QTLs on Chromosome 12 Responsible for Expressing Root Plasticity under Transient Soil Moisture Fluctuation Stress in Rice

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Abstract

Soil moisture fluctuations (i.e. transient waterlogged to drought) frequently occur in rainfed rice fields due to erratic rainfall pattern, and irrigated fields due to wetting and drying system practices and inefficient irrigation system. Such conditions are stressful for growth and development of rice. With the utilization of chromosome segment substitution line (CSSL), we previously demonstrated the significant roles of root plasticity as expressed by higher lateral root production in response to soil moisture fluctuation stress. Moreover, our molecular mapping analysis showed the presence of QTL that is associated with root plasticity, at short arm of chromosome 12 region. This QTL was found to control the productions of L-type lateral roots with the increase effect from Kasalath allele. In this study, we attempted to quantify the significant functions of QTL (qLLRn-12) on chromosome 12 in relation to the shoot dry matter productions and root system development. CSSL genotypes with QTL or substituted segment of Kasalath allele on chromosome 12 were evaluated and subjected to 38 days of transient soil moisture fluctuation stress. Results revealed that CSSL genotype with qLLRn-12 showed greater root system development as expressed its greater total root length than those without qLLRn-12 in response to moisture fluctuation stress. Longer total root length was attributed to higher production of L-type lateral roots, which can effectively maintain water and nutrient uptake in the soil. Such plastic root responses resulted in increased stomatal conductance and photosynthesis, eventually increased shoot dry matter production by 37% compared to its Nipponbare parent. Comparison of the location of the QTL across rice cultivars showed that there is no QTL reported in this region related to lateral root production. This may possibly be a putative QTL and potentially be used in the marker-aided breeding program to improve adaptation under fluctuating soil moisture environment.

Keywords: chromosome segment substitution lines (CSSL), lateral root, rice, root plasticity, soil moisture fluctuation

Introduction

With the scarcity of water for irrigation and continuing increase of world population, future increase in rice production will more heavily rely on rainfed rice ecosystem. Rainfed lowland conditions are characterized by frequent fluctuation in soil moisture at varying degrees throughout the cropping season, which have stressful effects on the growth and development of rice (Niones *et al*., 2009). Plant roots play an important role in water and nutrient acquisition. Both constitutive and adaptive root growth have been implicated in the improvement of plant performance under rainfed conditions (Kamoshita *et al*., 2002). A plant has an ability to change its phenotype, in response to soil's heterogeneous environment (Yamauchi *et al.,* 1996), which is termed as plasticity, and root plasticity is a key trait for plant adaptation to stressful conditions. Moreover, we reported that traits associated with developmental root plasticity triggered by mild drought stress played significant roles in water uptake and dry matter production in rice (Kano *et al*., 2011).

With the use of chromosome segment substitution lines (CSSL), we previously identified one line CSSL47 that consistently showed more plastic root growth than Nipponbare in terms of branching of lateral roots and aerenchyma formation at early vegetative stage (Suralta *et al.,* 2008,

2010) and until maturity (Niones *et al*., 2009) under transient soil moisture fluctuations. This line contains 10 substituted segments from Kasalath allele with Nipponbare genetic background. Niones *et al.* (2010) identified the location of the QTL at short arm of chromosome 12 region, which are responsible for controlling root developmental plasticity specifically the L-type lateral root production. The expression of such root plasticity QTL and its effect on dry matter production and root system development when rice plants are grown under transient soil moisture fluctuation conditions have not yet been further verified.

In this study, we examined the significant function of the QTL or substituted segment from Kasalath allele on chromosome 12 in relation to the shoot dry matter production at vegetative stage. Furthermore, we tested the variation in root plasticity expression of parents and CSSL genotype with QTL or substituted segment of Kasalath allele on chromosome 12 under 38 days of transient soil moisture fluctuation stress.

Materials and Methods

Plant materials

Four genotypes (Nipponbare, CSSL47, CSSL27, CSSL52) were used in this study. Nipponbare, an irrigated lowland japonica variety and recurrent parent of the 54 chromosome substituted segment line (CSSL) (Nipponbare/ Kasalath cross) population genotypes. CSSL47 genotype has ten substitute segments across genome from Kasalath allele with a unique characteristics of having greater plastic responses in root development under soil moisture fluctuations than the other CSSLs (Suralta *et al*., 2010; Niones *et al.* (2009). The CSSL27 and CSSL52 genotypes were selected from the 54 CSSL population. CSSL27 genotype contained no substituted segment of Kasalath in short arm of chromosome 12 region, and thus is hereon referred as ***- SL***, while CSSL52 (referred as ***+ SL***) has substituted segment of Kasalath in short arm of chromosome 12 region only. The seeds of CSSL population was provided by the Rice Genome Research Center of the National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan.

Treatment conditions

Seedlings were grown in soil-filled plastic root box and exposed to three different soil water conditions; the well-watered (WW), transient waterlogged to drought (W-D) and transient drought to waterlogged (D-W) conditions. Plants were exposed to waterlogged conditions for 17 days and thereafter the water was allowed to drain to the target soil moisture content (SMC) of 20%, which was then maintained for 21 days by watering every two days. Plant samplings were done at 38 DAS.

Root phenotyping

Lateral roots (LRn) were manually counted. Each nodal root was cut into 5-cm segments keeping the lateral roots intact. In this way, the number of each type of lateral root; L and S type of lateral roots in rice (Yamauchi *et al*., 1996) can be determined and expressed as linear frequency (number of lateral roots per unit length root axis; Ito *et al*., 2006). One of their major noticeable differences is that L type is branching while S type is non-branching. For total root length (TRL) measurements, root samples from the FAA were rinsed with running water and spread on transparent sheet without overlapping. The digitized images were taken using Epson scanner (ES2200) at 300 dpi resolution. TRL was analyzed using a NIH image software (ver.1.60), a public domain released by the National Institute of Health, USA.

Statistical analysis

The experiments were laid out in split-plot randomized complete block design with three replicates. The significance differences of four genotypes were compared at LSD > 0.05 significance level using the Cropstat software.

Results and Discussion

Plastic root development was mainly attributed to enhanced lateral root production particularly the L-type lateral (Bañoc *et al.,* 2002; Suralta *et al*., 2010), which is a key trait for the plant adaptation under transient soil moisture production. Lateral roots generally comprised the greater proportion of the whole root system (Yamauchi *et al.,* 1987), and thus promoted lateral root development in response to moisture stress directly reflects the performance of the entire root system. Enhanced lateral root development results in increased root surface area, and soil water extraction and water use. Therefore, the maintenance of stomatal conductance and photosynthesis could be a consequence of greater supply of water to leaves due to the enhanced lateral root development as a result of their plastic responses.

QTL reported by Niones *et al.* (2010) was located at the short arm of chromosome 12 region, which is hereon referred as qLLRn-12. This was associated with root developmental plasticity with the increase effect from Kasalath allele. The function of this QTL was to regulate the production of L-type lateral roots, which is associated with plastic root system development. It is assumed that the possible location of the qLLRn-12 may be near TG156 marker locus with the approximate distance of 17.6 cM between the adjacent markers RM6296.

In this study, greater lateral root development of ***+ SL*** genotype over Nipponbare and – ***SL*** genotypes was shown in higher linear frequency of lateral root number in response to transient soil moisture fluctuation stress. This result showed the evidence the importance of QTL or Kasalath segment that was present in chromosome 12 (Figure 1). Figure 2 shows the significant positive correlation between total root lengths and the production of lateral root under both transient soil moisture fluctuation stress treatments, with r-value of 0.624 for D-W and 0.646 for W-D. This result indicates that under transient fluctuations of soil moisture, higher production of lateral root had a significant contribution to greater root system development that eventually leads to enhanced water and nutrient uptake in the soil.

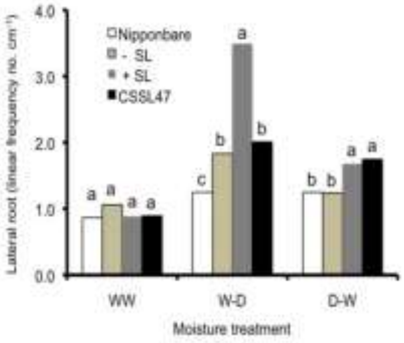

Figure 1. The linear frequency of lateral root number per cm of the four genotypes grown under different soil moisture conditions: i.e. WW, well-watered; W-D, transient waterlogging to drought; D-W, transient drought to waterlogging. (□)、Nipponbare; □□) - SL indicates the CSSL genotypes that do not contain QTL or introgressed segment from Kasalath, (□) + SL indicates the CSSL genotypes that do not contain QTL or introgressed segment from Kasalath and (□)、CSSL47. Same letter means that values are not significant at LSD > 5% level of significant level.

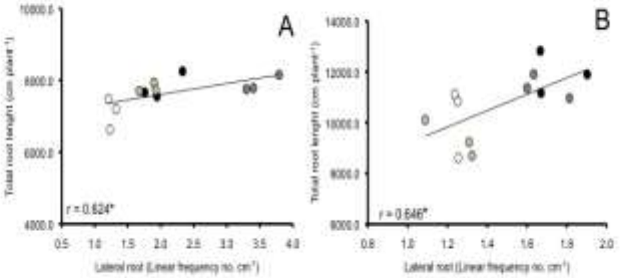

Figure 2. Relationships of linear frequency of lateral root with total root length under different soil moisture conditions; A, transient waterlogging to drought; B, transient drought to waterlogging. (□), - SL (CSSL28); (□), CSSL47; (□), + SL (CSSL52); (O), Nipponbare.

To further demonstrate the effect of the existence of QTLs in response to transient soil moisture fluctuation stress, ***+ SL*** genotypes were evaluated for plastic root system development in relation to shoot dry matter and root system development as shown in Figure3. The ***+ SL*** genotype showed significantly greater shoot dry matter production with the increase of 30% in W-D and 43% in D-W than Nipponbare. The increase in shoot dry matter of ***+ SL*** genotype was associated with a 10% (W-D) and 17% (D-W) increase in total root length compared to Nipponbare. On the other hand, these parameters were not significantly different between ***– SL*** genotype and Nipponbare parent. These facts clearly suggest that the function of QTLs at chromosome 12 was to regulate the production and branching of lateral roots (*i.e.*, L-type lateral root), which resulted in greater plastic root system development. This eventually led to the enhanced shoot dry matter production at vegetative stage under fluctuating soil moisture conditions.

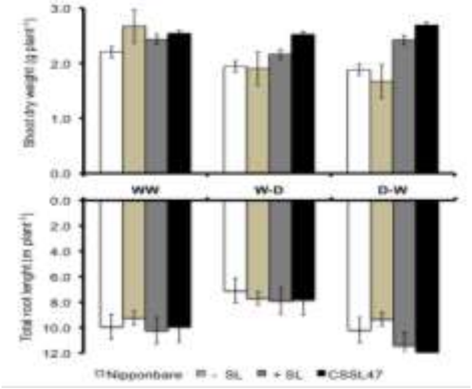

Figure 3. Effect of the presence of QTL at short arm of chromosome 12 on the shoot dry matter production and total root length under different transient soil moisture fluctuation conditions. Same letter means that values are not significant at LSD > 5% level of significant level.

We identified in short-arm of chromosome 12 region, the location of QTL that is responsible for regulating the production of L-type lateral roots with the increase effect from Kasalath allele. The use of the CSSL genotypes that contain substituted segment of Kasalath in chromosome 12 only further validated this QTL function. There have been reports on QTLs that are associated with root length, root number, root dry weight and branching index traits (Price *et al*., 2002; Horii *et al*., 2006; Gowda *et al*., 2011), however, with the best of our knowledge, there have been no reports on QTL in this region, which are related to lateral root production under soil moisture fluctuation stress. Fine mapping is necessary to narrow down the distance of linked markers to the target trait. Then, this could be potentially used in the marker-aided breeding program for enhanced plant adaptation under such environment.

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