QUANTITATIVE TRAIT LOCI (QTLs) MAPPING WITH SUBMERGENCE TOLERANCE IN RICE (*Oryza sativa*. L*)* IN THE MEKONG DELTA

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Abstract

SSR technique combined with selective genotyping was used to map quantitative trait loci (QTLs) associated with submergence tolerance in rice. One hundred sixty-eight lines (BC_2F_2) derived from the cross of OM1490/IR64Sub1 were evaluated for submergence at flowering. Microsatellite map of this population was used with 194 markers to detect the linkage to the target traits. The map covers 2,008 cM with an average interval of 10.97 cM between marker loci. Markers associated with submergence tolerance were located mostly on chromosomes 1, 2, 3, 8, 9, 11 and 12. QTL mapping was used to determine effects of loci associated with submergence tolerance traits: Plant height, tillers/panicle, unfilled grains/panicle, 1000 grains weight, yield and plant survival during flowering. We also mapped QTLs for morphological attributes related to submergence 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 obtained similar results. QTLs were identified for submergence tolerance with the emphasis on 26 QTLs related with 6 traits. Seven QTLs related to submergence at flowering focus on chromosome 8, 9 and 12. The proportion of phenotypic variation explained by each QTL ranged from 18.8 to 25.8% on chromosome 8, 14.8%, 24.2% to 32.4% on chromosome 9 and 19.0 to 25.9% on chromosome 12. Nine QTLs related with yield grain for Submergence at chromsome 1, 2, 3 and 11. Two QTLs related for 1000 grain weight on chromosome 2 and 11. Three QTLs for unfilled grain / panicle at chromosome 3, and 11. Two QTLs for plant height at chromosome 3. Three QTLs for tilling/plant on chromosome 3 and 11. This study has provided detailed information on the relative importance of marker-assisted selection of submergence tolerance in rice.

Keywords: Interval mapping (IM), quantitative trait loci (QTLs)**,** single marker analysis (SMA), submergence tolerance in rice

INTRODUCTION

Submergence is a severe problem affecting to rice production in flood-prone region in worldwide (Ismail *et al.,* 2009). Submergence can be caused by overflow by levees from the large river, too much rain and/or tidal flooding (Sairam *et al.,* 2008). The short-term high-yield rice varieties can be severely damaged by several days completely submerged due to lack of oxygen. In flooded conditions, gaseous diffusion is limited in comparison with air and O_2 depletion of oxygen can generate oxygen around the plant tissue (Setter *et al.,* 1989; Das and Uchimiya, 2002 (Ram *et al*., 2002). Furthermore, normal oxygen deficits trigger the process of anaerobic metabolism and affect the growth and development of the plants due to high energy needs, and can cause death when the plant was young. Identifying molecular markers associated with quantitative trait loci (QTLs) is linked with useful agronomic traits or adaptations will help accelerate progress in breeding once developed, because the DNA marker became effective tools to select. Furthermore, cloning location using DNA markers will make it possible to isolate useful agronomic genes. We have developed mapping populations using genotypes resistant to flooding and use the QTLs map associated with tolerance to flooding during the tillering stage. Two relatively large QTLs were identified, one on chromosome 9 (Ismail *et al*., 2009). In order to create multiple molecular markers used to select recombination, about 5Mb region on each side of the *Sub1* region, are targeted the regions bringing *sub1* gene. Microsatellite markers were identified from 20 BAC (chromosome bacterial artificial) clones flanking Sub1 locus (IRGSP 2005). Many submerged-tolerant varieties have been developed in different breeding programs (Mackill *et al.,* 1993). The deepwater rice varieties and floating rice in Mekong Delta were also researched by Buu et al. in 1997. But these varieties were local rice which have lost and no longer exist in the field of the Mekong Delta. Separately short-term high-yield rice varieties did not record with submergence gene according to Lang *et al.,* 2011. These varieties were developed through conventional breeding without a fully understanding of the genetic factors of the trait. Therefore, this subject needs to be studied in preparation for the next stage of the breeding program.

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MATERIALS AND METHODS

The population 168 plants of BC_2F_2 were developed from the cross of OM1490/IR64-Sub1of Lang and *et al.,* 2011. Each plant was harvested a panicle and selected two seeds for BC_2F_3 generation.

Flooding assessment

BC_3F_2 lines and parents were submergence screening at stage of 20 days after seedling with Sawana Sub 1 variety used as control. When testing vulnerability displayed 50% of damage, usually after about 14 days completely submerged.

QTL map is designed based on QTL software / map/QTL.

RESULTS AND DISCUSSION

Study set up map tolerant to submergence

Genetic engineering and biotechnology have created an immense potential for the plant breeding. The application of these techniques at the molecular level allows us to transfer the desired gene into the plant in the same species and we can introduce new genes from wild species which is close plant species. For traits of multi-gene as submergence gene on rice plant have many difficulties in analysis, when using traditional breeding methods. But this becomes easier because using of molecular marker-marker tool is very effective.

Evaluation of submerged tolerance on parents

The expression of flooding tolerance is observed through specific traits such as pollinating process at flowering, plant height, panicle length, filled-grains rate, unfilled grains per panicle, 1000-grain weight, yield and plant survival rate after 20 days of complete submergence. The results based on the 8 targets above. OM1490 is used as a mother. Sawana sub 1 and IR64 sub 1 used as father evaluating level 1-3 after 20 days of flooding purification. With resistant control was Sawana Sub1 resistant level 1. OM1490 control variety was recorded infectiously in level 9.

As analysis of OM1490/IR64Sub1 population recorded BC_1F_1 generation accounting for 85.6% was askew on father and 13.3% was askew infectious trait of level 7 as OM1490. In the BC_2F_1 generation, the rate of level 1 was still accounting for over 62.2% more than level 5 and level 7. Continuously in BC_3F_2 generation also recorded hybrids in level 1 and level 7 with decrease and level 5 in increasing. As research on backcross population recorded BC1 generation and BC2 generation in level 1 also recorded higher than F_2 and F_3 but lower than F1. This showed that exploitation in this crosses will be beneficial when flooding resistant rate is askew fairly hybrids.

Establishing genetic map by SSR molecular marker

Genetic linkage maps based on molecular marker was made with BC_3F_2 populations of crosses OM1490/ IR64Sub1. Using SSR molecular markers for the genetic map. For SSR molecular markers or microsatellites reflecting polymorphism on the basis of the repeating single number of a target region carrying submergence gene of rice genome. Polymorphism result to establish genetic map in rice more than 194 SSR molecular markers, average distance is 10.68 cM. The number and composition of microsatellites change on the different crosses. Repeat frequency of code sequence is longer than 20 bp. Nucleotide code sequence is adjacent repeat code sequence used to design primers in order to amplify a different number of repeat units in different varieties. Type of this polymorphism have highly reproducible. Such primers really need to detect rapidly for requirement and accurately polymorphism locations. This information was used to develop a physical map based on sequence tags. Genetic map on BC_3F_2 populations of OM1490/ IR64Sub1 combination recorded on Table 1 with 194 molecular markers located on chromosome 12.

Table 1. The alignment of the molecular markers on the chromosome of the BC_3F_2 population of OM1490/IR64Sub1 combination

Chromosome	Number of molecular marker	Map length (cM)	Mean
1	20	246.8	12.34
2	12	122.3	10.19
3	10	134.5	13.45
4	9	104.2	11.57
5	7	93.7	13.38
6	6	57.8	9.63
7	21	213.3	10.15
8	21	153.2	7.29
9	23	206.3	8.96
10	19	198.2	10.43
11	23	232	10.08
12	23	244.7	10.63
Total	*194*	*2.007*	*10.68*

Analyzing QTL-submergence

Most important agronomic traits such as yield, yield forming factors, flowering date, flood, etc. are

controlled by multiple genes. Multi-gene activity and mutual interaction, is still very little known while they control quantitative trait. Quantitative trait modifying factor (quantitative trait QTMF = modifying factor) was defined as the location affecting quantitative trait via epistasis. Meanwhile, QTL affecting phenotypic is relatively large and independent on genetic basis. Molecular markers tightly linked to the genes controlling submergence gene on evaluated submergence traits. For rice plant, submergence traits are on many targets. However in this subject focusing on four traits were analyzed applying QTL molecular markers for analysis. Genetic linkage map associated with high density has also been established with the help of software MAPMARKER / QTL (Yano *et al.*, 1995).

Normally it is difficult to determine the exact location and operation of the quantitative trait location. To overcome above disadvantages, the source of genetic material (genetic stocks) with clearly known traits was established, for instance near-isogenic line (NIL), carrying one or more chromosome segments of a parental line, the genetic background of different parent lines. Performing investigation on genome-wide to identify chromosome segments was introduced by means of SSR. By using NIL lines for certain chromosomal segment that can process a certain QTL as a single factor in Mendelian genetics. SSR maps are high qualitative, have high density of molecular markers, will help to define easier. It also helps to map and process quantitative trait into Mendelian genetic factors. The method includes: (i) searching for a combination between SSR molecular markers are segregating, and (ii) identifying the benefits of a segregating population to find linkage of molecular markers to QTL. On genetic mapping population of BC_2F_2 populations of OM1490/ IR64Sub1 combined with 168 individuals covering 194 molecular markers on 12 chromosomes. Having 26 QTLs located on chromosomes were evaluated focusing on 1, 2, 3, 8, 9, 11, and 12.

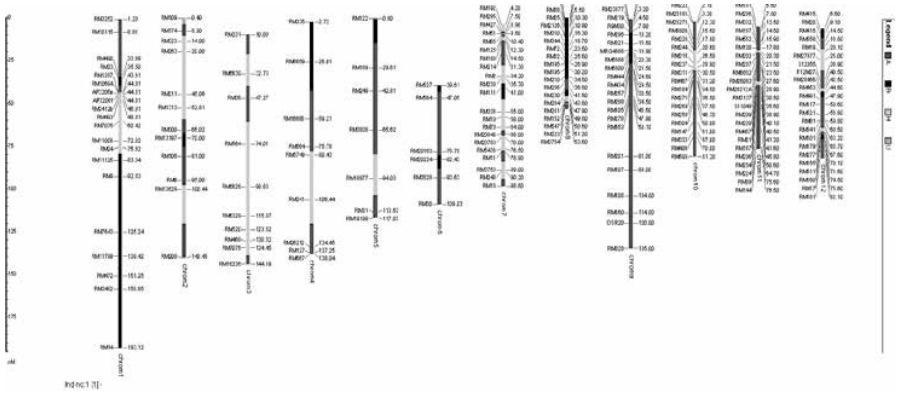

Figure 1. Genetic mapping tolerant to submergence

Analysing of QTL with submergence conditional tolerance

Affecting QTL and operation of submergence gene

The ability to detect QTL depending on possible conditions of molecular markers location, the combination in statistical analysis between the allele at the locations of molecular markers and the alleles at locations with quantitative properties (QTL). With the R^2 value was 0.207% and the linking contribution of QTL and associated phenotype contributes 20.73% on fluctuation of submergence tolerant content between parents OM1490/IR64sub1. This was also consistent with the results of previous reports (Steel *et al.*, 2006). Assessment was focused on 8 traits: submergence tolerance in flowering time, height, number of tillers per hill, grain length, number of filled-grain per panicle, percentage of unfilled-grain per panicle, 1000-grain weight and grain yield per hill and survival time after submerged stress in flowering stage (SF). Assumptive QTL mapping associated with flooding tolerant gene expressing on the chromosome of 1, 2, 3, 8, 9, 11 and 12 with LOD > 3.0. A total of 26 QTLs was recorded with 6 traits, two traits of filled-grains/panicle and grain length traits were recorded as association of genes in population's condition. 7 QTLs were related to plant survival after flooding stress focusing on chromosomes 8, 9 and 12. Phenotypic variation was explained 21.8 to 25.8% on chromosome 8; 14.8%, 24.2% and 32.4% on chromosome 9 and 19.0 to 25.9 appearing on chromosome 12. For QTL controls productivity trait had 9 QTLs focusing on chromosome 1 with 3 QTLs controlling yield on 3 molecular markers of RM10115, RM11125 and RM

9. Phenotypic variation was explained with 20.0, 11, 0 and 11, 0 in the order of molecular markers. Two QTLs control yield traits on chromosome 2 is RM323 and RM 263 with phenotypic variation is 13.73 and 20.01%, respectively. Two QTLs controlling yield traits associated with molecular markers RM 520 and RM6229 on chromosome 3 associated with phenotype and from 11.45 to 15.02% in order.

Remaining two QTL on chromosome 11 is RM 181 and RM 286 associated with phenotypic variation was 19.9 and 18.8% in order. For 1000-grain weight the factor contributing productivity affecting by two QTLs related to 1000-grain weight focused on chromosome 2 and 11 corresponding to molecular marker. Three QTLs related to % unfilled-grain per panicle were focused on chromosome 3, and 11. Two QTLs controlling plant height were focused on chromosome 3. Three QTLs controlling trait of number of tiller per hill were focused on chromosome 3 and 11. All these studies make opportunities for activity of breeding rice varieties tolerant to submergence via molecular marker (MAS).

Table 2. Identifying QTL of submerged tolerance in flowering stage by using SMA of 168 lines BC from combination OM1490/IR64SUB1

Molecular	Chromosome	Genotype	Mean of allele	F value	P value	R^2 (%)	DPE
RM72	8	A B	6.40±0.44	5.84	0.001	18.8	B
RM32	8	A B	5.40±0.44	6.50	0.000	25.8	B
RM328	9	A B	4.40±0.44	2.80	0.035	14.8	B
RM23818	9	A B	3.40±0.44	2.73	0.039	24.2	B
RM219	9	A B	5.40±0.44	3.38	0.016	32.4	A
RM28466	12	A B	4.40±0.44	3.87	0.008	25.9	A
RM277	12	A B	3.40±0.44	2.99	0.05	19.0	A

Note: Table 2, 3, 4, 5, 6, 7: DPE: (Direction of phenotypic effect) trend of phenotype in type of allele of A: OM 1490, B: IR64Sub1.

For quantitative trait QSTL is relatively influential average 14.8-25.9. For traits except on molecular markers RM219 (chromosome 9 QTL affecting the phenotype is relatively high 32.4%. This also contributes to assess polymorphism on the hybrid populations meeting highly requirement when separating dissociation of the next generation.

Among crosses of OM1490/IR64Sub1, morphological traits such as height, determined by two QTLs molecular markers, each molecular marker with relatively large influence of phenotype was relatively large. QTL is influential such like that often found in mapping technique on populations and in different environments. For height of this population was only recorded on chromosome 3 with RM520 and RM6329, indicated two molecular markers located near the QTLs controlling plant height trait of submergence tolerant plant on the same chromosome 3 with the affective contribution of phenotype is 35.0 to 10.6% in order of molecular marker of RM6329 and RM520.

Table 3. Identifying QTL of plant height (HP) by SMA method of 168 lines BC from combination OM1490/ IR64Sub1

Molecular marker	Chromosome	Genotype	Mean of allele	F value	P value	R^2 (%)	DPE
RM6329	3	A B	3±0.40	5.43	0.000	35.0	A
RM520	3	A B	3.3±0.40	3.40	0.002	10.6	B

Table 4. Identifying QTL of number of tiller per hill by SMA method of 168 lines BC from combination OM1490/IR64Sub1

Molecular marker	Chromosome	Genotype	Mean of allele	F value	P value	R^2 (%)	DPE
RM5639	2	A B	2±0.40	2,07	0.034	19,02	A
RM209	11	A B	2.3±0.40	3,08	0.002	30,9	B
RM2293	11		3.3±0.41	2,36	0.015	14,6	A

Table 5. Identifying QTL trait in 1000-grain weight by SMA method of 168 lines BC from combination OM1490/IR64Sub1

Molecular marker	Chromosome	Genotype	Mean of allele	F value	P value	R^2 (%)	DPE
RM 106	2	A B	7.85±0.44	3,22	0,003	18,5	A
RM167	11	A B	7.85±0.44	2,06	0.045	15,1	B

Table 6. Identifying QTL rate of unfilled-grain/panicle by SMA method of 168 lines BC from combination OM1490/ IR 64sub1

Molecular marker	Chromosome	Genotype	Mean of allele	F value	P value	R^2 (%)	DPE
RM 468	3	A B	8.85±0.41	2,98	0.004	15,33	B
RM202	11	A B	6.52±0.40	2.28	0.020	29.8	A

The quantitative traits such as yield and yield forming factors are normally controlled by the location with non-allelic interaction (epistasis) and QTL influencing relatively small fluctuating from 11% to 20.0%. QTLs influenced small and the non-allelic interactive position often recorded: interactive genotype × environment (G × E) and their impact often suffered the change on genetic background, can not predict it. Therefore, G × E interaction and epistasis is combined with QTL makes it more difficult in QTL applications - MAS to advance genetically of complex traits.

Table 7. Identifying QTL yield (YG) by SMA method of 164 lines BC from combination OM1490/IR64Sub1

Molecular marker	Chromosome	Genotype	Mean of allele	F value	P value	R^2 (%)	DPE
RM10115	1	A B	4,5 ±0,16	9.99	0.000	20.	A
RM11125	1	A B	5,0 ±0,14	3.77	0.001	11.0	B
RM9	1	A B	6,5 ±0.12	3.77	0.001	11.0	B
RM323	2	A B	2,5 ±0,17	2.65	0.009	13.75	A
RM263	2	A B	3,5 ±0,40	2.19	0.027	20.01	A
RM6229	3	A B	4,0 ±0,40	2.21	0.026	11.45	A
RM520	3	A B	6.0±0,20	2.19	0.030	15.02	A
RM181	11	A B	4,5 ±0,40	3.65	0.001	19.9	A
RM286	11	AB	6.8±0,10	3.99	0.001	18.8	B

The method which is more sensitive in detecting QTL is an analysis at the same time the influences of molecular markers linked on the same chromosome interval of the study of genetic tolerance to flooding conditions. Effect of different regions on each chromosome with 5 goal traits (selective standard) related to the phenomenon of submerged tolerance are shown in the related table.

Three regions: RM 11125 - RM 9 and RM 155 - RM 511 has significant influence on two yield target traits. Most target traits expressed significant influence on the identified region of the genome (Table 8), but SF and YG trait expressed only influence on an interval of genetic maps.

Table 8. QTL of traits determined by interval analysis method on genetic map

Index	Interval between of two molercular marker	Chromosome	P value	centi-Morgan
SF	RM72-RM32	8	0.001	10.8
YG	RM10115-RM11125	1	0.001	14.9

Genetic variance of additive is explained by molecular markers

The ability to detect QTL depending on possible conditions of molecular markers position, in an imbalance of the linkage, which we have discussed above. The contribution of genetic variance of an addition for a certain trait, was explained by the position of molecular marker, also depending on the influence of QTL and the magnitude of population denominator (number of individuals) was used for analysis.

Each QTL only involved in a small part of the phenotypic variation in general, and some molecular marker locations were closely linked with studied traits, there is enough to consider the additive variance at a certain particular QTL. QTL were detected at the level of statistical significance, if the contribution of genetic variance of additive is large.

Genetic variation of quantitative traits was usually created by a few locations with relatively large influence and many other positions with smaller influence (Shrimpton and Robertson, 1988; Paterson *et al.,* 1988). A simulation has been developed according to the geometric distribution of QTL affect to analyze the contribution of the variance due to additive.

From there, it can discover a range of locations of useful molecular markers in a denominator which magnitude were foregone. Prediction of above traits of genetic linkage maps were analyzed at molecular scale (molecular dissection). Based on the data of the table recorded linkage traits on the chromosomes is most significant from 0.5 to 1%.

CONCLUSION

QTLs were identified for submergence tolerance with the emphasis on 26 QTLs related with 6 traits:

- Seven QTLs related to submergence at flowering focus on chromosome 8, 9 and 12.

- Nine QTLs related with yield grain for Submergence at chromosome 1, 2, 3 and 11.

- Two QTLs related for 1000 grain weight on chromosome 2 and 11.

- Three QTLs for unfilled grain / panicle at chromosome 3 and 11.

- Two QTLs for plant height at chromosome 3.

- Three QTLs for tilling/plant on chromosome 3 and 11.

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EVALUATION AND TESTING OF TROPICAL RICE VARIETIES UNDER TEMPERATE CLIMATE IN RUSSIA

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Abstract

Breeding of rice varieties for Northern temperate regions always faces difficulty and therefore needs to have diverse sources of materials collected from tropical regions. A study of the collection of tropical rice materials with economically important quantitative traits has been carried out and a considerable variation was found. A positive correlation was recorded between the panicle length and plant height; weight of panicles and weight of 1000 grains; the number of grains per panicle and the ratio of fertile grains. The study results will provide pre-breeding materials for improving productivity and resistant ability of popular rice varieties in Russia.

Keywords: rice, collection, donors, sustainability, productivity

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food of more than half of the world's population. The global diversity of varieties is a genetic basis for breeding efforts aimed at maintaining the productivity of rice. In most Asian countries, it is known to be rich in rice germplasm. International Rice Research Institute (IRRI) has the most genetically diverse and comprehensive collection of rice in the world (Jackson MT, 2002).

Rice is grown in Northern zones of the Russian Federation. Rice is annually cultivated in Kuban, Don, Volga and in the Far East with approximately 200 thousand hectares. In Rostov region alone, rice is sown yearly with 14-15 thousand hectares (Kostylev PI. *et al*., 2004). In this regard, the main focus of rice breeding in this area is the generation of middle-stress resistant varieties with high productivity, well adapted to local conditions (Usatov AV. *et al*., 2004). The problem of their generation must be addressed through the study of various sources of materials and separated from the sources of agronomic characters for inclusion in the selection process, which will be allowed to more effectively develop new high-yielding varieties of rice.

The aim of this study was to conduct a comprehensive study on the collection of rice samples from IRRI (Philippines) and AGI (Institute of Agricultural Genetics, Vietnam) and to select pre-breeding materials for breeding of new tolerant lines under stress factors.

MATERIALS AND METHODS

50 donor samples of rice subspecies *indica* from the collection of AGI, carrying genes *Saltol* (salt tolerance), *Sub 1* (resistance to flooding), *Xa* (resistance to bacteriosis) *AG* (anaerobic germination), and others were used and control varieties were Boyarin and Uzhanin.

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