

Detection and Analysis of QILs Associated with Resistance to Ferrous Iron Toxicity in Rice (*Oryza sativa* L.), Using Recombinant Inbred Lines

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Abstract A mapping population of 81 F₁ lines (recombinant inbred lines: RILs), derived from a cross between a japonica variety Kinmaze and an indica variety DV85 by the single-seed descent methods, was used to detect quantitative trait loci (QILs) for leaf bronzing index (LBI) and plant height (PH) under Fe²⁺ stress condition. Two parents and 81 RILs were phenotyped for the traits by growing them in Fe²⁺ toxicity nutrient solution. A total of three QILs were detected on chromosome 3, with score LOD ranging from 3.79 to 5.89. Two QILs controlling LBI was located at the region of X279-C25 and X144-X362, and their contributions to total variation were 17.38% and 22.07%, respectively. One QIL for PH was located at the region of R1468A-R1468B, with contribution rate 23.18%. Comparing with the other mapping results, the QIL for LBI located at the region of X279-C25 on chromosome 3 was identical with the QIL for chlorophyll content on a rice function map. Another QIL for LBI located at the region of X144-X362 linked with QIL for chlorophyll content which located at the region of C136-C944 on a rice function map, the genetic distance between the two QILs was 25.6 cM. The results indicated that ferrous iron toxicity of rice was characterized by bronzing spots on the lower leaves, which spread over the whole leaves, causing the lower leaves to turn dark gray and producing chlorophyll catabolites or derivatives which reduced cytotoxicity of some heavy metals, such as ferrous iron.

Key words Rice (*Oryza sativa* L.); Resistance to ferrous iron toxicity; Recombinant inbred lines (RILs) population; Quantitative trait loci (QILs)

利用重组自交群体检测水稻抗亚铁毒胁迫的 QILs

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摘要 潜水性水稻广泛分布于中国、斯里兰卡、印度、印度尼西亚、塞拉里昂、利比亚、尼日利亚、哥伦比亚和菲律宾等国,其中我国南方稻区就有 670 万公顷低产潜育性水稻田。该类水稻田还原性强,矿质营养失调,尤以 Fe²⁺ 过量积累,对水稻生长发育产生不良的逆境胁迫作用。培育抗亚铁毒的水稻品种是简便、经济有效地提高稻谷产量的重要途径之一。本文利用 Kinmaze/DV85 的 81 个重组自交家系 (RILs) 的作图群体,采用水培鉴定方法,在亚铁毒条件下,检测叶片棕色斑点指数 (LBI)、株高 (PH) 的数量性状位点 (QILs)。结果表明,控制叶片棕色斑点指数、株高的数量性状位点都位于第 3 染色体上,各 QIL 的 LOD 值为 3.79~5.89。检测到与亚铁毒胁迫直接有关的性状叶片棕色斑点指数 QIL 2 个,分别位于第 3 染色体的 X279-C25 和 X144-X362 间,对应的贡献率分别为 17.38% 和 22.07%,其中位于第 3 染色体 X279-C25 间的叶片棕色斑点指数 QIL 与水稻功能图谱第 3 染色体上的控制叶绿素含量的 QIL 位置一致;另一个位于 X144-X362 间的叶片棕色斑点指数 QIL 与水稻功能图谱第 3 染色体上的另一个控制叶绿素含量的 QIL 连锁,相距 25.6 cM。表明在亚铁毒胁迫条件下,水稻在其叶片表面出现棕色斑点,叶片衰老,产生一些叶绿素降解物或衍生物,以提高叶片细胞对亚铁等重金属毒害的耐受能力。

关键词 水稻;抗亚铁毒;重组自交 (RILs) 群体;数量性状位点 (QILs)

中图分类号: S511

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The gleyed paddy soils are widely distributed in People's Republic of China, Sri Lanka, India, Indonesia, Sierra Leone, Libya, Nigeria, Columbia and Philippines^[1,2]. The gleyed paddy soils are estimated to cover 7.5 to 8.0 million hectares, in People's Republic of China, where ferrous iron toxicity in the gleyed paddy soils is reported to reduce rice yield by 10%—20% depending on the intensity of toxicity and the resistance of the rice cultivar^[3].

Rice genotypes differ widely in resistance to Fe^{2+} toxicity, which makes it possible to develop rice cultivars with enhanced resistance to Fe^{2+} through breeding program^[4-7]. The breeding effort could be facilitated by a better understanding of genetic background on the tolerance mechanism in rice.

In this study, based on a genetic map from a recombinant inbred lines (RILs) derived from Kinmaze/DV85 (F_{10}), QTLs associated with resistance to ferrous iron toxicity in rice were identified by using molecular markers, and their contributions to the total variation were evaluated, and the genetics and physiology of resistance to ferrous iron were discussed.

1 Materials and Methods

1.1 Plant materials

A set of recombinant inbred lines (RILs) was developed from F_1 to F_{10} , by the method of single seed descent (SSD) from a cross of Kinmaze/DV85, where "Kinmaze" was a variety belonging to *japonica* subspecies, and "DV85" was a variety belonging to *indica* subspecies. RILs (F_{10}) were provided by Professor Atsushi Yoshimura at Faculty of Agriculture, Kyushu University, Fukuoka, Japan. Two parents and eighty one plants of RILs were planted at the experimental station of Jiangsu Academy of Agricultural Sciences, People's Republic of China, and two parents and F_{11} seeds were harvested for evaluation of resistance to ferrous iron toxicity. IR26 and IR64 susceptible, IR74 and Mahsuri moderately tolerant and Suakoko 8 tolerant were used as control cultivars.

1.2 Solution culture experiment

The two parents, Kinmaze and DV85, five control cultivars, i.e., IR26, IR64, IR74, Mahsuri and Suakoko 8, and 81 RILs in F_{11} were evaluated for resistance to

their ferrous iron toxicity. Seeds were germinated after sterilization, and transplanted into quartz sand. After one week, six plants of each line and 12 plants each of parents and control cultivars were randomly selected and transferred to plastic culture containers containing 5 L nutrient solution. Individual seedlings were planted in a perforated sheet placed on the surface of solution in each container. Standard rice nutrient solution^[8] was used as the normal treatment. For Fe^{2+} stress treatment, 250 mg $\text{Fe}^{2+} \text{ L}^{-1}$ were added as FeSO_4 to the standard nutrient solution. The pH of the solution was daily adjusted to 4.5 using 1 mol/L NaOH or 1 mol/L HCl, and the solution was replaced every three days. The entire experiment was conducted in a glasshouse at Nanjing Agricultural University from June 10 to July 7, in 2002. The day temperature was 35 to 22 and the night temperature was 22 to 14 in the glasshouse during the experiment. On 7 July (28 days after treatment) each plant was harvested to test leaf bronzing index (LBI = bronzing leaf number/total leaf number) and PH, which were viewed as a direct indicator^[9,10] and an indirect indicator of resistance to ferrous iron toxicity in rice, respectively.

1.3 Genetic mapping and QTL detection

A linkage map used for QTL detection comprised 137 markers (1 386.2 cM) with an average marker density of 10.1 cM. One-way ANOVA (SAS/6.11, GLM) interval mapping analysis (Mapmaker/QTL)^[11] was performed for detecting molecular markers associated with the variations in LBI and PH and most likely positions of the gene loci. $P < 0.001$ for Type I error and \log_{10} -likelihood ratio (LOD) value of 3.0 were used as criteria to detect the significant association between marker loci and gene loci. Correlation analysis between the measurements was performed using correlation procedure of Microsoft Excel 97.

2 Results

2.1 Phenotypic response of two parents and control cultivars and correlation between traits

Ferrous iron toxicity of rice is characterized by bronzing spots on the lower leaves, which spread over the whole leaves, causing the lower leaves to turn dark gray. The two parents and five control cultivars were evaluated for resistance to ferrous iron toxicity in rice. The results

were summarized in Table 1. The LBI of Kinmaze (0.20) was approximately equal to that of control cultivar Suakoko 8 (0.08), and the LBI of DV85 (0.82) was equal to that of control cultivars IR26 (0.83) and IR64 (0.85). Therefore, Kinmaze and DV85 were evaluated to be tolerant and sensitive, respectively, to ferrous iron toxicity. Under ferrous iron toxicity stress, the PH of Kinmaze and DV85 were 54.9 cm and 65.9 cm, respectively.

Table 1 Mean LBI and PH of parents and CK

Cultivar	Mean LBI	Mean PH
Kinmaze	0.20	54.9
DV85	0.82	65.9
IR26	0.83	23.8
IR64	0.85	27.7
IR74	0.40	21.4
Mahsuri	0.35	31.2
Suakoko8	0.08	43.7

As the LBI was indicated as a phenotypic value for resistance to ferrous iron toxicity^[9,10], the correlation coefficient between LBI and PH were - 0.826. The LBI

and PH were significantly negatively correlated, which indicated that LBI was associated with PH reduction due to Fe^{2+} toxicity in this RILs population.

2.2 Phenotypic response of two parents and RILs

The distributions of the LBI and PH of the RILs were shown in Fig. 1. For the LBI counted there is clear difference between Kinmaze and DV85, but there wasn't very clear difference in PH between two parents.

2.3 The mapping of QIL for some traits associated with resistance to ferrous iron toxicity

A total of three QILs were detected on chromosome 3 (Fig. 2, Table 2), with LOD score ranging from 3.79 to 5.89. Two QILs controlling LBI was located at the region of X279-C25 and X144-X362, and their contribution to total variation were 17.38% and 22.07% respectively. Kinmaze alleles in these regions reduced the LBI on the heterozygous condition by 0.05 and 0.06, respectively. One QIL controlling PH was located at the region of R1468A-R1468B, with contribution of 23.18%. Kinmaze alleles in these regions increased PH on the heterozygous condition by 3.64 cm.

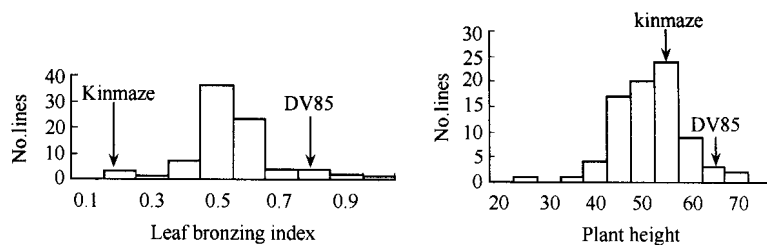


Fig. 1 Normal distribution of leaf bronzing index and plant height in RILs population

Table 2 QIL mapping of LBI and PH in rice seedlings

Traits	Marker interval	Chromosome	LOD score	PVE (%)	Additive
LBI	X279-C25	3	4.24	17.38	- 0.05
	X144-X362	3	3.79	22.07	- 0.06
PH	R1468A-R1468B	3	5.89	23.18	3.64

3 Discussion

In this study, the LBI was indicated as a phenotypic value for resistance to ferrous iron toxicity, the QIL located at the region of X279-C25 for LBI coincided with the QILs for chlorophyll content which located at the region of C515-C25 on a rice function map^[12]. Another QIL located at the region of X144-X362 for LBI on chromosome 3

linked with QIL for chlorophyll content which located at the region of C136-C944 on chromosome 3 on a rice function map^[12], the genetic distance between the two QILs was 25.6 cM. The results indicated that ferrous iron toxicity of rice was characterized by bronzing spots on the lower leaves, which spread over the whole leaves, causing the lower leaves to turn dark gray and producing chlorophyll catabolites or derivatives which reduced cytotoxicity

of some heavy metals^[13], such as ferrous iron.

Yan *et al.* (1998) used a doubled haploid population of 123 lines from IR64/Azucena to detect a conditional QTL for PH between markers CDO87 and RC910 on chromosome 3 only after 30 days of transplanting^[14]. In this study a QTL was also detected for PH at the region of

R1468A-R1468B on chromosome 3 after 30 days of transplanting under ferrous iron toxicity condition. Whether the QTL for PH at the region of R1468A-R1468B on chromosome 3 is identical with the conditional QTL at the region of CDO87-RC910 needs further study.

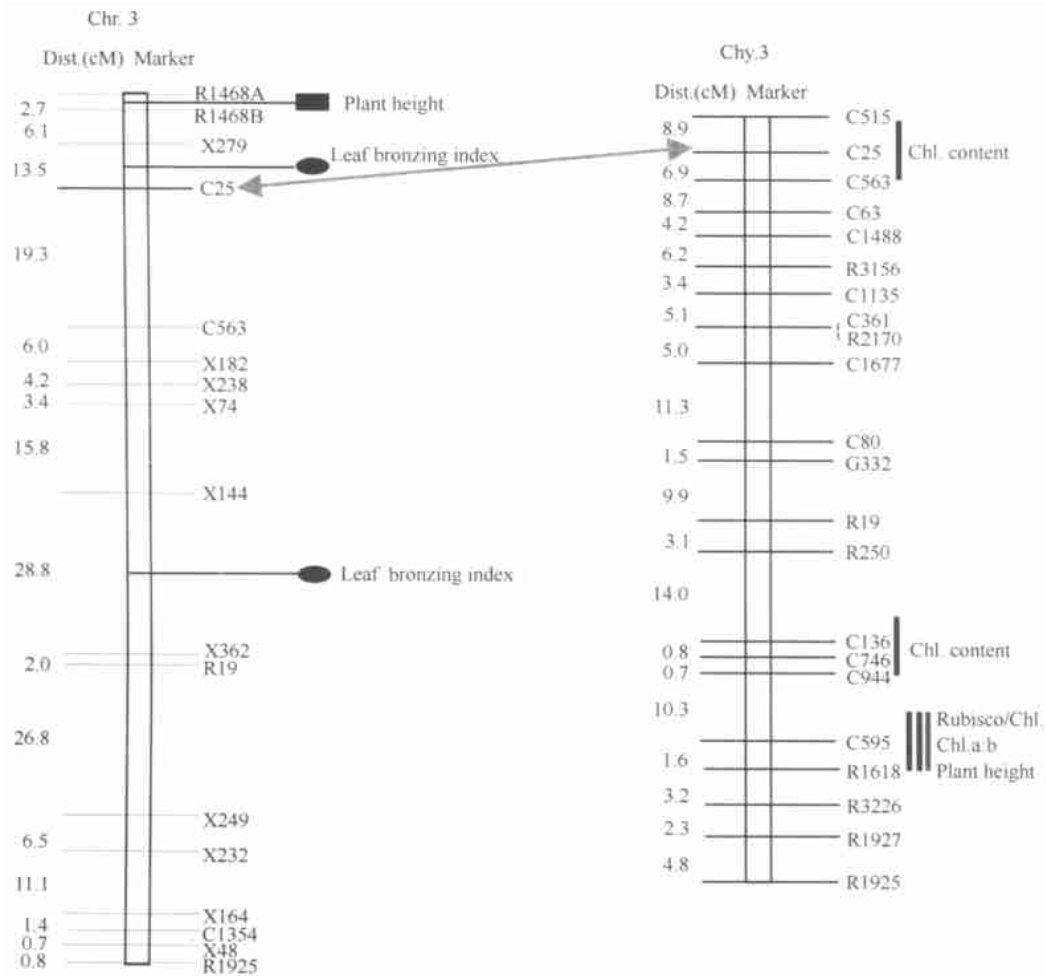


Fig. 2 QTLs identified for resistance to iron toxicity in Kinmaze/DV85 RIL population

A QTL was detected for PH at the region of R1468A-R1468B on chromosome 3 under ferrous iron toxicity condition. Wu *et al.* (1997) used a doubled haploid (DH) population consisting of 123 lines derived from a *japonica* cultivar, Azucena and an *indica* cultivar, IR64 and located a QTL for PH at the region of RZ248-RZ394 on chromosome 3 under potassium deficiency condition^[15]. This result verified that PH produced under Fe^{2+} toxicity stress was viewed as an indirect indicator of resistance to Fe^{2+} toxicity in rice, because a QTL for PH detected un-

der Fe^{2+} toxicity was also detected under potassium deficiency which appeared not related to resistance to Fe^{2+} toxicity.

A large population may provide greater insight about genetic basis of resistance to Fe^{2+} . We are developing a substitution population derived from extremely tolerant lines with minimum Kinmaze genome for the further study. Our goal is to identify breeding materials for resistance to Fe^{2+} toxicity through marker-assisted selection based on the detected markers.

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