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QTL mapping for traits at reproductive stage drought stress in rice using single marker analysis

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ABSTRACT

Drought is the major abiotic constraint leading to very high yield reduction in both rain-fed upland and lowland areas worldwide. Reproductive stage is the most sensitive to water deficiency as compared to vegetative stage of rice. In this present investigation, association between phenotype and genotype was carried out for the drought mapping population of CR143-2-2/ Krishnahamsa to detect QTLs through single marker analysis (SMA). A total of 21 polymorphic SSR markers were detected through bulked segeregant analysis approach. QTL mapping was performed through ICIM v4.0 software. Only phenotypic trait, days to 50% flowering (DFF) at reproductive stage stress showed association with chromosome 1 and 6. A significant higher peak was obtained in RM3825 marker linked in chromosome 1 detected to be a novel QTL, qDFF1.1 controlling flowering under drought stress. The other QTL on chromosome 6 is validated in this mapping population which controls flowering under terminal drought stress.

Key words: *Single marker analysis, bulked segeregant analysis, genetic variation, morpho-physiologic traits, reproductive stage drought stress*

INTRODUCTION

A major yield loss is being incurred in rainfed rice due to drought stress. The drought stress is responsible for the yield reduction of 44-71% worldwide (Pandey and Bhandari, 2009). Major effect of drought was observed mostly over the year in rainfed upland and low-land areas (Hijmas and Serraj, 2009). As rice is a staple food in the world, food security and lively hood activities were fully dependent on at least 70% of world population. Therefore, development of drought tolerant varieties is the most important task for the rice breeders. In most of the growth stages, drought affects rice plant growth and its production. Early season drought occurs at the vegetative stage of growth and it affects plant morphologies like leaf growth and stem elongation. Intermittent drought occurring in between the rainfall intervals which affects the developmental stage like root growth and architecture. But the most effective role plays by terminal drought which occurs at the end of the growing period particularly during the flowering stage, affecting grain filling, spikelet fertility and yield. So developing drought resistant cultivars especially with good performance under late season drought stress is one of the major challenges in rice breeding programs. Previous studies, a lot of experiments had been conducted on vegetative stage drought tolerance. But, very less information are available on reproductive stage drought tolerance. Efforts were made to incorporate the mechanisms like drought escape, drought avoidance and drought tolerance for developing drought tolerant rice varieties using both conventional and molecular breeding approaches (Basu et al., 2016). But parameter like drought avoidance by delaying the flowering time does not help the crop to minimize the losses due to water scarcity.

The analysis of association between phenotype and genotype in a population to detect QTLs through single marker analysis (SMA) was explained by Luo and Kearsey (1989). The simplest method for QTL mapping is single marker analysis, includes t-test,

ANOVA and simple linear regression, which asses the segregation of a phenotype with respect to a marker genotype (Soller et al., 1976; Nienhuis et al., 1987; Wang et al., 1994). A significant difference indicates that marker is linked to a QTL. This approach can indicate which markers linked to potential QTLs are significantly associated with quantitative trait investigated.

Therefore, the present study was conducted to screen out the interactions that perform more in reproductive stage drought stress and to find out all the effective morpho-physiological parameters. The results obtained from the experiment can be directly applicable in improving drought tolerance in rice. Correlating genetic information with physiological and morphological traits related to drought tolerance will allow the development of rice varieties tolerant to drought stress.

MATERIALS AND METHODS

Plant materials

The bi-parental mapping population of CR143-2-2/ Krishnahamsa comprising 190 recombinant inbred lines along with the parents were taken as the experimental material for the analysis. The experiment was conducted in the controlled facility of rain-out shelter of ICAR-National Rice Research Institute, Cuttack, Odisha during *kharif* season, 2014. The F_{7-8} generation recombinant inbred lines were subjected to phenotyping followed by genotyping.

Phenotyping for various morpho-physiological traits

Phenotyping for reproductive stage drought tolerance was conducted under control facility of rain-out shelter at ICAR-National Rice Research Institute, Cuttack during *kharif*, season 2014. The experiment was conducted by taking two parents CR143-2-2 and Krishnahamsa along with 190 RIL populations for twenty-two morpho-physiological traits *viz.,* days to 50% flowering, plant height, panicle length, canopy temperature, panicle emergence, relative chlorophyll content, leaf length, leaf width, leaf rolling, leaf drying, percentage of spikelet fertility, grain yield, biomass, harvest index, thousand seed weight, chlorophyll a, chlorophyll b, chlorophyll a+b, chlorophyll a/b, cell membrane stability, relative water content and proline content. All the pre-harvested data were collected during growth stage 6-9 (SES, 2014). Data for morphological trait like, leaf drying, leaf rolling and panicle emergence were recorded by standard evaluation system of rice (SES) scoring method developed by IRRI. Relative chlorophyll content was recorded by the instrument SPAD-502 plus chlorophyll meter from the widest part of the leaf in optimum day condition. Whereas canopy temperature was measured by the instrument VarioCAM, Infrared-Thermal Imager from portion of the plant exposed above the ground level and expose to sunlight. While post-harvested data were collected after growth stage 9. After maturation, 10 hill samples per each RIL lines were collected for post harvested traits like grain yield, biomass, harvest index and spikelet fertility. Separate samples were collected for physiological traits *viz.,* chlorophyll a, chlorophyll b, chlorophyll a+b, chlorophyll a/b, cell membrane stability, relative water content and proline content for wet lab experiment during mid-day situation within the growth stage 7-8 (SES, 2014).

Bulk segregant analysis using SSR primers

Bulked segregant analysis (BSA) was used to detect the major QTLs (Zhang et al., 2009). As per the protocol, two extreme gene pools of recombinant inbred line population with each bulk having 10 lines of DNA were selected. These selected lines were then bulked to detect the molecular polymorphism using SSR markers. The genomic DNA of the bulk genotypes were isolated by the following protocol described by Murray and Thomson (1980). The concentration of DNA was estimated by UV spectrophotometer. Quantification of DNA was accomplished by using UV-VIS spectrophotometer (UV-1201Schimazu Corp; Japan) and by analyzing the purified DNA on 0.8% agarose gels and using a double digest EcoRI, HindIII double digested of λ DNA as molecular weight marker. Approximate concentration of DNA 100ng/µl was used for PCR analysis. 201 linked SSR markers selected from different sources were collected (Table 1) and subjected to polymorphic analysis. The PCR reaction mixture contained 50ng template DNA, 5picoM of each of forward and reverse primers, 200µM dNTPs, 10X PCR buffer (10mM Tris-HCl, pH8.3, 2mM of $MgCl_2$ and 1U Taq (Thermus aquaticus) DNA Polymerase (Genaid). Amplification cycling was performed in a 96

Table 1. List of polymorphic SSR primers identified from BSA.

Primers Name	Chrom#	Position	Heterozygote	% of heterozygosis	% of segregation	
					Tolerant	Susceptible
RM495	1	2.8	3	0.01563	52.60	48.96
RM6703	1	139.1	4	0.02083	35.42	66.67
RM3825	1	143.7	0	0	29.69	70.31
RM327	2 ($qDTY_{2.1}$)	72.6	1	0.00521	45.31	55.21
RM341	2 ($qDTY_{2.1}$)	94.4	4	0.02083	24.48	77.60
RM263	2 ($qDTHI_{2.3}$)	127.5	8	0.04167	59.90	44.27
RM22	3 ($qDTY_{3.2}$)	7.2	14	0.07292	44.27	63.02
RM517	3 ($qDTY_{3.2}$)	30.3	9	0.04688	28.65	76.04
RM527	6 ($qDTY_{6.2}$)	61.2	2	0.01042	56.77	44.27
RM3	6 ($qDTY_{6.2}$)	92.4	0	0	52.60	47.40
RM337	8 ($MQTL_{8.1}$)	0.1	4	0.02083	42.71	59.38
RM72	8 ($qDTF_{8.1}$)	60.9	0	0	68.75	31.25
RM316	9	1.8	1	0.00521	7.29	93.23
RM257	9	79.7	0	0	52.08	47.92
RM271	10 ($qDTF_{10.1}$)	59.4	2	0.01042	29.17	71.88
RM171	10 ($qDTY_{10.1}$)	92.8	11	0.05729	79.17	26.56
RM484	10	102.9	3	0.01563	55.21	46.35
RM20A	12	0	0	0	50.52	49.48
RM511	12 ($qDTY_{12.1A}$)	59.8	1	0.00521	87.50	13.02
RM309	12 ($qDTY_{12.1}$)	74.5	5	0.02604	51.56	51.04
RM519	12 ($qDTY_{12.1}$)	94.8	2	0.01042	58.33	42.71

place programmable Thermal cycler (Applied Biosystems, California, United States) under following conditions. For PCR amplification, the reaction mixture was initially denatured for 4 min at 94ºC, then subjected to 35 cycles of 30 seconds denaturation at 94ºC , 1min annealing at 55^{0}C (Table 1) and 1.30 min extension at 72^{0}C; and a final extension for 10 min at 72^{0}C and hold at 4^{0}C. After the completion of the PCR, the products were stored at -20°C until the gel electrophoresis is done.

Statistical analysis

From the phenotypic value of *kharif*, 2014 generation, 190 recombinant lines and their parents were used for the analysis of variance, standard deviation, standard error of mean, skewness and kurtosis to determine the main effect of RILs with the relative traits by using software SPSS (Version 20.0, Chicago, USA). Normal distributions of all phenotypic traits were analysed and represented using the software SPSS v20.0. The effect of QTLs and their relation with phenotypic and molecular proportion was analysed using single marker analysis method using software ICIM v4.0 (Wang et al., 2014)

RESULTS AND DISCUSSION

Phenotypic variation of morpho-physiological traits

Availability of adequate genetic variations in a breeding population leads to effective selection and development of desired product. A mapping population with high genetic variation for traits within the population will give accurate mapping results. The analysis of the mean values of the traits obtained from RILs and parents showed significant differences among the traits under drought stress condition (Table 2). Morph-physiological traits like biomass (0.756), grain yield (0.746), leaf drying (2.758), leaf rolling (4.392), panicle length (1.93), relative water content (252.6), cell membrane stability (308.6) and proline content (3066.99) showed high phenotypic variance value (Table 2). A high range and high phenotypic coefficient and genotypic coefficient of variation were estimated for these traits under reproductive stage drought stress (Table 2). A wide variation for these traits was also reported earlier. A trend of low estimate values for these traits were obtained from the susceptible lines while a higher values were observed from tolerant lines. Therefore, the traits like biomass, grain yield, leaf drying, leaf rolling, panicle length, relative water content, cell membrane stability and proline content may be taken care during selection of drought tolerant lines.

Table 2. Genetic parameters and distribution of recombinant inbred lines used for marker analysis.

Sl. No.	Phenotypic Trait	Mean	Range	PCV	GCV	H2	Skewness	Kurtosis
1	Biomass	6.776	5.0021	34.072	3.274	0.0094	0.786	1.405
2	Harvest index	0.395	.083	1.205	1.164	0.9339	-2.3	0.71
3	Grain yield	2.754	5.0	34.215	2.461	0.0061	0.784	1.376
4	Days to 50% flowering	73.35	26.0	8.3778	8.075	0.9291	0.545	-.294
5	Leaf drying	1.57	7.0	86.285	84.827	0.7960	1.802	2.43
6	Leaf rolling	2.8	9.0	57.252	60.586	0.8348	0.918	0.122
7	Plant height	83.955	86.33	11.157	10.844	0.7342	0.008	0.156
8	1000 seed weight	20.25	27.85	14.758	17.651	0.9897	-0.797	1.978
9	% of spikelet fertility	74.024	67.6712	12.034	4.639	0.1355	-0.780	0.242
10	Canopy temperature	31.54	6.440	4.324	2.614	0.4607	0.053	0.867
11	Panicle emergence	95.63	25.0	3.196	1.970	0.2205	-1.254	3.546
12	Relative chlorophyll content	30.61	22.33	11.424	6.511	0.2091	0.542	1.602
13	Leaf length	31.61	39.0	15.421	6.387	0.2351	0.272	-0.477
14	Leaf width	0.9575	.800	18.457	6.486	0.1998	0.157	-0.123
15	Panicle length	20.12	13.0	10.841	5.640	0.3145	0.014	0.005
16	Chlorophyll a	2.43	3.7188	25.178	21.257	0.8875	-0.009	-0.994
17	Chlorophyll b	0.55	2.3806	44.521	43.514	0.9634	2.13	8.376
18	Chlorophyll a/b	5.18	23.0906	34.976	26.928	0.6741	2.313	8.468
19	Chlorophyll a+b	2.987	4.4675	23.171	21.836	0.9211	0.126	-0.852
20	Cell membrane stability	63.42	87.2318	18.588	16.777	0.8773	-0.783	0.4
21	Relative water content	75.52	89.362	17.198	15.896	0.9717	0.175	1.18
22	Proline content	74.88	221.2	64.903	62.989	0.9495	1.145	0.228

Results of Yue et al. (2006) emphasized the reduction in biomass under drought stress and played a key role in yield reduction. Balan et al. (2000) explains about the genetic variability and correlation for leaf drying and leaf rolling traits in upland drought stress. Variability and their association with drought stress of the traits like relative water content, leaf rolling, and leaf drying were reported by Babu et al. (2003). Cell membranes are one of the first targets and it is generally accepted that the maintenance of their integrity and stability under water stress conditions is a major component of drought tolerance in rice (Bajji et al., 2001). In drought stress condition, particularly at reproductive stage, proline content is greatly influence the performance under drought (Kumar et al., 2014).

Transgressive segregation was observed for the studied traits. A negatively skewed leptokurtic distribution of RILs was observed for harvest index, thousand grain weight, percentage of spikelet fertility, panicle emergence and cell membrane stability under drought stress condition whereas Chlorophyll a is the only trait which showed platykurtic distribution. Similarly positively skewed platykurtic distribution of RILs seen in days to 50% flowering, leaf length, leaf width and total chlorophyll content whereas positively skewed leptokurtic distribution observed in biomass, leaf rolling, leaf drying, plant height, grain yield, canopy temperature, relative chlorophyll content, panicle length, chlorophyll a, chlorophyll a/b, relative water content and proline content traits.

QTL analysis

In the present study, 201 rice microsatellite markers were used to study the parental polymorphism between two distinctly different rice varieties (CR143-2-2 and Krishnahamsa). Amongst these primers, 77 were polymorphic obtained from the survey of parental polymorphism were further subjected to bulked segregant analysis survey. Screening of the bulks with these 77 polymorphic markers resulted 21 SSR markers (Table 1), which were used in the single marker analysis study.

By analyzing the markers and phenotypic traits individually through Ici-Mapping, only phenotypic trait, days to 50% flowering (DFF) at reproductive stage stress showed association with chromosome 1 and 6.

Table 3. Significant markers associated with traits in single marker analysis.

Trait ID	Trait Name	Chromosome	Position	Marker Name	LOD	PVE (%)	Add
9	DFF	1	143.7	RM3825	3.839	8.7969	-1.97
9	DFF	6	61.2	RM527	2.0366	4.7676	-1.334

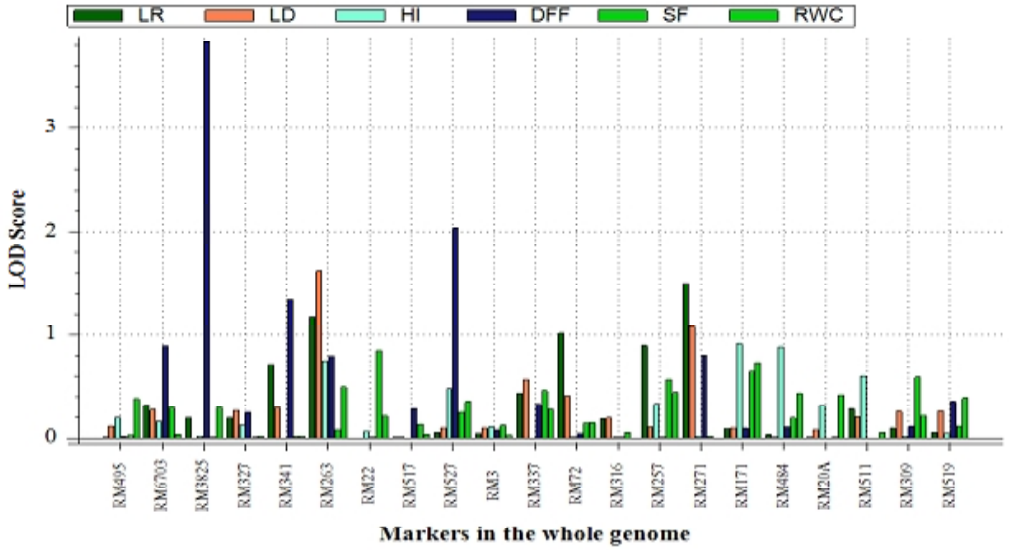

Fig. 1. Single marker analysis indicating the peak value of linked marker using *kharif*, 2014 phenotypic data.

In chromosome 1, the DFF was linked at 143.7cM position with primer RM3825 whereas in chromosome 6 the linkage was at 61.2cM position with primer RM527. The LOD value and PVE (%) were 3.84 and 8.80, respectively for marker detected in chromosome 1 with low additive effect of -1.97 (Figure 2). Similarly in chromosome 6 the peak detected with LOD value and PVE (%) of 2.04 and 4.77, respectively. Also, an additive effect of -1.33 was found for DFF trait linked in chromosome 6 (Table 3). A significant and higher peak is found in case of RM3825 marker linked with DFF trait in chromosome 1 (Fig. 1).

Previous publications on markers tightly linked to genes were found by using BSA (Xu et al., 1995; Mackay and Caligari, 2000; Zheng et al., 2002; Altinkue et al., 2003; Podlich et al., 2004; Govindaraj et al., 2005). BSA was firstly reported by Paran et al. (1991) to identify RAPD markers tightly linked to genes for resistance to lettuce downy mildew. Steele et al. (2006) conducted a marker-assisted back-crossing (MABC) breeding programme and found chromosome 7 responsible for delayed flowering. Kamoshita et al. (2008) identified four key genomic regions on chromosomes 1, 4, 8, and 9 which were co-located a number of QTLs including days to 50% flowering considered to be directly or indirectly responsible for grain yield under stress. However, our mapping results for DFF under reproductive stage drought stress showed the location of the QTL at 143.7cM which is quite away in the chromosome1, reported by Kamoshita et al. (2008). The identified QTL is designated as *qDFF1.1*. Lang and Buu (2010) used SSR marker combined with selective genotyping to map quantitative trait loci (QTLs) associated with drought tolerance in rice by considering the traits like drought at flowering (DRF), root dry weigh (RDW), and root length (RL) for the chromosomes 2, 3, 4, 8, 9, 10 and 12. MQTLs 3.1 identified for phenotypic trait days to flowering identified by Sellamuthu (2011) for drought stress and non-stress conditions. Suji et al. (2011) detected QTL region, RM204-RM197-RM217 on chromosome 6 linked to days to 50% flowering and grain yield per plant under both rain-fed and irrigated conditions. Our detected QTL on chromosome 6 for DFF under stress is located almost at equal distance as reported by Suji et al. (2011). Hence, the QTL reported earlier is validated in this mapping population which may be reliably useful in drought breeding program. Yadaw et al. (2013) also identified a large effect QTL, *qDTY3.2*, in co-localizing with the HD9 locus related to flowering time.

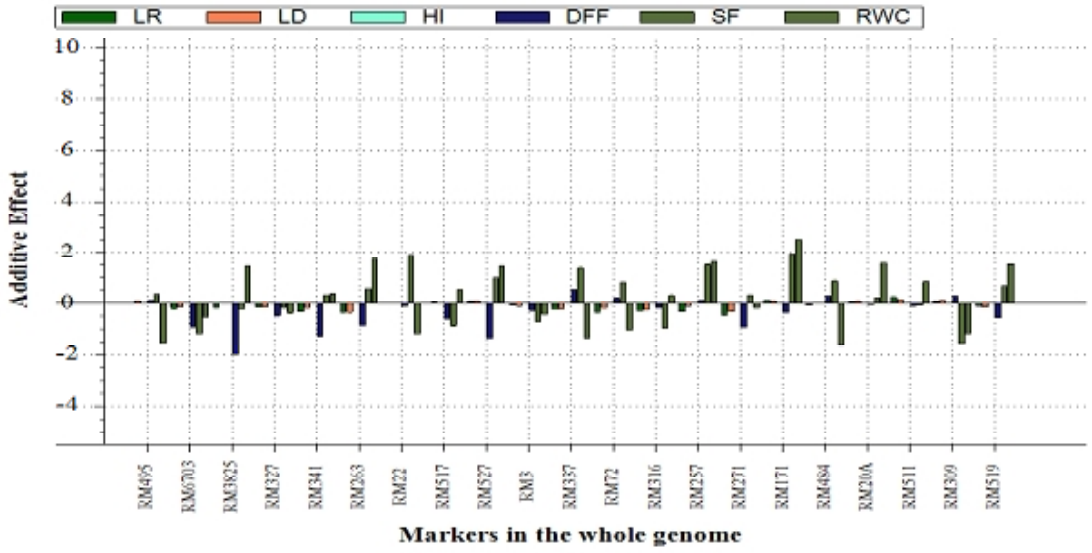

Fig. 2. Single marker analysis indicating the additive effects of the linked marker using *Kharif*, 2014 phenotypic data.

CONCLUSION

The phenotypic variation observed for various traits at reproductive stage drought stress was considerably adequate to study the QTL analysis for various morpho-physiological traits. Very high phenotypic, genotypic variance and heritability estimates were detected under drought stress for the traits like biomass, grain yield, leaf drying, leaf rolling, panicle length, relative water content, cell membrane stability and proline content which might be useful for selecting drought tolerant lines at reproductive stage. The phenotypic trait, days to 50% flowering was detected to be associated with reproductive stage drought tolerance in chromosome 1 and 6 using single marker analysis. The QTL located on chromosome1 at 143.7cM is detected to be a novel QTL controlling days to 50% flowering under reproductive stage drought tolerance.

REFERENCES

Altinkue A, Kazan K and Gozukirmizi N (2003). AFLP marker linked to water-stress-tolerant bulks in barley (*Hordeum vulgare* L.). Genetics and Molecular Biology 26: 77-81

Babu CR, Nguyen BD, Chamarerk V, Shanmugasundaram P, Chezhian P, Juyaprakash P, Ganesh SK, Palchamy A, Sadasivam S, Sarkarung S et al. (2003). Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. Crop Science 43: 1457-1469

Bajji M, Kinet JM and Lutts S (2001). The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regulation pp. 1-10

Balan A, Muthiah AR and Boopalthi SNMR (2000). Genetic variability, correlation and path coefficient analysis in upland early rice genotypes. Journal Asian Research 3: 87-93

Basu S, Ramegowda V, Kumar A and Pereira A (2016). Plant adaptation to drought stress. Version1.F1000Res5:F1000.FacultyRev.https://doi.org/ 10.12688/f1000 research. 7678.1

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

Hijmas RJ and Serraj R (2009). Modeling spatial and temporal variation of drought in rice production. In: Serraj R, Ben-nett J, Hardy B (eds) Drought frontiers in rice crop improvement for improved rainfed production. World Scientific Publishing, IRRI, Singapore pp. 19-31

Kamoshita A, Babu RC, Boopathi NM and FukaI S (2008). Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rain-fed environments. Field Crops Research 109: 1-23

Kumar A, Dixit A, Ram T, Yadaw RB, Mishra KK and Mandal NP (2014). Breeding high-yielding drought-tolerant

rice: genetic variations and conventional and molecular approaches. Journal Experimental Botany 65(21): 6265-6278

Lang NT and Buu BC (2008). Fine mapping for drought tolerance in rice (*Oryza sativa* L,). Omon rice 16: 9-15

Luo ZW and Kearsey MJ (1989). Maximum likelihood estimation of linkage between a marker gene and a quantitative locus. Heredity 63: 401-408

Mackay IJ and Caligari PDS (2000). Efficiencies in F_2 and backcross generations for bulked segregant analysis using dominant markers. Crop Science 40: 626-630

Murray MG and Thompson WF (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acid Research 8: 4321-4326

Nienhuis J, Sills GR, Martin B and King G (1994). Variance for Water Use efficiency among ecotypes and recombinant inbred lines of *Arabidopsis thaliana* (Brassicaceae). American Journal of Botany 81(8): 943-947

Pandey S and Bhandari H (2009). Drought: economic costs and research implications. In: Serraj R, Bennett J, Hardy B (eds) Drought frontiers in rice -crop improvement for improved rain-fed production. World Scientific Publishing, IRRI, Singapore pp. 3-17

Paran I, Kesseli R and Michelmore R (1991). Identification of restriction fragment length polymorphism and random amplified Polymorphic DNA markers linked to downy mildew resistance genes in lettuce using near isogenic lines. Genome 34: 1021:1027

Podlich DW, Winkler CR and Cooper M (2004). Mapping as you go: An effective approach for marker-assisted selection of complex traits. Crop Science 44: 1560-1571

Sellamuthu R, Liu GF, Ranganathan CB and Serraj R (2011). Genetic analysis and validation of quantitative trait loci associated with reproductive-growth traits and grain yield under drought stress in a doubled haploid line population of rice (*Oryza sativa* L.). Field Crops Research 124(1): 46- 58

Soller M, Brody T and Genizi A (1976). On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theoretical and Applied Genetics 47(1): 35-39

Standard evaluation system for rice (2014). International rice research institute (IRRI), Philippians

Steele KA, Price AH, Shashidhar HE and Witcombe JR (2006). Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. Theoretical Applied Genetics 112: 208-221

Suji KK, Biji KR, Poornima R, Silvas K, Prince J, Amudha K, Kavitha S, Mankar S and Babu RC (2011). Mapping QTLs for plant phenology and production traits using indica rice (*Oryza sativa* L.) lines adapted to rain-fed environment. Molecular Biotechnology 52(2): 151-160

Wang J, Li H, Zhang L and Meng L (2014). Users' Manual of QTL Ici-Mapping. The Quantitative Genetics Group, Institute of Crop Science , Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China, and Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico

Wang Z, Weber JL, Zhong Z and Tanksley SD (1994). Survey of plant short tandem DNA repeats. Theoretical Applied Genetics 88: 1-12

Xu BY, Shen ZT and Chen Y (1995). Molecular mapping for quantitative trait loci controlling rice yield. Acta Genetica Sinica 22: 46-52

Yadaw RB, Dixit S, Raman A, Mishra KK, Vikram P, Swamy BPM, Cruz MTS, Maturan PT, Pandey M, and Kumar A (2013). A QTL for high grain yield under lowland drought in the background of popular rice variety Sabitri from Nepal. Field Crop Research 144: 281-287

Yue B, Xue W, Xiong L, Yu X, Luo L, Cui K, Jin D, Xing Y and Zhang Q (2006). Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. Genetics 172(2): 1213-28

Zhang GL, Chen LY, Xiao GY, XiaoYH, ChenXB and Zhang ST (2009). Bulked segregant analysis to detect QTL related to heat tolerance in rice (*Oryza sativa* L.) using SSR markers. Agricultural Science in China 8: 482-487

Zheng XY, Wang YJ, Song SH, Li L and Yu SC (2002). Identification of heat tolerance linked molecular markers of Chinese cabbage Brassica campestris ssp. pekinensis. Agricultural Sciences in China 1: 309-313