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FINE MAPPING FOR DROUGHT TOLERANCE IN RICE (*Oryza sativa* L.)

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ABSTRACT

Drought is a more complex phenomenon than most other stresses, such as salinity, submergence, pests, and diseases. It can occur at any point during crop production and for any length of time, affecting a large array of physiological, biochemical, and molecular processes. These complexities, along with the uncertainty in drought timing, intensity, and duration, have posed a major challenge for agricultural scientists. Despite drought having been a focus of agricultural research for several decades, progress in delivering drought-adapted rice varieties and technologies has been relatively slow. In the study reported in this paper, we conducted a molecular marker-based genetic analysis of the drought stress. The objectives were: (1) to characterize the drought resistance of this line by determining the number and chromosomal locations of the drought resistance genes, (2) to determine the amounts and modes of the genetic effects of the resistance genes, and (3) to find molecular markers closely linked to the resistance genes that may be useful for cloning the genes and for improving drought resistance in rice breeding programs. Recent developments in molecular marker technologies, such as RFLP, PCR, RAPD, AFLP provide opportunities for analyzing both simply inherited and quantitative traits, and located and manipulate individual genetic factors associated with traits of interest. This report focuses on the mapping and marker-assisted selection of major genes for drought stresses in rice. Drought tolerance: the region indicates the occurrence of recombination between segments derived from 329 plants of BC_2F_2 from OM1490 / WAB880-1-38-18-20-P1-HB. Twenty markers were used to genotype the 329 BC_2F_2 plants. SSR markers located at the drought recovery score (DRS) genes between RM201, RM328 and that flanked 0.4cM and 13.8 cM, respectively on chromosome 9.

The target segment on chromosome 9 (RM201) significantly related to increased root length and drought tolerance under drought stress treatments. The implications of these results for developing an efficient strategy of marker-assisted selection for drought tolerance are discussed.

Keywords: DNA markers, linkage maps, marker-assisted selection (MAS), map-based cloning

INTRODUCTION

Drought is a more complex phenomenon than most other stresses, such as salinity, submergence, pests, and diseases. It can occur at any point during crop production and for any length of time, affecting a large array of physiological, biochemical, and molecular processes. These complexities, along with the uncertainty in drought timing, intensity, and duration, have posed a major challenge for agricultural scientists. The genetic mechanisms that condition the expression of drought tolerance in rice plants are poorly understood. Since drought tolerance is a complex trait controlled by polygenes, and is dependent on the phenotype evaluated it is one of the most-difficult traits to study and characterize. A linkage map using DNA markers was recently made for the rice plant (McCouch *et al*. 1998; Saito *et al*. 1991; Kurata *et al*. 1994). Utilization of DNA markers and the linkage map will clarify the linkage between gene-controlling characters and DNA markers; selection assisted by DNA markers

will be possible. Moreover, quantitative character locus (QTL) analysis will permit the selection of the quantitative character by DNA marker. Previously, genetic analysis with DNA markers on the drought had been conducted using

However, in this study, QTL was not detected; QTL analysis with DNA markers of the linkage between excellent agricultural characters and DNA markers requires further research; DNA markers will then become tools for selection. Moreover, positional cloning using DNA markers will make it possible to isolate agriculturally useful genes, and it will also contribute to breeding in the future. Understanding the genetic basis of drought tolerance in rice is fundamental to enable breeders and molecular biologists to develop new varieties with more drought tolerance characters. The objectives were: (1) to characterize the drought resistance of this line by determining the number and chromosomal locations of the drought resistance genes, (2) to determine the amounts and modes of the genetic effects of the resistance genes, and (3) to find molecular markers closely linked to the resistance genes that may be useful for cloning the genes and for improving drought resistance in rice breeding programs.

Materials and methods

Plant materials

A population of 229 plants by BC_2F_2 generations of parental lines OM1490 / WAB880-1-38-18-20-P1-HB, OM4495 / IR65195-3B-2-2-2-2 and OM1490 / WAB881 SG 9 were produced.

One hundred eighty BC_2F_2 lines derived from the cross between OM1490 / WAB 880-1-38-18-20-P1-HB were evaluated. BC_2F_2 lines were evaluated for seedling survival day (SD), dry root weigh (Rt.wt), dry shoot weight (St.wt) at seedling stage. STS and microsatellite map of this population was used with 300 markers to detect the linkage to target traits. A linkage map was constructed from 12-linkage groups based on the population. Screening drought tolerance was followed to IRRI 2006 and modified by Lang 2007.

DNA isolation

A crude DNA preparation suitable for PCR analysis was prepared using a simplified miniscale procedure (Lang 2002). A single piece of healthy young leaf was harvested and placed in a labeled 1.5 ml centrifuge tube in ice. The leaf sample was macerated using thick glass rod after adding 400 μl of extraction buffer (50 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 300 mM NaCl and 1% SDS). The leaf was grounded until the buffer turned into green colour. After grinding, another 400 μl of extraction buffer was added and mixed by pipetting. The contents were centrifuged at 12,000 g in micro centrifuge for 10min. Nearly 400 μl of lysate was extracted with 400 μl of chloroform. The top aqueous supernatant was transferred to another 1.5ml tube and DNA was precipitated with absolute ethanol. The contents were centrifuged for 3 min at full speed and the supernatants were discarded. The pellet was washed with 70% ethanol. The DNA was air dried and re-suspended in 50 μl of TE buffer (10mMTris-HCl, pH 8.0, 1 mM EDTA, pH 8.0). One ml of aliquot was used for PCR analysis and the remaining solution was stored at –20°C for any further use.

Polymerase chain reaction (PCR amplification)

A series of optimization experiments using parents and isogenic samples was carried out in which concentrations of template DNA, primers, dNTPs and *Taq* polymerase were varied to determine which conditions The PCR reaction mixture of 20 μl contained 25–50 ng template DNA, 50 ng of each primer obtained from CLRRI, 0.05 mM dNTPs, 1X PCR buffer (10 mMTris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.01% gelatin) and 1 unit of *Taq* polymerase obtained from Lang, Genome Mapping Laboratory, CLRRI. The template DNA was initially denatured at 94°C for 5 min followed by 35 cycles of PCR amplification under the following parameters: 1 min denaturation at 94°C, 1 min primer annealing at 55°C and 1.5–2.0 min primer extension at 72°C. A final 7 min incubation at 72°C was allowed for completion of primer extension on thermalcycler. Following amplification, the samples were run on a 1% agarose in 1X TBE buffer stained with 0.1μg/ml of ethidium bromide and visualized under ultraviolet light. For PCR-RFLP, initially 5 μl of PCR product was used for gel electrophoresis to determine successful of amplification. Seven to eight μl of the PCR product was then used for

restriction enzyme digestion if the amplification was successful. The total reaction volume was usually 20 μl. The reaction mixture consisted of 11.4 μl of double distilled water, 2μl of 10 X restriction buffer, 0.6 μl restriction enzyme (10 U/μl) and 6–8 μl of PCR products. The reaction mixture was incubated at 37°C for 6–8 h. The DNA fragments digested by restriction enzymes were separated on 1.5% agarose gel and visualized under UV light after staining with ethidium bromide.

Linkage analysis

Full multipoint linkage analysis for the segregating polymorphic markers and gene was conducted with MAPMAKER V. 2.0 (Lander *et al.* 1987) for each of the individual populations. For the population, the maximum-likelihood map order for markers was determined with a LOD score threshold of 3.0, and was used as a fixed sequence framework for integrating linkage data from the population. JOINMAP V. 1.4 was used to calculate the combined recombination frequencies for the two populations together and to align the markers into a consensus map. All map distances (centiMorgan, cM) are reported in Kosambi units, and critical LOD score thresholds of 3.0 and 0.05 were used for determining linkage groups and for the calculation of map distances in JOINMAP V. 1.4. The segregation ratios of individual markers were calculated with the software program QGENE V. 2.0 (Nelson 1987), and skewing was indicated when the ratio deviated significantly from the expected.

QTL analysis

QTL analysis was performed with software package Q-gene 1994 from Cornell University and MapL 1995 from Japan University. Q-gene was use to find the location of major and minor genes. QTL detection was performed by single marker analysis (SMA) and interval mapping (IM). One way ANOVA was performed for each single marker and each combination of two markers to be identified as putatively associated with salt tolerance (this was done to confirm the association between the marker and salt tolerance loci). The threshold for declaring a QTL for abiotic stress was LOD>3. All markers were tested for the expected 1:1. To identify the mode of inheritance, re-examination of putative QTL regions was carried out by three genetic components such as dominant, recessive, and additive with Q-gene software. Likelihood ratios (LRs) were calculated at 1 cM interval along the mapped genome. The proportion of phenotypic variation explained by significant marker was estimated as a coefficient of determination (R^2) for the single locus model.

RESULTS AND DISCUSSION

Phenotype evaluated

Rice is particularly sensitive to drought during the reproductive stage, when it can lead to various degrees of sterility. Crop tolerance to drought is complex both genetically and physiologically. Many morpho-physiological traits putatively contribute to drought tolerance (DT) and each of these traits is typically controlled by multiple genes or quantitative trait loci (QTLs), and is influenced by environment to a great extent. Developing DT rice varieties has not been very successful despite the efforts made by breeders, because in practical breeding programs, populations are typically segregating for maturity, making it difficult to accurately, repeatedly time, and manage a uniform and relevant water stress level for selection.

This variety, the recurrent parent in the ABC (advanced backcrossing), had not previously been used for quantitative trait locus (QTL) mapping. The donor parent was WAB 880-1-38-18-20-P1, IR65195-3B-2-2-2-2 and WAB881 SG9 from IRRI were crossed with OM1490 and OM4495 (indica genotypes).

Frequency

Phenotypic selection for completed in using 20 marker assays in 229 lines. BC_2F_2 were evaluated for root length (RL), spikelet fertility (SF), DRR (drought recovery score) and yield (Y) in CLRRI. The target segment on chromosome 9 (RM201) significantly increased root length and DT under drought stress treatments, confirming that this root length QTL from OM1490 / WAB 880-1-38-18-20-P1-; OM1490 / WAB881 SG9, OM4495 / IR65195-3B-2-2-2-2. The data suggested that drought tolerance for yield components is largely associated with genetic and physiological factors independent from those determining the traits *per*

se. The implications of these results for developing an efficient strategy of marker-assisted selection for drought tolerance are discussed.

Construction of a linkage map for drought tolerance

Linkage analysis was performed with microsatellite mapping data using MapMarker version 3.0 (Lander et al. 1987, 1989).

A molecular map was constructed according to published microsatellites from Cornell University. The 116 microsetellite markers, were assigned to linkage group. MapMarker was used to generate microsatellite. Figure 3 show the linkage map for 20 SSR markers employed in this study. Although there are a few gaps of more than 50 cM, the linkage map had a total map length of 2,905.50 cM. The average interval size was 23.05cM, the smallest size in chromosome 12 and chromosome 9 (12.50cM) (Table 2) and the largest in chromosome 9. There are a few gaps larger than 50 cM. It indicated that the genetically related parents cause the low turn of polymorphism for microsetellite markers.

A mapping population of 229 BC_2F_2 lines derived from a cross between OM1490 / WAB880-1-38-18-20-P1-HB, was used to detect quantitative trait loci (QTL) for traits associated with drought tolerance at 30 days after transplanting

From the random sample and SMA, F-tests were significant, indicating markers associated with drought tolerant. The results showed that individual putative QTL explained the average of phenotypic variation 20.78 % for drought. RM201, RM328 showed the highest F-value ($P<0.001$) and therefore are most likely to be linked drought tolerant trait (figure 1).

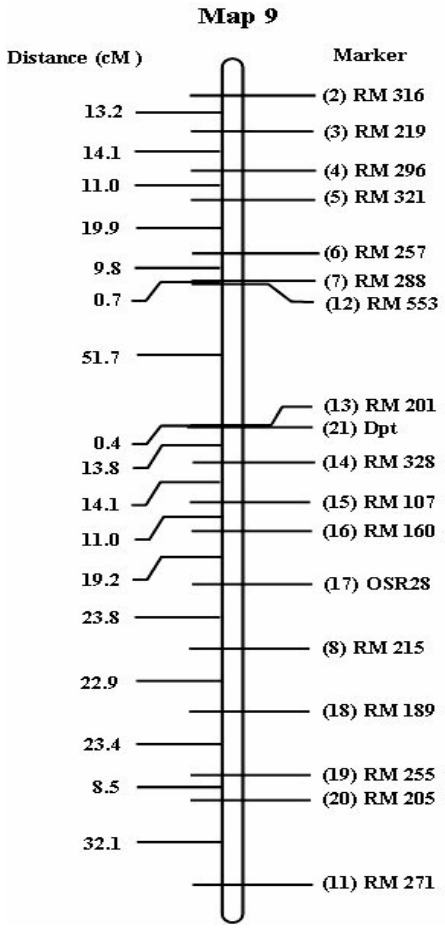

Figure 1: Drought tolerance fine mapping on chromosome 9, using BC_2F_2 population of OM1490 / WAB880-1-38-18-20-P1-HB.

PCR detected with primer RM201 as

F-5'CTCGTTTATTACCTACAGTACC-3'
R-5'CTACCTCCTTTCTAGACCGATA-3'

Marker RM201 linked to DRR, polymorphism exhibited at 201 bp DNA fragment of OM1490 and 225 bp of WAB880-1-38-18-20-P1-HHB

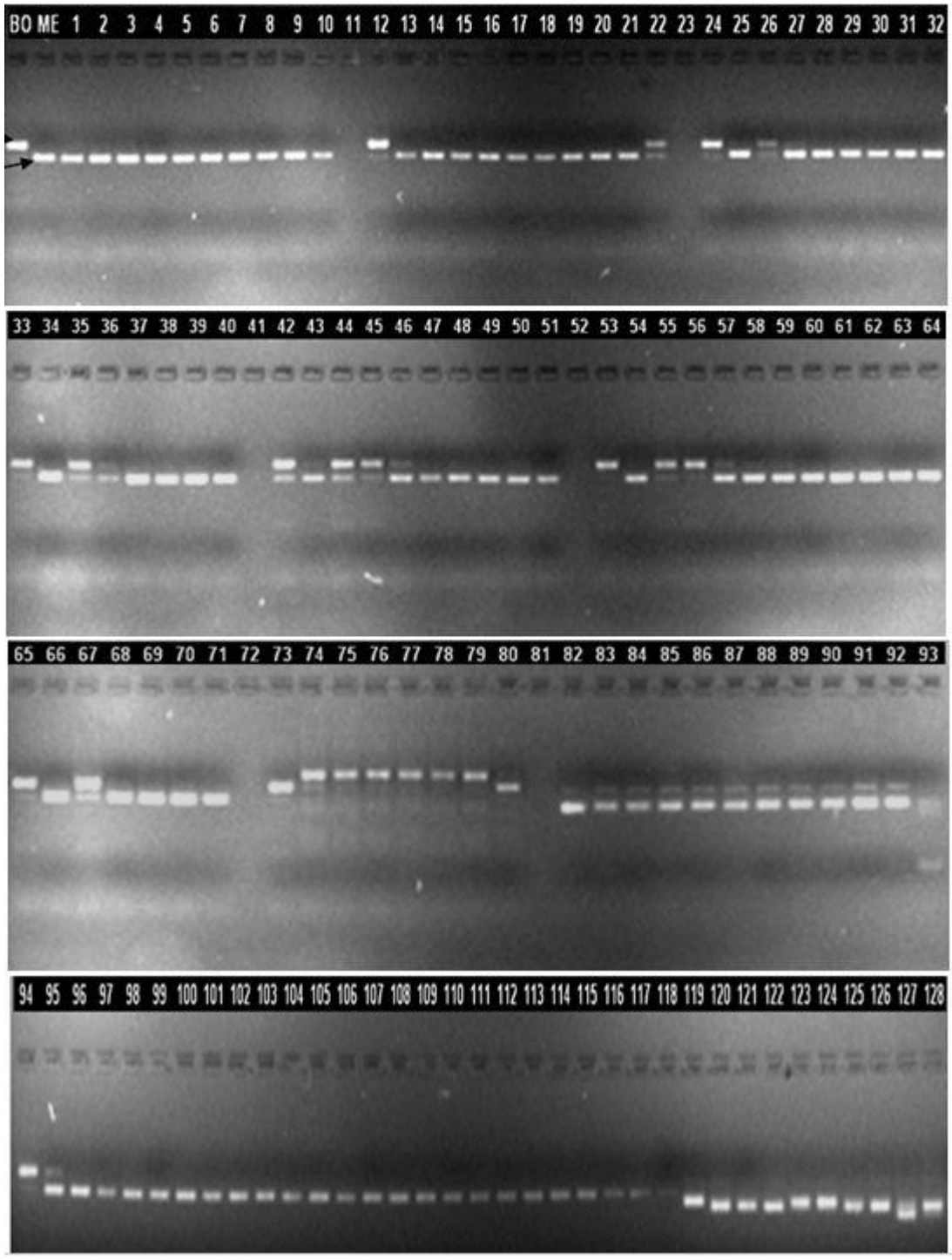

Figure 2. Polymorphism in BC_2F_2 population of OM1490 / WAB880-1-38-18-20-P1-HB at the locus RM201 on chromosome 9

QTL mapping on chromosome 9

The linkage map was constructed with SSR markers spanning 260.4 cM along chromosome 9 with the average interval of 16.13 cM and used for QTL mapping with MapMarker / QTL. LOD score of 3.0 was used as criteria to indicate the putative QTLs. QTLs for polygenic traits vary across populations. Mapping populations may not be representative of many breeding populations. Both parents contain favorable QTLs affecting this trait, suggesting the likelihood of recovering transgressive segregants (table 1)

Table 1: Quantitative analysis for drought tolerance

No.	Putative loci	QTL	R^2%	Population	Marker
1	RM201 - RM328	DRR	20.73	OM1490 / WAB880-1-38-18-20-P1-HB	RM201
2	RM201	RL	4.91	OM1490 / WAB880-1-38-18-20-P1-H	RM201
3	RM189	RL	5.01	OM1490 / WAB880-1-38-18-20-P1-H	RM189
4	RM201	DRR	9.95	OM4495 / IR65195-3B-2-2-2-2	RM201
5	RM201- RM328	DRR	32.38	OM1490 / WAB 881 SG 9	RM201
6	RM316	DRR	23.80	OM1490 / WAB 881 SG 9	RM316

CONCLUSION

The identification of QTLs affecting abiotic stress tolerance is a major step in understanding the genetic basis of plant response to salt, drought, submergence stresses and for the development of saline, drought, submergence tolerant cultivars. Results from this study indicate that at least eight genomic regions in these rice lines contain genes that confer salt tolerance during seed germination. Both parents contain favorable QTLs affecting this trait, suggesting the likelihood of recovering transgressive segregants **(**progenies derived from these parental lines). Such segregant lines may be identified through marker-assisted selection. In addition, because drought tolerance at one stage of plant development may not be correlated with tolerance at other stages, the utility of MAS may be even more if QTLs at all critical developmental stages are identified. This would also contribute to our knowledge of the genetic relationship between drought tolerance at different developmental stages. Simultaneous or sequential transfer of marker-linked QTLs at different developmental stages may lead to the development of cultivars with drought tolerance throughout the ontogeny of the plant.

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Thực hiện fine mapping gen chống chịu hạn cây lúa trên nhiễm sắc thể số 9

Sử dụng biện pháp chọn tạo giống lúa chống chịu khô hạn bằng chỉ thị phân tử (MAS) đã được thảo luận trong nghiên cứu này. Quần thể lai OM1490 / WAB880-1-38-18-20-P1-HB(với 229 cây BC_2F_2) và quần thể lai OM1490 / WAB881 SG9 229 BC_2F_2 và OM4495 / IR65195-3B-2-2-2-2 (100 F_2) được sử dụng. Sự thể hiện tính chống chịu khô hạn được quan sát thông qua những tính trạng cụ thể như hình thái rễ cây, lá, chồi thân, phản ứng co nguyên sinh, bao phấn, qúa trình trỗ bông. Khi phân tích quần thể OM1490 / WAB880-1-38-18-20-P1-HB ở thế hệ F_1, có 86,6% cá thể nghiêng lệch về bố và 15,3% nghiêng lệch về mẹ OM1490. Tần suất biến thiên của tính trạng DRR trong phân bố chuẩn. Locus RM201 trên nhiễm sắc thể số 9, được xác định liên kết chặt chẽ với tính trạng mục tiêu DRR, với gía trị R^2 = 20,73 %. Ở tổ hợp lai OM1490 / WAB881 SG9, biến thiên của kiểu hình được giải thích bởi quãng giữa RM201-RM238 là 32,28%, rất đáng chú ý. Quãng giữa này đều được ghi nhận trong cả hai quần thể của OM1490 / WAB880-1-38-18-20-P1-HB và OM1490 / WAB881 SG9. Tổng chiều dài được bao phủ bởi marker đa hình trên nhiễm sắc thể số 9 là 290,4 cM. Đa hình của quần thể phân ly tại locus RM201 trên nhiễm sắc thể số 9, với băng của bố ở vị trí 225 bp, và băng của mẹ ở vị trí 210 bp. RM201 được đề nghị sử dụng cho nội dung chọn tạo giống lúa chống chịu khô hạn nhờ chỉ thị phân tử