Root Development, Water Uptake, and Shoot Dry Matter Production under Water Deficit Conditions in Two CSSLs of Rice: Functional Roles of Root Plasticity

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Abstract: Root traits that can contribute to drought resistance have not been clearly indentified. We examined the role of root system development in enhancing water uptake and contribution to dry matter production by using the root box-pinboard method, with which quantitative assessment of root system development and the water uptake of root are possible. Chromosome segment substitution lines CSSL45 and CSSL50, and the recurrent parent Nipponbare were grown under continuously waterlogged conditions (control), and various intensities of water deficit in root boxes. There was no significant difference among the genotypes in shoot growth and root development, while CSSL45 and CSSL50 showed greater shoot dry weight than Nipponbare under water deficit conditions. This was due to their abilities to promote root system development as compared with Nipponbare, which facilitated greater water extraction than Nipponbare, especially under the mild water deficit condition of 20–25% w/w soil moisture contents. Furthermore, the increased root length density did not exceed the estimated critical value for water uptake, which indicates that plastic root system development was functionally effective and efficient for the enhancement of water uptake under mild water deficit conditions.

Key words: Chromosome segment substitution lines, Dry matter production, Rice, Root length density, Root plasticity, Water deficit.

Drought is the most important abiotic stress for rice production. For drought resistance, the consensus in 30 years of literature is that deep and thick roots are beneficial to drought avoidance, especially in upland conditions, where soil moisture is available mainly in a deeper soil layer (Yoshida and Hasegawa, 1982; Lilley and Fukai, 1994; Nguyen et al., 1997; Price et al. 2002; Kato et al., 2006, 2007; Uga et al., 2011). However, in rainfed lowlands that occupy about one third of the global rice area and are mostly prone to drought (Wade et al., 1999; MacLean et al., 2002), strong soil in the form of a hardpan can impede deep rooting (Clark et al., 1999; MacLean et al., 2002). These traits may not be exhibited because strong soil may prevent root development (Boonjung and Fukai, 1996a, 1996b), and the root traits responsible for the rice plant adaptation vary with the environment.

The root system structure and its response to various soil conditions have been studied intensively, including various soil moisture conditions (Galamay et al., 1992), water deficit and rewatered conditions (Azhiri-Sigari et al., 2000; Kamosita et al., 2000), fluctuating soil moisture conditions (Bañoc et al., 2000; Suralta et al., 2008a, 2008b, 2010; Suralta and Yamauchi, 2008) in rice, water deficit and different temperature conditions in legumes (Mia et al., 1996), fluctuating moisture conditions in sweet potato and cassava (Pardales and Yamauchi, 2003) and water deficit and rewatered conditions in cassava (Subere et al., 2009). The developmental responses of the root system to those stress conditions, due to greater contributions from seminal, nodal and adventitious roots elongation or lateral root development, or both, have been suggested to play significant roles in plant adaptation to the respective conditions (Yamauchi et al., 1996; Wang and Yamauchi, 2006). This ability has been termed phenotypic plasticity, which is the ability of the plant to change its morphology as environmental conditions change (O’Toole and Bland, 1987). Plasticity is a dynamic adaptive response to stress, such as water deficit.

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We have been examining the roles of root plasticity by using chromosome segment substitution lines (CSSLs) of rice that are genetically close to each other. Our previous study using CSSLs under field conditions, in which the soil depth was set at about 20 cm to eliminate the advantageous effect of deep rooting, showed that the plastic response in root system development to various intensities of drought stress is the key trait for plant adaptation to the drought stress conditions (Kano et al., 2011). In that study, we identified and selected two lines (CSSL45 and CSSL50) that resembled the recurrent parent Nipponbare in plant growth including its phenology under non-stressed condition but exhibited greater root system development than Nipponbare, especially under a soil moisture condition of 15–30% w/w, which was a mild water deficit condition. We evaluated plasticity as the ability to promote root system development, which is triggered by mild water deficit, and as a consequence, increases when the plant exhibits plasticity. However, the functional roles of root plasticity such as water uptake have not been quantitatively analyzed because of the difficulty of precise measurements of root development and water uptake in the field.

O’Toole (1982) emphasized the importance of root-related traits such as water uptake for improved rice growth under drought stress. In addition, the contribution of root system development to yield through water uptake may vary depending on the type of stress development (Fukai and Cooper, 1995; Acaña et al., 2008). Wade et al. (1999) pointed out that it is not clear whether the crop has sufficient roots at the depth to effectively extract soil water as drought progresses in rainfed lowlands. Plant water uptake increases through improved root length density (Kamoshita et al., 2000; Okada et al., 2002; Benier et al., 2009) and finally promote biomass production.

Materials and Methods

1. Plant materials

We used two CSSLs (CSSL45 and CSSL50) among 54 CSSLs derived from Nipponbare and Kasalath crosses reported previously (Kano et al., 2011). Seeds were provided by the Rice Genome Research Center, National Institute of Agrobiological Sciences, Japan. Soil moisture content (SMC) was kept at 15–30% w/w as a water deficit condition. Under this condition, two selected CSSLs (CSSL45 and CSSL50) exhibited pronounced root plasticity in our previous study (Kano et al., 2011).

This experiment was conducted in a vinyl house at the experimental field of Nagoya University, Japan (136°56′6″E, 35°9′5″N). The seeds of CSSL45, CSSL50 and recurrent parent Nipponbare were soaked in water with a fungicide (benomyl (benlate), 0.15% w/v) and incubated in seed germinator maintained at 28°C for 72 hr prior to sowing.
We used the root box-pinboard method (Kono et al., 1987). Three pre-germinated seeds of each genotype were sown in a PVC root box (25 cm×2 cm×40 cm, L×W×H) filled with 2.5 kg of air-dried sandy loam soil on 2 September 2007, which were placed in the vinyl house. The average solar radiation, and maximum and minimum temperature during the experimental period, as recorded by Japan Meteorological Agency (Nagoya, 136°57’9”E, 35°10’0”N), was 12.8 MJ m⁻² d⁻¹, 34.9°C and 16.7°C, respectively.

The soil in the box was first submerged overnight and then drained for 24 hr. SMC was measured to be 32.2% w/w. Complete fertilizer containing 60 mg nitrogen (N), 80 mg phosphorus (P), and 70 mg potassium (K) was mixed with the soil in each root box. The seedlings were thinned to one seedling per box at 5 d after sowing (DAS). The plants were grown for 38 d with three treatments: continuously waterlogged (control), 30% w/w SMC and 15% w/w SMC. Previously, we established the relationship between SMC and soil pH. The soil pH was monitored using tensiometers (DIK-8333, Daiki Rika Kogyo, Konosu, Japan) installed in the soil at a depth of 20 cm in a plastic pot (25.5 cm×30 cm, φ×H). The soil was first watered and then allowed to drain while its weight was monitored to determine SMC. Based on this relationship, the 30% w/w of SMC was found to have equivalent soil water potential of −0.003 MPa and 15% w/w of SMC, −0.050 MPa.

Water was supplied every two d or daily for all the treatments to maintain the target SMC. In the waterlogged control, water level was maintained at the soil surface in the root box. For the other two treatments, each root box was weighed daily and the amount of water lost through transpiration of the root box. For the other two, each root box was watered every two d or daily for all the treatments to maintain the target SMC. In the waterlogged control, water level was maintained at the soil surface in the root box. For the other two treatments, each root box was weighed daily and the amount of water lost through transpiration of the root box. For the other two, each root box was watered every two d or daily for all the treatments to maintain the target SMC.

The root system was stored in FAA solution (formalin: acetic acid: 70% ethanol =1:1:18 by volume) after taking photographs. Thereafter, the length of seminal roots and lateral root axes was counted and expressed as linear density, 1200 μmol m⁻² s⁻¹. The root system was stored in FAA solution (formalin: acetic acid: 70% ethanol =1:1:18 by volume) after taking photographs. Thereafter, the length of seminal roots and lateral root axes was counted and expressed as linear density, 1200 μmol m⁻² s⁻¹.

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Plants were sampled at 38 DAS. Four boxes (1 box=1 replication) were harvested for each treatment. Plants were cut at the stem base and oven-dried at 70°C for 3 d to measure shoot dry weight. The roots were sampled using a pinboard and transparent perforated plastic sheet following the methods of Kono et al. (1987).

The total root length was measured following the methods of Suralta et al. (2010). Each root sample that was sandwiched by the perforated plastic sheet was washed well and stained in 0.25% Coomassie Brilliant Blue R aqueous solution for 48 hr. The stained root samples were then rinsed with tap water and put on a lighted box for digital photographing using a Canon DSl26071 digital camera (Canon Ltd, Tokyo, Japan) at a resolution of 3,456×2,304 pixels. The digitized images of the root system in jpeg format were converted into tiff format for total root length determination using a macro program by Kimura et al. (1999) and Kimura and Yamasaki (2001) on NIH image software version 1.60 (public domain released by the National Institute of Health, U.S.A.) in a computer running G4 Macintosh.

3. Experiment 2

To validate the results of Experiment 1, we introduced various conditions, such as: different soil moisture treatments, shorter growth period, with season-influenced higher solar radiation and temperature. Specifically, the same set of genotypes used in Experiment 1 was grown for 31 d from 10 July 2008, when average solar radiation, maximum and minimum temperature during the experimental period was 20.7 MJ m⁻² d⁻¹, 37.9°C and 22.2°C, respectively. For soil moisture treatments, we intentionally set SMC at 25% w/w (−0.014 MPa) and 20% w/w (−0.040 MPa) that are in-between the range of Experiment 1 for water deficit treatments in Experiment 2, so that more precise effects can be examined.

In addition to the traits measured in Experiment 1, the number of tillers and leaf area were measured as developmental traits. Photosynthetic rate and stomatal conductance were measured using a portable photosynthesis analyzer (LI-6400, LI-COR, Lincoln, NE, USA) on abaxial side of the topmost fully-developed leaf on the main stem between 0900 and 1000 at 30 DAS under the following conditions: leaf temperature, 30°C; CO₂ concentration, 380 μL L⁻¹; relative humidity, 65–75%; quantum flux density, 1200 μmol m⁻² s⁻¹.

The root system was stored in FAA solution (formalin: acetic acid: 70% ethanol =1:1:18 by volume) after taking photographs. Thereafter, the length of seminal roots and nodal roots were measured using a ruler and the number of nodal roots was counted. Furthermore, the number of lateral root axes was counted and expressed as linear frequency (the number of lateral roots per unit length of seminal root axes; Ito et al., 2006).

4. Statistical analysis

Two-way analysis of variance (ANOVA) was conducted and Fisher’s LSD test at 5% level of confidence was used for the comparisons of the genotypes within each treatment by using Microsoft Excel Statistics 2006 for Windows. The relationships between root traits and shoot traits were determined using regression analysis.

Results

1. Experiment 1; Response of shoot and root growth to various levels of water deficit

Significant effects of soil moisture on shoot dry weight, total root length and water use were observed, while genotype had a significant effect only on water use. Interaction of the two factors was not significant in any of the traits (Table 1).
There was no significant difference among the genotypes in shoot dry weight, total root length or water use under waterlogged conditions (control). At 30% w/w SMC, shoot dry weight, total root length and water use were significantly lower than those in the controls, but without any significant difference among the genotypes. In contrast, CSSL45 and CSSL50 showed significantly higher shoot dry weight than Nipponbare at 15% w/w SMC. The total root length and water use also tended to be higher in CSSL45 and CSSL50 than in Nipponbare, but the difference was significant only in CSSL50.

2. Experiment 2

Due to higher radiation and air temperature during the experimental period, plants grew larger in this experiment than in Experiment 1. In Experiment 1, at 30% w/w SMC, shoot dry weight, total root length and water use were significantly lower than those in the controls, but without any significant difference among the genotypes. In contrast, CSSL45 and CSSL50 showed significantly higher shoot dry weight than Nipponbare at 15% w/w SMC. The total root length and water use also tended to be higher in CSSL45 and CSSL50 than in Nipponbare, but the difference was significant only in CSSL50.

| Soil moisture condition | Genotype  | SDW (mg plant⁻¹) | TRL (cm plant⁻¹) | WU (g plant⁻¹) |
|------------------------|-----------|------------------|------------------|---------------|
| Waterlogged            | Nipponbare| 1017.5 a         | 3300.0 a         | 552.5 a       |
|                        | CSSL45    | 1123.8 a         | 3285.5 a         | 593.3 a       |
|                        | CSSL50    | 957.7 a          | 3300.7 a         | 590.0 a       |
| 30% w/w                | Nipponbare| 805.0 a          | 2609.6 a         | 237.5 a       |
|                        | CSSL45    | 645.0 a          | 2028.5 a         | 210.0 a       |
|                        | CSSL50    | 785.0 a          | 2509.8 a         | 255.0 a       |
| 15% w/w                | Nipponbare| 516.7 b          | 1715.5 b         | 168.8 b       |
|                        | CSSL45    | 700.0 a          | 1923.8 b         | 190.0 b       |
|                        | CSSL50    | 957.7 a          | 2457.8 a         | 230.0 a       |

SM, soil moisture; G, genotype.
Values followed by the same letter in a column within each treatment are not significantly different at the 5% by Fisher’s LSD test.

Table 2. Shoot growth and water use of Nipponbare, CSSL45 and CSSL50 grown under different soil moisture conditions for 31 d in Experiment 2. Values are means of four replicates.

| Soil moisture condition | Genotype  | SDW (mg plant⁻¹) | TRL (no. plant⁻¹) | LA (cm² plant⁻¹) | WU (g plant⁻¹) |
|------------------------|-----------|------------------|------------------|-----------------|---------------|
| Waterlogged            | Nipponbare| 1538.6 a         | 7.0 a            | 222.2 ab        | 801.0 a       |
|                        | CSSL45    | 1700.9 a         | 7.0 a            | 256.2 a         | 828.3 a       |
|                        | CSSL50    | 1325.9 a         | 6.5 a            | 205.5 b         | 747.0 a       |
| 25% w/w                | Nipponbare| 573.3 b          | 2.3 b            | 83.1 b          | 322.8 b       |
|                        | CSSL45    | 797.9 a          | 4.3 a            | 111.9 b         | 374.0 ab      |
|                        | CSSL50    | 906.9 a          | 5.0 a            | 160.9 a         | 397.0 a       |
| 20% w/w                | Nipponbare| 466.3 b          | 1.8 b            | 66.5 b          | 229.7 b       |
|                        | CSSL45    | 674.6 a          | 3.3 a            | 100.4 a         | 291.8 ab      |
|                        | CSSL50    | 652.2 a          | 3.7 a            | 97.9 a          | 336.0 a       |

SDW, shoot dry weight; LA, leaf area; WU, water use; SM, soil moisture; G, genotype.
Values followed by the same letter in a column within each treatment are not significantly different at the 5% by Fisher’s LSD test.

* and ** indicates at the 5% and 1% level significance, respectively.
Response of shoot growth to various levels of water deficit

Table 2 shows shoot dry weight, number of tillers, leaf area and water use of the plants grown under waterlogged (control) and water deficit (25 and 20% w/w of SMC) conditions. The effects of soil moistures, genotypes and soil moisture × genotype interaction were significant for all the traits examined.

Under the control condition, there was no significant difference among the genotypes in shoot dry weight, number of tillers and water use, while CSSL50 showed significantly smaller leaf area than CSSL45. At 25% w/w SMC, shoot dry weight was 39.2% and 58.2% heavier in CSSL45 and CSSL50 than in Nipponbare, respectively. Furthermore, the number of tillers was 87.0% and 117.4% larger in CSSL45 and CSSL50 than in Nipponbare, respectively. Therefore, the number of tillers was 87.0% and 117.4% larger in CSSL45 and

(1) Response of shoot growth to various levels of water deficit

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Under the control condition, there was no significant difference among the genotypes in shoot dry weight, number of tillers and water use, while CSSL50 showed significantly smaller leaf area than CSSL45. At 25% w/w SMC, shoot dry weight was 39.2% and 58.2% heavier in CSSL45 and CSSL50 than in Nipponbare, respectively. Furthermore, the number of tillers was 87.0% and 117.4% larger in CSSL45 and
CSSL50 than in Nipponbare, respectively. In CSSL50, leaf area and water use were increased by 43.7% and 23.0%, respectively, as compared with Nipponbare, whereas in CSSL45, the difference from Nipponbare was not significant. At 20% w/w SMC, shoot dry weight was 44.7% and 39.9% heavier in CSSL45 and CSSL50 than in Nipponbare, respectively. Likewise, the number of tillers was 83.3% and 105.6% larger in CSSL45 and CSSL50 than in Nipponbare, respectively, and leaf area was 51.0% and 47.2% larger, respectively. Accordingly, water use was 43.6% higher in CSSL50 than in Nipponbare, but there was no significant difference between Nipponbare and CSSL45.

(2) Photosynthetic rate and stomatal conductance

Table 3 shows the photosynthetic rate and stomatal conductance for the plants grown under waterlogged (control) and water deficit (25 and 20% w/w SMC) conditions. The effects of SMC on photosynthesis and stomatal conductance were significant. Genotypic variations existed and the soil moisture × genotype interaction had a significant correlation with photosynthetic rate. Photosynthetic rate and stomatal conductance did not significantly vary with the genotype under the control condition.

At 25% w/w SMC, photosynthetic rate was significantly higher in CSSL45 and CSSL50 than in Nipponbare. Stomatal conductance was significantly higher in CSSL45 and tended to be higher in CSSL50 than in Nipponbare. At 20% w/w SMC, the photosynthetic rate was higher in CSSL45 and CSSL50 than in Nipponbare. CSSL45 had also significantly higher stomatal conductance, while there was no significant difference in stomatal conductance between Nipponbare and CSSL50.

(3) Response of root system development to various levels of water deficit

As shown in Fig. 1, under control conditions, the root system development of CSSL45 and CSSL50 appeared to be similar to that of Nipponbare. At 25 and 20% w/w SMC, the root system development was apparently inhibited in all genotypes with Nipponbare exhibiting greater inhibition than CSSL45 and CSSL50.

Table 4 shows the total root length, number of nodal roots, total nodal root length and total lateral root length in the plants grown under waterlogged (control) and water deficit (25 and 20% w/w of SMC) conditions. Soil moisture, genotype and soil moisture × genotype interaction had significant effects on total root length, while only soil moisture had a significant effect on the other root traits examined. Under the control condition, the parameters did not show any significant differences with the genotype.

At 25% w/w SMC, total root length was 29.8% and 27.1% longer in CSSL45 and CSSL50, respectively, than in Nipponbare. CSSL45 had 27.3% longer total nodal root length than Nipponbare, but there was no significant difference in the number of nodal roots and total lateral root length between CSSL45 and Nipponbare. In CSSL50,
The number of nodal roots and total nodal root length were increased by 40.2% and 45.6%, respectively as compared with those in Nipponbare, but total lateral root length was not significantly different.

At 20% w/w SMC, total root length was 52.2% and 51.7% longer, the number of nodal roots was 26.1% and 32.5% larger, and the total lateral root length was 74.9% and 61.5% longer in CSSL45 and CSSL50, respectively, than in Nipponbare. However, there was no significant difference in total nodal root length among the genotypes. There was no significant difference in number of lateral roots on segments along the seminal root with the genotype at any SMC (data not shown).

3. Relationship between root system development and shoot growth

As stated above, under the control (waterlogged) condition in both experiments and at 30% w/w SMC in Experiment 1, no significant difference or correlation was observed in water use or root system development expressed as total root length among the genotypes (Fig. 2). In contrast, under water deficit conditions (at 15% w/w SMC in Experiment 1, and 25 and 20% w/w SMC in Experiment 2), the root system development showed close correlations with water use, and the curvilinear regression was highly significant (Fig. 3). CSSL50 showed the longest total root length and also largest water use, which was followed by CSSL45 and then Nipponbare in both experiments (Fig. 3). Greater root system development as expressed in the greater total root length as compared with Nipponbare under water deficit conditions was also closely correlated with shoot dry matter production in a linear fashion (Fig. 4). CSSL50 showed the longest total root length and heaviest shoot dry weight, followed by CSSL45 and Nipponbare (Fig. 4).

Discussion

Consistent results obtained from two experiments showed that shoot and root growth in two selected CSSLs were not significantly different from those in their recurrent parent Nipponbare under the control, non-stressed conditions while the responses showed differences...
under water deficit conditions (Tables 1, 2, 3 and 4, Fig. 1).
In our previous study on 54 CSSLs in the field, the two
CSSLs showed no significant difference in plant growth
from Nipponbare under non-stressed conditions, but
exhibited greater root system development under water
deficit conditions (Kano et al., 2011). Furthermore,
CSSL50 consistently showed significantly greater shoot dry
matter production than Nipponbare for three years. This
was due to greater root system development in CSSL50
than in Nipponbare especially under mild water deficit
(15−30% w/w SMC) conditions in the field. CSSLs have
been useful to evaluate the root responses to transient
moisture stresses (Suralta et al., 2008b, 2010). Thus, studies
using the CSSLs have made it possible to evaluate the
contribution of root growth to shoot dry matter under
water stress conditions more rigorously and less
confoundedly than studies using varieties that are
-genetically diverse from each other.

In this study, we focused on the ability of the plant to
survive under severe drought stress to maintain water use
for growth and production under a mild level of water
deficit (Serraj et al., 2009, Gowda et al., 2011). Compared
with other studies on drought resistance in rice (e.g.,
MacMillan et al., 2006; Bernier et al., 2007; Venuprasad et
al., 2009), the target soil moisture in drought stress
treatment in our study was apparently milder (20−25% w/
SMC) compared to the studies using CSSLs and Nipponbare in the number of lateral roots (data not shown), while the two CSSLs showed greater ability than Nipponbare to elongate lateral roots at 20% w/w of SMC (Table 4). These results indicate that we need to pay close attention to the fact that nodal root development and lateral root development, each of which greatly affects the entire root system development, are under different genetic control (Horii et al., 2006; Qu et al., 2008; Xu et al., 2009). In this aspect, we previously found that the plastic responses to drought of different types of lateral root in rice are under different QTL controls (Wang et al., 2005a, 2005b). It is therefore important to take the heterorhizy in lateral roots

plant response to water deficit, per se.
Under the mild water deficit condition of 20−25% w/w
SMC, CSSL45 and CSSL50 showed significantly greater
shoot dry matter production than Nipponbare due to
greater tiller production and leaf expansion (Table 2).
This was attributed to the higher ability of CSSL45 and
CSSL50 than Nipponbare to maintain photosynthesis and
stomatal conductance and also to develop a greater root
system at 20−25% w/w SMC (Table 3) (Table 4 and Fig. 1).
The greater root system with increased total root length
resulted from increased nodal root production, and
promoted lateral root elongation, as compared with
Nipponbare. Especially CSSL50 showed greater ability to
minimize the reduction in nodal root production from the
tillers due to water deficit as compared with Nipponbare
(Table 4). For the lateral root development, we previously
showed that promoted lateral root production under water
stress conditions plays key role for rice adaptation to
drought (Bañoc et al., 2000; Suralta et al., 2010). In
contrast, in this study, there was no significant difference
between the two CSSLs and Nipponbare in the number of lateral roots (data not shown), while the two CSSLs showed greater ability than Nipponbare to elongate lateral roots at 20% w/w of SMC (Table 4). These results indicate that we need to pay close attention to the fact that nodal root development and lateral root development, each of which greatly affects the entire root system development, are under different genetic control (Horii et al., 2006; Qu et al., 2008; Xu et al., 2009). In this aspect, we previously found that the plastic responses to drought of different types of lateral root in rice are under different QTL controls (Wang et al., 2005a, 2005b). It is therefore important to take the heterorhizy in lateral roots

\[ WU = -0.0001 \text{TLL}^3 + 0.51 \text{TLL} - 220.1 \]
\[ R^2 = 0.77^{**} \]
\[ WU = -0.0002 \text{TLL}^2 + 0.16 \text{TLL} - 56.6 \]
\[ R^2 = 0.89^{**} \]

Fig. 3. Relationship of total root length with water use of
Nipponbare (●), CSSL45 (×) and CSSL50 (○) grown under
water deficit conditions (at 15% w/w of SMC in Experiment 1,
and 25 and 20% w/w of SMC in Experiment 2). Gray colors,
Experiment 1; black colors, Experiment 2. ** indicates
significant regression at 1%. Total root length was measured for
the plants grown for 38 d in Experiment 1 and 31 d in
Experiment 2. Water use is the accumulated amount of water
used by the plant during the growth period for each
experiment.

\[ SDW = 0.41 \text{TLL} + 23.57 \]
\[ R^2 = 0.71^{**} \]

Fig. 4. Relationship of total root length with shoot dry weight of
Nipponbare (●), CSSL45 (×) and CSSL50 (○) grown under
water deficit conditions (at 15% w/w of SMC in Experiment 1,
and 25 and 20% w/w of SMC in Experiment 2). Gray colors,
Experiment 1; black colors, Experiment 2. ** indicates
significant regression at 1%. Total root length and shoot dry
weight were measured for the plants grown for 38 d in
Experiment 1 and 31 d in Experiment 2.
Measurement of scanned root system as sampled in this study tends to underestimate the root length because of overlapping of roots, especially fine lateral roots. Nevertheless, results obtained with this method clearly showed that the difference between nodal and lateral roots in the developmental responses to mild water deficit were different. The length of the whole root system in was longer in CSSL 45 and CSSL50 than in Nipponbare, which was mainly due to the larger nodal roots under 25% w/w SMC while it was mainly due to the longer lateral root system at 20% w/w SMC (Table 4). These facts indicate that the degree of water deficit that triggers the developmental response may vary with the component roots, and requires further study. We previously reported that such plastic response would be advantageous for collection of soil water (Bañoc et al., 2000), and in fact, under both soil moisture conditions in this study, the longer root length substantially contributed to greater water uptake and dry matter production.

However, at 15% w/w SMC in Experiment 1, CSSL45 did not exhibit greater root growth than Nipponbare (Table 1). The 15% w/w SMC was probably too severe for CSSL45 to exhibit the plastic root system development. On the other hand, for CSSL50, plastic root response to water deficit effectively contributed to plant dry matter production through enhanced water uptake under water deficit conditions in both experiments.

Increase in root length under mild water deficit promoted water uptake, which was more clearly recognized in CSSL45 and CSSL50 than in Nipponbare (Fig. 3). However, the rate of water use with increasing total root length tended to slow down beyond approximately 1600 cm of root length per plant in Experiment 2. We then estimated the optimum value of water use (uptake) as the vertex of the parabola representing the quadratic equation between total root length and water use, which is shown in Fig. 3. As a result, the water use (uptake) is calculated to peak at around 3963 cm in Experiment 1 and 2568 cm in Experiment 2, which is equivalent to root length density (RLD) of 2.0 cm cm$^{-3}$ and 1.3 cm cm$^{-3}$, respectively (Fig. 3). These values have been reported as critical RLD for water uptake under upland conditions (Lilley and Fukai, 1994) and rainfed lowland conditions, when water deficit followed ponded water conditions (Siopongco et al., 2005).

A direct comparison of the absolute values of RLD may be difficult, but it is quite important to note that a critical RLD was observed in different experiments. Furthermore, the present study revealed that the length of root system of CSSL45 and CSSL50 that showed a greater root system development than Nipponbare did not exceed the critical RLD and thus effectively contributed to enhanced water uptake as shown by the positive and significant correlations of root length with water use (Fig. 3), which then contributed to greater shoot dry matter production under water deficit conditions (Fig. 4). This also indicates that root plasticity is important only under mild water deficit to benefit growth but not severe water deficit or non-stressed conditions. In other words, investment of roots to capture water is unnecessary under a water logged condition (Fig. 2), and may not be helpful when the resource is insufficient for growth under severe stress.

Utilization of the root box-pinboard method greatly helps us evaluate the relationship between root system development and shoot growth precisely. In wheat, Manschadi et al. (2008) quantified the root system architecture by using the digital images of the whole root system and demonstrated that the root growth angle was important for drought resistance, which contributed to deep root development. Similar relationships between root growth angle and drought resistance have been reported in rice (Kato et al., 2006). With our root box system, it is possible to precisely quantify the root growth angle (Yamauchi et al., 1987), and thus, will be useful for further study of this trait.

In conclusion, we confirmed the results of the previous study conducted in the field that the greater root system development under mild drought stress contributed to their increased dry matter production (Kano et al., 2011). The present study further evaluated quantitatively the functional roles of the root plasticity that contributed to greater shoot dry matter production. Although dry matter production in all the genotypes examined was generally reduced by the water deficit treatments regardless of the intensity, CSSL50 showed significantly less reduction in shoot dry weight than Nipponbare due to its ability to develop a larger root system, which was associated with greater water uptake and photosynthesis especially under mild water deficit conditions (20–25% w/w of SMC). These facts indicate that the chromosome segments in CSSL50 derived from Kasalath may be responsible for the expression of plasticity that promotes the root system development of Nipponbare in response to a mild water deficit.

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