



Short communication

Molecular mapping of quantitative trait loci for zinc toxicity tolerance in rice seedling (*Oryza sativa* L.)

YanJun Dong^{a,b,*}, Tsugufumi Ogawa^a, Dongzhi Lin^a, Hee-Jong Koh^c,
Hiroshi Kamiunten^a, Mitsuhiro Matsuo^a, Shihua Cheng^d

^aAgricultural Faculty, Miyazaki University, Miyazaki City 889-2192, Japan

^bCollege of Life and Environment Sciences, Shanghai Normal University, Shanghai 200234, China

^cDepartment of Plant Science, Seoul National University, Seoul 151-742, South Korea

^dChina National Rice Research Institute, Hangzhou 310006, China

Received 27 February 2005; received in revised form 16 March 2005; accepted 16 March 2005

Abstract

Excess zinc harms the growth of rice plants and zinc toxicity can easily occur in acid soils. The aim of the study was to map quantitative trait loci (QTLs) in rice for tolerance to zinc toxicity, using a recombinant inbred (RI) population derived from the cross of a *japonica* variety (Asominori: relatively tolerant to Zn²⁺ toxicity) with an *indica* variety (IR24, relatively susceptible), through 289 RFLP markers. The index scores of damage (representing Zn²⁺ toxicity tolerance), after irrigating rice seedlings with a 1000-ppm Zn²⁺ solution for 20 successive days, were examined for each RI line and its parental varieties. Continuous distributions and transgressive segregations of the index scores were observed in the RI population, suggesting that Zn²⁺ toxicity tolerance was a quantitatively inherited trait. Three QTLs for Zn²⁺ toxicity tolerance were detected on chromosomes 1, 3 and 10 and explained 21.9, 8.9 and 7.6%, respectively, of the total phenotypic variation. The results and the tightly linked molecular markers that flank the QTLs, detected in this study, will be useful in improving Zn²⁺ tolerance in rice. In addition, the genomic positions between QTLs for Zn²⁺ toxicity tolerance and the QTLs for other metal (Fe²⁺, Mn²⁺, Al³⁺) toxicity tolerances, from previous studies, are discussed.

© 2005 Published by Elsevier B.V.

Keywords: Rice (*Oryza sativa* L.); QTLs; Zn²⁺ toxicity tolerance; Molecular marker

1. Introduction

Zinc deficiency and excess, all harm the growth of rice plants and Zn²⁺ excess toxicity can easily occur in acid soil (Obata, 1995). No significant inhibitory effect on rice growth was observed below a 600-ppm Zn²⁺ concentration (Takenaga, 1995). In rice, the symptoms of Zn²⁺ toxicity occur first as leaf

* Corresponding author. Tel.: +81 985 58 58 7158;
fax: +81 985 58 58 7158.

E-mail addresses: dong@cc.miyazaki-u.ac.jp,
dongjapan@hotmail.com (Y. Dong).

discoloration and bronzing of older leaves. It then spreads to the whole plant. In addition, the degree of damage caused to seedlings has been suggested as a good measure of the degree of toxicity (Ota, 1968).

Recent advances in high-density marker linkage maps in rice have provided a powerful tool for elucidating the genetic basis of quantitatively inherited traits. Accordingly, many quantitative trait loci (QTLs) for toxicity tolerance to metals, such as Fe²⁺ (Wu et al., 1997; Wan et al., 2003) and Al³⁺ (Wu et al., 2000; Nguyen et al., 2001; Ma et al., 2002) and Mn²⁺ (Wang et al., 2002) in rice have been identified and mapped using DNA molecular markers. However, to the best of our knowledge, genetic analysis of QTLs associated with zinc toxicity tolerance has not been conducted in rice. The aims of this study are to identify QTLs for zinc toxicity tolerance using recombinant inbred (RI) lines from a *japonica* × *indica* cross and to understand the genetic relationships between QTLs for zinc toxicity tolerance and those for other metal toxicity tolerances, as found in previous studies.

2. Materials and methods

2.1. Plant materials

The recombinant inbred (RI) lines in this study were developed by single seed descent from the progeny of a cross of a *japonica* variety (Asominori), with an *indica* variety (IR24). A total of 165 F₆ lines were randomly selected from 227 original F₂ individual plants, 71 lines of which were used for mapping. The restriction fragment length polymorphism (RFLP) map, covering 1275 cM, was constructed with 375 RFLP markers from the F₆ and F₇ generations (Tsunematsu et al., 1996). In the past, the RI population was used successfully for mapping QTL for important agronomic traits (Yoshimura et al., 1998; Sasahara et al., 1999; Yamasaki et al., 1999, 2000; Kubo and Yoshimura, 2002; Dong et al., 2002, 2003a,b; Zheng et al., 2003). In the study, we used a subset of 289 RFLP markers, without overlapping, for all loci from the original genetic map (Tsunematsu et al., 1996) to map QTLs for zinc toxicity tolerance, for which the average interval distance between pairs of markers was 4.4 cM.

Table 1
The index score for Zn²⁺ toxicity in rice seedling

Scale	Symptoms
0	All green leaves and normal growth
1	Light-yellow discoloration on tips of old leaves, growth near normal
2	Yellow discoloration on tips of old leaves
3	Yellow discoloration on whole old leaves
4	Serious discoloration on all old leaves
5	Somewhat discoloration on all new leaves
6	Yellow discoloration on all new leaves
7	Serious discoloration on all new leaves, old leaves bronzing
8	Discoloration on stem and all leaves bronzing seriously
9	Serious discoloration on stem and leaves almost dead or dying
10	Whole seedling plant dead or dying

2.2. Cultivation and measurements

The seeds of all RI lines and their parents, ‘Asominori’ and ‘IR24’, were surface-sterilized for 7 min in 1% sodium hypochlorite solution and washed thoroughly with the distilled water. Then, they were soaked in distilled water and germinated for 48 h at 30 °C in a growth chamber. On 17 May 2004, 10 germinated seeds for each RI line, including ‘Asominori’ and ‘IR24’, were sown, in each hole (2.5 cm × 2.5 cm × 4.4 cm; *L* × *W* × *H*), in the nursery seeding beds (type: 128 holes/bed; Takii Seed, Japan), incorporated with commercial soil (Miyazaki Yamamune, Japan) containing basal fertilizer (N, P and K) for rice seedling growth. Irrigation began on the 20th day from sowing for 20 successive days. The Zn²⁺ treatment solution was applied every 2 days and the growth of rice seedlings was investigated 20 days after irrigation and classified into 11 (from 0 to 10) scores based on the symptoms of damage caused (Table 1), representing zinc toxicity tolerance. Experiments were conducted outdoors at Miyazaki University (Japan) with two replications. The average values of scores were used for QTL analysis.

2.3. Detection of QTLs

Two methods were simultaneously used to identify significant marker locus–trait associations: simple linear regression (single marker analysis) and composite interval mapping (CIM) analysis. The CIM

analysis was applied to trait average and marker data to more precisely identify the QTL locations (Zeng, 1994). Single marker analysis and CIM analysis were performed using the QTL Cartographer computer program software (Ver. 2.0) (Wang et al., 2003). (1) The linkages between respective marker loci and putative QTL are determined using single marker analysis. When F -values exceeded a value necessary for a probability value less than 0.005, the QTLs were considered to be significant. (2) CIM analyses were calculated using forward regression with a walk speed of 2 cM and window size of 10 cM. A locus with a LOD threshold value of more than 2.0 was declared a putative QTL.

In this study, only the QTLs detected by both methods were listed. In addition, the additive effect and percentage variation, explained by an individual QTL, were also estimated. The QTLs were named according to the suggestions of McCouch et al. (1997).

3. Results

3.1. Frequency distribution of the index scores representing the zinc toxicity tolerance in segregating RI population

Frequency distributions of the index score, representing the zinc toxicity tolerance, in the RI population is shown in Fig. 1. There is a clear difference ($P < 0.01$) for the Zn^{2+} toxicity tolerance between Asominori (relatively tolerant) and IR24 (relatively susceptible). In addition, the mean and median scores were 4.98 and 4.75, respectively, and both skew (0.08) and kurt (-0.06) values were less than 1.0, which showed that the Zn^{2+} toxicity

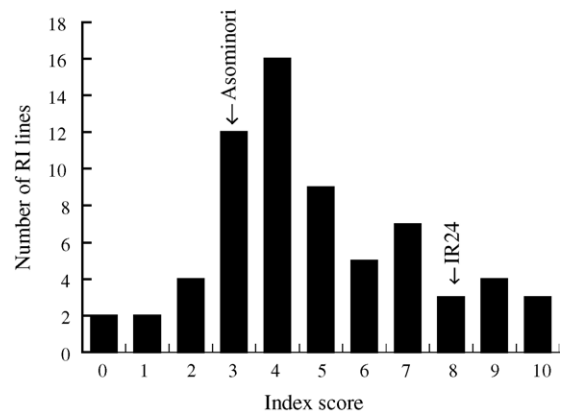


Fig. 1. Frequency distribution of the index scores of damage caused by zinc excess to rice seedlings in RI population from Asominori/IR24. Arrowheads indicate the average scores of Asominori and IR24.

tolerance of the RI population fitted normal distribution. Furthermore, continuous phenotypic variation of the index scores and transgressive segregation in both parental directions were observed, which suggested that zinc toxicity tolerance was a quantitatively inherited trait.

3.2. Mapping QTLs for zinc toxicity tolerance

Three QTLs associated with Zn^{2+} toxicity tolerance were identified and mapped to chromosomes 1, 3 and 10 (Table 2; Fig. 2) and tentatively designated as $qZNT-1$, $qZNT-3$, and $qZNT-10$, respectively. The $qZNT-1$, located near the *XNpb93* markers on chromosome 1, showed the largest effect on the trait with a LOD value of 6.0 and explained 21.9% of the total phenotypic variation. Another QTL, $qZNT-3$ (LOD = 3.2) was detected between *R1468B* and *C515* on chromosome 3 and accounted for 8.9% of total phenotypic variation. The remaining QTL, $qZNT-10$,

Table 2

QTLs associated with Zn^{2+} toxicity tolerance in rice based on CIM methods (Wang et al., 2003) using the RI population from a cross between Asominori and IR24

Name of QTL	Chromosome number	Marker interval ^a	Probability ^b	Peak LOD value	Additive effect ^c	Variation ^d (%)
<i>qZNT-1</i>	1	<i>XNpb93-C3029C</i>	0.000	6.0	1.76	21.9
<i>qZNT-3</i>	3	<i>R1468B-C515</i>	0.001	3.2	-0.85	8.9
<i>qZNT-10</i>	10	<i>C751B-C148</i>	0.003	2.2	-0.59	7.6

^a Italic markers indicate the nearest marker to putative QTL.

^b Refers the probability that the putative QTL is unlinked to the nearest marker by single marker analysis method.

^c Positive values indicate that Asominori alleles are in the direction of zinc toxicity tolerance.

^d Percentage of explained phenotypic variation.

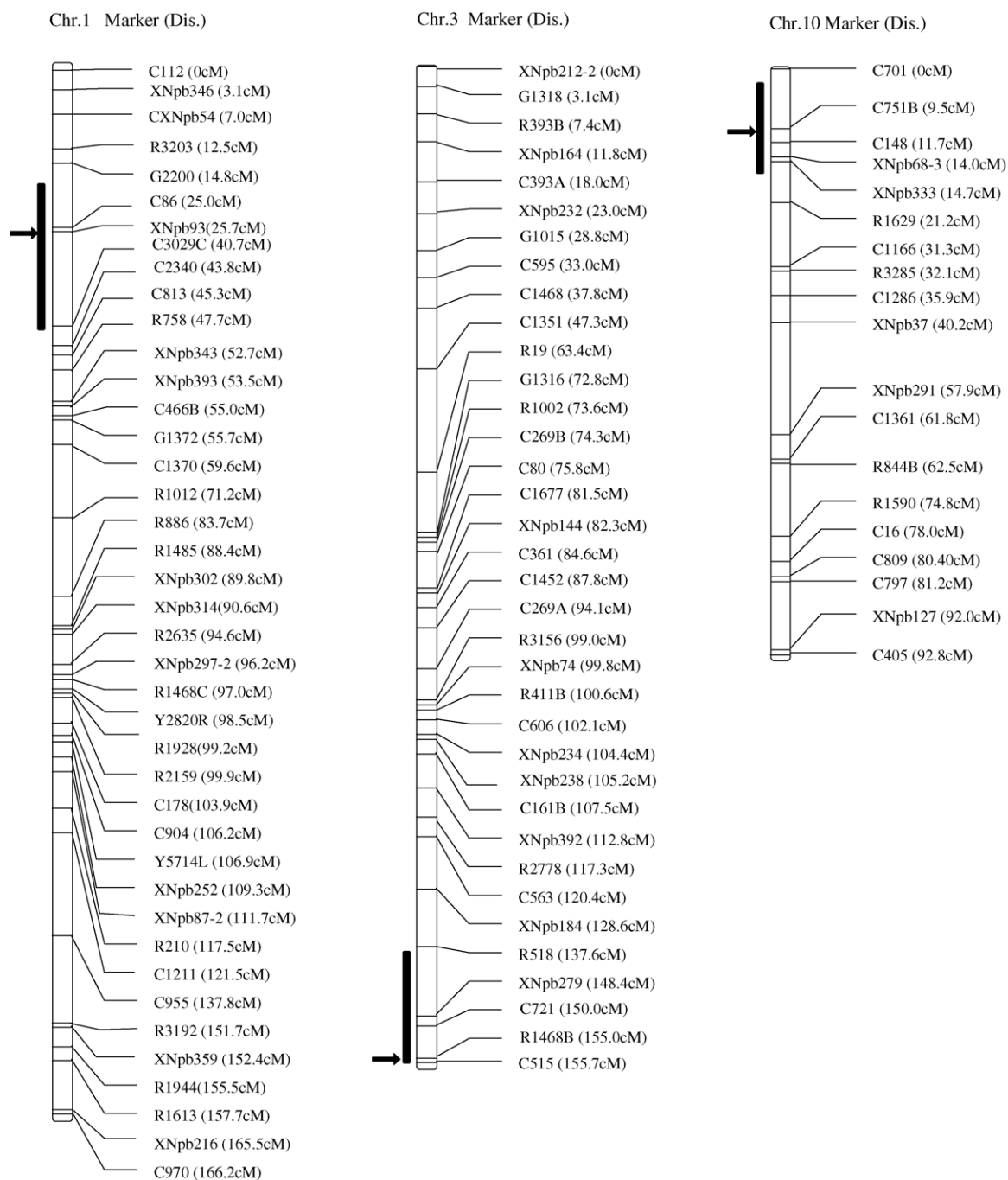


Fig. 2. Genomic positions of QTLs for Zn²⁺ toxicity tolerance in rice seedling. Black bars indicate the genomic regions with LOD > 1.5. Arrowheads indicate the location of peak LOD for QTL detected.

located near C751 and C148 on chromosome 10, with a LOD value of 2.2, explained 7.6% of total phenotype variation. In addition, the Asominori allele on *qZNT-1* contributed to the increase of Zn^{2+} toxicity tolerance, whereas *qZNT-3*, and *qZNT-10* alleles decreased the trait.

4. Discussion

QTL analysis of Zn^{2+} toxicity tolerance in rice has revealed the complexity of this trait and provided valuable information for breeding rice varieties tolerant to zinc toxicity. In the paper, we reported the results of QTL mapping for Zn^{2+} toxicity tolerance using the recombinant inbred lines derived from *japonica* Asominori and *indica* IR24 with 289 RFLP markers. Three QTLs for zinc toxicity tolerance were detected in rice and alleles with increasing and decreasing effects were detected from the both parents, which confirmed the continuous variation and transgressive segregation for zinc toxicity tolerance in RI population (Fig. 1). It was reported that inhibitory effects of metals on plant growth are closely related to the stability of metallo-organic complexes (Takenaga, 1995). Therefore, it is worth comparing the genomic positions (Table 3) of the three QTLs detected in our studies for Zn^{2+} toxicity tolerance with those for other metal (Fe^{2+} , Mn^{2+} , Al^{3+}) toxicity tolerances (Wu et al., 1997; Nguyen et al., 2001; Ma et al., 2002; Wang et al., 2002; Wan et al., 2003) to understand the interrelationship between metal toxicity tolerances in rice. Consequently, *qZNT-1*, located on chromosome 1, was a consistent/tight linkage with each QTL for Al^{3+} (Ma

et al., 2002) and Fe^{2+} toxicity tolerance (Wu et al., 1997) due to their location near the common C86 marker, which suggested that the *qZNT-1* allele might be involved in Fe^{2+} , Zn^{2+} and Al^{3+} excess tolerance in rice, whereas the *qZNT-1* allele was different from both QTL for Fe^{2+} toxicity tolerance (Wu et al., 1997; Wan et al., 2003) and one QTL for Al^{3+} toxicity tolerance (Wu et al., 2000). However, genomic positions of other QTLs for Fe^{2+} , Mn^{2+} and Al^{3+} excess tolerance in rice (Nguyen et al., 2001; Wang et al., 2002), on the same chromosomes, were not comparable with those QTLs for Zn^{2+} toxicity tolerance, found in this study, because of inadequate information about the relationship between molecular markers used. Further study on the genetic relationships between those QTLs is needed.

In addition, since excess Zn^{2+} concentration hampers nutrient uptake and, consequently, affects photosynthetic capacity in rice (Takenaga, 1995), it is significant to compare the genomic positions of the three QTLs for Zn^{2+} toxicity tolerance with those QTLs associated with photosynthetic rate and related physiological traits (Ishimaru et al., 2001). We found that *qZNT-3* coincided with the QTL for chlorophyll content (Ishimaru et al., 2001), due to the fact that they were tightly linked to the common C515 marker. More interestingly, *qZNT-3* coincided with one QTL for increased stem and root weights in excess Fe^{2+} conditions (Wan et al., 2003). From these results, it may be deduced that, in rice, the IR24 allele on *qZNT-3* expressed the tolerance to Zn^{2+} excess toxicity by maintaining a higher photosynthetic ability.

The results conclude that Zn^{2+} toxicity tolerance in rice is a complex quantitatively inherited trait. The molecular markers tightly linked to the three QTLs for

Table 3

Comparison of the genomic positions of QTLs for Zn^{2+} toxicity in the study with QTLs for metal toxicity (Fe^{2+} , Al^{3+} , Mn^{2+}) tolerance on the same chromosomes in previous studies

QTL for Zn toxicity	Chromosome	The linked marker	Comparison with location of QTLs for metal toxicity (Fe^{2+} , Al^{3+} , Mn^{2+}) tolerance
<i>qZNT-1</i>	1	XNpb93	Coincidence/tight linkage with each QTL for Al^{3+} toxicity tolerance (Ma et al., 2002) and Fe^{2+} toxicity tolerance (Wu et al., 1997); different from each QTL for Fe^{2+} toxicity tolerance (Wu et al., 1997 and Wan et al., 2003) and QTL for Al^{3+} toxicity tolerance (Wu et al., 2000); unknown relation to QTL for Al^{3+} toxicity tolerance (Nguyen et al., 2001)
<i>qZNT-3</i>	3	C515	Unknown relation to each QTL for Al^{3+} tolerance (Nguyen et al., 2001) and Mn^{2+} tolerance (Wang et al., 2002)
<i>qZNT-10</i>	10	C751B	Unknown relation to each QTL for Al^{3+} tolerance (Nguyen et al., 2001) and Mn^{2+} tolerance (Wang et al., 2002)

Zn²⁺ tolerance, detected in this study, might lead to the isolation of loci and will help to better understanding zinc tolerance mechanisms in rice.

Acknowledgements

We are greatly indebted to Professor A. Yoshimura (plant breeding laboratory, Agricultural faculty of Kyushu University, Japan) for kindly providing materials, molecular data and valuable advices. We want to thank Japan Society for the Promotion of Science (JSPS) providing the first author to post-doctoral fellowships.

References

- Dong, Y., Tusuzuki, E., Kamiunten, H., Terao, H., Lin, D., Matsuo, M., Zheng, Y., 2003a. Identification of quantitative trait loci associated with pre-harvest sprouting resistance in rice (*Oryza sativa* L.). *Field Crops Res.* 81, 133–139.
- Dong, Y., Tusuzuki, E., Kamiunten, H., Terao, H., Lin, D., 2003b. Mapping of QTL for embryo size in rice. *Crop Sci.* 43, 1068–1071.
- Dong, Y., Zheng, Y., Tsuzuki, E., Terao, H., 2002. Quantitative trait loci controlling steamed-rice shape in a recombinant inbred population. *Int. Rice Res. Note* 27, 19–20.
- Ishimaru, K., Yano, M., Aoki, N., Ono, K., Hirose, T., Lin, S., Monna, L., Sasaki, T., Ohsugi, R., 2001. Toward the mapping of physiological and agronomic characters on a rice function map: QTL analysis and comparison between QTLs and expressed sequence tags. *Theor. Appl. Genet.* 102, 793–800.
- Kubo, T., Yoshimura, A., 2002. Genetic basis of hybrid breakdown in a *Japonica/Indica* cross of rice, *Oryza sativa* L.. *Theor. Appl. Genet.* 105, 906–911.
- Ma, J.F., Shen, R.F., Zhao, Z.Q., Wissuwa, M., Takeuchi, Y., Ebitani, T., Yano, M., 2002. Response of rice to Al stress and identification of quantitative trait loci for Al tolerance. *Plant Cell Physiol.* 43, 652–659.
- McCouch, S.R., Cho, Y.G., Yano, M., Paul, E., Blinstrub, M., 1997. Report on QTL nomenclature. *Rice Genet. Newslett.* 14, 11–13.
- Nguyen, V.T., Burow, M.D., Nguyen, H.T., Le, B.T., Le, T.D., Paterson, A.H., 2001. Molecular mapping of genes conferring aluminum tolerance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 102, 1002–1010.
- Obata, H., 1995. Micro essential elements. In: Matsuo, et al. (Eds.), *Science of Rice Plant*, vol. 2 (Physiology). Food and Agriculture Policy Research Center, Tokyo, Japan, pp. 402–419.
- Ota, Y., 1968. Studies on the occurrence of the physiological disease called “bronzing”. *Bull. Natl. Inst. Agricult. Sci. (Japan)* 18, 31–104.
- Sasahara, H., Fukuta, Y., Fukuyama, T., 1999. Mapping of QTLs for vascular bundle system and spike morphology in rice, *Oryza sativa* L.. *Breed. Sci.* 49, 75–81.
- Takenaga, H., 1995. Nutrient absorption of rice plant. In: Matsuo, et al. (Eds.), *Science of Rice Plant*, vol. 2 (Physiology). Food and Agriculture Policy Research Center, Tokyo, Japan, pp. 247–294.
- Tsunematsu, H., Yoshimura, A., Harushima, Y., Nagamura, Y., Kurata, N., Yano, M., Iwata, N., 1996. RFLP framework map using recombinant inbred lines in rice. *Breed. Sci.* 46, 279–284.
- Wan, J., Zhai, H., Wan, J., Ikehashi, H., 2003. Detection and analysis of QTLs ferrous iron toxicity tolerance in rice. *Euphytica* 131, 201–206.
- Wang, S., Basten, C.J., Zeng, Z.B., 2003. Windows QTL Cartographer 2.0. Department of Statistics, North Carolina State University, Raleigh, NC, USA. , <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>.
- Wang, X.Y., Wu, P., Wu, Y.R., Yan, X.L., 2002. Molecular marker analysis of manganese toxicity tolerance in rice under greenhouse conditions. *Plant Soil* 238, 227–233.
- Wu, P., Luo, A., Zhu, J., Yang, J., Huang, N., Senadhira, D., 1997. Molecular markers linked to genes underlying seedling tolerance for ferrous iron toxicity. *Plant Soil* 196, 317–320.
- Wu, P., Liao, C., Hu, B., Yi, K., Jin, W., Ni, J., He, C., 2000. QTLs and epistasis for aluminum tolerance in rice (*Oryza sativa* L.) at different seedling stages. *Theor. Appl. Genet.* 100, 1295–1303.
- Yamasaki, M., Tsunematsu, H., Yoshimura, A., Iwata, N., Yasui, H., 1999. Quantitative trait locus mapping of ovicidal response in rice (*Oryza sativa* L.) against whitebacked planthopper (*Sogatella furcifera* Horvath). *Crop Sci.* 39, 1178–1183.
- Yamasaki, M., Tsunematsu, H., Yoshimura, A., Yasui, H., 2000. Mapping of quantitative trait loci of ovicidal response to brown planthopper (*Nilaparvata lugen* Stal) in rice (*Oryza sativa* L.). *Breed. Sci.* 50, 291–296.
- Yoshimura, A., Okamoto, M., Nagamine, T., Tsunematsu, H., 1998. Rice QTL analysis for days to heading-under the cultivation of Ishigaki island. *Breed. Sci.* 8 (Suppl. 1), 73.
- Zeng, B.Z., 1994. Precision mapping of quantitative trait loci. *Genetics* 136, 1457–1468.
- Zheng, Y., Dong, Y., Matsui, A., Udatsu, T., Fujiwara, H., 2003. Molecular genetic basis of determining subspecies of ancient rice using the shape of phytoliths. *J. Archaeol. Sci.* 30, 1215–1221.