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QUANTITATIVE TRAIT LOCI INFLUENCING DROUGHT TOLERANCE IN RICE (*Oryza sativa. L).*

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ABSTRACT

SSR technique combined with selective genotyping was used to map quantitative trait loci (QTLs) associated with drought tolerance in rice. Two hundred twenty nine (BC_2F_2) derived from the cross between OM1490 / WAB880-1-38-18-20-P1-HB were evaluated for drought at flowering (DRF), root dry weigh (RDW), and root length (RL). Microsetellite map of this population was used with 232 markers to detect the linkage to the target traits. A linkage map was constructed from 12-linkage groups based on the population. The map covers 2,553.7 cM with an average interval of 10.97 cM between marker loci. Markers associated with drought tolerance were located mostly on chromosomes 2, 3, 4, 8, 9, 10 and 12. QTL mapping was used to determine effects of loci associated with drought tolerance traits. We also mapped QTLs for morphological attributes related to drought tolerance. Chi-square tests (χ^2), single maker analysis (SMA), interval mapping (IM) were combined in the QTL analysis procedure. All approaches used for QTL detection gave similar results. Five QTLs were identified for DT(Drought Tolerance), two QTLs for RL, two QTLs for RDW. The proportion of phenotypic variation explained by each QTL ranged from 20.73% to 30.77% for DR, and from 6.23 to 3.39% for morphological characters related to drought at flowering. This study has provided detailed information on the relative importance of marker assisted selection of drought tolerance.

Keywords: drought, interval mapping (IM), QTL, rice, single maker analysis (SMA)

INTRODUCTION

Drought is a key factor affecting food security worldwide; its effects reduce 70% in crop's yield generally (Bray *et al*., 2000). Conventional plant breeding approaches for yield improvement under drought condition is time consuming and laborious, because carefully managed field conditions are required. Selection for a well-developed root system with long, thick roots should improve the drought tolerance of upland rice because the plant would avoid water stress by absorbing stored water in the deep soil layers (Yoshida and Hasegawa, 1982). Phenotypic selection for root morphological traits in conventional breeding programs is unfeasible. The use of molecular markers could provide a useful tool to support phenotypic selection. So, several mapped populations were developed to detect quantitative trait loci (QTLs) influencing root morphology and other drought-related traits that could then be used in marker-assisted selection (MAS) to improve upland varieties (Champoux *et al*., 1995; Yadav *et al.*, 1997; Price and Tomos, 1997).

So, we performed this study "**Marker assisted selection for drought tolerance rice in Vietnam**" with the purposes: (1) to introduce and to apply new approaches through molecular techniques; (2) to develop new rice varieties for drought tolerance.

MATERIALS & METHODS

2.1. Plant materials

- Two hundred twenty nine lines (BC_2F_2) which derived from crossing between OM 1490/WAB880-1-38-18-20-P1-HB (use for mapping all 12 chromosomes), 200 BC_2F_2 lines from OM4495/IR65195-3B-2-2-2-2 and 263 BC_2F_2 lines from OM1490/WAB881 SG 9 (mapping on chromosome 9).

- BC lines were tested for drought at flowering stage (DF), dry root weigh (DRW), root length (RL) and screened by using SSR markers
- Mapping of this population established with 232 SSR 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 by IRRI (2006) and modified by Lang (2007).
- The BC_2F_2 lines were screened by using microsatellite (SSR) marker.

Table 1: List and characterization of varieties used as the materials for crossing

No	Name of variety	Characterization	Gender
1	OM1490	High yield, good quality (the average amylose content) growth time for 90 days, susceptible to drought	Female
2	OM4495	High yield, good quality (the average amylose content), growth time for 90 days, drought tolerance moderately	Female
3	WAB880-1-38-18-20-P1-HB	Drought tolerance	Male
4	WAB881SG9	Drought tolerance, fragrance	Male
5	IR 65195-3B-2-2-2-2-2	Drought tolerance	Male

2.2. Methods

2.2.1. Evaluation of parents and BC_2F_2 for drought stress

The experiment was designed by random completely block, three replication with each experimental plot of 300 m^2. The BC_2F_2 lines and parents were soaked until germination, and sown into plastic trays. After 15 days, they were transplanted into basins which were built by cement. After transplanting about 10 days, the drainage through drain taps was performed; without provide the water until flowering rice. Subsequently, the samples were obtained, analysis of agronomic and yield traits as well as evaluation and screening of promising varieties for drought tolerance.

2.2.2. DNA extraction (CTAB)

DNA for PCR was prepared by using DNA extraction procedure with CTAB method which was modified by Lang (2002).

2.2.3. Amplification of microsatellites and detection of their polymorphisms

PCR amplification was performed in 10 mM Tris-HCl (pH=8.3), 50mM KCl, 1.5mM $MgCl_2$, 1 unit of TAKARA *DNA Taq Polymerase*, 4 nmole of dNTP, 10 pmole of primer, with 30ng of genomic DNA per 25 µl using a thermal cycler 9600 (Perkin-Elmer). The PCR reactions were denatured at 95°C for 4 min, followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minute. The final extension was at 72°C for 5 min. After PCR, 13µl of loading buffer (98% formamide, 10mm EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol) were added. Polymorphisms in the PCR products were detected by ethidium bromide staining after electrophoresis on 3% agarose gel.

2.2.4. Construction of SSR mapping and assignment of linked groups to chromosomes

To construct the SSR mapping and to assign the linked groups to chromosomes, a set of SSR markers which were present in the test population were first identified. Linkage groups were ordered by using MAPMARKER (Lander *et al.*, 1987). Linkage group was reconfirmed using the "GROUP" Map units (cM) were derived by using the Kosambi function (Kosambi, 1944).

2.2.5. QTL analysis

Single-marker QTL analysis using linear regression was done following Tanksley SD (1993). The marker alleles link to drought was

coded 1; and in contrast, coded 0 for conducting regression analysis.

- The data was analyzed using MAPMARKER to locate gene for protein.
- For QTLs, interval analysis was conducted with MAPMARKER/QTL on the quantitative. The threshold for declaring a QTL for protein was LOD> 3.0.

RESULTS & DISCUSSION

3.1. Phenotypic variation for drought tolerance

Figure 1 indicated that the phenotypic distribution of drought evaluation among the BC_2F_2 lines was continuous. This brings a good recombination for salinity reaction in the population. For dry shoot weight (DSW) and dry root weight (DRW), large differences were noticed in the population. DSW ranges from 45mg to 110mg and for DRW from 200mg to 350mg.

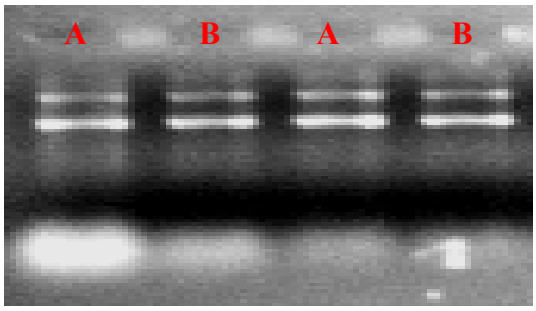

Figure 1: Screening for drought tolerance at seedling stage in rice

Tables 2: Genetic mapping for drought tolerance on 12 chromosomes of BC_2F_2 from OM1490/ WAB880-1-38-18-20-P1-HB

Chromosomes	Marker numbers	Length (cM)	Av. cM between two markers
1	24	355.5	14.81
2	25	327.0	13.08
3	19	221.8	11.67
4	18	187.9	10.43
5	17	183.2	10.77
6	20	120.9	6.04
7	18	189.0	10.50
8	17	180.9	10.64
9	20	290.4	16.13
10	15	133.3	8.88
11	18	177.2	9.84
12	21	186.6	8.88
Total	232	2,553.7	10.97

Note: *T: tolerance, MT: Moderate tolerance, S: susceptible*

Based on the result of evaluation for drought tolerance from parents and BC_2F_2 lines indicated that BC_2F_2 lines from crossing between OM4495/ IR 65195-3B-2-2-2-2-2 for drought tolerance moderately; BC_2F_2 lines from crossing between OM1490 / WAB880-1-38-18-20-P1-HB, OM1490 / WAB881SG9 for drought tolerance were very good. This demonstrated that tolerance genes for drought stress transferred from WAB880-1-38-18-20-P1-HB, WAB881SG9 into OM1490 variety.

Phenotyping

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).

3.2. Polymorphism of SSR markers

Fifty one out of 450 SSR markers were identical among these lines. The microsatellite markers have already been investigated before that, 82 primers showed no amplification, and 99 ones with amplification but monomorphism. However, polymorphism was noticed with 232 microsatellite markers. Among of 232 polymorphic SSR markers, 35 percentage SSR markers link to OM1490 and 65% others from WAB880-1-38-18-20-P1-HB. Amplified fragments ranged in size from 95 bp to 352 bp for all dissected primers in the analysis. The widest range of primers RM201, RM242 was 220bp

Construction of a linkage map

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

A molecular map was constructed according to published microsatellites from Cornell University. The 232 microsatellite markers were assigned to linkage group. MapMarker version 3.0 was used to generate microsatellite map for each linked group. Figure 2 shows the linkage map for 232 SSR markers employed in this study. Mapping covers 2,553.7 cM with an average interval of 10.97 cM between marker loci. Markers associated with drought tolerance were located mostly on chromosomes 2, 3, 4, 8, 9, 10 and 12

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OMDN110         AGGTTAAAGGGATAGTCGAGTTGGTTTCTGAT--AGATGATAGTAAGATTAAT-----AG 54
OMDN1           AGATAAAAAAAGTGTGTGAGTTATTTGGAGGTTTATTTATTCATAAGGTAAAT-----CA 143
S79982          TGGCAAAGAGGCCTCATCCATCGCCCTGGACGGCTCGTTTTGGTCAAATCAGTGATTGCG 599
S79983          TGGCAAAGAGGCCTCATCCATCGCCCTGGACGGCTCGTTTTGGTCAAATCAGTGATTGCG 567
                 *   **              *                  *  *  * *  * * *

OMDN110         AGTGA---TGTGAATGATGG---GGTGAAAGATGTG---GTGTAGCTGAATCTGGAAAT- 104
OMDN1           GGTGA---CTTGCACAGTTGATGGGAGGAGAAGAAA---GTGGATCTGGTTGGGGGAAT- 196
S79982          GCTAAACCCATCCATCATTTCATGGTGACACATGCTCCCGTGTGGGTATTTGAAGAGATC 659
S79983          GCTAAACCCATCCATCATTTCATGGTGACACATGCTCCCGTGTGGGTATTTGAAGAGATC 627
                  * *     *  *   *     ** *    *       ***    *   *   *  **

OMDN110         -----------GAA-TAGGGATGATGGATAGAAAGTGGATAAGTTGAGTGGTGTGAGGTG 152
OMDN1           -----------AAAATTTGGTCGGTGGGGGCAAAACAAAAAGAAAAAAAAATT---GTTA 242
S79982          ----------TGCGATCCTTCTTTTGGGCTGGCAAGGAAAAAATCCAACGGTGGCCAGTG 709
S79983          GAGCAGTGGATGGGATCCTTCTTTTGGGCTGGCAAGGAAAAG-TCCAACGGTGGCCAGTG 686
                          *        ***      *    * *     *    *      *

OMDN110         ATGGTTGAAATTGAGTTTGGTTGT--GCGTTGGTCGGGGTAAAG--TGGTGGGGTGAGAG 208
OMDN1           GGGGTTAAAATCCGTTCTCGCCCCCGGCCCCGAGTGATTTAAGGATAGGTTTGTTGGGGG 302
S79982          CTTGGTAACTTGGAAATCAGTCTGCAAACCCACTTCGCTTGGTGGTCTGGGAATCCGCAA 769
S79983          CTTGGTAACTTGGAAATCAGTCTGCAAACCCACTTCGCTTGGTGGTCTGGGAATCCGCAA 746
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Figure 2: Molecular linkage on 12 chromosome from BC_2F_2 population of **OM1490 / WAB880-1-38-18-20-P1-HB**

Construction of a linkage for fine map for drought tolerance

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

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.

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 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 (Lang et al 2008)

Phenotypic measurement is very important in tagging QTL, because quantitative traits are largely affected by environment and measuring characters like drought tolerance. It is complicated because there is little agreement about how to artificially impose the stress. Phenotypic frequency distributions support the quantitative inheritance of drought tolerance gene. There are large differences in those traits. Some lines are highly tolerant to drought, while others are very sensitive. This can be interpreted to mean that under certain drought condition, plant growth, dry shoot weight, and dry root weight are more severely affected by environment. Many of the drought tolerant varieties are traditional cultivars which tend to be tall and photoperiod sensitive. These maybe adaptive traits in many salt prone environments but modern plant breeders would like to be able to more genes controlling salt tolerance into short stature, high yielding varieties. There was no relationship between the number of shared QTL and presence/absence of phenotypic correlation. It is indicated that a large proportion of the phenotypic variance is explained by QTLs that were not detected or else by the presence of other genetic effects. Based on the QTLs for salinity tolerance excluding the major gene in chromosome 9, three microsatellite loci were tightly detected minor genes for drought tolerance. One of them is RM201 (chr.9). The ability to detect the tight linkage between markers and drought tolerance genes depends on the number of mapped markers that is available for populations. Results from this study indicate that at least eight genomic regions in these rice lines contain genes that confer drought 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 of 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. This study has provided much more detailed informations on the relative importance of genomic segment and has increased our understanding of the genetic basis of drought tolerance. QTL maps could help identify long-term strategies for drought tolerant breeding. Further studies are needed to confirm the estimation of fine mapping for markers assisted selection for drought tolerance.

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Phân tích qtl tính trạng chống chịu khô hạn của cây lúa

Trong phân tích các cặp lai đơn và hồi giao đều ghi nhận khả năng chống chịu khô hạn khá tốt của cá thể con lai trong tổ hợp lai BC_2F_2

Biến thiên kiểu hình được giải thích bởi liên kết giữa QTL mục tiêu và marker RM201 là 32,28%, 20,73% và 9,95%, theo thứ tự đối với cặp lai OM1490 / WAB881 SG9, OM1490/WAB880-1-38-18-20-P1-HB và OM4495 / IR65195-3B-2-2-2-2. Đối với tính trạng chiều dài của rễ, kết quả này biểu hiện thấp hơn 5,01 % tại locus RM189 đối với OM1490 / WAB880-1-38-18-20-P1-H. và tại locus RM316 kết quả đạt 23,80% đối với OM1490 / WAB881 SG9.

Nhìn chung tại locus RM201 của nhiễm sắc thể số 9, cả 3 quần thể con lai được nghiên cứu đều cho kết quả đa hình đáng tin cậy. Do đó, RM201 được đề nghị sử dụng cho nội dung chọn tạo giống lúa chống chịu khô hạn nhờ chỉ thị phân tử (MAS)