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ScienceDirect
J. Genet. Genomics 35 (2008) 299–305
JOURNAL OF GENETICS AND GENOMICS
www.jgenetgenomics.org

Identification of quantitative trait loci for the dead leaf rate and the seedling dead rate under alkaline stress in rice

Dongling Qi [a, b], Guizhen Guo [c], Myung-chul Lee [d], Junguo Zhang [c], Guilan Cao [a], Sanyuan Zhang [c], Seok-cheol Suh [d], Qingyang Zhou [e], Longzhi Han [a, c, *]

[a] *Institute of Crop Science, Chinese Academy of Agricultural Sciences/The National Key Facility for Crop Gene Resources and Genetic Improvement, NFCRI/Key Laboratory of Crop Germplasm Resources and Biotechnology, Ministry of Agriculture, Beijing 100081, China*
[b] *Rubber Research Institute of China, Chinese Academy of Tropical Agricultural Sciences/Key laboratory of Physiology for Tropical Crops, Ministry of Agriculture, Hainan 571737, China*
[c] *Institute of Rice Research, Jilin Academy of Agricultural Sciences, Gongzhuling 136100, China*
[d] *National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon 441-707, Korea*
[e] *Horticultural Department, Sichuan Agricultural University, Ya'an 625014, China*

Received for publication 9 October 2007; Revised 20 December 2007; Accepted 18 January 2008

Abstract

The quantitative trait loci (QTLs) for the dead leaf rate (DLR) and the dead seedling rate (DSR) at the different rice growing periods after transplanting under alkaline stress were identified using an $F_{2:3}$ population, which included 200 individuals and lines derived from a cross between two *japonica* rice cultivars Gaochan 106 and Changbai 9 with microsatellite markers. The DLR detected at 20 days to 62 days after transplanting under alkaline stress showed continuous normal or near normal distributions in F_3 lines, which was the quantitative trait controlled by multiple genes. The DSR showed a continuous distribution with 3 or 4 peaks and was the quantitative trait controlled by main and multiple genes when rice was grown for 62 days after transplanting under alkaline stress. Thirteen QTLs associated with DLR were detected at 20 days to 62 days after transplanting under alkaline stress. Among these, *qDLR9-2* located in RM5786–RM160 on chromosome 9 was detected at 34 days, 41 days, 48 days, 55 days, and 62 days, respectively; *qDLR4* located in RM3524–RM3866 on chromosome 4 was detected at 34 days, 41 days, and 48 days, respectively; *qDLR7-1* located in RM3859–RM320 on chromosome 7 was detected at 20 days and 27 days; and *qDLR6-2* in RM1340-RM5957 on chromosome 6 was detected at 55 days and 62 days, respectively. The alleles of both *qDLR9-2* and *qDLR4* were derived from alkaline sensitive parent "Gaochan106". The alleles of both *qDLR7-1* and *qDLR6-2* were from alkaline tolerant parent Changbai 9. These gene actions showed dominance and over dominance primarily. Six QTLs associated with DSR were detected at 62 days after transplanting under alkaline stress. Among these, *qDSR6-2* and *qDSR8* were located in RM1340–RM5957 on chromosome 6 and in RM3752–RM404 on chromosome 8, respectively, which were associated with DSR and accounted for 20.32% and 18.86% of the observed phenotypic variation, respectively; *qDSR11-2* and *qDSR11-3* were located in RM536–RM479 and RM2596–RM286 on chromosome 11, respectively, which were associated with DSR explaining 25.85% and 15.41% of the observed phenotypic variation, respectively. The marker flanking distances of these QTLs were quite far except that of *qDSR6-2*, which should be researched further.

Keywords: rice; alkaline stress; dead leaf rate; dead seedling rate; microsatellite marker; quantitative trait locus (QTL)

Introduction

Rice (*Oryza sativa* L.) is a crop, which is moderately sensitive to salt-alkaline. Therefore, the degree of salt-alkaline tolerance in rice has great effect on the rice production in sodic areas. It is of significance that the genetics and molecular mechanism of salt-alkaline tolerance in rice will be researched for rice breeding with salt-alkaline tolerance. Some researchers reported that salt-alkaline tolerance was a complicated phenomenon of various physiology reactions (Khatun and Flower, 1995; Zhao, 2002; Qi

* Corresponding author. Tel & Fax: +86-10-6217 6784.
E-mail address: lzhan58@yahoo.com.cn

et al., 2007), and the salt-alkaline tolerance was the quantitative trait controlled by several genes (Akbar and Yabuno, 1977; Flower, 2004). Gu et al. (2000) found four QTLs associated with the salt tolerance at the seedling stage and their whole alleles were from tolerant variety. Lin et al. (2004) identified 3 QTLs for the survival rate of seedling under salt stress on chromosomes 1, 6, and 7. Among these, *SNC-7* and *SKC-1* were the main genes, which explained 48.5% and 40.1% of the observed phenotypic variances, respectively. Lin et al. (1998) detected one QTL for survival days of seedling on chromosome 5, accounting for 11.6% of the observed phenotypic variance. Seven QTLs, which were associated with salt tolerance at the seedling stage, were detected on chromosomes 5, 6, 7, and 10 (Prasad et al., 2000). Koyama et al. (2001) identified eleven QTLs, which were associated with Na^+ concentration, K^+ concentration, Na^+ uptake, K^+ uptake, and Na^+:K^+ ratio. However, QTL analysis on the dead leaf rate (DLR) and the dead seedling rate (DSR) under alkaline stress were not reported.

In this study, genetic and QTL analysis of DLR and DSR were studied using SSR markers with Changbai 9 as the source of alkaline tolerance to provide theoretical basis for molecular marker-assisted breeding in rice.

Materials and methods

Mapping population

An $F_{2:3}$ population containing 200 lines derived from a cross between Gaochan 106 and Changbai 9, two *japonica* rice varieties, was used to construct the genetic linkage map. Gaochan 106 was an alkaline sensitive parent and Changbai 9 was an alkaline tolerant parent (Yang, 1997). Both of these were maintained in the Institute of Rice Research (IRR), Jilin Academy of Agricultural Sciences (JAAS), Gongzhuling, China.

Evaluation of the alkaline tolerance for the mapping population and its parents

The alkaline tolerance of the two parents and the F_3 lines were evaluated in the evaluation pool of alkaline tolerance at IRR, JAAS. The soil with pH value over 9.5 in the alkaline tolerance evaluation pool was derived from Baicheng heavy salt-alkaline area, Jilin Province. After running water irrigation, the pH value of the soil and water in the pool was adjusted to between 8.7 and 8.9 under 25°C. The depth of water was kept 3–5 cm above the soil during the rice growing period by the checking water depth level and the water was irrigated to the evaluation pool every 2 days. The water in the evaluation pool was kept still and reached immobility scale. To maintain the stabilization of the water pH value, the open canopy was placed 2 m above the evaluation pool to keep off rain fall.

The rice seeds were sown on 10 April, 2005, using the method of seedlings grown on the flat nursery with dry soil in a greenhouse. These seedlings were transplanted into the evaluation pools in the 4-leaf-stage. Twenty seeds of each line were planted in a single row 20 cm × 10 cm apart. Each treatment had two replicates. The DLR and the DSR of 15 plants for each line were investigated at 20 days, 27 days, 34 days, 48 days, 55 days, and 62 days after transplanting under alkaline stress, respectively. The means of DLR and DSR of each line were used as the statistic unit. The DSR per plant was investigated at 62 days under alkaline stress. The means were used for data analysis using the following method:

Dead leaf rate (%) = (Total number of dead leaf of plant/Total number of leaf of plant) × 100;

Dead seedling rate (%) = (Total number of dead seedling of plant/Total number of plants) × 100.

Extraction of DNA and SSR marker analysis

The total genomic DNA of both parents and 200 lines was extracted from 4–5 pieces of leaves at the tillering stage as described previously (Zhou et al., 2001). The concentration of DNA was determined using an ultraviolet spectrophotometer. Eight hundred and forty pairs of SSR primers were selected from the Website of Cornell University (http://www.cornell.edu/). Among these, 106 pairs of the primers selected were polymorphic between the two parents. PCR was carried out in a 20 μL reaction mixture containing 2 μL of 10 × PCR buffer with Mg^{2+}, 1.5 μL of 2.5 mmol/L dNTP, 0.5 μL of 5 U/μL *Taq* polymerase, 2.0 μL of 2 μmol/L SSR primers, 2.0 μL of 20 ng/μL DNA, and 12 μL of ddH_2O. The DNA amplification was performed for 38 cycles of 5 min at 94°C, 30 s at 95°C, 30 s at 60 °C, 1 min at 72 °C, and 10 min at 72°C. The PCR products were separated in 8% polyacrylamide gel electrophoresis and visualized following silver staining.

QTL analysis

The genetic linkage map was constructed using the MAPMAKER/EXP 3.0 software and mapping was done using a microsoft excel macro (Liu and Meng, 2003). A molecular linkage map of the $F_{2:3}$ population with 74 SSR markers evenly distributed on 12 linkage groups was constructed. The linkage map covered a total length of 1,246.2 cM with an average distance of 16.84 cM, and the genetic distance was calculated using the Kosambi function. QTL was analyzed using the WinQTLCartographer 2.0 software with an *LOD* threshold of 2.0 (http://statgen.ncsu.edu/qtlcart/). The nomenclature for QTLs was carried out according to McCouch et al. (1997). The gene action was