Available online at www.sciencedirect.com

ScienceDirect

Evaluation and Bulked Segregant Analysis of Major Yield QTL *qtl12.1* Introgressed into Indigenous Elite Line for Low Water Availability under Water Stress

N. Manikanda Boopathi[1], Gat Swapnashri[1], P. Kavitha[1], S. Sathish[1], R. Nithya[1], Wickneswari Ratnam[2], Arvind Kumar[3]

(*[1]Department of Plant Molecular Biology and Bioinformatics, CPMB&B, Tamil Nadu Agricultural University, Coimbatore-3, India; [2]Universiti Kebangsaan Malaysia, Bangi Selangor, Malaysia; [3]Division of Plant Breeding, Genetics and Biotechnology, International Rice Research Institute, Metro Manila, Philippines*)

Abstract: Near isogenic lines carrying large-effect QTL (*qtl12.1*), which has a consistent influence on grain yield under upland drought stress conditions in a wide range of environments, were evaluated under water stress in the fields. The line which gave higher yield under drought was crossed with a local elite line, PMK3, and forwarded to $F_{2:3}$ generation. Significant variation was found among the $F_{2:3}$ lines for agronomic traits under water stress in the fields. Low to high broad sense heritability (*H*) for investigated traits was also found. Water stress indicators such as leaf rolling and leaf drying were negatively correlated with plant height, biomass and grain yield under stress. Bulked segregant analysis (BSA) was performed with the markers in the vicinity of *qtl12.1*, and RM27933 was found to be segregated perfectly well in individual components of drought resistant and drought susceptible bulks which were bulked based on yield under water stress among $F_{2:3}$ lines. Hence, this simple and breeder friendly marker, RM27933, may be useful as a potentially valuable candidate marker for the transfer of the QTL *qtl12.1* in the regional breeding program. Bioinformatic analysis of the DNA sequence of the *qtl12.1* region was also done to identify and analyze positional candidate genes associated with this QTL and to ascertain the putative molecular basis of *qtl12.1*.

Key words: rice; bulked segregant analysis; water stress; quantitative trait locus; marker-assisted selection

Genetic improvement of rice largely focuses on breeding varieties suitable for water-limited environments since future increases in rice production will rely on these rainfed ecosystems (Blum et al, 1999). However, the progress in this direction was slow and more limited due to lack of knowledge on the mechanism of tolerance, poor understanding of the inheritance of tolerance, low heritability of yield under water stress and lack of efficient techniques for screening breeding materials for drought tolerance (Kamoshita et al, 2008). The advent of molecular markers has revolutionized the genetic analysis of complex traits such as drought resistance in crop plants (Boopathi et al, 2011). Such markers would be useful to enhance the efficiency of contemporary techniques such as quantitative trait loci (QTLs) mapping and marker-assisted selection (MAS)

besides understanding the molecular mechanism of drought resistance. QTLs have been detected for several root-related traits, osmotic adjustment, dehydration tolerance and other shoot-related drought resistance component traits in rice (Kamoshita et al, 2008).

Additionally, locating QTLs needs each rice line in a segregating population to be genotyped using numerous molecular markers. This is tedious, time and labour intensive and cost ineffective. Alternatively, bulked segregant analysis (BSA) has been proposed as an efficient strategy for identifying DNA markers linked to the genes or genomic regions of interest (Brauer et al, 2006). By making DNA bulks, all loci are randomised, except for the region containing the gene of interest. Markers are screened across the parents and two bulks. Polymorphic markers may represent markers that are linked to a gene or QTL of interest (Collard et al, 2005). BSA has been successfully used in rice for identifying markers linked to QTLs associated with grain quality parameters (Govindaraj et al, 2005), blast resistance

(Yang et al, 2009), mutant phenotype of twisted hulls (Li et al, 2009), gall midge resistance (Lima et al, 2007) and heat tolerance (Zhang et al, 2009), and it also shown as an effective method for identifying large effect QTLs linked to rice yield under severe drought stress (Venuprasad et al, 2009).

A QTL (*qtl12.1*) with a large effect on rice grain yield under stress was detected on chromosome 12, explaining 51% of the genetic variance. Under stress conditions, the locus also showed the potential to increase harvest index, biomass yield and plant height (Bernier et al, 2007). Further, the effects of *qtl12.1* on grain yield and its associated traits were measured in 21 field trials, and the results confirmed that *qtl12.1* has a large and consistent effect on grain yield under upland drought stress conditions in a wide range of environments (Bernier et al, 2008). Moreover, it also showed that *qtl12.1* enhanced water uptake in upland rice (Bernier et al, 2009). For successful MAS, the selected QTL should account for the largest proportion of phenotypic variance for the trait and MAS should be stable across environments (Collard et al, 2005). Thus, MAS for *qtl12.1* has the potential to generate upland adapted cultivars with a broad range of drought resistance. In order to understand the utility of *qtl12.1* in regional breeding applications, it is imperative to introgress this allele into local elite lines and to evaluate the resulting progenies under target environments. Hence, in this study, an attempt has been made to evaluate local elite rice lines introgressed with *qtl12.1* and identify molecular markers linked to yield under water stress using BSA for further genetic improvement in regional rice breeding program.

MATERIALS AND METHODS

Rice materials

A total of 60 rice lines introgressed with drought resistant QTLs were obtained from Dr. Arvind KUMAR, International Rice Research Institute (IRRI), the Philippines, and grown under irrigated and water stress conditions in the fields at Tamil Nadu Agricultural University (TNAU), Coimbatore, India during the summer, 2010 along with local elite cultivars, PMK3 and ADT43. Drought was imposed on 45 d after sowing (DAS) for water stress treatment and the plants were allowed to dry continuously for 15 d; whereas the control received regular irrigation. Regular irrigation was given to both the control and stress plots on 60 DAS. Among the 60 IRRI lines, IR84984-83-15-185-B performed well under water stress conditions and hence it was selected as male

parent. IR84984-83-15-185-B having *qtl12.1* was developed from Way-Rarem/Vandana back cross progenies (Arvind KUMAR, personal communication). Popularly grown rice line in this region, PMK3, which is well adapted to local rainfed rice cultivation, was used as a female parent. Crossing program and generation advancement to F_2 was executed at the Paddy Breeding Station (PBS), TNAU, Coimbatore during 2010 and 2011. Simple sequence repeat (SSR) markers were used for parental polymorphic survey. The polymorphic SSR markers *viz*., AC29 and RM3808 were used to fix F_1 progenies. The selected true F_1 progenies were forwarded to F_2.

$F_{2:3}$ phenotyping for drought tolerance

The $F_{2:3}$ progenies of PMK3/IR84984-83-15-185-B were evaluated under two different hydrological conditions *viz*., irrigated and water stress from March to July 2011 in 4.0 m × 0.2 m size plots at TNAU, Coimbatore. All the rice entries were replicated thrice both under irrigated and water stress conditions in Latin square design with a spacing of 4.0 m × 4.0 m. NPK fertilizers were applied at the rates of 50, 25 and 25 kg/hm^2, respectively. P and K were applied as base fertilizer, and N was applied in two splits as topdressing. Insect and weed control measures were applied periodically as and when required. Water stress was imposed on 45 DAS for a continuous 15 d in the drought treatment. There was no rain during these 15 d. The site, soil and characteristics of drought stress are summarized in Table 1.

Data on days to first flowering, 50 per cent flowering, leaf rolling, leaf drying, plant height, grain yield per plant, biomass per plant, harvest index and grain yield per plot (Venuprasad et al, 2009) were recorded. Basic statistical analysis of the data was done using the SAS Enterprise Guide 4.2 (http:// support. sas.com) using Descriptive Summary Statistics

Table 1. Site, soil and drought stress characterization for rice experimental trial (summer, 2011).

Particular	Data
Elevation (meters above sea level) (m)	427
Latitude and longitude	11° N, 77° E
Soil texture	Clay loam
Soil pH	8.4
Characterization of the stress	Severe
Timing of start to stress	45 d after sowing
Duration of stress period (d)	15
Rainfall during stress period (mm)	0.0
Rainfall during crop period (mm)	279.5
Number of rainy days (d)	26
Mean temperature (°C)	32.07
Maximum temperature (°C)	37.44
Minimum temperature (°C)	26.70

Tasks. Similarly, analysis of variance (ANOVA) was performed to check the genetic variance among the $F_{2:3}$ progenies for all the traits using the SAS Enterprise Guide 4.2. Broad sense heritability (H) was then computed using the following formula: $H = \sigma^2_G / (\sigma^2_G + \sigma^2_e / r)$. Where, σ^2_G and σ^2_e are the genetic and error variances, respectively, and r is the number of replications (Venupasad et al, 2009). Phenotypic correlations (Pearson correlation coefficients) among the traits within the treatments were computed using the SAS Enterprise Guide 4.2. Frequency distribution of each trait among $F_{2:3}$ rice lines under each treatment were drawn separately using Microsoft Office 2010 Excel utility.

Identification of molecular markers linked to yield under water stress

Based on grain yield under water stress, 10 drought resistant and 10 drought susceptible rice lines were selected among 251 $F_{2:3}$ progenies of PMK3/IR84984-83-15-185-B. Equal concentration (25 ng) of DNA of the selected rice lines were pooled into two separate bulks *viz.,* drought resistant bulk (DRB) and drought susceptible bulk (DSB). The details of the selected rice lines constituting DRB and DSB are given in Table 2. The *qtl12.1* was flanked by two SSR markers, RM28048 and RM511 (Bernier et al, 2007). Two map sets showing SSR markers RM28048 and RM511 were selected from the available map sets at Gramene Database (www.gramene.org) and 34 SSR markers mapped in the vicinity of RM28048 and RM511 were selected for BSA analysis. The SSR profile of each marker was analyzed for identification of polymorphic SSR markers between the parents, and the segregation pattern of those polymorphic SSR markers in DRB and DSB were also analyzed.

Bioinformatics analysis

The genes found between the markers RM27933–RM28048–RM511 on chromosome 12 were collected using marker/sequence tab in Gramene Database (http://www.gramene.org/markers/). PSI-BLAST was carried out against protein database (PDB) for those genes to find the closely related orthologs (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?CMD=Web&PAGE=Proteins&PROGRAM=blastp&RUN_PSIBLAST=on). The function(s) of those genes were known from PubMed (http://www.ncbi.nlm.nih.gov/pubmed). Approximately 1500 bp upstream of the identified genes were obtained and these promoter regions were analyzed using Plant Care Database (http://bioinformatics.psb.gent.be/webtools/plantcare/html/).

RESULTS

Phenotyping for agronomic traits under water stress conditions

All the $F_{2:3}$ progenies along with the parents were phenotyped for drought response and other agronomic traits under managed water stress conditions in the fields. Severe stress was noticed as the soil made deep cracks and in some of the lines there was up to 100% reduction in grain yields (Tables 2 and 3). Severe drought stress imposed on the plants was also indicated by average leaf rolling and leaf drying scores across the $F_{2:3}$ lines (Table 3). This confirmed that adequate slow progress of stress has been imposed during this trail which may be the true representation of natural drought that occurs at farmer's fields during terminal stage.

Significant variations were found among the $F_{2:3}$ lines for the investigated traits in both the treatments

Table 2. Drought resistant and drought susceptible $F_{2:3}$ rice lines of PMK3/IR84984-83-15-185-B selected for bulked segregant analysis and their everage grain yield per plant under irrigated and water stress conditions.

Rice entry name (Drought resistant)	Grain yield per plant (g)		Rice entry name (Drought susceptible)	Grain yield per plant (g)	
	Irrigated	Water stress		Irrigated	Water stress
PMK3/IR84984-83-15-185-B $F_{2:3}$: 23	4.90	2.27	PMK3/IR84984-83-15-185-B $F_{2:3}$: 101	5.70	-
PMK3/IR84984-83-15-185-B $F_{2:3}$: 24	1.91	2.83	PMK3/IR84984-83-15-185-B $F_{2:3}$: 116	4.76	-
PMK3/IR84984-83-15-185-B $F_{2:3}$: 50	3.65	3.50	PMK3/IR84984-83-15-185-B $F_{2:3}$: 145	15.38	-
PMK3/IR84984-83-15-185-B $F_{2:3}$: 51	6.50	3.00	PMK3/IR84984-83-15-185-B $F_{2:3}$: 147	10.70	-
PMK3/IR84984-83-15-185-B $F_{2:3}$: 133	6.80	2.71	PMK3/IR84984-83-15-185-B $F_{2:3}$: 169	6.25	0.67
PMK3/IR84984-83-15-185-B $F_{2:3}$: 158	11.66	3.43	PMK3/IR84984-83-15-185-B $F_{2:3}$: 179	7.13	0.40
PMK3/IR84984-83-15-185-B $F_{2:3}$: 177	4.94	4.00	PMK3/IR84984-83-15-185-B $F_{2:3}$: 240	11.83	-
PMK3/IR84984-83-15-185-B $F_{2:3}$: 178	6.50	2.58	PMK3/IR84984-83-15-185-B $F_{2:3}$: 243	7.27	0.43
PMK3/IR84984-83-15-185-B $F_{2:3}$: 221	12.95	3.58	PMK3/IR84984-83-15-185-B $F_{2:3}$: 244	6.03	-
PMK3/IR84984-83-15-185-B $F_{2:3}$: 238	6.33	2.47	PMK3/IR84984-83-15-185-B $F_{2:3}$: 264	11.06	-
PMK3 (Female parent)	6.85	0.82			
IR84984-83-15-185-B (Male parent)	3.38	2.09			

'-', No grain yield was noticed.

Table 3. Trait values for parents and 251 $F_{2:3}$ progenies.

Trait	Rice line	Mean		Range (Minimum–Maximum)		Standard deviation		Heritability (%)	
		Control	Water stress	Control	Water stress	Control	Water stress	Control	Water stress
Days to heading	PMK3	87.0	95.0	-	-	0.6	0.6	-	-
(d)	IR84984-83-15-185-B	66.0	73.0	-	-	1.4	2.5	-	-
	PMK3/IR84984-83-15-185-B $F_{2:3}$	73.0	83.0	63.0–91.0	67.0–104.0	7.1	7.6	97.5	93.7
Leaf rolling	PMK3	-	6.5	-	-	-	0.3	-	-
	IR84984-83-15-185-B	-	7.2	-	-	-	0.1	-	-
	PMK3/IR84984-83-15-185-B $F_{2:3}$	-	7.4	-	2.0–9.0	-	1.5	-	92.4
Leaf drying	PMK3	-	5.1	-	-	-	0.4	-	-
	IR84984-83-15-185-B	-	6.7	-	-	-	0.2	-	-
	PMK3/IR84984-83-15-1-85-B $F_{2:3}$	-	6.4	-	2.0–9.3	-	1.4	-	93.7
Plant height (cm)	PMK3	70.6	53.5	-	-	3.0	0.8	-	-
	IR84984-83-15-185-B	71.6	60.9	-	-	2.2	0.5	-	-
	PMK3/IR84984-83-15-185-B $F_{2:3}$	75.5	58.1	51.5–98.0	36.0–63.0	7.2	19.1	77.4	10.0
Biomass per plant	PMK3	46.6	16.9	-	-	7.4	2.6	-	-
(g)	IR84984-83-15-185-B	13.0	11.4	-	-	0.4	1.3	-	-
	PMK3/IR84984-83-15-185-B $F_{2:3}$	30.8	16.1	5.0–153.6	0.8–46.0	16.3	6.6	81.9	69.9
Harvest index	PMK3	14.4	4.9	-	-	1.6	1.0	-	-
	IR84984-83-15-185-B	25.6	18.5	-	-	1.5	0.6	-	-
	PMK3/IR84984-83-15-185-B $F_{2:3}$	20.8	12.0	3.0–82.8	0.0–92.6	7.0	8.2	67.7	56.7
Grain yield per plot	PMK3	137.1	16.4	-	-	35.3	4.5	-	-
(g)	IR84984-83-15-185-B	67.6	41.9	-	-	4.8	2.8	-	-
	PMK3/IR84984-83-15-185-B $F_{2:3}$	124.5	36.6	10.0–626.7	0.0–140.0	76.0	21.8	73.7	73.3

'-' refers to not applicable.

(Table 3). This indicated that there were genetic variations for drought response between the parents and among the $F_{2:3}$ progenies. IR84984-83-15-185-B had higher plant height, grain yield and harvest index than PMK3 under water stress conditions in the fields. This reveals the superiority of these IRRI donor lines over PMK3 in drought resistance under field conditions. Some of the $F_{2:3}$ lines produced higher yield than the parents even under this severe water stress (Table 2), which showed that these progenies performed better than the parents under water stress. This may be due to the formation of recombinants by employing best genomic segments of both the parents. Transgressive segregation of traits was observed for several traits and they were approximately fitted to the normal distribution (data not shown here).

The broad sense heritability (H) of grain yield under water stress was 73.3% in PMK3/IR84984-83-15-185-B $F_{2:3}$ lines. Further, days to heading and leaf drying scores recorded the highest H (93.7%), and the lowest H was noticed for plant height (10.0%) under water stress conditions (Table 3).

Even after the severe water stress, several lines of PMK3/IR84984-83-15-185B $F_{2:3}$ lines could produce grains. Significant positive correlations were observed between grain yield per plant and harvest index (r = 0.56**), grain yield per plant and biomass per plant (r = 0.45**), and leaf rolling and leaf drying (r = 0.89**) in PMK3/IR84984-83-15-185B $F_{2:3}$ lines under water

stress conditions (Table 4). However, significant negative correlations were noticed between days to heading with plant height (r = -0.25**), grain yield per plant (r = -0.37**) and harvest index (r = -0.19**) under water stress.

Identification of markers linked to yield under water stress in field conditions

BSA was performed with the SSR markers in the vicinity of *qtl12.1* which was introgressed into the IRRI donor line. Ten drought resistant and 10 drought susceptible rice lines were selected out of 251 $F_{2:3}$ progenies derived from PMK3 and IR84984-83-15-185-B and the selection was based on their yields under a managed field stress condition. Among the 34 SSR markers, 12 were polymorphic between parents (35%) and were further used for screening the bulks.

Table 4. Pearson correlation coefficients among agronomic traits in PMK3/IR84984-83-15-185-B $F_{2:3}$ rice population under water stress conditions.

Trait	LR	LD	PH	DTH	GY/Plant	Biomass/ Plant
LD	0.89**					
PH	-0.05	-0.05				
DTH	0.08	0.02	-0.25**			
GY/Plant	0.02	-0.01	0.01	-0.37**		
Biomass/Plant	-0.10**	-0.15**	0.03	0.02	0.45**	
HI	0.06	0.07	-0.02	-0.19**	0.56**	-0.28**

LR, Leaf rolling; LD, Leaf drying; PH, Plant height, DTH, Days to heading; GY/Plant, Grain yield per plant; HI, Harvest index.
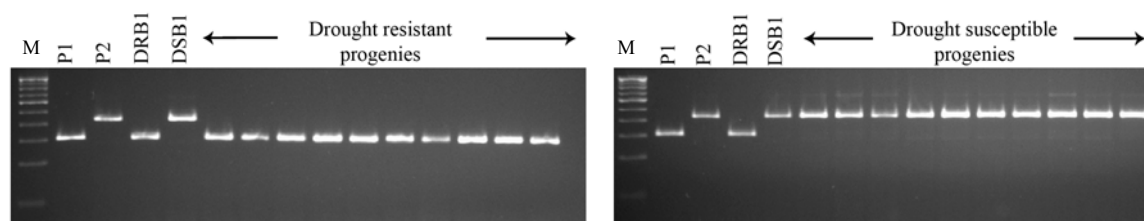**, $P < 0.01$.

Fig. 1. Alleles showing co-segregation of RM27933 among individual F$_{2:3}$ rice lines and bulks.
M, 100 bp ladder; P1, PMK3; P2, IR84984-83-15-185-B; DRB, Drought resistant bulk; DSB, Drought susceptible bulk.

As reported previously (Venuprasad et al, 2009; Boopathi et al, 2011), SSRs are sufficient enough to map the QTLs linked to drought resistance traits.

Four polymorphic primers, RM27920, RM27933, RM27962 and RM27981, were found to be exactly segregating between the bulks DRB and DSB as that of the parents. Hence, all these four primers were used to screen the individual components of DRB and DSB along with their parents. Though all the markers were segregated in the F$_{2:3}$ lines constituting both DRB and DSB, three of them have represented mixed alleles of both the parents. However, RM27933 alleles have segregated perfectly well in individual components of DRB and DSB with respect to the alleles of recurrent parent and donor parent, respectively (Fig. 1).

DISCUSSION

Developing rice cultivars with inherent capacity to withstand drought stress would help to stabilize rice production especially in rainfed ecosystems. Molecular markers help to track the QTLs controlling drought resistance without having to measure the phenotype, thus reducing the need for extensive field testing over space and time (Boopathi et al, 2011). Locating QTLs needs each rice line in a segregating population to be genotyped using numerous molecular markers and phenotyped for the given traits. The mapping population used in this study has shown severe yield reduction under water stress conditions. Similar kind of results has also been noticed in the rice mapping populations (Babu et al, 2003; Venuprasad et al, 2009). Further, as that of this study, significant differences for plant phenology and production under stress and for indicators of plant water stress have been reported among a subset of 100 doubled haploid (DH) rice lines of CT9993/IR62266 (Blum et al, 1999). This elucidated that there might be different genetic mechanisms involved in control of these traits. In addition, similar level of low *H* was found earlier for

spikelet fertility rate under water stress in CT9993/ IR62266 DH line population of rice (Babu et al, 2003) and similar correlations were found by Champoux et al (1995) as that of this study. In general, water stress indicators such as leaf rolling and leaf drying are negatively correlated with plant height, biomass and grain yield under stress. Negative correlations of water stress indicators with biomass and positive correlations of plant production traits were also observed in rice (Babu et al, 2003).

In this investigation, BSA has identified RM27933 as a marker linked to the yield under water stress in field conditions. It is interesting to note that this marker is located in the *qtl12.1* (Bernier et al, 2007). Remarkably, the allele conferring improved drought resistance is contributed by PMK3, the inferior parent, suggesting that its effect is in some way not expressed under that background. Similar kind of contribution of inferior parent (Way Rarem) in yield under water stress was also reported (Bernier et al, 2007). Hence, *qtl12.1* deserves a promising future research on contribution of a poor parent allele in yield enhancement under water-limited environments.

The identified SSR marker, RM27933, linked to grain yield under water stress in the field conditions is consistent with the *qtl12.1* that have been constantly reported previously for the same trait in several environments (Bernier et al, 2008). Identification of common location under different water stress environments confirms the strength of the QTLs *qtl12.1* and it is essential for effective marker-assisted selection. Hence, the marker RM27933, consistently identified across the trials may be useful as a potentially valuable candidate marker for the transfer of these QTLs into elite background through marker-assisted selection.

The markers RM27933–RM28048–RM511 were found from 10 426 617 bp to 13 777 049 bp on rice chromosome 12 and there are 126 putative coding genes. PSI-BLAST analysis of these genes showed that 12 of them have high similarity with proteases,

peptidases, oxidases and enzymes involved in detoxi-fication processes (data not shown here). Approximately 1 500 bp upstream of the above 126 genes were obtained and promoter analysis showed that there were several abiotic stress responsive elements such as *cis*-acting element involved in the abscisic acid response and light response, *cis*-acting regulatory element essential for the anaerobic induction, MYB binding site involved in drought-inducibility, and *cis*-acting element involved in heat stress response (data not shown here). Thus, it is likely that this genomic segment on chromosome 12 may have genes that are involved in increasing grain yield under water stress in the fields. However, detailed investigation is necessary to establish the function(s) and utilize these genes using transgenic or mutagenesis strategies.

We believe that several other factors will greatly affect the efficiency and effectiveness of MAS research. Among them, new types of markers and high-throughput marker techniques should play an important role in the construction of second-generation maps, provided that these methods are not too expensive. Due to the abundance of single nucleotide polymorphisms (SNPs) and the development of sophisticated high-throughput SNP detection systems, it has recently been proposed that SNP markers will have a great influence on future mapping research and MAS studies. Fine mapping of the *qtl12.1* with candidate genes/SNPs may resolve the functional relationship between the QTL and the investigated traits in rice under water stress in the fields and research is progressing in this direction at this laboratory.

ACKNOWLEDGEMENT

REFERENCES

Babu R C, Nguyen B D, Chamarerk V, Shanmugasundaram P, Chezhian P, Jeyaprakash P, Ganesh S K, Palchamy A, Sadasivam S, Sarkarung S, Wade L J, Nguyen H T. 2003. Genetic analysis of drought resistance in rice by molecular markers: Association between secondary traits and field performance. *Crop Sci*, **43**: 1457–1469.

Bernier J, Kumar A, Venuprasad R, Spaner D, Verulkar S, Mandal N P, Sinha P K, Peeraju S, Dongre S R, Mahto R N, Atlin G. 2008. Characterization of the effect of a QTL for drought resistance in rice, *qtl12.1*, over a range of environments in the Philippines and eastern India. *Euphytica*, **166**(2): 207–217.

Bernier J, Kumar A, Ramaiah V, Spaner D, Atlin G. 2007. A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Sci*, **47**: 505–516.

Bernier J, Serraj R, Kumar A, Venuprasad R, Impa S, Oane V R R, Spaner D, Atlin G. 2009. The large-effect drought-resistance QTL *qtl12.1* increases water uptake in upland rice. *Field Crops Res*, **110**(2): 139–146.

Blum A, Mayer J, Golan G, Sinmena B. 1999. Drought tolerance of a doubled-haploid line population of rice in the field. *In*: Ito O. Genetic Improvement of Rice for Water Limited Environments. Los Banos, Philippines: International Rice Research Institute: 253–262.

Boopathi N M, Thiyagu K, Urbi B, Santhoshkumar M, Gopikrishnan A, Aravind S, Swapnashri G, Ravikesavan R. 2011. Marker-assisted breeding as next-generation strategy for genetic improvement of productivity and quality: Can it be realized in cotton? *Int J Plant Genom*, **2011**: 1–16.

Brauer M J, Christianson C M, Pai D A, Dunham M J. 2006. Mapping novel traits by array-assisted bulk segregant analysis in *Saccharomyces cerevisiae. Genetics*, **173**: 1813–1816.

Champoux M C, Wang G, Sarkarang S, Mackill D J, O'Toole J C, Huang N, McCouch S R. 1995. Locating genes associated with root morphology and drought avoidance in rice *via* linkage to molecular markers. *Theor Appl Genet*, **90**: 961–981.

Collard B C Y, Jahufer M Z Z, Brouwer J B, Pang E C K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker assisted selection for crop improvement: The basic concepts. *Euphytica*, **142**: 169–196.

Govindaraj P, Arumugachamy S, Maheshwaran M. 2005. Bulked segregant analysis to detect main effect QTL associated with grain quality parameters in Basmathi 370/ASD16 cross in rice (*Oryza sativa* L.) using SSR markers. *Euphytica*, **144**: 61–68.

Kamoshita A, Babu R C, Boopathi N M, Fukai S. 2008. Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crops Res*, **109**: 1–23.

Li J B, Xia M Y, Wan B L, Du X S, Zha Z P, Yu D Z, Qi H X. 2009. Genetic analysis and mapping of *TWH* gene in rice twisted hull mutant. *Rice Sci*, **16**(1): 79–82.

Lima J M, Dassa A, Sahub S C, Beherab L, Chauhan D K. 2007. A RAPD marker identified a susceptible specific locus for gall midge resistance gene in rice cultivar ARC5984. *Crop Prot*, **26**: 1431–1435.

Selvakumar P, Ravikesavan R, Gopikrishnan A, Thiyagu K, Preetha S, Boopathi N M. 2010. Genetic purity analysis of cotton (*Gossypium* spp.) hybrids using SSR markers. *Seed Sci Technol*, **38**: 358–366.

Venuprasad R, Dalid C O, Del Valle M, Zhao D, Espiritu M, Sta Cruz M T, Amante M, Kumar A, Atlin G N. 2009. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theor Appl Genet*, **120**: 177–190.

Yang Q, Lin F, Wang L, Pan Q. 2009. Identification and mapping of *Pi41*, a major gene conferring resistance to rice blast in the *Oryza sativa* subsp. *indica* reference cultivar, 93-11. *Theor Appl Genet,* **118**: 1027–1034.

Zhang G L, Chen L Y, Xiao G Y, Xiao Y H, Chen X B, Zhang S T. 2009. Bulked segregant analysis to detect QTL related to heat tolerance in rice (*Oryza sativa* L.) using SSR markers. *Agric Sci China*, **8**(4): 482–487.