Utilizing Chromosome Segment Substitution Lines (CSSLs) for Evaluation of Root Responses to Transient Moisture Stresses in Rice

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Abstract: Drought and waterlogging that occur sequentially under field conditions are important abiotic stresses affecting plant growth and development. The ability to maintain the root system development during the contrasting moisture stresses may be one of the key traits for plant adaptation. This study aimed to identify the key root traits that contributed to the above ability by comparatively examining the effects of the two moisture stresses in succession on root system development. The chromosome segment substitution lines (CSSLs) from the crosses between the japonica rice cultivar Nipponbare and indica rice cultivar Kasalath were used for precise comparison of root system development. The rice seedlings were grown by hydroponics under a continuously well-aerated condition for 14 days (non-stressed), a drought condition for 7 days followed by an oxygen (O₂)-deficient (stagnant) condition for 7 days (drought-to-stagnant, D-S), or a stagnant condition for 7 days followed by drought condition for 7 days (stagnant-to-drought, S-D). CSSL43 and 47 did not show any significant differences in growth from Nipponbare under the non-stressed condition, but exhibited greater lateral root production under the stresses. Lateral root production was most closely related to faster seminal root elongation mediated by higher aerenchyma formation in the D-S condition, and to more branching of lateral roots on the seminal root axis in the S-D condition. The D-S condition severely affected lateral root production due to reduced seminal root elongation and aerenchyma formation. These results confirmed the fact that those root traits previously identified using different cultivars greatly contribute to plant adaptation. Oxygen deficiency preceded by drought (D-S) was more stressful to roots than drought preceded by O₂ deficiency (S-D), because drought reduced root aerenchyma formation during the subsequent stagnant condition.

Key words: Aerenchyma, Chromosome segment substitution line (CSSL), Drought, Lateral roots, Oryza sativa, Rice, Soil moisture, Stagnant.

Soil moisture under field conditions fluctuates due to the intermittent nature of rainfall and irrigation system. These fluctuations trigger the alternate occurrences of transient drought and waterlogging, which are two of the most important abiotic stresses affecting plant growth. For plant adaptation to such conditions, we have shown that roots play key roles in the developmental and functional responses to water deficit or excess (Yamauchi et al., 1996; Wang and Yamauchi, 2006). Previously, (Suralta and Yamauchi, 2008), we found that the aerobic rice genotypes showed greater shoot dry matter production than irrigated lowland genotypes under constant drought due to their ability to maintain root system development under the condition. In contrast, under constant waterlogged condition, shoot dry matter production of aerobic genotypes was comparable with that of irrigated lowland genotypes because of their similar ability to enhance aerenchyma to maintain root growth under O₂-deficient soil conditions. When these genotypes were grown under drought followed by O₂-deficient condition or reverse sequence, the aerobic genotypes showed greater ability than irrigated lowland genotype in terms of root system development that was evaluated based on lateral root production (Suralta et al., 2008). The key root traits that contributed to the greater lateral root production of aerobic genotypes than irrigated lowland genotypes under the O₂-deficient condition followed by drought were faster seminal root elongation associated with more branching of lateral roots and greater nodal root production. On the other hand, the key root traits that contributed to greater lateral root production of aerobic genotypes than irrigated lowland genotypes under the drought followed by O₂-deficient condition were faster seminal root elongation associated with higher aerenchyma formation, and greater nodal root production (Suralta et al., 2008). However, these key root traits identified with genetically diverse rice genotypes need to be further validated and confirmed.
using sets of nearly isogenic rice lines because the confounding effects of other traits are expected to be much less than comparing among different cultivars. Furthermore, in the above study, we did not examine the differences between the effects of two transient moisture stress treatments on root system development because the inherent genotypic differences among cultivars in root responses to both constant and transient contrasting moisture stresses may make the precise interpretation of the results difficult.

The deficit (drought) and excess (O₂ deficiency) of moisture are two contrasting stresses, each of which requires specific plant adaptations (Wang and Yamauchi, 2006). The effects of these stresses occurring in sequence on root system development may vary with the order in which they occur (i.e., O₂ deficiency followed by drought or drought followed by O₂ deficiency). This is because the occurrence of the first stress could affect the root adaptation to the subsequent stress. Whether preceding O₂ deficiency or drought would have a greater effect is unknown.

Recent progress in DNA markers and their linkage maps has provided powerful tools for mapping quantitative trait loci (QTLs) (Yano et al., 2001). This has also provided opportunities for studying the genetics and physiology of root growth, and functional contribution to dry matter production under abiotic stresses, by precisely identifying the QTLs that control important phenotypes. Kamoshita et al. (2002a, 2002b) emphasized the difficulty in phenotyping for correct identification of QTLs in abiotic stresses where both constitutive and adaptive traits are present, and each interacts with both the stress conditions prevailing and the genetic background. Several improved genetic materials for QTL analysis may help examine more precisely the physiology and phenotype with reduced confounding by genetic backgrounds. These genetic materials include the novel mapping populations of backcross-inbred lines (BILs) (Lin et al., 1998; Ma et al., 2002; Nagata et al., 2002), chromosome segment substitution lines (CSSLs) (Kubo et al., 2002; Ebitani et al., 2002, 2005) and anther culture-derived doubled-haploid lines (DHLs) (Siopongco et al., 2005, 2006). The DHLs are proven to be useful for precise evaluation of root traits with reduced effects of genetic confounding of other traits (Siopongco et al., 2005, 2006). For CSSLs, several sets from different parents have been developed in rice by the Rice Genome Resource Center (RGRC), National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan. The mapping population carries a few chromosome segments of Kasalath (indica) in each line overlapping with segments on a Nipponbare (japonica) genetic background.

The seeds of CSSLs and Nipponbare parent were soaked in water solution pre-mixed with fungicide (0.15% w/v) and incubated in seed germinator maintained at 28°C for 24 hours prior to sowing. The seedlings were grown in a growth chamber for two weeks, one week each under either a drought or O₂-deficient condition. The growth chamber was programmed to maintain day (0600–2100) and night (2100–0600) time temperatures at 30°C and 22°C, respectively, and under a 12-hr photoperiod with photosynthetically active radiation of 175 μmol m² s⁻¹ and 70% constant relative humidity.

2. Transient moisture stress treatments

The seedlings of each genotype were grown by hydroponics and placed in a O₂-deficient (stagnant) or drought condition right after sowing (0 day after sowing, DAS). Oxygen deficiency was induced by mixing water solution with agar (0.1% w/v) (herein referred to as ‘stagnant’) (Wiengweera et al., 1997) and flushed with N₂ gas to reduce O₂-concentration in the water from 8.0 ppm (well-aerated) to between 0.14 and 0.16 ppm. This agar-mixed water solution was not aerated and remained ‘stagnant’ during the growing period. Water culture with 0.1% agar prevents turbulence (convection), thus simulating the slow gas movement found in waterlogged soils (Wiengweera et al., 1997). On the other hand, drought was induced by adding polyethylene glycol 6000 (PEG 6000) (4%, w/v) to attain a -0.13MPa water potential. Polyethylene glycol has no apparent toxic effects under well-aerated conditions (Verslues et al., 1998; Raymond and Smirnoff, 2002; Ober and Sharp, 2003; Ogawa et al., 2005, 2006a, 2006b) hence, can mimic the drying effects of soil environment. Seven days after the onset of the initial moisture stress treatment (7 DAS), the
seedlings of each genotype were transferred to the contrasting moisture stress treatment for another 7 days (8-14 DAS). The seedlings that were grown under stagnant condition initially were transferred to the drought conditions (herein referred to as stagnant-to-drought, S-D), while those grown under drought initially were transferred to the stagnant condition (referred to as drought-to-stagnant, D-S). Another group of seedlings that were grown by hydroponics under a well-aerated (non-stressed) condition for 14 days from sowing served as a control.

Three plants were collected at each sampling period.

3. Shoot and root growth measurements

The shoots were separated from the roots, oven dried at 70°C for 2 days and weighed. The roots were stored in an FAA solution consisting of formalin: acetic acid: 70% ethanol, FAA at 1: 1: 18 by volume for further measurements. The length of seminal and nodal roots was measured using a ruler. The number of lateral roots along the seminal and nodal root axes was manually counted. From these data, the linear frequency of lateral roots was calculated as the number of lateral roots per unit length of parental root axis (Ito et al., 2006).

4. Root porosity measurements

The internal gas space (porosity) may not be identical with aerenchyma. For instance, rice roots have root porosity of ~9% at 20–25 mm from the root tips even in the absence of aerenchyma (Armstrong, 1971). This means that the estimates of aerenchyma based on porosity may overestimate the size of aerenchyma. However, there is no effective method to distinguish the aerenchyma from total porosity, and thus we used total porosity as an estimate for aerenchyma development.

The seminal root axis of plant grown under the D-S condition was used for root porosity measurement following the microbalance method (Visser and Bögenmann, 2003). Briefly, seminal root axis was divided into 1-cm segments from the base acropetally. Each root segment was cut with a sharp razor blade and gently blotted by rolling it with a small brush on tissue paper for about 2 seconds to remove adherent water. Then, to prevent weight loss by evaporation, we transferred the segments into a capsule with a cover that had been tared on a microbalance. After closing the capsule, the weight of the segments was measured (\(w_1\) in \(\mu g\)), transferred to a holder with small vials filled with water, and stored for a maximum of 30 minutes. In this way, up to 36 samples were weighed before they were infiltrated with tap water twice under vacuum for 30 minutes each. After water infiltration, the root segments were blotted again on a tissue paper for about 2 seconds and weighed in a capsule (\(w_2\) in \(\mu g\)). Using the specific weight (SW) obtained from larger samples (1.04 g mL\(^{-1}\)) (Visser and Bögenmann, 2003), the porosity was calculated using the formula:

\[
\text{Porosity (\%: \(v:v\)} = 100 \cdot (w_2 - w_1) \cdot \text{SW}/w_2
\]

with the specific weight of water being 1.00 g mL\(^{-1}\).

5. Statistical analysis

The difference between the two average values was subjected to the LSD test at \(P<0.05\). The difference between root porosity of seminal root segments grown under different stress conditions was subjected to \(t\)-test at \(P<0.05\). The relationships between total lateral root production and other related root components were determined using correlation analysis.

### Results

1. **Selected CSSLs under continuously well-aerated (non-stressed) conditions**

At 14 DAS, shoot dry weight ranged from 6.1 to 14.5 mg plant\(^{-1}\) and the total number of lateral roots per plant ranged from 613.0 to 1208.0 in the 54 CSSLs grown under the continuously well-aerated (non-stressed) condition, while these values were 7.1 mg plant\(^{-1}\) and 815 in Nipponbare, respectively (data not shown).

Table 1 shows the 13 CSSLs that were initially selected under non-stressed conditions for further evaluation under D-S and S-D conditions. These selected CSSLs showed no significant difference from Nipponbare in shoot dry weight (6.5–8.2 mg plant\(^{-1}\)) and total number of lateral roots (710.0–948.8).

| Genotypes  | Shoot dry weight (mg plant\(^{-1}\)) | Number of lateral roots (roots plant\(^{-1}\)) |
|------------|-----------------------------------|---------------------------------------------|
| Nipponbare | 7.1                               | 815.0                                       |
| CSSL16     | 8.1 ns                            | 906.0 ns                                    |
| CSSL20     | 8.0 ns                            | 710.0 ns                                    |
| CSSL21     | 7.8 ns                            | 765.3 ns                                    |
| CSSL30     | 7.6 ns                            | 852.4 ns                                    |
| CSSL33     | 7.1 ns                            | 814.7 ns                                    |
| CSSL34     | 6.8 ns                            | 747.7 ns                                    |
| CSSL37     | 6.8 ns                            | 798.3 ns                                    |
| CSSL41     | 7.6 ns                            | 791.0 ns                                    |
| CSSL43     | 8.2 ns                            | 930.7 ns                                    |
| CSSL45     | 7.8 ns                            | 741.3 ns                                    |
| CSSL47     | 6.5 ns                            | 855.7 ns                                    |
| CSSL48     | 7.7 ns                            | 948.8 ns                                    |
| CSSL51     | 7.7 ns                            | 844.0 ns                                    |

ns: not significant.
2. Shoot and root growth of selected CSSLs under transient moisture stresses

(1) Drought followed by stagnant (D-S) condition

This condition tended to inhibit the plant growth in all of the genotypes examined. Table 2 shows the shoot dry weights at 14 DAS of selected CSSLs that were not significantly different from that of Nipponbare (8.6 mg plant$^{-1}$) under the D-S condition, which ranged from 7.0 to 9.0 mg plant$^{-1}$. Among those selected CSSLs, CSSL47 had 47.4% more lateral roots than Nipponbare, while the rest were not significantly different (Table 2). Among the root traits that contributed to lateral root production, CSSL47 had 59.8% longer seminal roots and 64.9% greater linear frequency of lateral roots on the nodal root axis than Nipponbare (Table 3). The linear frequency of lateral roots on the seminal root axis and mean nodal root length in CSSL47 were not significantly different from those in Nipponbare (Table 3).

In the D-S condition, the average porosity of the seminal root axis of Nipponbare and CSSL47 was 6.0 and 8.0%, respectively, at 7 DAS and 10.0% and 20.0%, respectively, at 14 DAS (Fig. 1). Higher mean root porosity increase in CSSL47 under this condition was due to the significant increases in root segment porosity in almost all segments in the seminal root axis (Fig. 1). In Nipponbare, significant increase in root porosity was limited only to a few root segments.

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Table 2. Effect of D-S conditions on the shoot growth and lateral root production in Nipponbare and selected CSSLs at 14 DAS.

| Genotypes | Shoot dry weight (mg plant$^{-1}$) | Number of lateral roots (roots plant$^{-1}$) |
|-----------|---------------------------------|---------------------------------|
| Nipponbare | 8.6                             | 437.7                           |
| CSSL16    | 7.0 ns                          | 399.7 ns                        |
| CSSL20    | 7.4 ns                          | 320.3 ns                        |
| CSSL21    | 8.0 ns                          | 332.0 ns                        |
| CSSL30    | 9.0 ns                          | 522.7 ns                        |
| CSSL33    | 8.1 ns                          | 376.7 ns                        |
| CSSL34    | 8.0 ns                          | 431.0 ns                        |
| CSSL37    | 7.6 ns                          | 395.7 ns                        |
| CSSL41    | 8.5 ns                          | 448.3 ns                        |
| CSSL43    | 7.7 ns                          | 463.3 ns                        |
| CSSL45    | 8.0 ns                          | 515.0 ns                        |
| CSSL47    | 7.2 ns                          | 645.0 *                         |
| CSSL48    | 7.7 ns                          | 450.3 ns                        |
| CSSL51    | 7.3 ns                          | 401.7 ns                        |

ns, not significant; *, significantly different from Nipponbare at $P<0.05$.

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Table 3. Effect of D-S conditions on the total number of lateral roots, length of seminal and nodal roots, number and length of nodal roots and linear frequency of lateral roots on the seminal and nodal roots axes of Nipponbare and selected CSSL47 at 14 DAS.

| Genotypes | Lateral roots (LR) (roots plant$^{-1}$) | Seminal root (SR) | Nodal roots (NR) |
|-----------|---------------------------------|-----------------|-----------------|
|           | Length (cm) | Linear frequency (number LR cm$^{-1}$ SR) | Number | Mean length (cm) | Linear frequency (number LR cm$^{-1}$ NR) |
| Nipponbare | 437.7       | 11.7            | 10.3            | 6.3   | 6.7       | 7.4    |
| CSSL47    | 645.0 *     | 18.7 *          | 12.8 *          | 4.7 * | 7.6 ns    | 12.8 * |

ns, not significant; *, significantly different from Nipponbare at $P<0.05$. 

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Fig. 1. Root porosity along the seminal root axes of Nipponbare and CSSL47 at 7 DAS (○, drought conditions) and 14 DAS (●, drought to stagnant conditions). Numbers inside the graph indicate the means of root porosity for each treatment. Dr=drought; Dr-stag=drought to stagnant. Data are means of three replicates. ns, not significant; *, significantly different at $P<0.05$. 

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located in the middle of seminal root axis.

The length of seminal root was positively and significantly correlated with the extent of root porosity in the D-S conditions (Fig. 2), indicating that aerenchyma development contributed to O₂ diffusion supporting the higher rate of continuous root elongation.

The correlation between the root porosity and the number of lateral roots at each segment along seminal root axes of CSSL47 and Nipponbare was positive and significant at 7 DAS (Fig. 3). At 14 DAS, the correlation between root porosity and number of lateral roots remained significant in CSSL47 but not in Nipponbare (Fig. 3).

(2) Stagnant followed by drought (S-D) condition

This condition also generally tended to inhibit the plant growth. Table 4 shows the shoot dry weights of selected CSSLs under the S-D condition, which ranged from 9.6 to 11.4 mg plant⁻¹. These values were not significantly different from that of Nipponbare (9.4 mg plant⁻¹). Lateral root production in these selected CSSLs was not significantly different from that in Nipponbare with the exception of CSSL43 and CSSL47, which produced 29.3 and 36.8% more lateral roots than Nipponbare, respectively (Table 4).

CSSL43 showed 26.0% more nodal roots than Nipponbare (Table 5). CSSL47 on the other hand, showed a significantly 12.6% longer seminal root, 25.2% greater linear frequency of lateral roots on the seminal root axis and 56.6% greater mean length of nodal roots than Nipponbare (Table 5).

![Fig. 2](image2.png)

**Fig. 2.** The relationship between mean root porosity and length of seminal roots under D-S conditions at 14 DAS. Nipponbare (●) and CSSL47 (●). Values from each point are from each replication of each genotype. In CSSL47, the values for the length of seminal root and percent root porosity were almost the same for two of the three replications, and thus, only two points seemed to appear in the graph. *, significant at P < 0.05.

![Fig. 3](image3.png)

**Fig. 3.** The relationship between root porosity of seminal roots and number of lateral roots in segments along the seminal root axis at 7 DAS (○, drought conditions) and 14 DAS (●, drought to stagnant conditions). ***, significant at P < 0.01.

### Table 4. Effect of S-D conditions on shoot growth and lateral root development in Nipponbare and selected CSSLs at 14 DAS.

| Genotypes     | Shoot dry weight (mg plant⁻¹) | Number of lateral roots (roots plant⁻¹) |
|---------------|-------------------------------|----------------------------------------|
| Nipponbare    | 9.4                           | 684.0                                  |
| CSSL16        | 10.3 ns                       | 525.7 ns                               |
| CSSL20        | 11.4 ns                       | 834.7 ns                               |
| CSSL21        | 9.9 ns                        | 648.7 ns                               |
| CSSL30        | 10.0 ns                       | 715.0 ns                               |
| CSSL33        | 9.6 ns                        | 613.5 ns                               |
| CSSL34        | 10.0 ns                       | 808.0 ns                               |
| CSSL37        | 10.4 ns                       | 707.3 ns                               |
| CSSL41        | 9.6 ns                        | 635.3 ns                               |
| CSSL43        | 10.1 ns                       | 884.3 *                                |
| CSSL45        | 10.1 ns                       | 540.3 ns                               |
| CSSL47        | 10.8 ns                       | 936.0 **                               |
| CSSL48        | 10.8 ns                       | 827.0 ns                               |
| CSSL51        | 10.9 ns                       | 581.0 ns                               |

ns, not significant; *, **, significantly different at P < 0.05 and P < 0.01, respectively.
3. Comparison of root system development between D-S and S-D conditions

The value of each root parameter at 14 DAS was averaged across the selected CSSLs and Nipponbare to compare the effects of the D-S and S-D conditions on the root system development (Table 6). Relative to the continuously well-aerated (control) condition, D-S condition inhibited lateral root production by 40.7%, whereas the S-D condition did not significantly inhibit lateral root production (Table 6). Significant inhibition of lateral root production by the D-S condition was attributed to a significant reduction in seminal root elongation and less branching of its lateral roots, reduced mean nodal root elongation and less branching of lateral roots on the nodal root axis (Table 6). On the other hand, the S-D conditions significantly inhibited the seminal root elongation and branching of lateral roots on the seminal and nodal root axes, but it significantly promoted mean nodal root elongation (Table 6).

Correlation analysis showed that under the D-S condition, the lateral root production had the closest relationship with faster seminal root elongation. On the other hand, lateral root production under the S-D condition had the closest relationship with the frequency of lateral roots on the seminal root axis (Table 7).

Table 5. Effect of S-D conditions on the total number of lateral roots, length of seminal and nodal roots, number and length of nodal roots and linear frequency of lateral roots on the seminal and nodal root axes in Nipponbare and selected CSSLs at 14 DAS.

| Genotypes | Lateral roots (LR) (roots plant⁻¹) | Seminal root (SR) | Nodal roots (NR) | |
|-----------|-----------------------------------|------------------|-----------------|---|
|           | Length (cm) | Linear frequency (number LR cm⁻¹ SR) | Number | Mean length (cm) | Linear frequency (number LR cm⁻¹ NR) | |
| Nipponbare | 650.7 b | 17.5 b | 10.7 b | 5.0 b | 10.6 b | 9.2 a |
| CSSL43    | 884.3 a | 17.2 b | 11.8 ab | 6.3 a | 11.8 b | 9.3 a |
| CSSL47    | 936.0 a | 19.7 a | 13.4 a | 4.7 b | 16.6 a | 9.6 a |

Means within a column followed by the same letter are not significantly different at P < 0.05.

Table 6. Effect of transient moisture stress treatments on the total number of lateral roots, length of seminal and nodal roots, number and length of nodal roots and linear frequency of lateral roots on the seminal and nodal root axes which were averaged across selected CSSLs and Nipponbare at 14 DAS.

| Moisture treatment | Lateral roots (LR) (roots plant⁻¹) | Seminal root (SR) | Nodal roots (NR) | |
|                   | Length (cm) | Linear frequency (number LR cm⁻¹ SR) | Number | Mean length (cm) | Linear frequency (number LR cm⁻¹ NR) | |
| CWA              | 890.7 a | 21.9 a | 16.9 a | 4.5 a | 9.5 b | 11.8 a |
| Dr→stag          | 516.1 b | 14.3 c | 11.6 b | 5.3 a | 6.9 c | 9.1 b |
| Stag→dr          | 823.2 a | 18.1 b | 12.0 b | 4.9 a | 13.4 a | 9.3 b |

Means within a column followed by the same letters are not significantly different at P < 0.05.

CWA: continuously well-aerated; Dr→stag: D-S condition and Stag→dr: S-D condition.

Table 7. Correlation coefficient between the total number of lateral roots and other root traits of Nipponbare and selected CSSLs grown under D-S and S-D conditions (14 DAS).

| Parameter                                      | Drought to stagnant | Stagnant to drought |
|------------------------------------------------|---------------------|---------------------|
| Seminal root (SR) length                       | 0.92**              | 0.43 ns             |
| Linear frequency of lateral roots (no. cm⁻¹ SR) | 0.80*               | 0.76*               |
| Number of nodal roots (NR)                     | −0.60 ns            | −0.01 ns            |
| Mean nodal root length                         | 0.14 ns             | 0.65*               |
| Linear frequency of lateral roots (no. cm⁻¹ NR) | 0.82*               | 0.26 ns             |

ns, not significant; *, **, significant at P < 0.05 and P < 0.01, respectively.

Discussion

The 13 selected CSSLs that showed no significant differences in shoot growth and lateral root production from Nipponbare under non-stressed condition were assumed to be under similar genetic control of such traits at this developmental stage. Any of these selected CSSLs, which produce more lateral roots than Nipponbare under transient moisture stresses, carry one or more substituted chromosome segments from Kasalath that are responsible for the response.
1. Key root traits for greater lateral root production under transient moisture stresses

The key root traits that significantly and highly contributed to more lateral roots under the D-S condition were faster seminal root elongation (Table 3) mediated by increased root porosity (Figs. 1, 2) and more branching of lateral roots on the seminal and nodal root axes, as expressed by linear frequency roots (Table 3). On the other hand, the key root traits that significantly and highly contributed to more lateral roots under the S-D condition included more branching of lateral roots on the seminal root axis and faster nodal root elongation (Table 5).

The above results confirmed most, but not all, of the key root traits responsible for the response to each transient moisture stress previously identified using genetically diverse cultivars (Suralta et al., 2008). The nodal root was previously identified as an important trait under transient moisture stresses. However, in this study, there was no clear relationship between nodal root production and lateral root production under both transient moisture stresses (Table 7).

2. Comparison between D-S and S-D conditions

Using the data of the selected CSSLs and Nipponbare parent, this study showed that D-S conditions had severe effect on lateral root production while S-D conditions had no effect (Table 6).

Under the D-S conditions, the lateral root production was greatly influenced by faster seminal root elongation (Table 7) mediated by the ability to increase root porosity (Fig. 2). Rice roots normally have porosity range of 30-40% for the whole root system when grown either directly under O₂-deficient (Colmer et al., 1998) or well-aerated then transferred to O₂ deficient conditions (Colmer, 2003a; Insulad et al., 2006). The reported root porosity values in the previous studies were higher than the 10-20% average porosity on the seminal roots of Nipponbare and CSSL47 grown under D-S conditions in this study (Fig. 1). This indicates that the increase in root porosity during the stagnant period of the D-S condition may not be enough to facilitate sufficient O₂ diffusion to the root tip. This was evident in CSSL47, which was able to increase root porosity during the stagnant period of D-S condition but its seminal root elongation was still significantly reduced as compared with that of its continuously well-aerated counterpart. These results show that drought stress limits the ability to enhance aerenchyma in the seminal roots, thus significantly lowering their elongation ability under subsequent stagnant conditions.

Furthermore, root porosity was also observed under initial drought stress (Fig. 1) although the volume was usually smaller than those in O₂-deficient environments. Many wetland and non-wetland plant species constitutively form small volume of gas space in roots even when grown under non-flooded conditions, i.e. 15-30% in rice (Colmer, 2003b). Kono et al. (1972), and Kono and Yamada (1972) studied earlier the physiological significance of root porosity in relation to root development in addition to O₂ supply to the root tips. They reported that cortical disintegration has a synchronized pattern with lateral root initiation and growth in seminal roots of rice. Furthermore, cortical disintegration in the seminal root axis caused the translocation of about 50 to 60% of nitrogen and phosphorous from the root axis to the younger root parts, which may be required for new lateral root production (Kono and Yamada, 1972). In this study, we also clearly showed that the root porosity formed during the drought period of the D-S condition had positive and significant correlations with the linear frequency of lateral roots on the root segment along the seminal root axis regardless of genotypes (Fig. 3).

Furthermore, root porosity also showed positive and significant correlations with seminal root elongations (Fig. 2) and thus, contributed to greater lateral root production under this condition.

The above facts suggest the importance of root porosity for rice adaptation to D-S conditions through the following possible mechanisms: a) under drought condition, the root porosity especially as a result of cortical disintegration is constitutive and may provide materials needed for lateral root production while b) under the event of sudden stagnant condition, the root porosity is increased by further cortical disintegration resulting in aerenchyma formation, which facilitates effective diffusion of atmospheric O₂ to the root tips.

Such internal atmospheric O₂ diffusion to the roots supports continuous growth of the existing lateral roots produced during drought and the continuous elongation of root axis to produce new lateral roots under stagnant condition. Additionally, aerenchyma formation as a result of further cortical disintegration under stagnant condition may also provide materials required for continued elongations of root axis and lateral root production.

Why did S-D conditions not significantly affect the lateral root production even though it has the same combination of stresses with D-S conditions? This is because rice plants have the ability to enhance aerenchyma in roots when directly grown under anaerobic condition. Such adaptation enabled rice to maximize its potential constitutive root development, which may be an advantage for plant adaptation during the periods of transient drought. Several studies in rice have shown that constitutive root system development under anaerobic, well-watered conditions had a positive effect on subsequent growth of during progressive drought stress (Hoque and Kobata, 1998; Azhiri-Sigari et al., 2000; Kamoshita et al., 2000).
3. Comparison of markers contained in the substituted segments of selected lines to those reported QTLs detected from other rice mapping populations

CSSL47 consistently produced more lateral roots than Nipponbare under both transient moisture stress conditions (Tables 3, 5), while CSSL43 showed such ability only under S-D conditions (Table 5). The specific root traits that contributed to greater lateral root production than Nipponbare under S-D conditions differed between the two selected CSSLs (Table 5). This result was also supported by the fact that none of their substituted segments overlap in the same chromosome regions, indicating different putative QTLs controlling lateral root production.

Some of the QTL markers contained in the substituted segments of CSSL43 and CSSL47 coincided with the reported QTLs found in other rice mapping populations. These root traits include root to shoot ratio, maximum root length and root thickness at the base and below 90-cm soil depth (Champoux et al., 1995; Price et al., 2002; MacMillan et al., 2006). The QTL markers of the substituted chromosome segments are also subject for meta-QTL analysis to determine the number of true QTLs in those areas and the estimated positions (Ahmadi et al., 2007).

There are few studies on the QTLs controlling lateral root development under flooded and upland conditions (i.e. Zheng et al., 2003; Horii et al., 2006) but none of these marker-linked QTLs did match with any of the markers contained in the substituted chromosome segments of CSSL43 and CSSL47. Therefore, these facts suggest that the differences in putative QTLs controlling lateral root development are either due to the differences in types of moisture stress imposed (i.e. constant versus transient moisture stresses) and/or rice mapping populations used between studies. To the best of our knowledge, QTLs controlling aerenchyma development under waterlogging as well as under transient drought to waterlogged conditions has not been reported in rice. Therefore, putative QTLs for aerenchyma formation need to be identified under such transient moisture stress condition. Further genetic studies to elucidate putative QTLs under transient moisture stresses will be carried out using the selected CSSLs as soon as the contributions of their root responses specifically in lateral root production and aerenchyma formation to the maintenance of shoot dry matter production are precisely quantified, which are currently in progress.

Conclusions

One CSSL (47) produced more lateral roots than Nipponbare under D-S conditions while two CSSLs (43 and 47) under S-D conditions. The key root traits that showed close relationship with lateral root production were faster seminal root elongation mediated by higher aerenchyma formation under D-S conditions and more branching of lateral roots on the seminal root axis under S-D conditions. These results confirmed the key root traits reported previously with genetically diverse rice cultivars (Suralta et al., 2008) such as linear frequency of lateral roots on the nodal roots axis, and nodal root elongation but not nodal root productions.

The degree of the effect of transient drought and O$_2$ deficiency depended on which of the two stresses occur first in the sequence. Root system development was generally inhibited when drought preceded O$_2$ deficiency but not vice versa, mainly because drought reduced the plant ability to enhance aerenchyma formation under the subsequent sudden O$_2$ deficiency.

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