



Mapping QTLs Related to Zinc Deficiency Tolerance under Aerobic Rice Ecosystem

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Abstract

Rice (*Oryza sativa* L.) is the "Global Grain" because of its use as prime staple food in about 100 countries of the world. Aerobic rice provides for effective use of water as the concept of flooding paddy fields is abandoned in this ecosystem. But due to the transition from flooding of paddy fields to aerobic system of rice cultivation many factors that determine nutrient (Zinc) bioavailability changes in addition to water deficit. Achieving high yields under aerobic conditions requires new varieties of "aerobic rice" combining both the water stress tolerance and nutrient (especially Zinc) deficiency tolerance traits. The present study was undertaken to know the genetic and molecular basis of zinc (Zn) deficiency tolerance using an *indica/indica* F₄ RIL population of CO51 (A high yielding low land variety with low zinc efficiency) and CB-06-803-2 (An improved upland culture with high zinc efficiency) comprising of 246 RILs. These RILs were phenotyped under aerobic system in control (+Zn) and stress (-Zn) plots and genotyped using 80 SSR markers specific to zinc transporter genes (*ZIP* family) and root related traits. The parents for the mapping population were selected by screening 60 rice germplasm lines for ZE under aerobic condition. The reported map position of gramene database was used to estimate the QTLs and followed inclusive composite interval mapping of additive and dominant (ICIM-ADD) method. The QTL analysis resulted in the identification of three QTLs viz., zinc efficiency in shoot (ZES), days to 50 per cent flowering in control (DFFC) and shoot zinc content in stress (SZCS) condition.

Keywords: Aerobic rice; Zinc deficiency tolerance; ICI mapping; QTL

Introduction

The Wonder Cereal, Rice (*Oryza sativa* L.) is the heart of our culture and the staple food crop consumed by more than 50 per cent of the world's population. It is estimated that, with every additional 1 billion people, 100 million additional tons of rice need to be produced but with limited and highly competitive land and water resources [1]. There is an urgent need to increase rice production to meet global demand. With the grain yield in irrigated areas reaching stagnation, a large portion of the predicted increase has to come from the rain fed lowland and upland rice areas. Irrigated rice has very low water-use efficiency as it consumes 3000–5000 litres of water to produce 1 kg of

rice. The traditional rice production system not only leads to wastage but also causes environmental degradation and reduces fertilizer use efficiency. Along with high water requirement, the traditional system of transplanted rice production in puddled soil on long run leads to destruction of soil aggregates and reduction in macro pore volumes, and to a large increase in micro pore space which subsequently reduce the yields of post rice crops [2].

In rice, among the several production constraints, availability of irrigation water is a major factor as it consumes about 70 percent of the water available for agriculture [3]. Therefore, ways must be sought to reduce water requirements in rice and increase its productivity. Water requirements can be lowered by reducing water losses by seepage, percolation, and evaporation. Promising technologies developed are saturated soil culture, intermittent irrigation and the system of rice intensification. However, these technologies still use prolonged periods of flooding, so water loss remains high. So that, an aerobic rice production is developed which is a fundamentally different approach where rice is grown like an upland crop, such as wheat, on non flooded aerobic soils, thereby eliminating continuous seepage and percolation and greatly reducing evaporation [4]. In India, aerobic rice varieties are bred by crossing lowland and upland varieties. But due to the transition from flooding of paddy fields to aerobic system of rice cultivation many factors that determine nutrient (Zinc) bioavailability changes in addition to water deficit. This includes change in bulk soil pH which may either increase or decrease depending on the original soil pH [5]. Increase in redox potential causing Fe oxidation, with concomitant acidification, precipitation of Fe(OH)₃ and adsorption of Zinc (Zn) on these oxides. Increased nitrification may cause plants to take up NO₃⁻ instead of NH₄⁺ which also causes the rhizosphere pH to increase. Organic matter on to which Zn can be adsorbed will be oxidized [6]. Furthermore, under oxidized condition, the production of phytosiderophores essential for chelating zinc and making it available to rice plants is low [7].

Therefore achieving high yields under aerobic conditions requires new varieties of "aerobic rice" combining both the water stress tolerance and nutrient (especially Zn) deficiency tolerance traits. Thus there is an immediate need to accelerate the genetic improvement of rice for aerobic cultivation, which until recently has received less attention compared to lowland rice. This could be hastened through the identification of molecular markers linked to traits associated with water use efficiency and zinc deficiency tolerance under aerobic condition in rice and deployment *via* marker assisted breeding. To date, several QTLs were reported to be linked to traits associated with water stress in rice but markers/QTLs have not been reported for zinc deficiency tolerance under aerobic condition in rice. Zinc (Zn) deficiency is the most widespread micronutrient disorder in rice, but efforts to develop cultivars with improved tolerance have been hampered by insufficient understanding of genetic factors contributing to tolerance. Among several strategies, mapping quantitative trait loci (QTLs) associated with tolerance has greater promise for identifying the most informative traits that would provide realistic information for rice breeders based on marker assisted selection. [8]. With this informational background, the present study was undertaken to evaluate the RIL (derived out of a cross between zinc efficient and inefficient genotypes) for yield and

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zinc deficiency tolerant traits under aerobic system in control (+Zn) and stress (-Zn) condition.

Materials and Methods

Plant material and development of mapping population

The 246 F₄ RILs derived from the cross involving CO51 x CB-06-803-2 (Table 1) were phenotyped along with the parents in a zinc deficient soil under aerobic condition at Wetland, Dept. of Farm management, TNAU, Coimbatore. The DNA was extracted from 246 F₄ individuals and parents at Marker assisted selection lab, Department of Rice, Centre for Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University, Coimbatore for genotyping using SSR markers.

Phenotypic data analysis

The 246 F₄ RILs were evaluated along with the parents in a zinc deficient soil (0.8 ppm) under aerobic condition at Wetland, Dept. of Farm management, Tamil Nadu Agricultural University, Coimbatore. To raise the crop under aerobic condition, land was prepared similar to upland condition and direct seeding was done. A control plot (+Zn) was maintained by treating the soil with recommended dose of 25 Kg/ha ZnSO₄. Each entry was direct sown in a plot size of 3.6 m x 0.8 m by adopting a spacing of 20 cm x 20cm. The entries were replicated twice in Randomized block design. Single seedling per hill was maintained. The crop was irrigated everyday till establishment and thereafter the crop was irrigated once in 4-5 days (when hairline crack occurs).

Data were recorded on different characters viz., Shoot zinc content (ppm), Root zinc content (ppm), Days to 50% flowering (days after sowing), Zinc efficiency in shoot, Zinc efficiency in root and plot yield (g). Variability parameters, viz., phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability, genetic advance and genetic advance as per cent of mean (GAM), were estimated using the standard methods for the mapping population under aerobic field condition at +Zn (control) and -Zn (stress) levels.

Estimation of shoot/root zinc content

Five plants from 246 F₄ RILs were harvested 28 days after germination, because Zn deficiency problems are usually most severe in the first 2-4 weeks of growth [9]. Shoots were cut off at ground level and soil was washed from the roots with tap water. Shoots and roots were rinsed in deionized water, oven dried at 70°C for 48 h, and weighed. Zn in plant digests was analyzed with an atomic absorption spectrophotometer (Varian SPECTRAA-200).

Estimation of zinc efficiency

Zinc Efficiency (ZE) was calculated as the ratio of shoot (root) dry weight under Zn deficiency over that under adequate Zn supply [10,11].

Estimation of plot yield (g)

The plot yield was calculated by weighing the cleaned dried grains harvested from the entire plot of every genotype and expressed in grams.

Genomic DNA isolation

DNA was extracted from 15-20-day-old fresh fully expanded leaves of the parental genotypes and from their respective F₄s using the modified cetyl trimethyl ammonium bromide (CTAB) extraction method as described in Hoisington et al. [12]. The DNA quality and quantity were checked on 0.8 % agarose gel and DNA concentration was normalized to 10 ng/L.

QTL analysis

Genotyping and phenotyping data obtained from the mapping population was subjected to QTL analysis using ICIM through the QTL ICI mapping software version 3.2. The ICIM-ADD was performed with mapping parameters as scan interval of 1cM, 0.001 probability in step-wise regression and LOD threshold of 2.5. Graphic presentation of the linkage groups and QTLs was obtained by using the Map chart version 2.2 [13].

Results and Discussion

Plot yield of the mapping population under aerobic field condition at +Zn (control) and -Zn (stress) levels

CB-06-803-2 (0.88 and 0.81 kg) had higher plot yield than CO51 (0.44 and 0.38 kg) in both control and stress conditions respectively. Among the 246 RILs there were a wide range of variation for plot yield, ranging from 0.12 to 1.13 kg under control condition with the average yield of 0.55 kg whereas under stress, the plot yield ranged from 0.08 to 1.08 kg with the average yield of 0.28 kg. Out of 246 RILs, 2 genotypes in control and 3 genotypes in stress conditions had significantly higher plot yield than better parent CB-06-803-2. Tolerant cultivars may have lower Zn requirements or translocate relatively more Zn from roots to shoots [14]. Staple crops with high Zn efficiency (ZE) could offer a sustainable and cost-effective way to overcome Zn deficiency problems.

Phenotypic and genotypic analysis of the mapping population under aerobic field condition at +Zn (control) and -Zn (stress) levels

The results of phenotypic analyses of the F₄ population are given in Table 2. In the present study, moderate to high PCV and GCV were observed for the traits viz., shoot zinc content, root zinc content and plot yield in both control (+Zn) and stress (-Zn) conditions under aerobic system. These results indicated the presence of wider variability for these traits, and hence selection could be effective for these traits under aerobic system for zinc efficiency. Days to 50 per cent flowering showed low PCV and GCV, this might be due to the similar genetic profile of the parents for this trait. Similar findings were earlier reported by Ogunbayo et al. [15] and Tandekar et al. [16]. Heritability and genetic advance are useful tools for breeders in determining the direction and magnitude of selection. Johnson et al. [17] suggested that heritability in combination with genetic advance was more effective and reliable in predicting the resulting effect of selection than heritability alone. High heritability together with high genetic advance as percent of mean (GAM) for any characteristic indicates additive gene action, and selection will be rewarding for

Table 1: Parents of the mapping population.

Parents	Parentage	Varietal group	Origin	Features
CO51	ADT43 x RR272-1745	<i>Indica</i>	Tamil Nadu	Semi-dwarf, fine grain, high yielding low land variety with low zinc deficiency tolerance
CB-06-803-2	PMK3 x Norungan	<i>Indica</i>	Tamil Nadu	Tall, bolded grain, high yielding improved upland culture with high drought resistance and zinc deficiency tolerance

improvement of such traits. High heritability and high GAM were observed in the F₄ generation for the traits shoot zinc content, root zinc content and plot yield in both control (+Zn) and stress (-Zn) conditions under aerobic system, hence these traits were expected to respond to selection with greater efficiency under aerobic system for zinc efficiency.

Polymorphism survey and molecular analysis

In this study, the reported map position of Gramene database was used to estimate the QTLs following inclusive composite interval mapping of additive and dominant (ICIM-ADD) method. The parental survey was performed for the two parents CO51 and CB-06-803-2 using 80 SSR markers specific to Fe/Zn transporter (*ZIP*) family [18] and root traits [19] for amplifying genomic DNA fragment by polymerase chain reaction. The parental polymorphism survey identified 22 (27.50 %) polymorphic markers (17 markers specific to *ZIP* family and 5 markers related to root traits) between the parents CO51 and CB-06-803-2.

QTL analysis

QTL mapping provides a powerful selection tool for conducting physiological and genetical research to understand and possibly improve zinc deficiency tolerance under aerobic condition. Reports on QTLs associated with zinc deficiency tolerance in rice are very few. However, several *ZIP* transporter genes in rice have been reported, e.g., *OsZIP1*, *OsZIP2*, *OsZIP3*, *OsZIP4*, *OsZIP5*, *OsZIP6*, *OsZIP7*, *OsZIP8* and *OsZIP9* [20]; *OsZIP1*, *OsZIP3*, *OsZIP4*, *OsZIP5* and *OsZIP8* [18]. Under zinc deficit condition, root traits play an inevitable role in the acquisition of zinc for enhanced uptake and translocation to shoot. Several QTLs are reported for root traits in rice [6,19,21,22,23]. So in the present study, CO51 and CB-06-803-2 were surveyed for parental polymorphism using 80 SSR markers specific to *ZIP* transporter gene and root traits. Among the 80 SSR markers studied, 22 (27.50 per cent) were found polymorphic between parents. Out of 22 polymorphic markers, 17 markers were specific to *ZIP* family genes and 5 markers were related to root traits. These polymorphic markers were utilized for profiling the F₄ progenies.

Composite interval mapping

One of the most important uses of QTL mapping is to apply them in marker-assisted selection (MAS) for genetic improvement of quantitative traits. Once the tightly linked markers have been identified, the traits can be selected indirectly using MAS. The reported map position of gramene database was used to estimate the QTLs following the inclusive composite interval mapping of additive and dominant (ICIM-ADD) method. The QTL analysis resulted in the identification of three QTLs viz., zinc efficiency in shoot (ZES), days to 50 per cent flowering in control (DFFC) and shoot zinc content in stress (SZCS) condition (Table 3 and Figure 1). QTL for the foremost trait zinc efficiency in shoot (ZES) was identified on chromosome 1 with flanking markers RM3694 and RM8136 and a LOD value of 2.50 explaining 13.19 per cent of phenotypic variation for this trait. The distance between the flanking markers is 1.0cM. Hence this QTL may be useful in Marker Assisted Selection programme (MAS) for zinc deficiency tolerance in aerobic system [24,25].

The QTLs for days to 50 per cent flowering in control (DFFC) and shoot zinc content in stress (SZCS) condition were formed on chromosome 5 with the flanking markers RM1187 and RM87. These QTLs had 2.57 and 2.58 as LOD with 11.83 and 11.95 per cent phenotypic variation respectively (Figure 1). The marker RM1187 was specific to *ZIP* family and the marker RM87 was related to root traits. The QTLs identified for shoot zinc content under stress (-Zn) between these two flanking markers showed that putative effect of (*ZIP*) zinc transporter and root related genes. For enhanced uptake of zinc from soil under zinc deficit condition and effective translocation to shoot for the maintenance of higher shoot zinc content. This further confirms the earlier reports that increased root uptake of zinc is one of the mechanism involved in zinc deficiency tolerance. Inclusion of some more markers in this region will help improve the success of MAS for zinc deficiency tolerance under aerobic system.

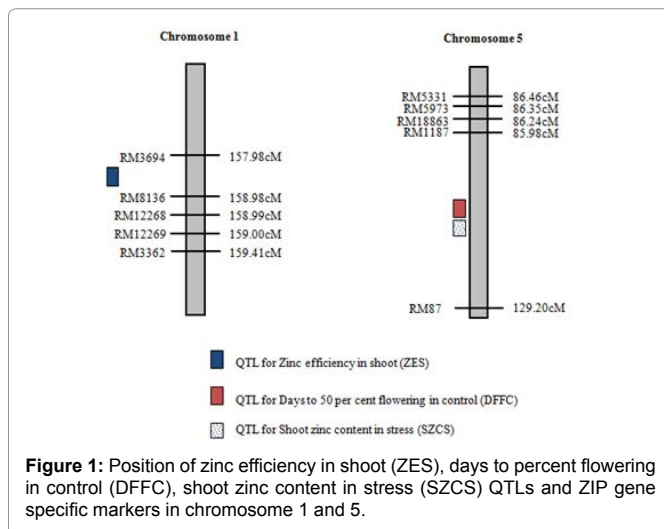
The QTLs identified in the present study is the first reported QTLs for zinc deficiency tolerance under aerobic condition in rice. In aerobic rice besides water stress, zinc deficiency is a major problem

Table 2: Estimation of genetic parameters in F₄ progenies for traits associated with zinc deficiency tolerance and yield component traits under aerobic field condition at +Zn (control) and -Zn (stress) levels.

Characters		Maxi-mum	Mini-mum	Mean	PCV	GCV	h ²	GAM
Days to 50 per cent flowering (days)	+Zn	100.00	80.00	89.62	8.20	8.06	97.12	16.32
	-Zn	105.00	80.00	91.83	7.89	7.67	94.35	15.33
	-Zn	28.25	13.75	18.11	19.85	15.94	91.00	23.82
Shoot zinc content (ppm)	+Zn	69.77	30.10	55.77	37.61	31.92	72.00	55.79
	-Zn	43.42	5.15	27.70	66.43	64.62	64.94	48.74
Root zinc content (ppm)	+Zn	84.80	39.95	64.69	28.92	24.55	72.04	42.92
	-Zn	58.79	13.40	31.24	26.42	24.82	88.27	48.04
Plot yield (kg)	+Zn	1.13	0.12	0.55	33.78	28.83	72.80	50.68
	-Zn	1.08	0.08	0.28	33.37	30.06	81.10	55.76

Table 3: QTL mapping of F₄ population of CO51 x CB-06-803-2 cross for yield and zinc deficiency tolerance traits in rice.

Character	QTLs	Chromosome	Position (cM)	Left marker	Right marker	LOD	R ²	Additive	Dominance
ZE in shoot	ZES	1	158.97	RM3694	RM8136	2.50	13.19	-1.05	-2.35
Days to 50 per cent flowering in control	DFFC	5	110.98	RM1187	RM87	2.57	11.83	-1.33	-2.10
Shoot zinc content in stress	SZCS	5	109.98	RM1187	RM87	2.58	11.95	-2.08	-4.47



resulting in poor establishment of crop and reduced yield [4]. Hence the QTL identified for zinc deficiency tolerance with closely linked markers may be exploited for the genetic improvement of rice genotypes under aerobic condition through marker assisted selection.

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