

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), these traits may not be exhibited because strong soil in the form of a hardpan can impede deep rooting (Clark et al., 2002; Samson et al., 2002). In addition, the effect of drought varies with the plant variety, degree and duration of stress and the growth stage of the plant (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; Kamoshita 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.

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; Acuña 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), but some studies showed that root length density was not a factor for enhanced plant water uptake when the critical value for water uptake (Lilley and Fukai, 1994; Siopongco et al., 2005) was high, or there was insufficient additional water to gain from the investment in extra roots.

To quantify the responses in root system development and identify the exact nature of the architecture required for efficient water uptake, we need to improve the sampling method and clarify the differences in the rooting system among genotypes with different water uptake abilities. In the present study, we used the root box-pinboard method to collect the whole root system with minimum impairment to its structure under regulated soil moisture conditions, and evaluated the root system development in response to plant water use precisely (Kono et al., 1987; Suralta et al., 2010).

The rice root system of an individual plant consists of different component roots that differ in morphology, anatomy, physiological functions (Yamauchi et al., 1996) and genetic regulation for development (Wang et al., 2005a, 2005b). The adaptive responses of plastic root

system development to various environments also vary with the plant (Yamauchi et al., 1996; Bañoc et al., 2000; Wang and Yamauchi, 2006). We have examined the root system structure and its plasticity as expressed by the combination of production and elongation of component roots of a root system such as nodal roots and their concomitant lateral roots, which are associated with their functional roles in water uptake (Yamauchi, 1998).

Many studies on drought resistance have evaluated the putative traits under severe water deficit conditions. For example, the soil water potential was -0.07 MPa at a 15 cm depth, a condition under which severe leaf rolling were observed in the field (Bernier et al., 2007; Venuprasad et al., 2009) and -0.7 MPa at 15 cm depth in soil culture using a growth box (MacMillan et al., 2006). Even relatively mild water deficit was found to substantially reduce dry matter production (Kano et al., 2011).

In this study, we examined the root plasticity as defined above, which follows the definition by O'Toole and Bland (1987) and our previous study (Kano et al., 2011). We paid special attention to the quantitative evaluation of response of root system development and the precise measurement of water uptake by using the root box-pinboard method. The plasticity is genetically controlled (Wang et al., 2005a, 2005b) and we thus used the difference in root length between CSSLs and the recurrent parent, Nipponbare for the quantification of the root plasticity as in our previous study (Kano et al., 2011). Through these measurements, we examined whether or not the plastic root system development under a mild water deficit would enhance water uptake, stomatal opening, and photosynthesis, 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.

2. Experiment 1

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 ($136^{\circ}56'6''$ E, $35^{\circ}9'5''$ 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, $L \times W \times H$) 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 m² 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 pF. The soil pF 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, $\Phi \times H$). 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 replenished, which was recorded as evapotranspiration. Four root boxes without plants were also prepared to measure the amount of evaporation from the soil surface so that the amount of water lost through transpiration of the each plant could be estimated.

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 DS126071 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 m² 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).

Table 1. Shoot dry weight (SDW), total root length (TRL) and water use (WU) of Nipponbare, CSSL45 and CSSL50 grown under different soil moisture conditions for 38 d in Experiment 1. Values are means of four replicates.

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	740.0 a	2457.8 a	230.0 a
SM		**	**	**
G		ns	ns	*
SM×G		ns	ns	ns

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 ns indicates at the 5%, 1% level and no significance, respectively.

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)	Tillers (no. plant ⁻¹)	LA (cm ² 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	250.2 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
SM		*	**	**	**
G		**	**	**	*
SM×G		*	*	**	*

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.

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,

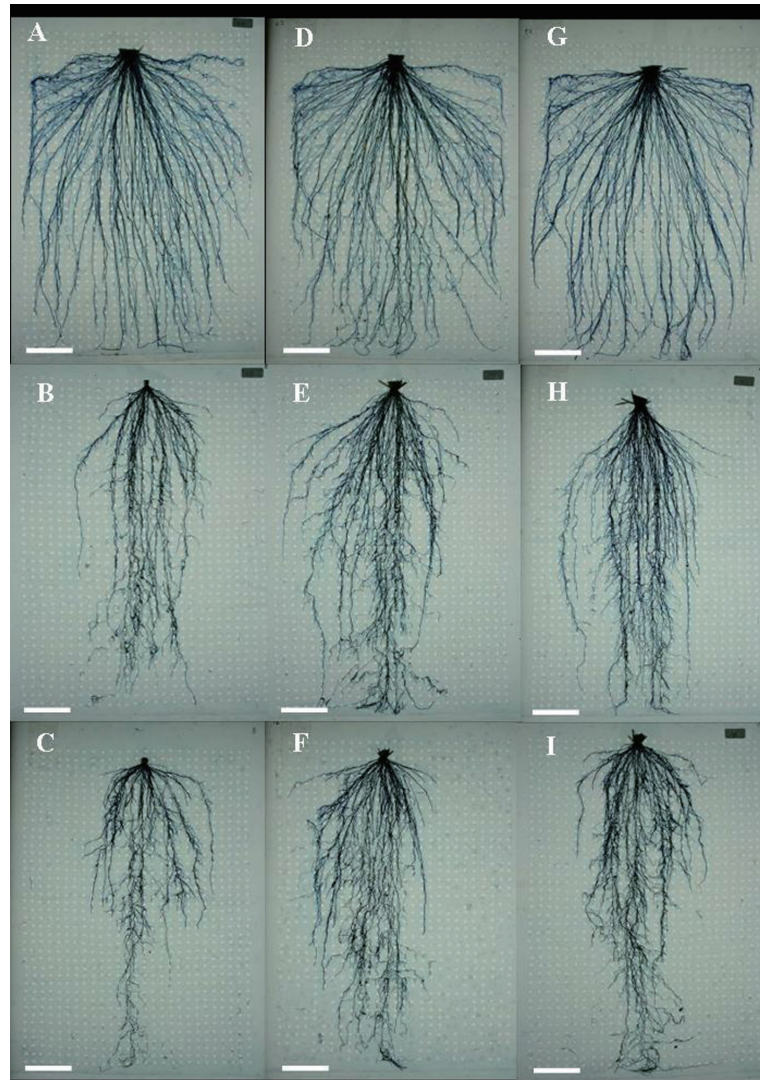


Fig. 1. Root system profiles of Nipponbare (A-C), CSSL45 (D-F) and CSSL50 (G-I) grown under waterlogged (control; A, D, G), 25% w/w of SMC (B, E, H) and 20% w/w of SMC (C, F, I) for 31 d in Experiment 2. Root systems were sampled with rootbox-pinboard method (Kono et al., 1987). Bars=5 cm.

the two CSSLs did not differ in their growth responses from Nipponbare. At 15% w/w SMC, probably because the intensity of drought stress was too severe, root system development in CSSL45 was not greater than that in Nipponbare. Based on these results, two other soil moisture contents (25 and 20% w/w SMC) were used as water deficit treatments in this experiment. The results also showed that the imposed water deficit significantly reduced both shoot and root growth as compared with their controls. Generally, there was no significant difference among the genotypes in shoot and root growth under the waterlogged control condition, but CSSL45 and CSSL50 showed significantly heavier shoot dry weight than Nipponbare under the two water deficit conditions (Tables 2 and 4, Fig. 1).

(1) 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 \times 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

Table 3. Photosynthetic rate (Pn) and stomatal conductance (g_s) of Nipponbare, CSSL45 and CSSL50 grown under different soil moisture conditions measured between 0900 and 1000 at 30 DAS in Experiment 2. Values are means of four replicates.

Soil moisture condition	Genotype	Pn ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)
Waterlogged	Nipponbare	31.3 a	1.276 a
	CSSL45	29.9 a	1.240 a
	CSSL50	29.7 a	1.545 a
25% w/w	Nipponbare	16.7 c	0.352 b
	CSSL45	23.4 b	0.465 ab
	CSSL50	27.3 a	0.611 a
20% w/w	Nipponbare	14.9 b	0.282 b
	CSSL45	24.9 a	0.467 a
	CSSL50	22.0 a	0.355 b
SM		**	**
G		**	ns
SM \times G		**	ns

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 ns indicates at the 1% level and no significance, respectively.

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 \times 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 \times genotype interaction had significant effects on total root length, while only soil moisture had a significant 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,

Table 4. Root system development 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	TRL (cm plant ⁻¹)	NRN (no. plant ⁻¹)	NRL (cm plant ⁻¹)	LRL (cm plant ⁻¹)
Waterlogged	Nipponbare	2635.9 a	127.3 a	2336.6 a	262.9 a
	CSSL45	2823.5 a	126.7 a	2384.9 a	403.1 a
	CSSL50	2492.7 a	118.5 a	2264.7 a	196.4 a
25% w/w	Nipponbare	1485.6 b	56.0 b	775.6 b	682.9 a
	CSSL45	1928.1 a	69.0 ab	987.3 a	903.4 a
	CSSL50	1888.8 a	78.5 a	1129.2 a	721.8 a
20% w/w	Nipponbare	1058.3 b	49.8 b	508.6 a	512.4 b
	CSSL45	1611.0 a	62.8 a	676.3 a	896.2 a
	CSSL50	1605.1 a	66.0 a	736.6 a	827.5 a
SM		**	**	**	**
G		**	ns	ns	ns
SM×G		*	ns	ns	ns

TRL, total root length; NRN, number of nodal roots; NRL, total nodal root length; LRL, total lateral root length; 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 ns indicates at the 5%, 1% level and no significance, respectively.

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

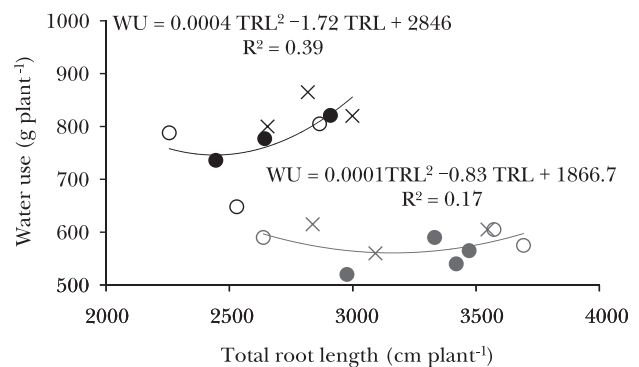


Fig. 2. Relationship of total root length with water use of Nipponbare (●), CSSL45 (×) and CSSL50 (○) grown under waterlogged (control) conditions. Gray colors, Experiment 1; black colors, Experiment 2. Total root length was measured for the plants grown for 38 d in Experiment 1 and 31 d in Experiment 2. Water use is the accumulated amount of water used by the plant during the growth period for each experiment.

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

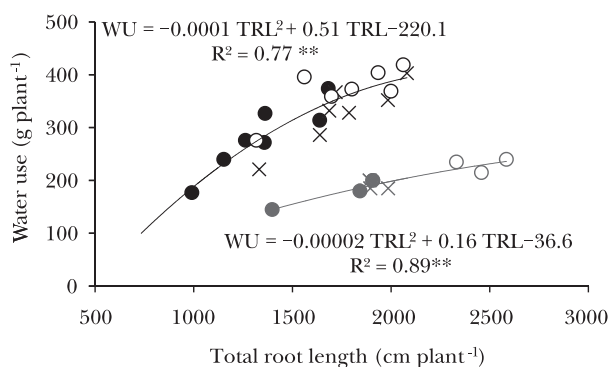


Fig. 3. Relationship of total root length with water use of Nipponbare (●), CSSL45 (×) and CSSL50 (○) grown under water deficit conditions (at 15% w/w of SMC in Experiment 1, and 25 and 20% w/w of SMC in Experiment 2). Gray colors, Experiment 1; black colors, Experiment 2. ** indicates significant regression 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.

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/w SMC (equivalent to -0.040 to -0.014 MPa)). Our results showed that such mild water deficit significantly reduced both shoot and root growth as compared with their controls consistently (Tables 1, 2, 3 and 4). It indicates that we need to pay attention not only to the indicator of drought intensity like soil water potential but also to the

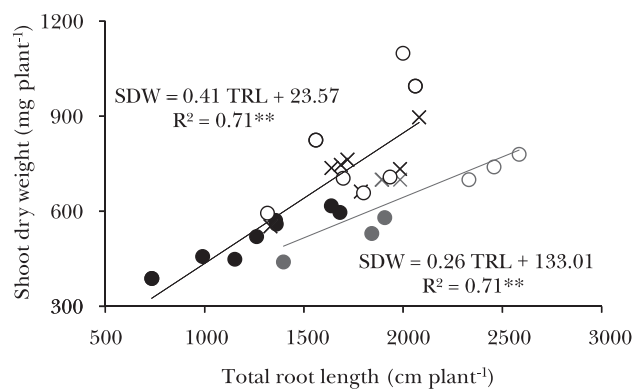


Fig. 4. Relationship of total root length with shoot dry weight of Nipponbare (●), CSSL45 (×) and CSSL50 (○) grown under water deficit conditions (at 15% w/w of SMC in Experiment 1, and 25 and 20% w/w of SMC in Experiment 2). Gray colors, Experiment 1; black colors, Experiment 2. ** indicates significant 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.

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 significantly 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

(Yamauchi et al., 1996) into account when discussing the genetic control of plastic root system development.

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 CSSL45 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 2565 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.

Acknowledgments

We thank Dr. Roel Rodriguez Suralta of Philippine Rice Research Institute (PhilRice) for assistance in statistical analysis. This research was supported by Grant-in-Aid for Scientific Research (No. 22380013), Grant-in-Aid for JSPS Fellows (No. 21007569) from the Japan Society for the Promotion of Science, and a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Genomics for Agricultural Innovation, QTL-4004), and contributed to the Generation Challenge Project under the Consultative Group for International Agricultural Research on

“Targeting drought avoidance root traits to enhance rice productivity under water-limited environments”.

References

- Acuña, B.T.L., Lafitte, H.R. and Wade, L.J. 2008. Genotype × environment interactions for grain yield of upland rice backcross lines in diverse hydrological environments. *Field Crops Res.* 108: 117-125.
- Azhiri-Sigari, T., Yamauchi, A., Kamoshita, A. and Wade, L.J. 2000. Genotypic variation in response of rainfed lowland rice to drought and rewatering. II. Root growth. *Plant Prod. Sci.* 3: 180-188.
- Bañoc, D.M., Yamauchi, A., Kamoshita, A., Wade, L.J. and Pardales, J.R.Jr. 2000. Genotypic variations in response of lateral root development to fluctuating soil moisture in rice. *Plant Prod. Sci.* 3: 335-343.
- Bernier, J., Kumar, A., Venuprasad, R., Spaner, D. and Atlin, G.N. 2007. A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Sci.* 47: 507-516.
- Bernier, J., Serraj, R., Kumar, A., Venuprasad, R., Impa, S., Gowda, V., Owane, R., Spaner, D. and Atlin, G. 2009. Increased water uptake explains the effect of *qtl12.1*, a large-effect drought-resistance QTL in upland rice. *Field Crops Res.* 110: 139-146.
- Boonjung, H. and Fukai, S. 1996a. Effects of soil water deficit on growth stages on rice growth and yield under upland conditions. 1. Growth during drought. *Field Crops Res.* 48: 37-45.
- Boonjung, H. and Fukai, S. 1996b. Effects of soil water deficit at different growth stages on rice growth and yield under upland conditions. 2. Phenology, biomass production and yield. *Field Crops Res.* 48: 47-55.
- Clark, L.J., Cope, R.E., Whalley, W.R., Barraclough, P.B. and Wade, L.J. 2002. Root penetration of strong soil in rainfed lowland rice: comparison of laboratory screens with field performance. *Field Crops Res.* 76: 189-198.
- Fukai, S. and Cooper, M. 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. *Field Crops Res.* 40: 67-86.
- Galamay, T.O., Yamauchi, A., Nonoyama, T. and Kono, Y. 1992. Acropetal lignifications on cereal nodal root axes as affected by different soil moisture conditions. *Jpn. J. Crop Sci.* 61: 511-517.
- Gowda, V.R.P., Henry, A., Yamauchi, A., Shashidhar, H.E. and Serraj, R. 2011. Root biology and genetic improvement for drought avoidance in rice. *Field Crops Res.* 122: 1-13.
- Horii, H., Nemoto, K., Miyamoto, N. and Harada, J. 2006. Quantitative trait loci for adventitious and lateral roots in rice (*Oryza sativa* L.). *Plant Breed.* 125: 198-200.
- Ito, K., Tanakamaru, K., Morita, S., Abe, J. and Inanaga, S. 2006. Lateral root development, including responses to soil drying, of maize (*Zea mays*) and wheat (*Triticum aestivum*) seminal roots. *Physiol. Plant.* 127: 260-267.
- Kamoshita, A., Wade, L.J. and Yamauchi, A. 2000. Genotypic variation in response of rainfed lowland rice to drought and rewatering. III. Water extraction during the drought period. *Plant Prod. Sci.* 3: 189-196.
- Kano, M., Inukai, Y., Kitano, H. and Yamauchi, A. 2011. Root plasticity as the key root trait for adaptation to various intensities of drought stress water stresses in rice. *Plant Soil.* 342: 117-128.
- Kato, Y., Abe, J., Kamoshita, A. and Yamagishi, J. 2006. Genotypic variation in root growth angle in rice (*Oryza sativa* L.) and its association with deep root development in upland fields with different water regimes. *Plant Soil.* 287: 117-129.
- Kato, Y., Kamoshita, A. and Yamagishi, J. 2007. Evaluating the resistance of six rice cultivars to drought: root restriction and the use of raised beds. *Plant Soil.* 300: 149-161.
- Kimura, K., Kikuchi, S. and Yamasaki, S. 1999. Accurate root length measurement by image analysis. *Plant Soil.* 216: 117-127.
- Kimura, K. and Yamasaki, S. 2001. Root length and diameter measurement using NIH Image: application of the line-principal for diameter estimation. *Plant Soil.* 234: 37-46.
- Kono, Y., Yamauchi, A., Nonoyama, T., Tatsumi, J. and Kawamura, N. 1987. A revised experimental system of root-soil interaction for laboratory work. *Environ. Contor. Biol.* 25: 141-151.
- Lilley, J.M. and Fukai, S. 1994. Effect of timing and severity of water-deficit on four diverse rice cultivars. 1. Rooting pattern and soil-water extraction. *Field Crops Res.* 37: 205-213.
- Maclean, J.L., Dawe, D.C., Hardy, B. and Hettel, G.P. 2002. Rice Almanac, 3rd edition. IRRI, WARDA, CIAT, FAO. Manila. 1-253.
- MacMillan, K., Emrich, K., Piepho, H.P., Mullins, C.E. and Price, A.H. 2006. Assessing the importance of genotype environment interaction for root traits in rice using a mapping population. I. A soil-filled box screen. *Theor. Appl. Genet.* 113: 977-986.
- Manschadi, A.M., Hammer, G.L., Christopher, J.T. and deVoil, P. 2008. Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). *Plant Soil.* 303: 115-129.
- Mia, M.W., Yamauchi, A. and Kono, Y. 1996. Plasticity in taproot elongation of several food legume species. *Jpn. J. Crop Sci.* 65: 368-378.
- Nguyen, H.T., Babu, R.C. and Blum, A. 1997. Breeding for drought resistance in rice: Physiological and molecular genetics considerations. *Crop Sci.* 37: 1426-1434.
- Okada, K., Kondo, M., Ando, H. and Kakuda, K. 2002. Water uptake under water stress at panicle initiation stage in upland rice as affected by previous soil water regimes. *Soil Sci. Plant Nutr.* 48: 151-158.
- O'Toole, J.C. 1982. Adaptation of rice to drought-prone environment. *In* Drought Resistance in Crops with Emphasis on Rice. IRRI, Los Baños, Philippines. 195-213.
- O'Toole, J.C. and Bland, W.L. 1987. Genotypic variation in crop plant root system. *Adv. Agron.* 41: 91-145.
- Pardales, J.R.Jr. and Yamauchi, A. 2003. Regulation of root development in sweetpotato and cassava by soil moisture during their establishment period. *Plant Soil.* 255: 201-208.
- Price, A.H., Steele, K.A., Moore, B.J. and Jones, R.G.W. 2002. Upland rice grown in soil filled chambers and exposed to contrasting water-deficit regimes. II. Mapping QTLs for root morphology and distribution. *Field Crop Res.* 76: 25-43.
- Qu, Y., Mu, P., Zhang, H., Chen, C.Y., Gao, Y., Tian, Y., Wen, F. and Li, Z. 2008. Mapping QTLs of root morphological traits at different growth stages in rice. *Genetica* 133: 187-200.
- Samson, B.K., Hasan, M. and Wade, L.J. 2002. Penetration of hardpans by rice lines in the rainfed lowlands. *Field Crops Res.* 76: 175-188.
- Serraj, R., Kumar, A., McNally, K.L., Slamet-Loedin, I., Bruskiewich, R., Mauleon, R., Cairns, J. and Hijmans, R.J. 2009. Improvement

- of drought resistance in rice. *Adv. Agron.* 103: 41-99.
- Siopongco, J.D.L.C., Yamauchi, A., Salekdeh, H., Bennett, J. and Wade, L.J. 2005. Root growth and water extraction responses of doubled-haploid rice lines to drought and rewatering during the vegetative stage. *Plant Prod. Sci.* 8: 497-508.
- Subere, J.O.Q., Bolatete, D., Bergantin, R., Pardales, A., Belmonte, J.J., Mariscal, A., Sebidos, R. and Yamauchi, A. 2009. Genotypic variation in responses of cassava (*Manihot esculenta* Crantz) to drought and rewatering. I. Root system development. *Plant Prod. Sci.* 12: 462-474.
- Suralta, R.R. and Yamauchi, A. 2008. Root growth, aerenchyma development, and oxygen transport in rice genotypes subjected to drought and waterlogging. *Environ. Exp. Bot.* 64: 75-82.
- Suralta, R.R., Inukai, Y. and Yamauchi, A. 2008a. Genotypic variations in responses of lateral root development to transient moisture stresses in rice cultivars. *Plant Prod. Sci.* 11: 324-335.
- Suralta, R.R., Inukai, Y. and Yamauchi, A. 2008b. Utilizing chromosome segment substitution lines (CSSLs) for evaluation of root responses under transient moisture stresses in rice. *Plant Prod. Sci.* 11: 457-465.
- Suralta, R.R., Inukai, Y. and Yamauchi, A. 2010. Dry matter production in relation to root plastic development, oxygen transport and water uptake of rice under transient soil moisture stresses. *Plant Soil.* 332: 87-104.
- Uga, Y., Okuno, K. and Yano, M. 2011. *Dro1*, a major QTL involved in deep rooting of rice under upland field conditions. *J. Exp. Bot.* 62: 2485-2494.
- Venuprasad, R., Dalid, C., Del Valle, M., Bool, E., Zhao, D., Espiritu, M., Sta Cruz, M. T., Amante, M., Atlin, G. and Kumar, A. 2009. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress and aerobic conditions in rice using bulk-segregant analysis (BSA). *Theor. Appl. Genet.* 120: 177-190.
- Wade, L.J., Fukai, S., Samson, B.K., Ali, A. and Mazid, M.A. 1999. Rainfed lowland rice: Physical environment and cultivar requirements. *Field Crops Res.* 64: 3-12.
- Wang, H., Inukai, Y., Kamoshita, A., Wade, L.J., Siopongco, J., Nguyen, H. and Yamauchi, A. 2005a. QTL analysis on lateral root development of rice under different water conditions. *Jpn. J. Crop Sci.* (Extra 1) 74: 300-301.
- Wang, H., Inukai, Y., Kamoshita, A., Wade, L.J., Siopongco, J., Nguyen, H. and Yamauchi, A. 2005b. QTL analysis on plasticity in lateral root development in response to water stress in the rice plant. In Toriyama, K., Heong, K.L. and Hardy, B. eds., *Rice is life: scientific perspectives for the 21st century.* 464-469.
- Wang, H. and Yamauchi, A. 2006. Growth and Function of Roots under Abiotic Stress in Soil. In Huang, B. eds., *Plant-Environment Interactions.* 3rd eds. CRC Press, New York. 271-320.
- Xu, Y., This, D., Pausch, R.C., Vonhof, W.M., Coburn, J.R., Comstock, J.P. and McCouch, S.R. 2009. Leaf-level water use efficiency determined by carbon isotope discrimination in rice seedlings: genetic variation associated with population structure and QTL mapping. *Theor. Appl. Genet.* 118: 1065-1081.
- Yamauchi, A., Kono, Y. and Tatsumi, J. 1987. Comparison of root system structures of 13 species of cereals. *Jpn. J. Crop Sci.* 56: 618-631.
- Yamauchi, A., Pardales, J.R. Jr. and Kono, Y. 1996. Root system structure and its relation to stress tolerance. In Ito, O., Katayama, K., Johansen, C., Kumar, R.J.V.D.K., Adu-Gtamifi, J.J. and Rego, T.J. eds., *Dynamics of roots and nitrogen in cropping systems of semi-arid tropics.* Japan International Research Center for Agricultural Sciences, Tsukuba, Japan. 211-234.
- Yamauchi, A. 1998. Root traits for measurement. In *Root Encyclopedia* Editorial Committee. eds., *Root. Encyclopedia.* Asakurashoten, Tokyo, Japan. 74-375*.
- Yoshida, S. and Hasegawa, S. 1982. The rice root system: its development and function. In *Drought Resistance in Crops with Emphasis on Rice.* International Rice Research Institute, Los Baños, Philippines. 97-114.

* In Japanese.