QTL analysis on rice genotypes adapted to acid sulfate soils in the Mekong river delta, Vietnam

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Abstract:

Three target points in acid sulfate soils have been identified as: 1) Aluminum (Al) toxicity; 2) Iron (Fe) toxicity; 3) Phosphorous (P) deficiency; and 4) Droughts at the seedling stage. The exploitation of gene pools from wild rice species fruitfully obtained a true introgression of desirable traits into high yielding varieties (HYVs), such as AS996 (IR64/*Oryza rufipogon*), which are tolerant to Al-toxicity, have short durations, high yields, and adaptability to acid sulfate soils. Major QTLs on chromosome 3 were detected to control Al-toxicity as identified through an analysis of the RIL population of IR64/*O. rufipogon* on control relative root length (RRL). RM232 was considered as a good marker linked to the target quantitative trait locus (QTL) on chromosome 3, then SR28 and OSR29 on chromosome 9 were also used.

QTL mapping by 126 SSRs through 225 individuals of the F_6 RILs population of AS996/OM2395 was carried out to find the *P-uptake* gene on chromosome 12. The promising genotype of OM4498 from the BC population of IR64/OMCS2000 was selected through MAS with RM235 and RM247 on chromosome 12 linked to QTL, which controls the P-deficiency tolerance.

Based on the leaf bronzing index (LBI), SSR markers were used to select promising genotypes tolerant to iron-toxicity, such as RM315 and RM212 on chromosome 1, and RM252 and RM211 on chromosome 2. The intervals among RM315-RM212 on chromosome 1, RM6-RM240 on chromosome 2, and RM252-RM451 on chromosome 4, were continually studied through further fine mapping.

A backcrossing mapping population that included 217 individuals of BC_2F_2, was set up from OM1490/WAB880-1-38-18-20-P1-HB to detect the QTLs relating to drought tolerance (DT). The QTL was located in the intervals between RM201-RM511 on chromosome 9. BAC clones $13A_9$ and $7O_3$ were used as pinpoints on the high solution map for new markers designed from their sequences. The markers became useful to help rice breeders possibly select the improved genotypes adapting to drought stress in the seedling stage.

Keywords: ***aluminum tolerance, drought tolerance, iron-tolerance, P-deficiency tolerance.***

Classification number: ***3.1***

Introduction

Acid sulfate soils (*Sulfaquefts* and *Sulfaquents*) account for 30.1 and 48.5% in the Mekong River Delta and Red River Delta, respectively [1]. Thus, acid sulfate soils have become the main constraint for rice production in the Mekong delta.

Four target points in acid sulfate soils have been identified as aluminum (Al) toxicity, iron (Fe) toxicity, phosphorous (P) deficiency, and drought stress at the seedling stage. The problems and constraints vary across ecosystems; therefore, the solutions to the problems will vary accordingly. The research thrushes each ecosystem to address these particular problems. Currently, water management and agronomic practices have been recommended. Rice varietal improvement is also considered as a key approach. QTL analysis was performed using the software package QGEN from Cornell University and MapL from Japan University. MapMarker/QTL (IRRI) was also used to find the location of major and minor genes. The threshold for declaring a QTL for P deficiency tolerance was at LOD > 3. All markers were tested for the expected 1:1 ratio.

Tolerance to Al-toxicity

Since the aluminum (Al) forms of soils and their solubility have a high pH of 5 or less, Al-toxicity is one of the major growth limiting factors of acidic soils [2]. Roots injured by high Al-concentrations are usually stubby, thick, dark-colored, brittle, poorly branched, and have reduced root length and volume.

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Al-toxicity may inhibit shoot growth by limiting the supply of nutrients and water due to poor subsoil penetration or lower root hydraulic conductivity. Y. Tang, et al. (2000) [3] mapped a gene for Al-tolerance on the long arm of chromosome 4H of barley, 2.1-cM proximal to the marker Xbcd117, and 2.1-cM distal to the markers Xwg464 and Xcdo1395. P. Wu, et al. (2000) [4] identified several QTLs conferring Al-tolerance in a random inbred mapping-population derived from Azucena and IR1552. V.T. Nguyen, et al. (2001) [5] also detected five QTLs for Al-tolerance scattered across five chromosomes with a major QTL located on chromosome 1. V. Nguyen, et al. (2002) [6] found ten QTLs located on nine chromosomes for Al-tolerance using a doubled-haploid population derived from the cross of CT9993 x IR62266. Mapping using Indica x japonica populations identified QTLs associated with a transgressive variation where alleles from a susceptible *aus* or *Indica* parent enhanced Al-tolerance in a tolerant *Japonica* background [7].

Three populations of *O. rufipogon* were collected by Duncan Vaughan and Bui Chi Buu in 1989 at Tram Chim - bird sanctuary (Dong Thap Muoi), which area has strong acid sulfate soils, and its pH varies from 2.8 to 3.2 [8].

A total of 274 RFLPs from Cornell University and RGPs digested by *Eco*RI, *Eco*RV, *Dra*I, *Hin*dIII, and *Xba*I exhibited 14.0, 12.5, 19.8, 27.7, and 19.5% degrees of polymorphism, respectively. A population of 171F_6 recombinant inbred lines were derived from the cross of IR64 x *O. rufipogon* (acc. 106412). A genetic map, consisting of 151 molecular markers covering 1,755 cM with an average distance of 11.6 cM between loci, was constructed (Table 1). The seedling stage, a major QTL for RRL, explained 24.9% of the phenotypic variations, and was found on chromosome 3 of the rice varieties (Fig. 1 and 2). These results indicated the possibilities to use MAS and pyramiding QTLs for enhancing Al-tolerance in

Table 1. QTL mapping by 126 SSRs through 225 individuals of the F_6 RIL population of AS996/OM2395 [10, 11].

Chromosome	cM	Number of SSRs	Mean of genetic distance between two markers
1	507.5	18	28.19
2	206.7	14	14.76
3	795.9	12	66.33
4	216.7	13	16.70
5	196.6	11	17.87
6	101.4	6	16.90
7	319.2	13	24.55
8	115.7	7	16.52
9	99.9	8	12.48
10	79.9	5	15.98
11	115.9	7	16.55
12	150.1	12	12.50
Total		**126**	**23.05**

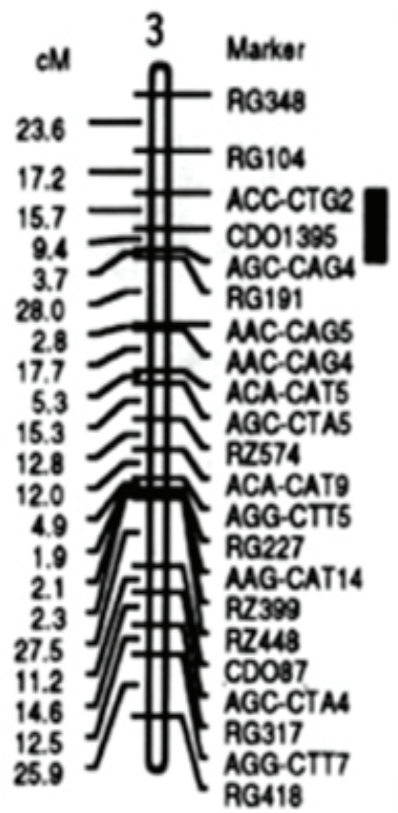

Fig. 1. QTLs controlling Al-tolerance related to RRL on chromosome 3.

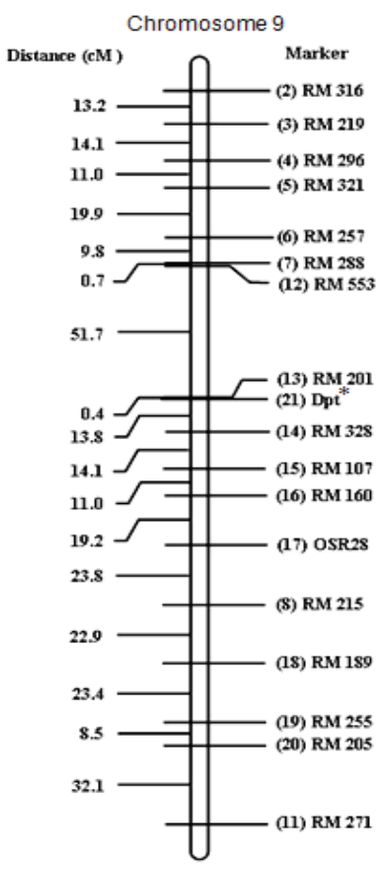

Fig. 2. Fine mapping on chromosome 9 from BC_2F_2 of OM1490/WAB880-1-38-18-20-P1-HB [12, 13].

Table 2. Putative QTLs detected for RRL by interval mapping analysis [9].

Interval	Chromosome	Length (cM)	Additive effect (DPE)	LOD	R^2
RG406-RZ252	1	6.5	0.100 (O)	2.4	9.0
CDO1395-RG391	3	0.6	0.167 (O)	8.3	24.9
RZ629-RG650	7	29.8	0.126 (O)	5.4	22.5
RG28-RM223	8	31.0	0.104 (O)	2.5	20.8
RM201-WALI7	9	10.0	0.109 (O)	2.6	9.9

DPE (direction of phenotypic effect): The allelic genetic effect and the O and I observed shows that the favorable alleles were derived from *O. rufipogon* and IR64, respectively; LOD: The maximum-likelihood of LOD score for the individual QTL; R2: Phenotypic variation explained by the individual QTL.

Table 3. Nature of gene variation for important characters under P-stress [20].

Trait	$(H_1/D)^{1/2}$	$2\sigma^2gca/(2\sigma^2gca + \sigma^2sca)$	H^2ns(%) (Narrow sense heritability)
Tilling capacity	1.94	0.16	19.70
Growth duration	0.98	0.56	33.90
Filled grains/pan.	5.80	0.01	3.10
Root dry weight	0.81	0.03	20.90

rice varieties [9]. AS997 was officially released and has become a leading variety adapted to acid sulfate soil areas in the Mekong river delta so far. The exploitation of the gene pool from wild rice species fruitfully displayed a true introgression of desirable traits into high-yielding varieties (HYVs), such as AS996 (IR64/*O. rufipogon*), which is tolerant to Al-toxicity and has short duration, high yield, and adaptability to acid sulfate soils.

Major QTLs on chromosome 3 were detected to control Al-toxicity, and this was observed through the analysis of the RIL population of IR64/*O. rufipogon* on RRL (Table 2) [9].

Tolerance to P-deficiency

P-deficiency in soils is a major yield-limiting factor for rice production. Increasing the P-deficiency tolerance of rice cultivars may represent a more cost effective solution than relying on fertilizer application [14]. The QTL linked to marker C443 on chromosome 12 displayed a major effect. Two of the three QTLs were detected for internal P-use efficiency, which included a major one on chromosome 12, that coincided with QTLs for P-uptake; however, whereas Indica alleles increased P-uptake they reduced P-use efficiency [14]. Three QTLs that were identified for dry weight and four QTLs for P-uptake together explained 45.4 and 54.5% of the variation for the respective traits. M. Wissuwa, et al. (2002) [15] finally identified the gene *Pup1,* which controls P-deficiency tolerance on chromosome 12, in acidic soils. Y.J. Zhang, et al. (2010) [16] identified the interval of R3375-R367 on chromosome 12, which controls P-deficiency tolerance. Common quantitative trait loci (QTLs) for P-deficiency tolerance have been mapped on chromosomes 6 and 12 [14, 15, 17]. P-deficiency has been identified as the main factor in preventing the realization of high-yielding potentials of modern varieties in lowland rice production as well [18]. This problem is aggravated by the high P-fixing financial capacity of many soils commonly found in rice growing regions [19].

The allelism test and QTL map analysis were conducted among progenies of mapping populations of Kasalath 47/OM4495 (BC_2F_3) and AS996/OM2395 (BC_2F_3).

The genetic nature of some characters related to P-deficiency tolerance was studied using diallele analysis. Suitable materials were chosen as OM723-11, OM850, IR64, IR50404, OM997, and IR59606. The tillering ability was considered as a good selection criteria. Maximum tiller numbers were scored at 45 days after transplanting the hybrids and their parents, constituting a 6 x 6 diallel set. However, shoot dry weight is the most sensitive plant parameter to P-deficiency, followed by root dry weight and the number of tillers. The proportion of dominant and recessive genes in the parent (K_D/K_R = 1.6) was more than one unit, which means that the dominant gene actions were more important under P-stress. The tendency of + ve alleles was clear ($H_2/4H_1$ = 0.37) showing the higher the root dry weight, the better tolerance to P-deficiency.

The variance ratio $2\sigma^2gca/(2\sigma^2gca + \sigma^2sca)$ was computed from expected components of the mean square assuming a fixed model to access the relative importance of additive and non-additive gene effects in predicting progeny performance (Table 3).

The tolerance variety of AS996 to P-deficiency is one derivative of *O. rufipogon,* whereas high-yielding varieties of OM2395 are sensitive. The SSR linkage map consisted of 116 polymorphic SSR markers which showed the location of QTLs associated with relative shoot length, RRL, relative shoot dry weight, relative root dry weight under the Yoshida solution treatments of P-deficiency (0.5 mg P/liter), and P-adequate (10.0 mg P/liter). The map length was 2,905.5 cM with an average interval size of 23.05 cM. Based on the constructed map, a major QTL for P-deficiency tolerance was located on chromosome 12. Several minor QTLs were mapped on chromosomes 1, 2, 5, and 9. The study indicated that the candidate genes linked to RM235 and RM247 on chromosome 12, had an interval distance of 0.2 cM (Fig. 3 and Table 4) [10, 11].

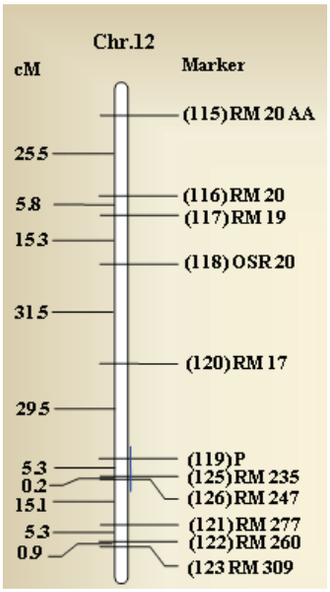

Fig. 3. QTL controlling P-uptake under acidic soils on chromosome 12.

Phosphorous-uptake 1 (*Pup*-1) controlling P-deficiency tolerance was considered as one of the most promising QTLs to develop rice genotypes (*Oryza sativa* L.) that are tolerant to abiotic stress. Gene-based molecular markers which were distributed among QTLs were fine-mapped as a 278-kb region [21] to be useful for rice breeders.

DT at the seedling stage

Acid-sulfate toxicity normally combines with drought stress at the seeding stage in dry seasons (April-May) to be harmful to rice crop in the Mekong River Delta. Crop tolerance connected to drought is genetically and physiologically complicated. Many morpho-physiological traits putatively contribute to DT, and multiple genes or quantitative trait loci (QTLs) typically control each of these traits. It is influenced by the environment to a great extent. Developing DT rice varieties has not been very successful despite the efforts made by breeders because they are done through practical breeding programs. Populations are typically segregating for maturity, making it difficult to accurately, repeatedly, and uniformly time and manage relevant water stress levels for selections. In most rice growing areas, yield reductions due to drought have been observed. To overcome this problem, it was proposed to improve DT by marker-assisted selection (MAS) for DT. A marker-assisted back-crossing (MABC) breeding program was conducted to improve the root morphological traits. This variety, the recurrent parent in the MABC, was not previously used for QTL mapping. The donor parents as WAB880-1-38-18-20-P1, IR65195-3B-2-2-2-2, and WAB881 SG9 from IRRI, and were crossed with OM1490 and OM4495 (Indica genotypes). Using 20 marker assays in a total of 229 lines of BC_2F_2 were evaluated for root length (RL), spikelet fertility (SF), DRR (drought recovery score), and yield (Y). The target segment on chromosome 9 (RM201) was significantly related to root length and DT under drought stress treatments, confirming that this root length QTL from OM1490/WAB880-1-38-18-20-P1, OM1490/WAB881 SG9, and OM4495/IR65195-3B-2-2-2-2 (Table 5). The data suggested that DT 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 DT are discussed.

BAC clones $13A_9$ and $7O_3$ were used as pinpoints on the high solution map for new markers designed from their sequences. The markers became useful to help rice breeders possibly select

Table 4. Interval mapping analysis of the target characters.

Index	Interval marker	Chromosome	P-value	Centi-Morgan
8-9 (RSL)	RM307-RM237	1	0.001	10.8
63-64 (RSDW)	RM291-RM261	5	0.000	12.0
125-126 (RSDW and RSL)	RM235-RM247	12	0.001	0.2

RSL: Relative shoot length; RSDW: Relative shoot dry weight.

Table 5. QTL mapping by 232 SSRs through 225 individuals of a BC_2F_2 population of OM1490/WAB880-1-38-18-20-P1 [12, 13].

Chromosome	cM	Number of SSRs	Mean of genetic distance between two markers
1	355.5	24	14.81
2	337.0	25	13.08
3	221.8	19	11.67
4	187.9	18	10.43
5	183.2	17	10.77
6	120.9	20	6.04
7	189.0	18	10.50
8	180.9	17	10.64
9	290.4	20	16.13
10	133.3	15	8.88
11	177.2	18	9.84
12	186.6	21	8.88
Total	**2,553.7**	**232**	**10.97**

improved genotypes that are adapting to drought stress in the seedling stage (Fig. 2 and 5) [22]. The rice variety OM6162 was well-adapted to drought prone areas, and has been released by MARD through the marker-assisted backcrossing (MAB) approach from C50/Jasmine 85/C50 [22]. Molecular breeding approaches, such as marker-assisted backcrossing, marker-assisted recurrent selection, and genome-wide selection, have been suggested to be integrated into crop improvement strategies to develop drought-tolerant cultivars that will enhance food security in a changing and more variable climate [23].

Iron-toxicity tolerance

'Bronzing', the symptom of iron-toxicity in rice, is caused by high ferrous (Fe^{2+}) concentrations found in flooded soils in many of the lowlands and swamps in India, West Africa, and other regions. Molecular markers linked to genes for tolerance to ferrous (Fe^{2+}) toxicity in rice seedlings were identified by using 175 DNA markers mapped on all of the chromosomes of a double haploid population derived from a cross between an upland variety, Azucena, and the Indica variety, IR64 [24]. In preliminary screening using toxic and non-toxic solution cultures, no leaf bronzing was observed in Azucena under Fe^{2+} stress with 250 mg Fe^{2+}/L at pH 4.5 for four weeks, but clear symptoms appeared in the IR64 variety [25].

Based on the leaf bronzing index, SSR markers were used to select promising genotypes tolerant to iron-toxicity, such as RM315 and RM212 on chromosome 1, and RM252 and RM211 on chromosome 1. The intervals between RM315-RM211 on chromosome 1 (Fig. 4), RM6-RM240 on chromosome 2, and RM252-RM451 on chromosome 4 (Table 6) were continued studied through further fine mapping (Fig. 5). Marker RM252 was finally recommended (Table 7).

J.L. Wan, et al. (2005) [27] conducted the study on F_2 and equivalent F_3 populations derived from Japonica/ Indica crosses of rice and Longza 8503/ IR64, and they were raised under iron-enriched solution cultures, and are used to map QTLs that control ferrous iron-toxicity tolerance. Leaf bronzing index, plant height (PH), and maximum root length (MRL) were evaluated. QTLs controlling LBI were located at the region of RM315-RM212 on chromosome 1, RM6-RM240 on chromosome 2, and RM252-RM451 on chromosome 4.

Ethylene production of rice roots significantly increased when grown under

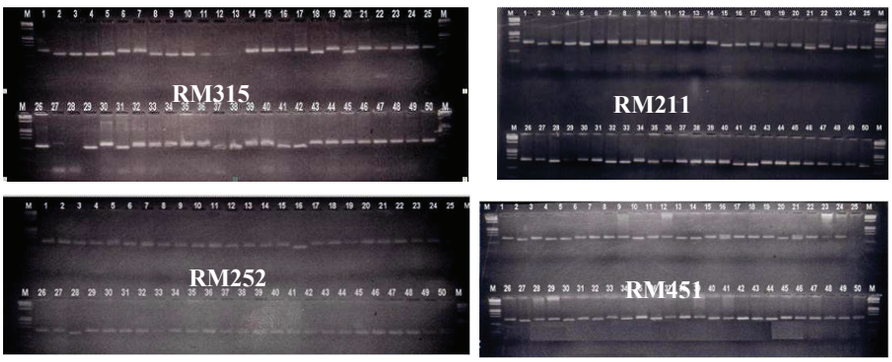

Fig. 4. PCR products at the loci RM315 (left) and RM211 (right) on chromosome 1; loci RM252 (left) and RM451 (right) on chromosome 4.

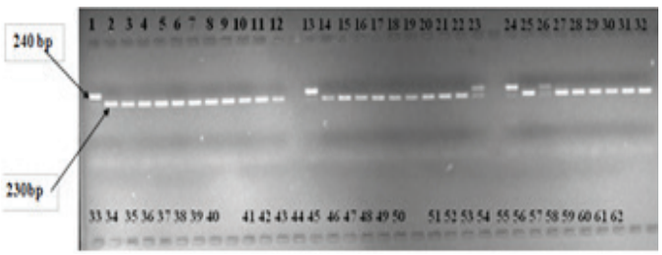

Fig. 5. PCR products at the locus RM23805 on chromosome 9 from BC_2F_2 of OM1490/WAB880-1-38-18-20-P1-HB [12, 13, 22].

Table 6. SSRs linked to the putative QTLs concerning to iron-toxicity tolerance under the iron concentration of 100 ppm in Yoshida nutrition solution [26].

Chrm.	Marker	F- primer	R-primer	Motif
1	RM315	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG	(AT)4 (GT)10
2	RM6	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC	(AG)16
4	RM252	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	(CT)19
9	RM201	CTCGTTTATTACCTACAGTACC	TACCTCCTTTCTAGACCGATA	(CT)17

Table 7. Phenotypic and genotypic assessment to estimate the accuracy of the SSR markers related to iron-tolerance.

Marker	Number of individuals	Homozygous R	Homozygous S	Heterozygous	Predictability (%)
RM6	24	22	2	0	91.67
RM240	24	16	8	0	66.67
RM252	24	20	2	2	83.33
RM451	24	12	12	0	50.00

Fe-depleted conditions. Fe-limiting conditions increased ethylene production and signaling in rice varieties [28]. Molecular properties of GR (glutathione reductase) (gene *OsGR*) from rice (*Oryza sativa* L.) was considered as reducing the deleterious effects of unfavorable abiotic conditions such as iron-toxicity [29].

Rice breeding for acid sulfate soils will be considered as a key activity in the coming years when considering how to narrow yield gap in less favorable areas. Priorities will be considered as marker-assisted selection combined to the advantages of conventional breeding methods. Vietnam needs to increase capacity building biotechnology to rice improvement and to receive assistance in preparing pre-breeding materials especially by IRRI. The integration of biotechnology tools with conventional breeding methods offers new opportunities to increase rice productivity and sustainability, achieve better progenies tolerant to acid sulfate toxicity.

The potential of genetic diversity has not been adequately utilized. We need the collaboration to make better use of this potential latest biotechnological methods employed in conjunction with conventional rice breeding program.

Conclusions

QTL mapping is an important activity connecting genome research to varietal improvements, which is a key application to be applied to breeding for acid sulfate soil adaptations.

PCR-based markers in MAS are to be identified to have high levels of accuracy and efficiency with the emphasis on chromosomes 3 and 9 for Al-toxicity tolerance, then chr. 9 for drought tolerance, chr.12 for P-deficiency tolerance, chr.1 and 4 for iron-toxicity tolerance.

One of the important applications on molecular linkage map is to allow "molecular dissection" of complex traits through design and analysis of QTL mapping experiments. Drought and iron-stresses have been considered as the most difficult traits to be phenotyped.

Potential GxE interactions and epistasis associated with QTLs make it more difficult to apply QTL-MAS to genetic improvement of the complex trait.

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