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Identification of QTL associated with silicon and zinc content in rice (*Oryza sativa* L.) and their role in blast disease resistance

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Abstract

QTL associated with zinc (Zn) and silicon (Si) content in rice were identified and mapped on different rice chromosomes. Based on interval mapping, a QTL was detected for silicon content on chromosome three and this QTL showed positive additive effect of 0.054 and explained 12.9% phenotypic variation. Additionally, single marker analysis results identified eighteen QTL for silicon content on different chromosomes. Similarly, a total of six QTL were identified for zinc content in rice seeds using single marker analysis and mapped one each on chromosome number 1, 4, 5, 8, 9 and 11. These QTL individually explained 4.4% to 9.5% phenotypic variation. The identified QTL were mapped on chromosome regions having blast resistance QTL and candidate genes for blast resistance. The overlapping of silicon and zinc QTL and disease resistance loci on same chromosome regions suggested their positive role in blast disease resistance.

Key words: Silicon, zinc, mapping, micronutrients and quantitative trait loci.

Introduction

Micronutrients are essential for balanced nutrition in plants and animals [1, 2]. Apart from this, some micronutrients play vital role in biotic and abiotic stresses tolerance in crop plants. They are essential in chlorophyll synthesis, activation of enzymes responsible for growth hormone production and carbohydrate transformation. Silicon (Si) is a common constituent of plants, and its mean content ranges from 0.3% to 1:2 % in the dry matter of crop plants. The accumulation of silicon in plants helps in disease resistance, amelioration of abiotic stresses, and increased growth in some plants [3, 4]. The relationship between silicon content and blast susceptibility in rice was first reported by Isenosuke Onodera in 1917 [5] that stimulated further research on role of silicon content in biotic stress tolerance. Later on it was demonstrated that Si content in rice straw and husks were inversely proportional to the severity of blast disease and number of blast lesions on leaves. In rice blast disease, Si mediated resistance is conditioned by two mechanisms, one by forming a physical barrier in leaf epidermis to impede fungal penetration and the other is an active role in response to pathogen attack [5]. Resistance through physical barrier mechanism is conditioned by the existence of a layer beneath the cuticle of rice leaves and sheaths. This cuticle-Si double layer can impede *Magnaporthe grisea* penetration and, consequently, decrease the number of blast lesions on leaf blades [6]. In case of active mechanism, momilactones is synthesized in response to infection by *Magnaporthe grisea*, and express their fungitoxicity within the zone of the infection [7, 8].

Zinc is an essential trace element for microorganisms, plants and animals. For humans, sufficient zinc is needed to maintain health and plays a role in many major metabolic pathways. In plants, it is involved in a large number of enzymes, carbohydrate metabolism and protein synthesis. In biotic and abiotic stress tolerance, it plays critical roles in the defense system of cells against reactive oxygen species (ROS), and thus represents an excellent protective agent against the oxidation of several vital cell components such as membrane lipids and proteins, chlorophyll, SH-containing enzymes and DNA. The cysteine, histidine and glutamate or aspartate residues represent the most critical Zn binding sites in enzymes, DNA-binding proteins (Zn-Finger proteins) and membrane proteins. Pater *et al.* [9], reported first time a cDNA clone Zinc-dependent Activator Protein-1 (ZAP1), which is expressed during pathogenesis. It has also been reported that WRKY factors with zinc finger motif, comprise a large gene family of plant-specific transcriptional regulators (WRKY6, *TRANSPARENT TESTA GLABRA2* gene and AtWRKYIS) and control several types of plant stress responses like pathogen attack, mechanical stress, and senescence in *Arabidopsis* [10-12]. Crop growth, quality,

yield, and biotic and abiotic resistance may be affected if any one of the eight essential micronutrients are lacking.

The inheritance of most of the agronomically important characteristics in rice is known and saturated molecular maps have made it possible to dissect the agronomically important traits. Extensive studies on mapping major genes and quantitative trait loci (QTL) have been reported for major agronomic traits in a number of crop species [13-16]. However, till today no research reports are available on mapping of micronutrient content in rice. Therefore, considering the vital role played by essential micronutrients in biotic and abiotic resistance mechanism, present study was conducted to identify and map the QTL associated with silicon and zinc content in rice grains and to associate their relationship with disease resistance. Consequently, the micronutrient mapping knowledge can augment in the development and identification of new genotypes with enhanced micronutrient content. The identification of QTLs responsible for micronutrient content and their enhancement in rice can be an effective strategy to address widespread dietary deficiency in human populations as well as this will enhance the plants resistance against biotic and abiotic stresses.

Materials and methods

In the present study, a subset of ninety-three double haploid lines (DHLs) developed from the cross between IR64, an *indica* variety and Azucena, a traditional aromatic *japonica* variety, was used in mapping silicon (Si) and zinc (Zn) content in rice grain. Existing molecular map of IR64 and Azucena, double haploid mapping population with 254 markers data of both RFLP and micro satellite markers was used in mapping the micronutrients. Grain samples of each double haploid line were analyzed to estimate the silicon content using wet oxidation method and zinc content was estimated using atomic absorption spectrophotometer [17]. The micronutrient values were transformed with appropriate statistical transformation procedure to have normal distribution and then QTL analysis was done. Identification and mapping of QTL was carried out by interval mapping using Mapmaker/QTL software [18] and also by single marker analysis [19].

Evaluation for blast resistance was carried out in the disease hot spot condition at Agricultural Research Station (ARS), Ponnampet, Karnataka, India. The DH lines, two parents and susceptible checks (IR50 and HR12) were sown during wet season of 2004 in the Uniform Blast Nursery. All the entries were sown in single rows of 30 cm length with 5 cm row spacing by following recommended cultural practices. All around the blast nursery, two rows of spreaders (susceptible cultivars) IR50 and HR12 were sown in order to trap the fungal spores and to enhance the natural inoculum. The observations on disease resistance and disease susceptible parameters were recorded by both visual scoring and actual infection. Disease reaction of each genotype was scored using a 0-5 scale [20] after 34 and 58 days of sowing by both visual scoring and actual infection, when all the border rows (susceptible checks) were completely infected with blast disease.

Results and discussion

The three important components of partial resistance (field resistance) to leaf blast disease in rice are Disease Leaf Area (DLA), lesion number and lesion size [21]. The QTL associated with these components, which were determined by single marker analysis and interval analyses are presented in Table 1.

Table 1. QTL associated with Leaf blast disease resistance in rice DH population derived from the cross between IR64 and Azucena identified by interval mapping (Threshold LOD > 1.50)

Sl. No.	Traits	QTLs	Chro.	Flanking markers	% variation	LOD score
1	DLAV1	1	3	RG 910-RZ 519	10.30	1.9830
2	DLAA1	1	5	RG 556-RZ 390	7.30	1.5550
3	LSNN1	3	4	RG 218-RG 190	15.50	1.7510
			7	RG 488-RG 769	19.40	2.0450
			9	RG 451-RZ 404	24.80	2.9620
4	LSSI1	3	4	RG 218-RG 190	13.70	1.5400
			7	RG 488-RG 769	18.60	1.9710
			9	RG 451-RZ 404	19.20	2.2130
5	DLAV2	2	4	RG 218-RG 908	10.90	2.6320
			4	RG 449-RZ 675	8.40	1.9490
6	DLAA2	1	8	A10K 250-RZ 617	7.40	1.7490
7	LSNN2	2	1	K5-W 1	14.00	2.0920
			4	RG 143-RG 620	12.50	1.8330

DLAV = Diseased Leaf Area (visual); DLAA = Diseased Leaf Area actually computed by using 0-5 scale; DLAV1 = DLA scored visually on 34th day after sowing DLAA1 = DLA computed for the above data LSNN1 = No. of susceptible lesions LSSI1 = Size (cm2) of susceptible lesions; DLAV2 = DLA scored visually on 58th day after sowing DLAA2 = DLA computed for the above data LSNN2 = No. of susceptible lesions

Based on interval mapping results one QTL was detected for silicon content in rice grains on chromosome 3 between RG191 and RZ678 RFLP markers at 2.475 LOD. This QTL showed no dominance effect but had showed positive additive effect of 0.054 and it explained 12.9% phenotypic variation. Eighteen QTL were identified using single marker analysis for silicon content on chromosomes 2, 3, 4, 5, 6, 7 and 12. Maximum of six QTL were identified and mapped for silicon content

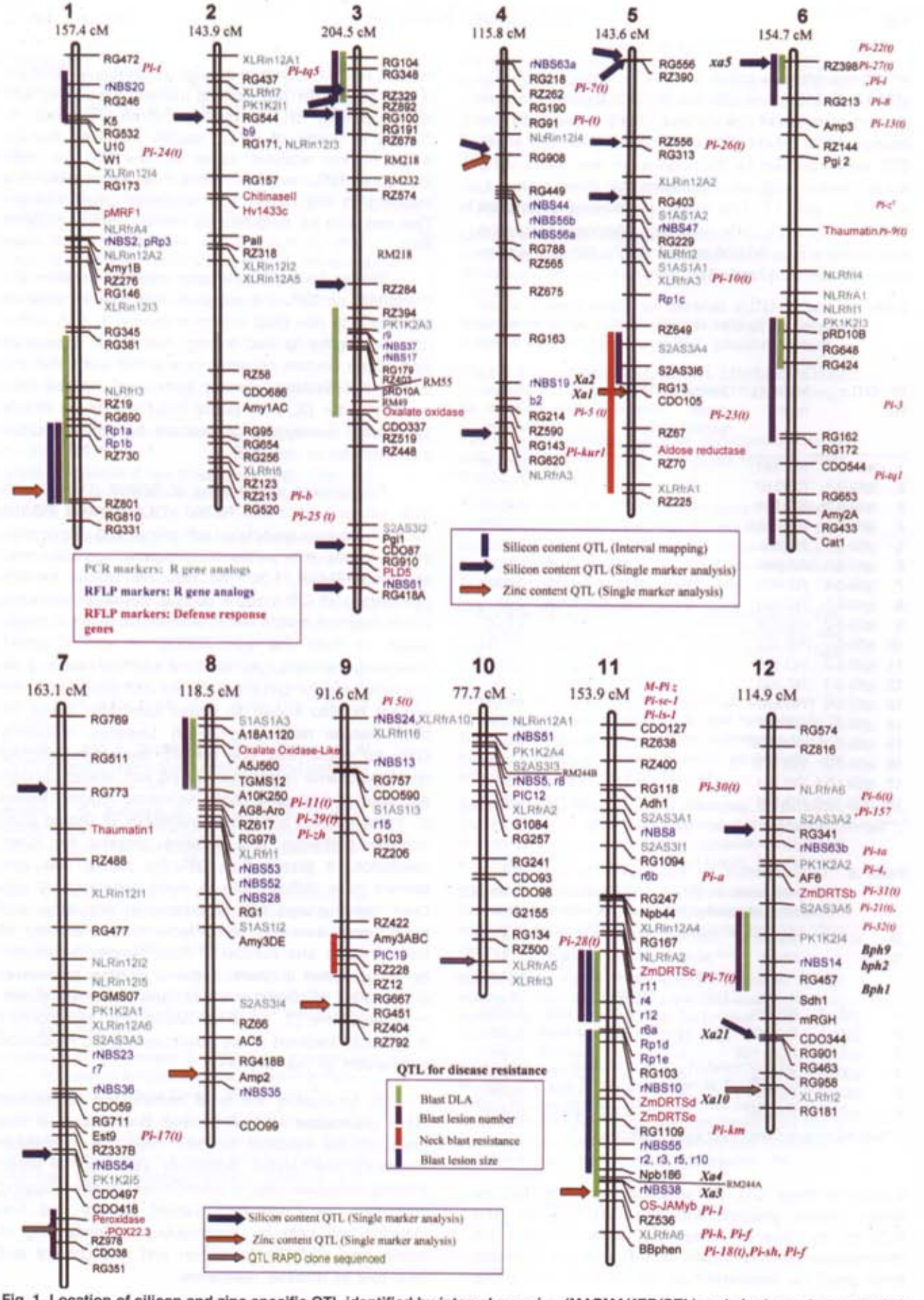

Fig. 1. Location of silicon and zinc specific QTL identified by interval mapping (MAPMAKER/QTL) and single marker analysis in IR 64 × Azucena DH mapping population of rice (map position of candidate genes/markers as in Ramalingam *et al.*, 2003)

on chromosome number 3. Out of which, two QTL, qtSi-3-2 (RG191) and qtSi-5-3 (RZ390) explained higher percent of variation (11.0% and 16.0% respectively) as scampered to others (Table 2). Similarly, a total of six QTL were identified for Zinc content in rice seeds using single marker analysis, one each on chromosome 1, 4, 5, 8, 9 and 11. The range of phenotypic variation was 4.4% to 9.5%. The maximum phenotypic variation was explained by RZ536 marker (9.5% R2) present on chromosome 11 (Table 3).

Table 2. List of QTL's detected for silicon content in rice grain on percent basis in IR64 × Azucena double haploid mapping population using single marker analysis

Sl. No.	QTLs	Marker's name	Chromosome number	R2 (% phenotypic variation)	F value	Probability values
1	qtSi-2	RG 544	2	4.9*	4.3553	0.0399
2	qtSi-3-1	CDO 87	3	5.8*	5.172	0.0255
3	qtSi-3-2	RG 191	3	11.0**	10.4554	0.0017
4	qtSi-3-3	RG 418A	3	5.5*	4.9432	0.0289
5	qtSi-3-4	RZ 329	3	8.0*	7.3486	0.0081
6	qtSi-3-5	RZ 284	3	6.3*	5.7023	0.0192
7	qtSi-3-6	RZ 892	3	5.5*	4.9177	0.0293
8	qtSi-4-1	RG 143	4	7.6*	6.9436	0.01
9	qtSi-4-2	RG 908	4	6.9*	6.3011	0.014
10	qtSi-5-1	RG 403	5	6.4*	5.7893	0.0183
11	qtSi-5-2	RG 556	5	7.1*	6.4587	0.0129
12	qtSi-5-3	RZ 390	5	16.0**	16.6934	0.0001
13	qtSi-5-3	RZ 556	5	6.3*	5.6439	0.0198
14	qtSi-6	RZ 398	6	5.1*	4.517	0.0365
15	qtSi-7-1	CDO 497	7	6.61*	5.942	0.0169
16	qtSi-7-2	RG 773	7	6.0*	5.4361	0.0221
17	qtSi-12-1	RG 341	12	5.7*	5.1532	0.0258
18	qtSi-12-2	RG 901	12	6.2*	−5.5718	0.0206

*,**Significance at 5 and 1 per cent, respectively

Table 3. QTL identified for zinc content in rice grain on percent basis in IR64 × Azucena double haploid mapping population using single marker analysis

Sl. No.	QTLs	Marker's name	Chromosome number	R2 (% phenotypic variation)	F value	Probability values
1	qtZn-1	RZ 801	1	5.0*	4.8867	0.0295
2	qtZn-4	RG 908	4	4.8*	4.6465	0.0337
3	qtZn-5	CDO 105	5	4.4*	4.0328	0.0403
4	qtZn-8	Amp_2	8	5.2*	5.0958	0.0263
5	qtZn-9	RG 451	9	4.6*	4.4941	0.0367
6	qtZn-11	RZ 536	11	9.5**	9.6676	0.0025

*,**Significance at 5 and 1 per cent, respectively

Cluster of three QTL for silicon content identified by single marker analysis and one QTL (RG191 and RZ678) by interval mapping were mapped on chromosome 3. This cluster of QTLs on chromosome three could be considered as one QTL as one of the marker, RG191, identified through single marker analysis is also part of the two flanking markers associated with silicon content on chromosome three. However, to classify a cluster of QTLs, usually identified through single marker analysis, either as one QTL or more than one QTL requires further saturation and fine mapping in this region with additional DNA markers. This can also be simplified by interval marker analysis also.

Similar findings have been reported to show the clustering of QTLs associated with partial disease resistance to rice blast on chromosome 3, 4, 5 and 8 [22]. Analogous to this finding, two QTLs associated with silicon content on chromosome five were also the part of two flanking markers associated with the QTL governing the DLA for partial blast resistance (Table 1). Similar overlapping of markers for different QTLs was reported in rice [23].

Furthermore, overlapping of RG908 (DLAV2 with both silicon and zinc), RZ390 (DLAA1) and RG556 (DLAA1) markers associated with silicon and zinc content in rice grains with partial resistance to rice blast was observed (Tables 1-3). The plausible reason for this overlapping of QTLs could be due to trait correlations, which may result from either pleiotrophic effect of single genes or from the tight linkage of several genes controlling the traits [24, 25]. The clustered regions on chromosomes for QTLs associated with silicon and zinc content is also known to harbor candidate genes for blast disease resistance [26-28]. Likewise, remaining QTL, for silicon and zinc content, were also mapped on chromosome region associated with already known rice blast and bacterial blight resistance regions. Based on these findings and earlier evidences of shared plant defense pathways and defense proteins for biotic resistance, it shows that QTL for silicon and zinc content have definite role in biotic resistance. It has been demonstrated that Si content in rice straw and husks were inversely proportional to the severity of blast disease and number of blast lesions on leaves. Its accumulation in plants helps in disease resistance, amelioration of abiotic stresses, and increased growth in some plants [3, 4]. Thus, silicon and zinc content is indirectly involved in the abiotic and biotic resistance mechanism of plants [10-12].

In conclusion, the initial identification of putative QTLs associated with silicon and zinc content in rice grain can be validated for their clear role in disease resistance mechanism. Additional saturation of either existing molecular map or development of new mapping population for these micronutrient content and fine mapping will help in unambiguous understanding of overlapping of QTLs for silicon and zinc content and their role in disease resistance.

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