential under temperate environmental conditions in Korea. This suggests that it may be more efficient to introduce the drought tolerance QTLs of those lines into the background of Korean Tong-il types in temperate regions. Varieties with an improved drought tolerance and high yield potential are likely to reduce production risk and contribute to food security through more stable production when affected by drought.

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