Mapping QTLs for Physiological Traits Associated with Drought Tolerance in Rice (*Oryza Sativa* L.)

Abstract

The present investigation mainly focused to study the physiological and molecular characterization of 101 Backcross Inbred Lines (BILs) of rice (BC1F5) for drought tolerance derived from the cross combination of Norungan // TKM9/ Norungan. The traits like photosynthetic rate, transpiration rate and stomatal conductance were observed using infrared gas analyzer (IRGA) and the population was phenotyped for other traits. Estimation of genetic parameters revealed that, among the physiological traits observed, the trait transpiration rate recorded higher value of heritability (0.28) and genetic advance (17.49). Association analysis recorded that, all the physiological traits studied under severe water deficit condition had high and significant positive correlation with plot yield. The trait photosynthetic rate had exhibited significant positive correlation with transpiration rate and stomatal conductance but recorded negative correlation with relative water content (RWC). Single marker analysis was performed with the genetic data and the trait mean values observed under stress condition. Among 167 SSR (microsatellite) markers surveyed, around 15 markers were identified and found to be linked to at least one of the investigated trait and many markers were reported to be linked with more than one trait. Characterization of Quantitative trait loci (QTLs) for drought tolerance in this study provides insight into an understanding of the mode of drought tolerance in rice and to perform MAS (Marker aided selection) aimed at the efficient pyramiding of favorable QTL alleles to improve drought tolerance in elite background of popular rice varieties.

Keywords: Drought; Backcross inbred lines; Infra red gas analyzer; Rice; Microsatellites; Quantitative trait loci (QTLs)

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Abbreviations: RWC: Relative Water Content; BILs: Backcross Inbred Lines; QTL: Quantitative Trait Loci; ROS: Rain Out Shelter; IRGA: Infrared Gas Analyser; MABC: Marker Assisted Backcrossing; MAS: Marker Aided Selection

Introduction

Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world [1]. According to FAO statistics, the percentage of drought affected land areas more than doubled from the 1970s to the early 2000s in the world [2]. Drought is a world-spread problem seriously influencing grain production and quality and with increasing population and global climate change the situation is becoming more serious [3]. Rice (*Oryza sativa* L.) is particularly susceptible to water stress [4,5]. It is estimated that 50% of the world rice production is affected more or less by drought [6].

To improve crop productivity, it is necessary to understand the mechanism of plant responses to drought conditions with the ultimate goal of improving crop performance in the vast areas of the world where rainfall is limiting or unreliable. In addition to the complexity of drought itself [1], whole plant responses to drought are complex as different mechanisms are adopted when drought is encountered [7]. Developing rice cultivars with inbuilt drought tolerance mechanism(s) will increase rice production under rainfed environments. However, conventional breeding has met with little success due to complexity of stress tolerance mechanisms and low heritability of yield under stress. Putative traits conferring drought tolerance in rice have been proposed, but incorporation of these traits in breeding programme is limited due to difficulty in screening large germplasm for these secondary traits [8]. Many studies reported various strategies, including the measurement of net photosynthetic rate, leaf water potential, water use efficiency, osmotic adjustment, stomatal tolerance, strength and distribution of root systems and the level of leaf rolling under drought stress [9,10], to evaluate drought tolerance under water stress conditions. Until recently, lack of concept, direction, and protocol has remained as a significant obstacle to genetic improvement of drought tolerance [11].

Drought tolerance is a complex trait, and a number of quantitative trait loci (QTL) for drought tolerance in rice have been identified [12]. Because of the complexity of drought stress and the range of conditions to which rainfed rice crops are exposed, a multifaceted breeding strategy including marker-assisted approaches is necessary to manipulate drought tolerance in rice [13]. In the past decade, the availability of molecular markers coupled with precision phenotyping has led to the identification of

several quantitative trait loci (QTLs) for grain yield under drought qDTYs from Apo, Adaysel, N22 and Wayrarem [14]. Most of these QTLs have been discovered through single marker analysis and fine mapping of these QTLs and identifying candidate genes underlying these QTLs will help in deployment of these QTLs in breeding programs. Selecting rice for drought tolerance based on unknown genetic mechanisms will result in inefficient genetic improvement. Molecular tools facilitate the identification and genomic localization of genes controlling traits related to drought tolerance using quantitative trait loci (QTL) analysis.

Materials and Methods

The present study involved in phenotyping of different physiological traits and genotyping of an indigenously developed advanced backcross population of rice for drought tolerance. Backcross Inbred Lines (BILs) were developed using Norungan and TKM9 as the parents. TKM9 was crossed with Norungan and F_1 was backcrossed to Norungan once. BC_1F_1 of Norungan / TKM9// Norungan were selfed and a large population of BC_1F_2 was selected upon for dwarf/semi-dwarf plants to improve the agronomy of the population. Selected BC_1F_2 progenies were selfed further and a set of 289 BILs were developed in BC_1F_4 generation. The population was screened under irrigated condition during Kharif 2009 and a total of 101 lines which yielded significantly higher than population mean were chosen for further study in BC_1F_5 generation.

Phenotyping of physiological traits in BILs

The BILS along with the parents were phenotyped for various physiological attributes under severe water stress (SS) condition under Rain out Shelter (ROS) at Tamil Nadu Agricultural University, Coimbatore. Plant samples were taken at both vegetative and maturity stages for recording various physiological characters under severe moisture stress conditions. Physiological traits were estimated with the help of Infrared gas analyzer (IRGA) and estimation of relative water content (RWC) was based on the method of Barrs et al. [15] under stress condition in ROS. The correlations were computed by using the physiological traits in stress condition for yield traits and also between derived traits.

Measurement by infrared gas analyzer (IRGA)

Infrared Gas Analyzer (IRGA) is a portable photosynthetic system (LICOR- Model LI 6400 version.5) and major component used for the measurement of different parameters like photosynthetic rate, stomatal conductance and transpiration rate. The parameters measured by Infrared Gas Analyzer (IRGA) and their units are Photosynthetic rate (A, μ mol CO_2 m^{-2} s^{-1}), Stomatal Conductance (gs, mol H_2O m^{-2} s^{-1}) and Transpiration rate (E, mmol H_2O m^{-2} s^{-1}).

Estimation of relative water content (RWC)

Relative water content is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit while water potential as an estimate of plant water status is useful in dealing with water transport in the soil-plant-atmospheric continuum. The basic principle [15] of this technique consists essentially in comparing the water content of leaf tissue when fresh leaf sampled with the fully turgid water content and expressing the results on percentage basis.

Relative water content was estimated by Weatherley method and expressed in percentage. Leaf RWC was calculated using the formula presented below

RWC = (Fresh weight –Dry weight / Turgid weight-Dry weight) X 100

Genotyping of BILs

The DNA was extracted from the leaf samples of parents and BILs using liquid nitrogen and CTAB method [16] with suitable modifications [17]. The nucleic acids were quantified by the methods of Spectrophotometric determination and gel electrophoresis (0.8% agarose gel). DNA concentration for PCR amplification was estimated by comparing the band intensity of a sample with the band intensities of known dilutions that have good amplification. The dilutions were carried out by dissolving the genomic DNA in appropriate volume of TE buffer. The population (BILs) was genotyped using 167 SSR (Simple Sequence Repeats) primer pairs for amplifying genomic DNA fragment by polymerase chain reaction. The amplification was carried out in an Eppendorf master cycler provided with gradient option. The procedure includes initial denaturation at 94 degree centigrade for 5 minutes, followed by denaturation at 94 degree centigrade for 1 minute, annealing at 55 degree centigrade for 1 minute, extension at 72 degree centigrade for 2 minutes and final extension at 72 degree centigrade for 5 minutes. The resultant PCR products of SSR primer pairs were resolved by agarose gel electrophoresis on 1.5% to 3% agarose gel to understand the difference in molecular weight of the products. Bands were visualized by staining the gels with ethidium bromide and photographed under UV light using Alpha Imager 2200.

Single marker analysis

Single marker analysis was carried out using the software, QTL CARTOGRAPHER v2.5. This analysis fitted into the linear regression model: y = b0 + b1 x + e.

The results were obtained as the estimates for b0, b1 and the F statistic for each marker. Since we are interested in whether the marker is linked to a QTL for a trait we tested the hypothesis if b1 is significantly different from zero. The F statistic compares the hypothesis H0: b1 = 0 to an alternative H1: b1 not 0. The pr (F) is a measure of how much support there is for H0. A smaller pr (F) indicates less support for H0 and thus more support for H1. Likelihood ratio test statistic compared two nested hypotheses and was two times the negative natural log of the ratio of the likelihoods. For example, assume that hypothesis H0 is nested within H1 and that they have likelihoods L0 and L1 respectively. Then, the "Likelihood Ratio Test Statistic" is -2ln (L0/L1).

Results and Discussion

Variation in physiological traits of BILs

The experiment under rain out shelter was conducted to impose severe drought stress on the plants, thereby enabling to observe significant variation among the BILs for all investigated

component traits, which indicated the presence of genetic variation for drought response among the parents and BILs in the study. Among the physiological traits studied under severe stress condition, the trait relative water content (RWC) had recorded the highest phenotypic and genotypic variance of 45.52 and 19.03 which was followed by photosynthetic rate has the phenotypic and genotypic variance of 10.54 and 2.40 respectively. The trait stomatal conductance had registered the lowest phenotypic and genotypic variance of around 0.02 and 0.002 respectively. The trait plot yield had recorded the highest phenotypic and genotypic variance (2197.05 and 1468.58) (Table 1). These findings are similar to the assumption that traits are much significantly influenced by the environmental factors under moisture stress condition [18].

Heritability is the ability of the characters to inherit into the subsequent generation. Among the physiological traits, the trait relative water content had recorded the highest heritability of 0.42 with the genetic advance as percentage of mean of 7.04 whereas the trait transpiration rate had registered a heritability of 0.28 and genetic advance of 17.49 which was higher when compared to other physiological traits. The trait plot yield had recorded the highest heritability value of 0.67 and genetic advance of 54.82 followed by the trait harvest index (0.60 and 46.26) [19,20](Table 1).

Table 1: Estimation of genetic parameters for different physiological traits under severe stress condition.

Traits	TKM9	Norungan	BILs			V_g	V_p	GCV	PCV	h^2	GA% Mean
			Mean	Range	CD%						
Photosynthetic rate (PR)	18.52	24.83	21.83	16.88-32.69	4.61	2.4	10.54	7.09	14.85	0.23	6.97
Transpiration rate (TR)	4.58	6.74	5.64	3.37 - 9.90	2.27	0.8	2.82	15.91	29.84	0.28	17.49
Stomatal conductance (SC)	0.31	0.56	0.38	0.21-0.69	0.23	0.002	0.02	10.33	37.6	0.07	5.85
Relative water content (RWC)	76	89.86	82.49	69.22-94.42	8.28	19.03	45.52	5.29	8.18	0.42	7.04
Harvest index (HI)	13.99	32.49	24.59	5.39- 48.16	9.22	50.58	83.69	28.89	37.16	0.6	46.26
Plot yield (PY)	65	148.33	117.48	23.3-221.67	43.1	1468.58	2197.05	32.55	39.81	0.67	54.82

Trait associations

Correlation study was made to establish the extent of association between yield and other component traits in rice [21]. These studies help the plant breeder to understand the relationship among the component traits of yield. Correlating genetic information with physiological traits related to drought tolerance will allow the development of rice cultivars tolerance to drought through indirect selection.

Genotypic correlation for physiological traits associated with drought tolerance was studied under severe water stress regime to study the relationships between the physiological traits and some other yield traits. All the physiological traits recorded significant and positive correlation with the trait plot yield (Table 2) and the same kind of result was reported earlier [22,23]. The trait photosynthetic rate exhibits positive and significant correlation with the traits transpiration rate, stomatal conductance and harvest index while it showed significant negative correlation with RWC [24]. The trait stomatal conductance and transpiration rate showed significant and negative correlation with relative water content [25]. Stomatal conductance had significant positive association with harvest index [26]. A very strong correlation was observed between grain yield and harvest index under severe stress indicating that yield differences observed under drought were mostly the result of a large difference in the capacity of the plants to maintain seed set under stress rather than to accumulate biomass. Therefore the genetic improvement of harvest index would improve grain yield greatly [27-29].

Mapping QTLs for physiological traits associated with drought tolerance

Molecular marker technology facilitates the identification of genomic locations linked to traits of interest and helps in indirect selection of such complex traits without the need for difficult phenotypic measurements. Marker assisted backcrossing (MABC) combines 'foreground' selection of donor alleles linked to QTLs and 'background' selection of recurrent parent alleles in the later generation. Quantitative trait loci (QTL) have been identified for several drought tolerance component traits in rice [30,31].

Single marker analysis performed with the help of QTL cartographer had provided information on the genetic architecture of complex traits which estimated that the number of QTL and magnitude of their estimated additive, dominance and epistatic effects in multiple environments [32,33]. In the present study, 167 SSR markers were selected based on their position in the target genomic region and subjected to single marker analysis (Figure 1). Among the polymorphic markers, 15 markers were identified as putatively associated to at least one of the investigated trait. Many of the markers showed linkage or pleiotropic effect on the investigated traits since these were known to control more than one trait.

Table 2: Genotypic correlation coefficient of physiological traits under severe stress condition.

	PR	TR	SC	RWC	HI	PY
PR	1	0.579 **	0.431**	-0.032**	0.137**	0.324**
TR		1	0.740**	-0.167**	0.262**	0.351**
SC			1	-0.437**	0.448**	0.501**
RWC				1	0.043**	0.163**
HI					1	0.898**
PY						1

*, ** Significant at 0.05 and 0.01 probability level

The marker RM3165 on chromosome 9 was linked with the character RWC in this study (Figure 2). The same region flanked by the markers RM215- RG667 on chromosome 9 [28]. The marker RM315 on chromosome 1 was linked with the trait harvest index under stress condition [28]. He also reported two QTLs for grain yield on chromosome 1 and 4 flanked by the markers EM18_10-L107 and RG476-RG939. The same region was also reported by this study also. The marker RM315 under stress on chromosome 1 was linked with the character grain yield (Figure 3) [34-37]. These QTL regions on chromosome 1 are associated with various physio-morphological and plant production traits under drought stress in rice [38,39].

The marker RM17 on chromosome 12 was linked with the physiological trait photosynthetic rate in this study (Table 3). One QTL namely qPn12 on chromosome 12 flanked by the markers OSR20- RM277 nearer to the marker region RM17 [40]. The marker RM470 on chromosome 4 had association with the trait transpiration rate under stress condition. Identified one QTL qTR-4 on chromosome 4 flanked by G177-CT206 nearer to the region of RM470 [41]. Characterization of QTLS for drought tolerance in this study provides insight into an understanding of the mode of drought tolerance in rice. The most practical application of the identified QTL for drought resistant components is to perform MAS (Marker aided selection) aimed at the efficient pyramiding of favorable QTL alleles to improve drought tolerance in rice.
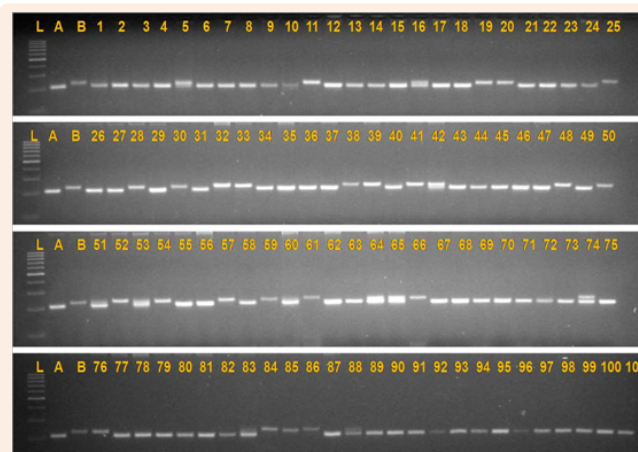
Figure 1: BILs segregating for marker allele amplified with the SSR primer pair RM515.

(L – 100 base pair ladder, A- TKM9 and B-Norungan respectively, 1 to 101- Backcross Inbred Lines)
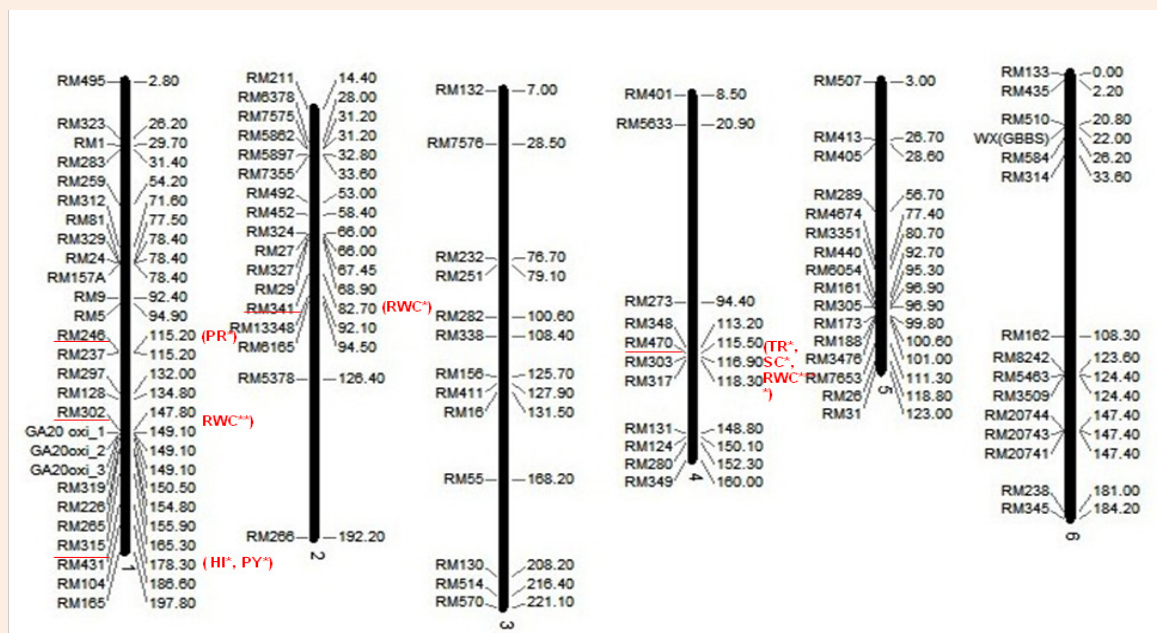
Figure 2: Marker loci associated with drought tolerance physiological traits.

Table 3: Marker trait association evolved through QTL cartographer.

| Trait		Marker	Chromosome	Position	LOD score	Pr(F)
Photosynthetic rate	(S)	RM246 RM17	1 12	115.2 109.1	5.329 7.706	0.023* 0.006**
Transpiration rate	(S)	RM470 RM515 RM264 RM20a RM17	4 8 8 11 12	115.5 80.5 128.6 3.2 109.1	4.999 4.356 5.203 4.373 4.571	0.027* 0.039* 0.024* 0.039* 0.035*
Stomatal conductance	(S)	RM470 RM152 RM17	4 8 12	115.5 9.4 109.1	5.119 5.094 4.075	0.025* 0.026* 0.046*
RWC	(S)	RM302 RM341 RM470 RM152 RM44 RM331 RM105 RM3165 RM20a RM17	1 2 4 8 8 8 9 9 11 12	147.8 82.7 115.5 9.4 60.9 60.9 32.1 65.1 3.2 109.1	10.479 5.447 18.178 10.903 6.674 8.041 11.633 5.717 12.517 7.337	0.001** 0.021* 0.000**** 0.001** 0.011* 0.005** 0.001*** 0.018* 0.000*** 0.007**
Harvest index	(S)	RM315 RM536	1 11	165.3 55.1	6.352 6.392	0.013* 0.013*
Plot yield	(S)	RM315	1	165.3	5.439	0.021*

S- Severe stress condition
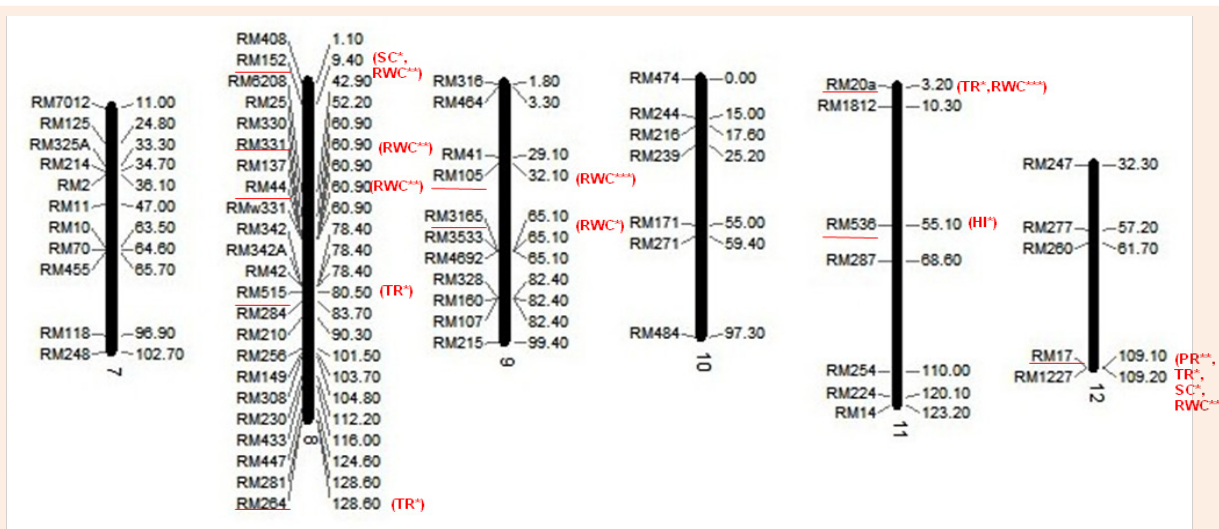
Figure 3: Marker loci associated with drought tolerance physiological traits.

Conclusion

The study on molecular genetic analysis of drought tolerance in rice using an indigenously developed backcross Inbred population has brought out relationship among the drought tolerance physiological traits and key genomic introgressions of drought tolerance and related traits were mapped using SSR markers. The result would help in fine tuning the breeding strategies and application of SSR markers for the development of the drought resistant rice varieties through marker assisted pyramiding multiple QTLs.

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