Validation of SSR markers associated with drought tolerant QTLs in rice (Oryza sativa L.)

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Abstract

Water stress, one of the devastating abiotic stresses in rain fed rice ecosystems causes huge loss to rice yields. Enhancement of production as well as productivity in rain fed areas requires development of improved varieties for such regions. Slow progress in drought breeding is an impediment in this way. Maker assisted approach is an alternative for fast track improvements of crops for drought. Validation of markers associated with drought related traits across the populations would add value to their application. In the present study, four SSR markers viz., RM 263, RM 3825, RM 212 and RM 22 associated with drought tolerance QTLs, qDTY2.3, MQTL1.1, MQTL1.2 and qDTY 3.2 respectively, were evaluated for their use in marker assisted selection (MAS) in BC1F1 individual plants, derived from the cross (HUR 3022 × Nagina 22) × HUR 3022, (HUR 3022 × Birsa Gora) × HUR 3022, (Sarjoo 52 × Nagina 22) × Sarjoo 52 and (Sarjoo 52× Birsa Gora) × Sarjoo 52. Marker RM 263 (qDTY2.3) and RM 3825 (MQTL1.1) were consistently associated with yield per plant in all the cultivars and their derived backcross populations. Therefore, these two SSR markers RM 263 and RM 3825 were found suitable for selection of drought tolerant rice lines in marker assisted backcross breeding (MAB) programs. Besides, SSR marker RM 212 (MQTL1.2) was reported to validate in BC1F1 population of (Sarjoo 52 × Nagina 22) × Sarjoo 52 and (Sarjoo 52 × Birsa Gora) × Sarjoo 52 whereas, SSR marker RM 22 (qDTY3.2) was validated in (HUR 3022 × Nagina 22) × HUR 3022 and (Sarjoo 52 × Nagina 22) × Sarjoo 52. Thus, these SSR markers would also be useful for improvement of drought tolerance in rice through MAS.

Introduction

Rice, one of the most important food crops for over half of the world's population accounts for around 23% of the global calorie intake (Bernier et al., 2008; Li et al., 2011). It has been estimated that rice supplies 35 to 60% of the total calorie intake at any given day in Asia where 90% of world's rice is grown (Khush, 1997). Increasing population pressure, global warming and unpredictable rainfall patterns have induced severe drought spells in the major rice growing areas of world in past few years. To meet the growing demands of global population rice productivity needs to be significantly

increased. Rice is a semi aquatic plant that can be grown under irrigated as well as rainfed conditions. Poehlman and Sleper (1995) described four different types of rice growing environments i.e., irrigated, rain fed lowland, deep-water and rain fed upland. Irrigated rice is by far the most common rice ecosystem constituting 55% of the world area providing 75% of the rice production globally (Khush, 1997). Of the total 150.1 million hectare of world rice acreage, the rain fed rice shares 30.9% whereas upland rice is about 9% (Bernier et al., 2008). Rain fed low land rice occupies about 32.1% of the total rice grown area in Asia and 35.4% in Africa. For rain fed upland and rain fed lowland rice, drought stress is the most devastating abiotic factor that affects rice yields severely. It has been estimated that losses due to drought stress are about 18 million tons annually (O'Toole, 2004). In Asia alone, it is estimated that a total of 23 million hectare of rice fields are drought prone (Pandey et al., 2009). Drought can be simply defined as reduction in yield due to shortage of water (Bernier et al., 2008). Drought tolerance, a well-known complex trait is associated with a number of physiological and biochemical phenomena (Kamoshita et al., 2008). Traits associated with such phenomena are usually considered as secondary traits. Secondary traits used for selection purpose are mainly the root traits,

osmotic adjustment and traits governing maintenance of plant water potential like relative water content (Kato et al., 2006; Nguyen et al., 1997; Babu et al., 2001). Responses of plants to cope up drought situation are drought escape, drought recovery, drought tolerance, drought recovery and drought avoidance.

A QTL is fit for marker assisted breeding, if it is consistently expressing in several genetic backgrounds. Such QTLs have also been identified in rice for grain yield under drought indicating presence of major genes associated with drought tolerance (Vikram et al., 2009). For the grain yield major QTLs linked to SSR markers RM 263 on chromosome 3 (Vikram et al., 2012), Meta QTL RM 3825 and RM 212 on chromosome 1 (Salunkhe et al., 2011) and RM 22 on chromosome 3 (Vikram et al., 2012) were identified. The present study was aimed at validating these four major drought QTL markers RM263, RM 3825, RM 212 and RM 22 respectively, associated with grain yield in BC1F1 of different populations of HUR 3022 and Sarjoo 52 with Nagina 22 and Birsa Gora growing well in the states of Uttar Pradesh and Bihar.

Materials and Methods

The present study was conducted at Department of Genetics and Plant Breeding,

Banaras Hindu University, India during Kharif, 2010 and 2011 under irrigated and rain fed conditions.

Plant materials

The study of validating SSR markers associated with drought tolerance was conducted on BC1F1 populations derived from crossing between four parents. The following BC1F1 populations were used for validation: (HUR 3022 × Nagina 22) × HUR 3022, (HUR 3022 × Birsa Gora) × HUR 3022, (Sarjoo 52 × Nagina 22) × Sarjoo 52 and (Sarjoo 52 × Birsa Gora) × Sarjoo 52. HUR 3022 and Sarjoo 52 are drought-susceptible indica cultivars while Nagina 22 and Birsa Gora are drought-tolerant indica cultivars. Details of the parental materials are given in Table 1. Leaf rolling and Stay Green traits remain to be desirable for phenotyping of the lines for drought. Measurement of stay-green is done as an independent visual estimation of the retention of the green-area for leaves on a 1 to 5 scale. A rating of 1 indicated complete or nearly complete leaf death, while rating 5 corresponded to a complete green leaf (Jiang et al., 2004). Leaf rolling as estimated by visual estimation depicted that the susceptible varieties were first to start the rolling symptoms, in the morning. Rolling in other parents or lines started at around 8 a.m. as the transpiration demand increased. A rating of 1

exhibited no rolling (resistant) whereas scale 9 indicated fully rolled leaves. Leaf rolling was recorded based on method proposed by Abd Allah (2009).

DNA extraction and SSR marker assay

The genomic DNA was extracted using modified CTAB method (Panaud et al., 1996) and PCR reactions were performed 15μl volume. Inside the reaction mixture 1μl DNA , 1.5 μl 10x buffer, 0.2 μl MgCl2, 0.2 μl dNTps, 0.2 μl Taq polymerase and 1μl forward and reverse primers each were used. PCR profile used for amplification was - initial denaturation at 94°C for 3 minutes, followed by 39 cycles of denaturation at 94°C for 30 seconds, optimum annealing temperature for 30 seconds, extension at 72°C for 1 minute and final extension at 72°C for 7 minutes. The PCR products were separated electrophoretically in 2.5% (w/v) agarose gel using 0.5 x TBE buffer and visualized under a UV light source in a gel documentation system (Gel DocTM EZ Imager). The following four SSR primers linked to the grain yield were used in the present study.

S. No.	SSR Loci	Position on LG	Associated with QTL	Phenotypic variation explained (%)	Reference
1.	RM 3825	1	MQTL 1.1	12.1	Salunkhe et al., 2011
2.	RM 212	1	MQTL1.2	12.1	Salunkhe et al., 2011
3.	RM 263	2	qDTY 2.3	4.6	Vikram et al., 2012
4.	RM 22	3	qDTY 3.2	34.9	Vikram et al., 2012

Results and Discussion

SSR markers RM 263 (qDTY2.3) and RM 3825 (qDTY1.1) were consistently associated with yield per plant in all the four parents viz., HUR 3022, Sarjoo 52, Nagina 22 and Birsa Gora. These markers clearly distinguished drought susceptible and tolerant parents. Marker RM 263 was reported to amplify a PCR product of approx. 190 bp in HUR 3022 and Sarjoo 52 (drought susceptible parents) whereas, ~160 and ~170 bp bands were visualised in drought tolerant parents Nagina 22 and Birsa Gora, respectively. RM 22 amplified at ~200 bp in HUR 3022, Sarjoo 52 and Birsa Gora but at ~ 190 in Nagina 22. RM 3825 amplified at ~175 bp in HUR 3022, ~160 bp in Sarjoo 52 and ~ 140 bp in Nagina 22 and Birsa Gora. RM 212 was reported to amplify at ~130 bp in HUR 3022, Nagina 22 and Birsa Gora whereas in Sarjoo 52 it produces a band of ~ 150 bp (Fig. 1).

Besides, RM 263 and RM 3825 was also reported to validate in the derived BC1F1 populations of the drought tolerant and susceptible parents i.e., (HUR 3022 × Nagina 22) × HUR 3022, (HUR 3022 × Birsa Gora) × HUR 3022, (Sarjoo 52 × Nagina 22) × Sarjoo 52 and (Sarjoo 52 × Birsa Gora) × Sarjoo 52 (Fig. 2 and Fig. 3). Whereas SSR markers RM 212 (MQTL1.2) was reported to validate in (Sarjoo 52 × Nagina 22) × Sarjoo 52 and (Sarjoo 52 × Birsa Gora) × Sarjoo 52. SSR marker RM 22 (qDTY3.2) was validated in (HUR 3022 × Nagina 22) × HUR 3022 and (Sarjoo 52 × Nagina 22) × Sarjoo 52.

In the present era of global climatic change where crops are more likely to undergo abiotic stresses, drought becomes quite important particularly for high water consuming plant like rice (Wassmann et al., 2009). Rice is a semi-aquatic plant that requires huge amount of water to complete its life cycle. Water stress is one of the most important abiotic stresses in rice causing severe reduction in grain yield. Drought stress

affects the key physiological phenomena of rice plant which ultimately affects the overall grain yield. Understanding the mode of physiological mechanism help breeders and physiologists to develop appropriate selection and breeding strategies (Blum 1988). There is an urgent need to breed drought-tolerant rice varieties with high yield potential. Over the last few years, two to three varieties in each country have become popular among farmers and are grown on millions of hectares because of their high yield potential and preferred grain quality. But, these varieties are highly susceptible to RS. The adaptability of such farmer-preferred varieties could be increased by incorporating drought tolerance.

In our present study, four SSR markers linked to the grain yield trait were validated in order to further study the ability of these markers to distinguish genotypes derived from genetically diverse indica × indica crosses. Salunkhe et al. (2011) reported R2 = 12.1% for RM 3825 and RM 212 (MQTL 1.1 and MQTL1.2) whereas Vikram et al. (2012) reported R2 = 34.9% for RM 22 and R2 = 4.6% for RM 263. SSR marker RM 263 and RM 3825 were consistently polymorphic and reliably distinguished in all parents and their derived populations in the present study. Consistent effect of drought QTLs is of utmost importance from the introgression

point of view. Recently a large effect QTL for grain yield under drought on chromosome 1 has been reported in rice (Vikram et al., 2011). At the same locus QTLs for secondary traits like root thickness, root length, root number, root depth, root penetration ability, osmotic adjustment and dehydration tolerance have been reported in several rice populations (Gomez et al., 2009; Kanagaraj et al., 2010). Meta-analysis studies, of drought yield QTLs in rice, also consolidated the fact that there are QTLs/ genes consistently effective across backgrounds/ populations (Swamy et al., 2011). A large number of root QTLs have been identified but their background specificity is a major drawback and therefore meta-analysis is required to identify the consistent ones (Gowda et al., 2011). Unlike the most QTL regions that are specific to particular backgrounds such QTLs are quite valuable. The other QTL locus used in our study was RM 212 on chromosome 1 and RM 22 on chromosome 3 which were not found to be consistently effective in population lines as well as random cultivars. The marker RM 212 differentiated in (Sarjoo 52 × Nagina 22) × Sarjoo 52 and (Sarjoo 52 × Birsa Gora) × Sarjoo 52 whereas, SSR marker RM 22 (qDTY3.2) was validated in (HUR 3022 × Nagina 22) × HUR 3022 and (Sarjoo 52 × Nagina 22) × Sarjoo 52. In addition to the application of these QTL alleles for marker

assisted breeding, such studies would also be helpful to workout allelic pattern. Random and diverse rice genotypes were used for QTL validation. QTL markers identified in one population could be useful for marker assisted breeding with other populations also. Already identified QTLs in several backgrounds could be screened in several other backgrounds for their effect. Valuable QTL markers with consistent effect could be worked out as was the case in RM 263 and RM 3825 in our study. This approach of validating QTL markers would be useful in identifying and introgressing QTLs in multiple backgrounds

Conclusion

The most significant application of the so far identified major QTLs for drought tolerance is to pyramid those favorable alleles into an elite local rice line through marker assisted breeding. Thus, the markers RM 263, RM 3825, RM212 and RM 22 will be useful for more efficient way for selecting drought tolerant lines through MAS approach especially in those regions growing rice with irrigated ecosystem. In addition, the well conserved QTL associated with drought tolerance and other agronomically important traits can be used as tools to localize syntonic regions in other cereal crops through comparative genomics. Thus, those drought tolerant genes or other important genes

isolated in rice can be used to facilitate the isolation of corresponding genes in other crops such as maize or wheat.

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Fig.1: DNA banding profile using RM 263, RM 22, RM 3825 and RM 212 differentiating drought susceptible lines (P₁ = HUR 3022, P₂ = Sarjoo 52) from drought tolerant lines (P₃ = Nagina 22, P₄ = Birsa Gora)

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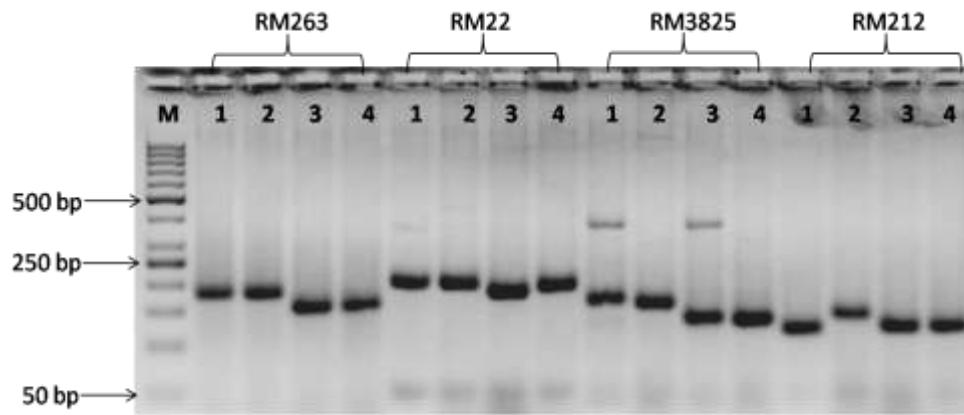
Fig.2. Foreground analysis of BC_1F_1 population derived from the cross (HUR3022 × Birsa Gora) × HUR3022 with SSR marker RM 263. Lane M = 50 bp DNA size ladder; P1 = HUR3022; P2 = Birsa Gora; 1-17 are individual backcross plants; * (asterisk sigh) indicates some of the heterozygous plants.
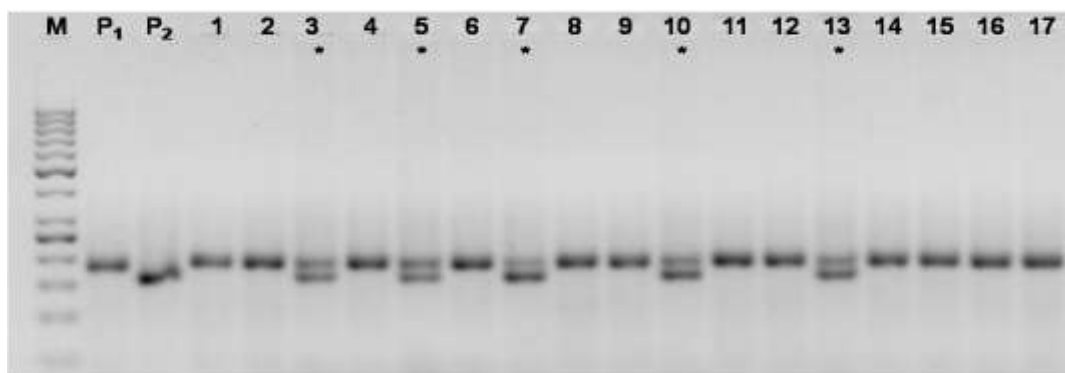
Fig.3. Foreground analysis of BC₁F₁ population derived from the cross (HUR3022 × Birsa Gora) × HUR3022 with SSR marker RM 263. Lane M = 50 bp DNA size ladder; P1 = HUR3022; P2 = Birsa Gora; 1-17 are individual backcross plants; * (asterisk sigh) indicates some of the heterozygous plants.
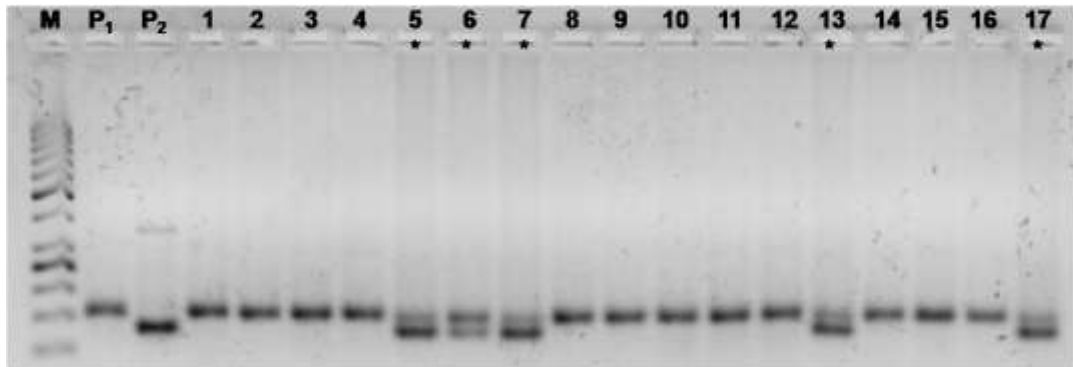
Table 1. Rice cultivars used for validation of molecular markers associated with drought tolerance:

S. No.	Cultivar	Pedigree	Drought Reaction	LR[*]	SG	Approx. amplification product size (bp)				Characteristic Features
						RM 263	RM 22	RM 3825	RM 212	
1.	HUR 3022	IR 36 × HR 137	S	9	5	190	200	175	130	Semi dwarf, 50-55 q/hq out yielded Saket4 & NDR 80, Lodging resistant, large (30-35 cm,) panicles, 105-110 Days maturity, high tillering (15-25)
2.	Sarjoo 52	T(N) 1 × kashi	S	9	5	190	200	160	150	Semi dwarf, yield 50- 60 quintal ha$^{-1,}$ 130-133 days maturity, moderately resistant to BLB
3.	Nagina 22	Selection from Rajbhog	R	1	1	160	190	140	130	Tall (115–120 cm), yield 20- 25 quintal ha^{-1}, 85-102 days maturity, presence of red awns, grains are short, bold and white
4.	Birsa Gora	Selection from germplasm collection of Gora	R	1	1	170	200	140	130	Tall (165- 180 cm), yield 18- 20 quintal ha^{-1}, 95-100 days maturity, tall and pubescent (120-140 cm), grain is medium sized, bold and red, moderately resistant to major diseases and pests

[*]LR = Leaf rolling (1–9 scale); SG = Stay green (1-5 scale).