Genotypic Variations in Responses of Lateral Root Development to Transient Moisture Stresses in Rice Cultivars

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Abstract: Soil water regimes under field conditions inevitably tend to fluctuate ranging from drought to waterlogging. Genotypes that adapt better to such changing hydrologic conditions are assumed to have the ability to maintain root system development under such conditions. This study aimed to evaluate the responses of root system development based on lateral root production to transient moisture stresses, and the contribution of the elongation of seminal and nodal root axes and their lateral, root branching, and aerenchyma development in the seminal root axis, to root system development. The seedlings of two aerobic genotypes (UPLRi7 and NSICRc9) and one irrigated-lowland genotype (PSBRc82), and two parental genotypes (Nipponbare and Kasalath) of chromosome segment substitution lines (CSSLs) were grown by hydroponics. The seedlings were exposed to a drought condition by adding polyethylene glycol to the solution for 7 days and then to an O₂-deficient stagnant condition for 7 days (drought-to-stagnant condition), or to reverse successive conditions (stagnant-to-drought condition). Under both conditions, the aerobic genotypes showed greater ability to produce lateral roots than the irrigated-lowland genotype. Under the transient stagnant-to-drought condition, the root traits that contributed to greater lateral root production in the aerobic genotypes were faster seminal root elongation that was closely associated with branching of lateral roots, and greater nodal root production. Under transient drought to stagnant condition; these were faster seminal root elongation mediated by higher aerenchyma formation, and greater nodal root production. Kasalath showed much greater ability to produce lateral roots under both transient moisture stress conditions than Nipponbare. This indicates the potential utility of the CSSLs for precise identification of desirable root traits with less genetic confounding.

Key words: Aerenchyma, Aerobic rice, Chromosome segment substitution lines, Drought, Lateral root production, O₂ deficiency.

Under field conditions, the soil moisture fluctuates due to the intermittent nature of rainfall patterns and irrigation systems. These environments are common in some tropical savannas and temperate sub-humid grasslands (Sarmiento, 1984; Casas and Ninot, 2007; Ferreira et al., 2007), rainfed lowlands (Boling et al., 2004) and flood-prone rice fields (Khush, 1997). Such fluctuations are also inevitable even in irrigated rice fields (Belder et al., 2005) where water saving production technology, such as alternate wetting and drying systems and aerobic rice production systems are practiced (Bouman et al., 2005; Toung et al., 2005). Common to these environments are the transient anaerobic and flooded condition followed by aerobic and drought stressed conditions, and vice versa, which are crucial for plant productivity. In rice water-saving systems with intermittent irrigations, for example, the soil water potential fluctuated between 0 and -30 KPa (Belder et al., 2005). We assumed that such soil moisture fluctuations partially contributed to the observed rice yield reductions by 15-19% under water-saving systems compared to continuously flooded system (Belder et al., 2005; Bouman et al., 2005) because severe changes in soil moisture have marked effects on the soil condition, availability of nutrients and water, root development and functions (Kondo et al., 2005; Iijima et al., 2007; Kato et al., 2007; Richards, 2008; Siopongco et al., 2008), and thus, are very crucial for crop growth and yield. In this aspect, the ability of root traits to change developmentally and functionally in response to the changing conditions was suggested to be one of the most important traits for adaptation (Ingram et al., 1994; Yamauchi et al., 1996; Wang and Yamauchi, 2006).

In rice, the whole root system consists of different component roots such as seminal root, nodal roots and lateral roots of various branching orders. Among these root components, the lateral roots comprised more than 90% of the total root system in terms of root length and more than 99.9% in terms of root number (Yamauchi et al., 1987), which are common to various crop species grown under both excess and deficit soil moisture environments (Yamauchi et al., 1996; Bañoc et al., 2000b; Armstrong and Armstrong,
The role of target root traits under moisture stresses can be precisely evaluated by using sets of genetically homozygous lines such as chromosome segments substitution lines (CSSLs) (Kubo et al., 2002; Ebitani et al., 2005) because the confounding effects of other traits are expected to be much less than comparing among varieties. The additional advantage of utilizing CSSLs is that they facilitate the identification of target trait loci for further genetic analysis (Ebitani et al., 2005). In this series of studies, we will be using the 54 CSSLs from Nipponbare and Kasalath crosses for further precise evaluation of root response to transient moisture stresses and their analysis of associated QTLs. For this purpose, we need to test first whether the CSSL parents have wide contrasting responses to transient moisture stresses.

In this study, we mainly used the seminal root for the analysis. The seminal root system is the most mature root in the whole root system and is at the same age regardless of genotypes. Therefore, using the seminal root system can minimize the confound effect of age, and improve the precision of the analysis.

This study therefore aimed to 1) compare the root system development in terms of lateral root production between the aerobic and irrigated-lowland genotypes, and between Nipponbare and Kasalath under transient moisture stresses; 2) evaluate the contribution of seminal and nodal root elongation and their branching of lateral roots; and 3) evaluate the contribution of aerenchyma development to lateral root production under transient drought followed by stagnant conditions through its effect on seminal root elongation or branching of lateral roots.

**Materials and Methods**

1. **Plant cultivation**

Five rice genotypes were used in this study. UPLRi7 and NSICRc9 (Apo) (herein referred to as ‘aerobic1’ and ‘aerobic2’ genotype, respectively) were identified as high-yielding rice genotypes (Cruz and Atlin, 2003) under aerobic conditions. Aerobic2 was also identified as a high-yielding genotype based on its consistent good performance under aerobic conditions and the responsiveness to nutrients (George et al., 2002). PSBRc82 (herein referred to as ‘irrigated-lowland’ genotype) is a high-yielding irrigated-lowland genotype. This genotype was chosen in this study because it consistently produced the highest grain yields among recently released irrigated-lowland cultivars in the Philippines during two wet season evaluations at IRRI (Laza et al., 2004).

Kasalath (indica) and Nipponbare (japonica) are the set of parents for 54 CSSLs generated by the Rice Genome Research Center (RGRC) of the National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan. These two CSSL parents showed wide genotypic differences when tested under constant drought stress.
condition. Confirmation of their wide genotypic differences under transient moisture stresses is the important first step to get high probability of selecting appropriate lines when evaluating their corresponding CSSLs as the next step under such transient moisture stress conditions.

The seeds 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. The growth chamber was programmed to maintain day (0600–2100) and night (2100–0600) time temperatures of 30°C and 22°C, respectively, with 70% constant relative humidity.

2. Transient moisture stress treatments

The seedlings from each genotype were grown by hydroponics, and initially exposed to stagnant, drought or well-aerated (control) conditions right after sowing (0 days after sowing, DAS). Oxygen deficiency was induced by mixing the water solution with agar (0.1% w/v) (referred herein as ‘stagnant’ treatment) (Wiengweera et al., 1997) and flushed with N2 gas to reduce O2 concentration in the water solution for measurements. The length of seminal and nodal roots was measured using a ruler. The number of lateral roots per unit length of parental root axis was manually counted. From these data, the linear frequency of lateral roots on the seminal and nodal root axes at 14 DAS were analyzed using paired t-test of statistical software packages statistiXL version 1.8 (statistiXL, Kalamunda, Western Australia) and determined the significance of differences between transient moisture stress treatments and control within each genotype at P < 0.05.

The seminal root of plants grown under simple initial drought followed by stagnant condition was used for root porosity measurement following the microbalance method (Visser and Bögenmann, 2003). Briefly, a seminal root axis was transversely cut 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 vaporization, the segments were transferred into a capsule with cover that had been tared on a microbalance. After closing the capsule, the weight of the segments was measured (w1 in µg), transferred to a holder with small vials filled with water, and stored for maximally 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 tissue paper for about 2 seconds and weighed in a capsule (w2 in µg). 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:

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

with the specific weight of water being 1.00 g mL−1.

3. Root growth measurements

The roots were collected and stored in FAA (formalin: acetic acid: 70% ethanol = 1: 1: 18 by volume) solution for 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 because even rice roots grown under well-drained conditions have porosity of 10–12% (W. Armstrong, personal communication). This means that the estimates of aerenchyma based on porosity may possibly overestimate the size of the aerenchyma. However, there is no effective method to distinguish this aerenchyma from total porosity, and thus we used total porosity as an estimate of aerenchyma development in this study.

5. Statistical Analyses

Data on the total number of lateral roots and the linear frequency of lateral roots on the seminal and nodal root axes at 14 DAS were analyzed using paired t-test of statistical software packages statistiXL version 1.8 (statistiXL, Kalamunda, Western Australia) and determined the significance of differences between transient moisture stress treatments and control within each genotype at P < 0.05.

The data on growth increments (8-14 DAS) were used to compare between the transient moisture stresses and control for each genotype. The growth increments were computed as the difference between the mean values measured at 14 DAS and 7 DAS and thus, statistical analyses were not possible. Therefore, the observed differences in increments between transient moisture stresses and control, expressed as
ratio, had no accompanying statistical information.

Results

1. Effect of transient stagnant-to-drought condition on root development

(1) Aerobic versus irrigated-lowland genotypes

Under a transient stagnant-to-drought condition, the lateral root production was not significantly affected in aerobic2, while it was significantly reduced 19% in aerobic1 and 36% in irrigated-lowland genotype (Table 1).

The number of lateral roots produced during the drought period (8-14 DAS) under the transient stagnant-to-drought condition was 10-17% higher than in the control in aerobic1 and aerobic2, but it was 17% lower in the irrigated-lowland genotype (Table 1). The promoted lateral root production in aerobic1 and aerobic2 under the transient stagnant-to-drought condition was associated with their ability to maintain seminal root elongation and the linear frequency of lateral roots on the seminal root axis (Table 1). The length of seminal roots was reduced in all of the genotypes under the transient stagnant-to-drought condition but the reduction was less in aerobic1 and aerobic2 (29% and 26%, respectively) than in the irrigated-lowland genotype (62%). The linear frequency of lateral roots on the seminal root axis was also reduced in all genotypes but the reduction was less in the aerobic (34%) than in irrigated-lowland genotype (42%). The differences in the number of lateral roots between the aerobic and irrigated-lowland genotype under the transient stagnant-to-drought condition were also associated with their differences in nodal root production (Table 1). The number of nodal roots was promoted 40% in aerobic1 and 140% in aerobic2, whereas it was reduced 65% in irrigated lowland compared with the continuously well-aerated control (Table 1). Furthermore, the genotypic differences between the aerobic and irrigated-lowland genotypes in mean length of nodal roots and linear frequency of lateral roots on the nodal root axis did not contribute to their differences in lateral root production under this condition (data not shown).

(2) Kasalath versus Nipponbare (CSSL parents)

The transient stagnant-to-drought condition promoted lateral root production 25% in Kasalath while it reduced 19% in Nipponbare, although the differences were not statistically significant (Table 2). The number of lateral roots produced during the drought period (8-14 DAS) of the transient stagnant-to-drought condition was higher than that in the control in both Kasalath and Nipponbare (Table 2), but the increase was greater in Kasalath (138%) than in Nipponbare (24%). The genotypic differences in number of lateral roots between Kasalath and Nipponbare were associated with the differences in their ability to maintain seminal root elongation and the linear frequency of lateral roots on the seminal root axis (Table 2). Specifically, the length of seminal root was increased 140% in Kasalath, whereas it was reduced 10% in Nipponbare (Table 2). The linear frequency of lateral roots on the seminal root axis was maintained in Kasalath, whereas it was reduced 30% in Nipponbare, a significant difference (Table 2).
2). The genotypic differences in the number of lateral roots between the two parental genotypes were also associated with their differences in nodal root production (Table 2). The number of nodal roots was increased 30% in Kasalath, whereas it was reduced 53% in Nipponbare (Table 2). The differences between Kasalath and Nipponbare in mean length of nodal roots and linear frequency of lateral roots on the nodal root axis did not contribute to their differences in lateral root production under this condition (data not shown).

2. Effect of transient drought-to-stagnant condition on root development

(1) Aerobic versus irrigated-lowland genotype

Under the transient drought-to-stagnant condition, the lateral root production was reduced 37% in aerobic1, 40% in aerobic2 and 48% in the irrigated-lowland genotype compared with the control, the differences being significant (Table 3).

The number of lateral roots produced during the stagnant period (8-14 DAS) of the transient drought-to-stagnant condition was also reduced in both the aerobic and irrigated-lowland genotypes (Table 3).
However, the reduction in total lateral root production was less in aerobic1 and aerobic2 (38% and 59%, respectively) than in the irrigated-lowland genotype (65%).

The genotypic differences in the reduction of total number of lateral roots between the aerobic and irrigated-lowland genotypes were associated with their differences in the ability to maintain seminal root elongation under the transient drought-to-stagnant condition. Both the aerobic and irrigated-lowland genotypes showed reduction in seminal root elongation during the period of transient stagnant condition, but the reduction was less in aerobic1 and aerobic2 (13 and 35%, respectively) than in the irrigated-lowland genotype (76%). The aerobic genotypes also maintained (aerobic2) or even 100% higher nodal root production (aerobic1) than in the control, whereas it was reduced 15% in the irrigated-lowland genotype (65%).

The genotypic differences in the reduction of seminal root length (369%) than that of Nipponbare (171%). The genotypic differences between Kasalath and Nipponbare in other root traits such as the number of nodal roots and linear frequency of lateral roots on the seminal and nodal root axes did not contribute to their differences in lateral root production under this condition (data not shown).

### 3. Aerenchyma development in the seminal root under the transient drought-to-stagnant condition

#### (1) Aerobic versus irrigated-lowland genotype

Root porosity was measured only under the transient drought-to-stagnant condition (Fig. 1) because the ability of aerenchyma development is more functional under this transient condition than under the transient stagnant-drought condition. The aerobic1 and aerobic2 genotypes under the drought-to-stagnant condition had 48 and 31%, respectively, higher mean root porosity at 14 DAS than at the end of initial drought condition at 7 DAS (Fig. 1). Such significant difference was not observed in the irrigated-lowland genotype. Furthermore, the significant difference in root porosity in seminal root was mostly found in the middle and more apical portions of the seminal root in aerobic1 and aerobic2 genotypes (Fig. 1).

#### (2) Kasalath versus Nipponbare (CSSL parents)

The lateral root production in Kasalath was not significantly affected by the transient drought-to-stagnant treatment, whereas it was reduced 40% (significantly different) in Nipponbare (Table 4).

The number of lateral roots produced during the stagnant period (8-14 DAS) of the transient drought-to-stagnant condition was 90% higher than in the control in Kasalath, whereas it was 25% lower in Nipponbare (Table 4). The genotypic differences in the production of lateral roots between Kasalath and Nipponbare under transient drought-to-stagnant condition were associated with their differences in the ability to maintain seminal root elongation (Table 4). The length of seminal root was promoted 115% in Kasalath, whereas it was inhibited 50% in Nipponbare. Furthermore, Kasalath also showed greater promotion in nodal root length (369%) than that of Nipponbare (171%). The genotypic differences between Kasalath and Nipponbare in other root traits such as the number of nodal roots and linear frequency of lateral roots on the seminal and nodal root axes did not contribute to their differences in lateral root production under this condition (data not shown).

### Table 4. Effect of transient drought to stagnant condition on the total number of lateral roots (14 DAS) and the increments of total number of lateral roots, and the length of seminal and nodal roots during the stagnant period (8-14 DAS) of Kasalath and Nipponbare CSSL parents.

| Genotypes/ Water Treatments | No. of LR (roots plant⁻¹) (14 DAS) | Increment of LR (roots plant⁻¹)* (8-14 DAS) | Increment of SRL (cm)* (8-14 DAS) | Mean Length of NR (cm)* (8-14 DAS) |
|----------------------------|------------------------------------|-----------------------------------------------|-----------------------------------|-----------------------------------|
| Kasalath                   | 643.0                              | 269.0                                         | 4.0                               | 1.3                               |
| % of the control           | 107.0                              | 189.7                                         | 215.0                             | 469.2                             |
| Drought→stagnant           | 688.0 ns                           | 510.3                                         | 8.6                               | 6.1                               |
| Nipponbare                 | 727.0                              | 362.3                                         | 3.0                               | 1.4                               |
| % of the control           | 60.0                               | 74.9                                          | 50.0                              | 271.4                             |

Abbreviations: LR, lateral roots; SRL, seminal root length; NR, nodal roots. * Increments were computed as the difference in growth between 14 DAS and 7 DAS. * and ns indicate significantly and not significantly different from the control, respectively, at P<0.05, respectively, at P<0.05.
On the other hand, the mean root porosity in Nipponbare after drought-to-stagnant treatment was not significantly different from that at the end of the initial drought condition at 7 DAS (Fig. 1). Significant difference mentioned for Kasalath was mostly found within the 9-cm apical portion of the seminal root (Fig. 1).

4. Relationship of root porosity with seminal root elongation and lateral root production under transient drought to stagnant condition

The increments of root porosity, length of seminal root and the number of lateral roots on the seminal root axis during the stagnant period after initial drought were pooled for aerobic, irrigated-lowland and CSSL parents to evaluate the relationship of the root porosity with the two root traits under transient drought to stagnant condition (Fig. 2). Scatter diagram showed positive and linear relationships between increments of root porosity and the length of seminal roots \((r=0.94**, \text{Fig. 2A})\) and the number of lateral roots on seminal root axes \((r=0.93**, \text{Fig. 2B})\). These significant relationships indicate that genotypes that had a higher ability to enhance aerenchyma under the stagnant condition had higher ability to elongate the seminal root and to produce lateral roots. Aerobic1, aerobic2, and Kasalath had higher ability to increase aerenchyma than the irrigated-lowland genotype and Nipponbare, and thus, showed greater seminal root elongation (Fig. 2A) and lateral root production (Fig.
2B). The relationship between root porosity and the number of lateral roots per cm-segment of seminal root was also examined (Fig. 3). At the end of the initial drought (7 DAS), the relationship between root porosity and the number of lateral roots was positive and linear in both the aerobic and irrigated-lowland genotypes (Fig. 3). At the end of drought-to-stagnant condition (8-14 DAS), the linear relationships were clear in aerobic genotypes but not in the irrigated-lowland genotype (Fig. 3). A similar observation was also made between the two CSSL parents, Kasalath showed a linear relationship between root porosity and number of lateral roots at both the end of the initial drought (7 DAS) and drought-to-stagnant conditions (14 DAS) (Fig. 3). In Nipponbare, however, the linear relationship between root porosity and the number of lateral roots disappeared during the stagnant condition after initial drought stress (Fig. 3)

**Discussion**

1. **Genotypic differences**

Consistent genotypic differences were found between aerobic and irrigated-lowland genotypes, and between Kasalath and Nipponbare in lateral root production in response to transient moisture stresses. Under the transient stagnant-to-drought condition, the lateral root production was less inhibited in the aerobic genotype than in the irrigated-lowland genotype (Table 1), and in Kasalath than in Nipponbare (Table 3), indicating wide genotypic differences between the two CSSL parents in lateral root production under this type of transient moisture stresses.

Under the transient drought-to-stagnant condition, the aerobic genotypes produced a larger number of lateral roots than in the lowland genotype (Table 2). Further, Kasalath and Nipponbare showed wide contrasting genotypic responses with the former genotype showing promoted lateral root production while the latter showed reduction (Table 3).

2. **Root traits that contributed to lateral root production under transient moisture stresses**

Under the transient stagnant-to-drought condition, the root trait that mainly determined the genotypic differences between the aerobic and irrigated-lowland genotypes, and between Kasalath and Nipponbare was lateral root production and the ability to maintain seminal root elongation (Table 1). The linear frequency of lateral roots is determined by a combination of the elongation of parent root and the root branching (Pages and Serrà, 1994). The linear frequency of lateral roots on the seminal root axis was higher and the seminal root was longer in aerobic genotypes than in irrigated-lowland genotype and in Kasalath than in Nipponbare, indicating that aerobic1 and aerobic2 had higher root-branching ability than the irrigated-lowland genotype, and Kasalath had higher root-branching ability than Nipponbare.

Furthermore, the ability to maintain nodal root production and elongation also contributed to the promotion of lateral root production under the transient stagnant-to-drought condition, which was exhibited by aerobic genotypes (Table 1) and Kasalath (Table 2). The above-mentioned root traits that played important roles under the transient stagnant-to-drought condition were also reported to be important for plant adaptation to constant drought stress and the recovery from drought injury (Ludlow and Muchow, 1990; Bañoc et al., 2000a; Davis and Bacon, 2003). However, whether the expression of such root traits under drought shown here was affected by the preceded stagnant condition still remains to be examined further.
On the other hand, under transient drought-to-stagnant condition (Table 3), the differences in lateral root production between aerobic and irrigated-lowland genotypes were mainly determined by the differences in their ability in maintaining seminal root elongation and nodal root production, which were both higher in the aerobic than irrigated-lowland genotype (Table 3). Between the two CSSL parents, Kasalath showed higher ability than Nipponbare in maintaining seminal root elongation and nodal root production under transient drought-to-stagnant condition, which explained greater lateral root production in the former than the latter genotype (Table 4).

These facts show that the difference in the ability of seminal root to elongate under transient drought-to-stagnant condition mainly contributed to the differences in lateral root production. At the end of initial drought stress (7 DAS) prior to stagnant condi-
tion, the seminal roots were the longest in the root system regardless of the genotypes (data not shown). Under O₂ deficiency, the aerenchyma development is an important root trait for facilitating effective O₂ diffusion to the roots (Colmer, 2003) to sustain root growth. Therefore, we examined the effect of initial drought stress on aerenchyma formation under the subsequent stagnant condition in the seminal root (Fig. 1). Our results showed that the seminal roots of aerobic genotypes subjected to the initial drought had the ability to enhance root aerenchyma under the subsequent stagnant condition, but those of the irrigated-lowland genotype had no such ability (Fig. 1). Kasalath also had the ability to enhance its aerenchyma in the seminal root under the transient drought-to-stagnant condition (Fig. 1), whereas Nipponbare completely lost such ability when subjected to drought in advance (Fig. 1).

The lack of ability to enhance the aerenchyma under a sudden stagnant condition in the irrigated-lowland and Nipponbare (Fig. 1) appeared to limit the diffusion of internal O₂ to the seminal roots. Such limitations in internal O₂ diffusion within the root due to low root porosity would reduce root respiration, which might have then resulted in reduced root elongation (Fig. 2A) and reduced the formation of new lateral roots (Fig. 2B). This was clearly shown by shorter seminal roots in the irrigated-lowland and Nipponbare under the transient drought-to-stagnant condition (Tables 3 and 4, Fig. 1). The genotypic differences in linear frequency of lateral roots on seminal root axis did not contribute to those in lateral root production under the transient drought-to-stagnant condition, which means that low root porosity affected lateral root production (Fig. 2B) through reduced root elongations (Fig. 2A).

Our results also showed that constitutive root porosity present in the drought stressed seminal root showed a positive linear relationship with the number of lateral roots regardless of genotypes (Fig. 3). Kono et al. (1972) and Kono and Yamada (1972) showed a synchronized pattern between cortical disintegration, and lateral root initiation along rice seminal root axis. This cortical disintegration caused the translocation of about 50 to 60% of nitrogen and phosphorous from the matured to younger portions along the root axes. They discussed a possibility that those materials from disintegrated cortex may be inherently required for new lateral root production (Kono and Yamada, 1972).

When drought stressed seminal roots were transferred to the stagnant condition, a strong relationship between root porosity and the number of lateral roots was maintained in aerobic genotypes and Kasalath, whereas such relationship was lost in irrigated-lowland genotype and Nipponbare (Fig. 3). Furthermore, the regression line of the relationship between the root porosity and the number of lateral roots under the transient drought-to-stagnant condition was different from that under initial drought condition (Fig. 3). In aerobic genotypes and Kasalath, these differences were attributed to the increased porosity in the part of the previously drought stressed seminal root and in the newly elongated seminal root, all of which in response to sudden stagnant condition (Fig. 3). In contrast, it was attributed to the inability of the drought-stressed seminal root to increase root porosity when transferred to sudden stagnant condition in irrigated-lowland and Nipponbare (Fig. 3). The above facts indicate that the increased root porosity by cortical disintegration resulting in aerenchyma formation would support the lateral root development through facilitating effective diffusion of atmospheric O₂ to the root tips (Gibberd et al., 2001) and providing a source of materials required for continued elongation of root axis and lateral root production (Kono and Yamada, 1972).

Rice plants generally showed an enhanced aerenchyma formation when roots were either directly subjected to O₂ deficiency (Colmer, 2003) or initially grown under well-aerated condition prior to O₂ deficiency (Insalud et al., 2006). However, in this study, we showed that the ability of rice roots to enhance aerenchyma formation when subjected to sudden O₂ deficiency is lost if the roots are exposed to drought precedingly. This was observed in the irrigated-lowland genotype examined and in Nipponbare (Fig. 1), which may be because they are less tolerant to fluctuating moistures. On the other hand, the aerobic genotypes (Atlin et al., 2006) and Kasalath might have preserved their ability to enhance their aerenchyma for the transient drought-to-stagnant conditions, which may be one of the desirable root traits for transient soil moisture conditions that normally occur in the fields.

Aerenchyma development, elongation and branching of roots in response to moisture stress were examined mainly on the seminal root axis in this study. However, the seminal and nodal roots have dissimilar morphology, anatomy, and physiological functions, and thus may differ in developmental responses to various environments (Yamauchi et al., 1996; Zobel, 2005). Further study is therefore required to examine if the above results on seminal root would also hold for the nodal roots to fully understand the roles of response of the entire root system to transient moisture stress particularly the drought-to-stagnant condition.

Conclusions

On the basis of lateral root production ability under both transient stagnant-to-drought and transient drought-to-stagnant conditions, the root system development of aerobic genotypes was found to be greater than that of irrigated-lowland genotype. Under transient stagnant-to-drought condition, the root traits that contributed to greater lateral root production in the aerobic genotypes was faster seminal root...
elongation that was closely associated with branching of lateral roots, and greater nodal root productions. Under the transient drought-to-stagnant condition, it was faster seminal root elongation mediated by higher ability to form aerenchyma, and greater nodal root productions.

Kasalath had much greater ability to produce lateral roots under both transient moisture stresses than Nipponbare. This indicates the potential usefulness of CSSLs for precise identification of desirable root traits with less genetic confounding.

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Under the transient drought-to-stagnant condition, it produced aerenchyma, and greater nodal root productions. Kasalath had much greater ability to produce lateral roots under both transient moisture stresses than Nipponbare. This indicates the potential usefulness of CSSLs for precise identification of desirable root traits with less genetic confounding.

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* In Japanese with English summary.