Marker-Trait Association for Physiological Traits Associated with Drought Resistance in Rice under Different Water Regimes

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Rice is the principal food crop for majority of the global population and its production is frequently affected by drought. The objective of this study was to have improved understanding on drought resistance as a trait, study its molecular genetics and also to identify the potential drought resistant segregants. The genetic material used in this study comprised of near-isogenic lines of IR64 (BC_2F_7 generation) with random introgression from drought resistant land race Norungan. There was a significant reduction of 25 per cent and 75 per cent in mean plot yield under moderate and severe stress conditions respectively from that under irrigated control which reflected the varied stress intensities between the treatments. The genetic material exhibited significant phenotypic variation for 16 traits studied under three experimental conditions. Marker - trait association was assessed by single marker analysis. Totally 121 markers were used out of that eighteen markers showed putative association with at least one of the investigated trait. A candidate gene based marker for DREB 1 positioned in chromosome 8 was associated with yield under severe stress whereas SSR markers *viz*, RM 256 in chromosome 8; RM 245 in chromosome 9 were linked with yield under moderate stress. These markers were also linked with other associated traits *viz*, harvest index, leaf senescence and leaf rolling. These might be useful for marker assisted selection for rainfed rice improvement.

Key words: Rice, drought resistance, molecular marker, component traits

Rice is grown on more than 154 million hectares in the world in a wide range of ecosystems under varying temperatures and water regimes. About 28 per cent of the global rice is grown in rainfed lowlands. These areas frequently experience severe water deficit due to uncertain and uneven rainfall distribution and yields are seriously affected by drought. Another 13 per cent of the rice is grown under upland conditions without any surface water accumulation and is always prone to water stress during some part of the growing season. Drought stress is the major constraint to rice production affecting yield stability in the rainfed regions (Kole, 2006). There are worrying signs in some areas that groundwater resources are being overexploited, with groundwater levels falling, which could develop into a crisis. To meet the increasing demand of rice in the future, therefore, we can not continue to depend solely on irrigated area. There is a compulsory need to improve the yield potential of rice crop in rainfed areas. As world population grows to an estimated 8.9 billion in 2030, agriculture must respond to the increasing demand for food and compete for scarce water with other users. Many of the over 800 million

people in the world who still go hungry live in water scarce regions (FAO 2002). Marker assisted backcrossing (MABC) combines 'foreground' selection of donor alleles linked to QTLs and 'background' selection of recurrent parent alleles in the BC_2 and later generations. Advanced backcross methods were developed by Tanksley and Nelson (1996) that allow simultaneous analysis and introgression. Near-isogenic lines (NILs) are important genetic stocks for investigating the function and regulation of single genes. In the case of stress-related QTLs, the results of MAS are limited (Tuberosa *et al.*, 2002; Steele *et al.*, 2006) owing to difficulties such as QTLs that have epistatic interactions and do not contribute significantly in a novel genetic background. Much genetic analysis has been done in the past 20 years to identify QTLs for traits related to drought resistance (Nguyen *et al.* 1997). The advanced back-cross strategy appeared to be an appropriate method to accelerate the process of introgressing interesting traits into elite material (Robin *et al.,* 2003). Progress in the improvement of drought-resistant rice cultivars has been slow, mainly because of tremendous diversity of environments which complicate selection of new

cultivars (Fukai and Cooper, 1995). Use of molecular markers is considered helpful to plant breeders because it is complementary to the existing practices and can be used to assign the number, chromosomal locations and genetic contributions of genes controlling intricate or quantitative traits as well as simply-inherited traits. Present study was undertaken to carry out trait association with SSR markers for physiological traits associated with drought resistance in a backcross inbred population of rice under different water regimes.

Materials and Methods

Plant material

A backcross inbred population (BC$_2$F$_7$) comprising of 65 back cross inbred lines (BILs) derived from the cross IR64/Norungan//IR64///IR64 was used in the study. The parent, Norungan, an extremely drought resistant landrace known for its long term adaptation in drought prone ecosystem, was crossed with high yielding variety IR64, a popular variety which possesses many agronomical superior traits but sensitive to drought stress.

Phenotyping

Field experiments were conducted using a randomized complete block design with three replications. The inbred lines along with their parental lines were raised in a plot size of 2.4m x 4 rows with a row spacing of 20 cm and intra row spacing of 10 cm. Three treatments followed were ; irrigated control, moderate stress and severe stress. Under moderate stress condition, stress was imposed by withholding irrigation from 60$_{th}$ day of crop age. In severe stress, the trial was raised as a delayed wet season crop during end of October 2008 to February 2009 to push the flowering of the crop to coincide with rain –free period of the season based on the 100 years rainfall probability data. Totally sixteen characters were studied in these three environments. This phenotypic data were used for single marker analysis.

Genotyping

DNA was extracted from the leaf samples of the two parents and 65 BILs following CTAB method developed by Saghai-Maroof *et al* . (1984) with suitable modifications by Hoisington *et al* . (1994). The parental survey was performed for the two parents, IR64 and Norungan using 115 SSR and 6 DREB (Transcription factors) gene specific primers. Sixty five BILs were surveyed with 24 polymorphic markers. Bands were visualized by staining the gels with ethidium bromide and photographed under UV light using Alpha Imager 2200 (Alpha Innotech Corporation, San Leandro, CA). The bands on gels were scored as corresponding to the parental alleles as homozygote or heterozygote allele (Fig 1).

Single marker analysis

Single marker analysis was performed to the phenotypic and genotypic data generated from the three treatments. To discover markers that are closely describing the trait distribution the basic model looked into was, $y_i = ì + f(M) + å_j$, where, y_i is the trait value of the j_{th} individual in the population,

ì is the population mean, $f(M)$ is the function of the marker genotype and $å_j$ is the residual associated with the j_{th} individual. To resolve the above relation, single marker analysis has been employed with help of software, QTL Cartographer v2.5 (Wang *et al.*, 2005).

Results and Discussion

Marker allele constitution of BILs

Among the BILs, segregation of parental alleles varied widely with a mean segregation percentage of 87.58 of IR64 alleles as homozygote, 9.62 percentage of Norungan alleles as homozygote and 2.79 per cent of heterozygotes. The introgression of Norungan alleles in the BILs ranged from 0 to 41.67 per cent (data not shown).

Marker - trait analysis

The results of single marker analysis provided information on the genetic architecture of complex traits i.e., estimated number of QTL and magnitude of their estimated additive, dominance, and epistatic effects in multiple environments (Macky, 2001; Holland, 2007). In this study, 24 polymorphic markers selected based on their position in the target genomic region were used for single marker analysis. Among them 18 markers were identified as putatively associated to at least one of the investigated trait. Many of the markers were known to control more than one trait indicating either linkage or pleiotropic effect (Table 1). In particular the marker RM 232 on chromosome 3 was associated with seven traits *viz.,* days to 50% flowering and CATD under control, plant height and number of tillers under moderate stress, plot yield, harvest index and osmotic potential under severe stress. This indicated that chromosome 3 harbouring this marker might either contain genes which were involved in drought resistance in rice under field conditions. Similar to this finding, plot yield under severe stress was linked to this marker as previously reported by Venuprasad *et al.* (2002) and Subashiri *et al.* (2009). Singh *et al.* (1996) and Chen *et al.* (2001) reported this marker for days to 50 per cent flowering. Chromosome 8 was linked with many traits under three environments. In that RM256 on chromosome 8 was associated with five traits across the environments namely, days to 50% flowering (control), panicle length (MS), plot yield (control), moderate stress and severe stress, harvest index and leaf rolling under severe stress. This region was also mapped for yield and panicle exsertion by Subashiri *et al.* (2009) and plot yield by Singh *et al.* (1966) and Cheng *et al.* (2001). Another marker, RM

Table 1. Marker – Trait association revealed by single marker analysis using QTL cartographer

Traits	Environment	Marker name	chromosome	LOD score	Pr(F)
Days to 50% flowering	(C)	RM232	3	5.758	0.019*
		RM256	8	5.003	0.028*
	(MS)	RM225	6	4.271	0.043*
	(SS)	RM225	6	6.470	0.013*
Plant height	(C)	RM246	1	4.379	0.040*
		RM102	1	6.350	0.013*
		RM303	4	4.185	0.045*
	(MS)	RM246	1	6.378	0.013*
		RM102	1	7.424	0.008**
		RM157A	3	4.276	0.043*
		RM303	4	4.016	0.049*
	(SS)	RM102	1	5.251	0.025*
		RM303	4	4.208	0.044*
Number of Tiller	(C)	RM280	10	4.091	0.047*
		RM107	9	6.397	0.013*
		RM245	9	8.724	0.004**
	(MS)	RM232	3	4.977	0.029*
		RM80	8	4.938	0.029*
Panicle length	(MS)	RM303	4	7.115	0.009*
		RM256	8	5.362	0.023*
	(SS)	RM246	1	5.842	0.018*
		RM303	4	5.279	0.024*
Panicle exertion	(C)	RM246	1	5.149	0.026*
	(MS)	RM246	1	7.123	0.009**
	(SS)	RM152	8	4.151	0.046*
1000 grain weight	(C)	RM225	6	7.546	0.007**
		RM254	11	8.178	0.005**
	(MS)	RM303	4	6.360	0.013*
		RM280	4	5.210	0.025*
	(SS)	RM225	6	4.796	0.032*
		RM264	8	9.399	0.003**
CATD	(C)	RM157A	3	7.474	0.007*
		DREB 1I	8	6.048	0.016*
	(MS)	RM303	4	5.390	0.023*
Single plant yield	(C)	RM280	4	18.34	0.000****
		RM225	6	18.99	0.000****
	(SS)	DREB 1 I	8	6.446	0.013*
		RM254	11	8.262	0.005*
Plot yield	(C)	RM225	6	7.811	0.006**
		RM256	8	6.235	0.014*
	(MS)	Rm256	8	5.884	0.017*
		RM245	9	7.136	0.009**
	(SS)	RM232	3	7.820	0.006**
		RM256	8	6.812	0.010*
		RM80	8	8.273	0.005**
		RM149	8	4.320	0.042*
		RM264	8	8.422	0.004**
Spikelet sterility	(SS)	RM 84	1	4.146	0.046*
		RM 264	1	6.341	0.014*
Harvest index	(SS)	RM232	3	8.570	0.004**
		RM256	8	9.005	0.003**
		RM80	8	8.047	0.005**
		DREB1 I	8	4.801	0.032*
Leaf rolling	(SS)	RM84	1	4.011	0.050*
		RM245	9	4.934	0.29*
Leaf senescence	(SS)	RM256	8	6.890	0.010*
		RM80	8	4.349	0.041*
RWC	(SS)	RM80	8	4.142	0.046*
Osmotic potential		RM232	3	4.811	0.031*
		RM251	3	5.088	0.027*
		RM303	4	4.107	0.047*
		RM245	9	4.372	0.040*
Osmotic adjustment	(SS)	RM80	8	4.740	0.033*
		RM254	11	4.993	0.028*

*, ** Significant at 0.05 and 0.01 probability level
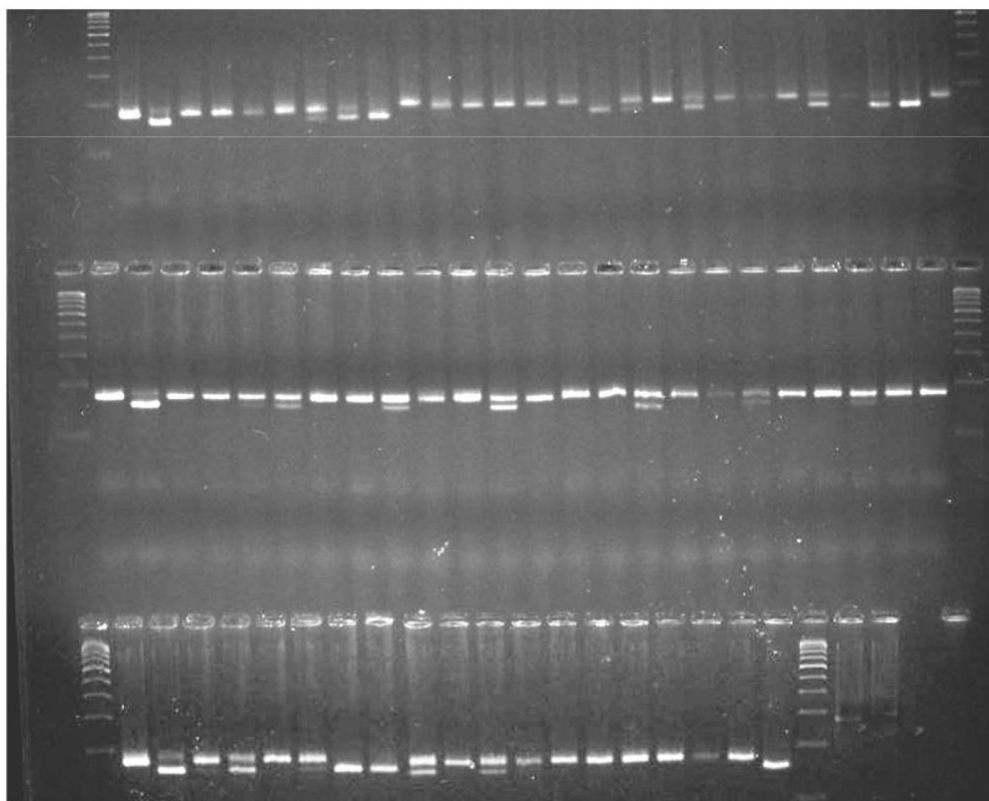
(lane 2 & 3 correspond to IR64 and Norungan respectively and the rest belong to BILs)

Fig.1. BILs segregating for marker allele amplified with the SSR primer 264

80 on chromosome 8 was putatively linked with number of tillers (MS), harvest index, leaf senescence and osmotic adjustment under severe stress. The same region was previously mapped for OA by Robin *et al.* (2003) and for several traits by Subashiri *et al.* (2009). RM 264 on chromosome 8 was associated with three traits such as 1000 grain weight, plot yield and spikelet sterility. This region was reported by Jiang *et al.* (2004) for single plant yield. RM 152 on chromosome 8 was linked to panicle exsertion and this region was previously mapped by Yue *et al.* (2005) for panicle exsertion. RM 303 was associated with panicle length (MS) and (SS), 1000 grain weight and CATD under moderate stress. RM102 was previously mapped for 1000 grain weight by Singh *et al.* (1996) and Cheng *et al.* (2001). These two regions RM102 and RM 303 were already mapped for stomotal conductance and panicle length by Subashiri *et al.* (2009). The linkage of panicle length with RM 303 was similar to Subashiri *et al.* (2009). RM 254 on chromosome 11 was found to be associated with 1000 grain weight (C), single plant yield and OA (SS). This region was previously mapped for single plant yield and 1000 grain weight by Jiang *et al.* (2004) and for 1000 grain weight by Brondani *et al.*

(2002). DREB 1 I on chromosome 8 was putatively linked with CATD under control, single plant yield and harvest index under severe stress condition

thus signifying the relevance of transcription factors in drought resistance. Most of the markers - trait associations were consistent with Subashiri *et al.* (2009) using the same parents but in the RIL population. The results indicated that the backcross inbred population inherited many of the useful alleles from Norungan which could be pyramided through intermating among the selected BILs to improve drought resistance in this population beyond the donor parent, Norungan.

References

Brondani, C., Rangel, N., Brondani, V. and Ferreira, E. 2002. QTL mapping and introgression of yield related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. *Theor. Appl. Genet.*, **104**: 1192-1203.

Cheng, Z., Presting, G.G., Buell, C.R., Wing, R.A. and Jiang, J. 2001. High-resolution pachytene chromosome mapping of bacterial artificial chromosomes anchored by genetic markers reveals the centromere location and the distribution of genetic recombination along chromosome 10 of rice. *Genetics*, **157**: 1749-1757.

Food and Agricultural organization. 2002. Crops and Drops – making the best use of water for agriculture. Food and Agriculture Organization of the United Nations. Rome.

Fukai, S. and Cooper, M. 1995. Development of drought-resistant cultivars using physiomorphological traits in rice. *Field Crops Res.,* **40***:* 67-86.

Hoisington, D., Khairallah, M. and Gonzalez-de-Leon, D. 1994. *Laboratory protocols*: CIMMYT applied molecular genetics laboratory. CIMMYT, Mexico.

Holland, J.B. 2007. Genetic architecture of complex traits in plants. *Current Opinion* in *Plant Biol.*, **10**:156-161.

Jiang, G.H., He, Y.Q., Xu, C.G., Li, X.H. and Zhang, Q. 2004. "The genetic basis of stay-green in rice analyzed in a population of doubled haploid lines derived from an indica by japonica cross", *Theor. Appl. Genet.*, **108:** 688-698.

Kole, C. 2006. Genome mapping and molecular breeding in plants, In: Cereals and millets. Springer-Verlag Berlin Heidelberg. **1**: 1-27.

Mackey, T.F. 2001. The genetic architecture of quantitative traits. *Annu. Rev. Genet.*, **35**: 303–339.

Nguyen, H.T., Babu, R.C. and Blum, A. 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. *Crop Sci.,* **37***:* 1426-1434.

Robin, S., Pathan, M.S., Courtois, B., Lafitte, R., Carandang, S., Lanceras, S., Amante, M., Nguyen, H. T. and Li, Z. 2003. Mapping osmotic adjustment in an advanced back-cross inbred population of rice. *Theor. Appl. Genet.*, **107:** 1288-1296.

Saghai-Moroof, M.A., Soliman, K., Jorgensen ,R.A. and Allard, R.W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci.,* USA, **81:** 8014-8018.

Singh, K., Ishii, T., Parco, A., Huang, N., Brar, D.S. and Khush, G.S. 1996. Centromere mapping and orientation of the molecular linkage map of rice (*Oryza sativa* L.)", *Proc. Natl. Acad. Sci.,* **93**, pp: 6163-6168.

Subashri, M., Robin, S., Vinod, K.K., Rajeswari, S., Mohanasundaram, K. and Raveendran, T.S. 2009. Trait identification and QTL validation for reproductive stage drought resistance in rice using selective genotyping of near flowering RILs. *Euphytica,* 10681-008-9847-6.

Steele, K. A., Price, A. H., Shashidhar, H. E. and Witcombe, J. R. 2006. Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety? *Theor. Appl. Genet.*, **112**: 208–221.

Tanksley, S.D. and Nelson, J.C. 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor. Appl .Genet.*, **92**:191–203.

Tuberosa, R.S. Salvi, Sanguinet,i M. C., Landi, P., MacCaferri, M. and Conti,S. 2002. Mapping QTLs regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives in drought-stressed maize. *Ann. Bot.,* **89:** 941–963.

Venuprasad, R., Shashidar, H.E., Hittalmani, S. and Hemamalini, S. 2002. Tagging quantitative trait loci associated with grain yield and root morphological traits in rice (*Oryza sativa* L.) under contrasting moisture regimes. *Euphytica,* **128**: 293-300.

Wang, S., Basten, C.J., Gaffney, P. and Zeng, Z.B. 2005. Windows QTL cartographer 2.5. Bioinformatics research center. North Carolina State University, p: 80.

Yue, B., Xue, W., Xiong, L., Yu, X., Luo, L., Cui, K., Jin, D., Xing, Y. and Zhang, Q. 2005. Genetic basis of drought resistance at reproductive stage in rice: Separation of drought tolerance from drought avoidance. *Genetics,* **172:**1213–1228.