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## Genotypic variations in the plasticity of nodal root penetration through the hardpan during soil moisture fluctuations among four rice varieties

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### ABSTRACT

Rainfed lowland rice fields are characterized by soil moisture fluctuations (SMF) and the presence of hardpan that impedes deep rooting and thus limits water extraction from deep soil layer during the periods of drought. In this study, we used rootboxes with three layers; shallow layer, artificial hardpan, and deep and wet layer below the hardpan, to evaluate differences in the plasticity of nodal roots elongation through the hardpan and promote root branching below the hardpan in response to SMF among four rice varieties; Sasanishiki, Habataki, Nipponbare, and Kasalath. Experiments were conducted during the summer and autumn seasons. Plasticity was computed as the difference in root traits within each variety between the SMF and continuously well-watered treatments. In both experiments, Habataki consistently tended to exhibit higher root plasticity than the other three varieties by increasing number of nodal roots that penetrated the hardpan during rewatering period in SMF, when the soil moisture increased and penetration resistance decreased. This root plasticity then contributed to greater water use at the deeper soil during the subsequent drought period and overall shoot dry matter production. Habataki had significantly higher  $\delta^{13}\text{C}$  value in roots at deep layer than roots at the shallow and hardpan layers under SMF, which may indicate that these were relatively newly grown roots as a consequence of root plasticity. This study also indicates that CSSLs derived from Sasanishiki and Habataki varieties may be suitable for the analysis of QTLs associated with root plasticity expression in rainfed lowland with hardpan and experiencing SMF.

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### Introduction

Approximately 19% of the world's rice production comes from 52 million ha of rainfed lowlands (IRRI, 2013). Rainfed rice environments experience multiple abiotic stresses and high uncertainty in the timing, duration, and intensity of rainfall. In rainfed lowland ecosystems, soil moisture fluctuation (SMF) or the alternating recurrence of drought and rewetting depending on the rainfall patterns, are two of the main limiting factors that reduce production (Suralta et al., 2016). Typical rainfed lowland fields are characterized by a 5-cm high bulk density hardpan layer underneath the cultivated layer at approximately 20 cm depth (Samson et al., 2002; Yano et al., 2006). The presence of hardpan is important for reducing the loss of irrigated water in lowland fields but it can also hinder both the penetration of root to deeper soil layer as well as the movement of water between soil layers (Cairns et al., 2011). In rainfed lowlands, the soil generally tends to start drying from the surface

while there is availability of water in the deep soil horizon, which could be accessed by the roots (Kameoka et al., 2015, 2016; Yoshida & Hasegawa, 1982). Therefore, the ability of roots to penetrate the hardpan is one of the key traits for deep root system development (Fukai & Cooper, 1995), where water supply at the shallow layer is limited during periods of drought (Henry et al., 2011; Siopongco et al., 2009).

The penetration resistance of soil including that of hardpan is inversely related with the prevailing soil moisture content (SMC) (Cairns et al., 2011), because soil strength increased with decrease in soil moisture (Tuzet et al., 2003). Several studies have demonstrated that increased soil water extraction from the deeper soil layers is associated with the increase in root length density at this layer (Henry et al., 2011; Kameoka et al., 2015, 2016) and contributed to the maintenance in dry matter production (Gowda et al., 2011; Henry et al., 2012; Uga et al., 2011). Therefore,

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the plasticity of roots to penetrate the hardpan is essential for rice plant to adapt to rainfed lowlands with cycles of wet and dry conditions (Bañoc et al., 2000a, 2000b; Cairns et al., 2011; Samson et al., 2002).

Roots play significant roles in plant adaption to various stress soil conditions such as unsaturated (Kato & Katsura, 2014) and water-deficit soils (Kato et al., 2006) by responding developmentally to such conditions. Under SMF conditions in rainfed lowlands, it is generally believed that root penetration through the hardpan happened in response to drought conditions (Samson et al., 2002). With the progress of understanding the functional significance of root plasticity, we examined the timing of root plasticity expression in relation to the soil moisture and penetration resistance interaction by conducting a series of experiments using our newly developed rootbox-hardpan experimental system with three layers: water-fluctuating topsoil, heavily compacted artificial hardpan, and a wet soil below the hardpan (Suralta et al., *in press*). Using this system, we clearly showed that hardpan penetration resistance interacted with the changes in soil moisture during SMF (Suralta et al., *in press*) as opposed with the previously reported experimental systems that have hardpan with fixed penetration resistance regardless of prevailing soil moisture conditions (Clark et al., 2002; Kamoshita et al., 2002; Price et al., 2000; Samson et al., 2002). We also proved that deep root system development was a result of the plasticity in nodal root elongation through the hardpan, which was triggered by rewatering that makes the hardpan relatively softer during SMF. Such root plasticity enhanced water uptake in the deep soil layer during the drought periods and contributed to the increase in overall dry matter production (Suralta et al., *in press*). In that same study, we used a recurrent parent IR64 and its introgression line, YTH304 that are highly genetically similar with each other showing similar shoot and root growth performance under non-stress conditions. These two genotypes did differ only under stressed conditions such as rainfed lowland field conditions (Kano-Nakata et al., 2013) and experimentally controlled SMF stress with YTH 304 showing greater performance. Thus, the utilization of those genetic materials was effective to explicitly show the functional contribution of the plasticity in nodal root elongation through the hardpan to dry matter production through enhanced water uptake in the deep layer under SMF. Further, we pointed out the need to further confirm such root plasticity and its functions using other genetic mapping populations with parents that have wider genotypic variations and thus can be useful for future breeding program in rainfed lowland rice (Suralta et al., *in press*).

The plasticity in root branching in the deeper soil layer where the soil moisture is more available during

drought could help plants maintain dry matter production (Kameoka et al., 2015) through more efficient root exploration in deeper soil and greater water acquisition, which may be closely linked with promoted carbon assimilate allocation to the deep roots during soil drying (Gowda et al., 2011; Zhu et al., 2010). Drought stress had stronger effects on  $\delta^{13}\text{C}$  levels in sink parts such as roots and panicles than the other plant parts (Grant et al., 2012; Monti et al., 2006; Peuke et al., 2006), indicating that roots are the prominent sink for photo-assimilated carbon (Kano-Nakata et al., 2014). Kano-Nakata et al. (2014) also reported that the plants that are well adapted to mild drought stress showed greater variance in carbon isotope ratio ( $\delta^{13}\text{C}$ ) value among different plant parts. It is well established that such variation in  $\delta^{13}\text{C}$  among plant parts is related to the isotope fractionation during transport, the synthesis of metabolites, and the chemical composition such as the amounts of lipids, lignin and cellulose contents (Brugnoli & Farquhar, 2000) while the mechanism for the isotope fractionations is yet the subject for further study. Several researchers also used  $\delta^{13}\text{C}$  for evaluating belowground carbon allocation (Meier & Leuschner, 2008; Scartazza et al., 2015; Yao et al., 2016). Meier and Leuschner (2008) reported that higher  $\delta^{13}\text{C}$  values were found for roots grown under drought relative to those grown under well-watered conditions in European beech (*Fagus sylvatica* L.). This was attributed to higher carbohydrate demand of the droughted root system, which led to an increased rate of fine root mass turnover (Meier & Leuschner, 2008). These facts also indicate that the newly produced roots tend to have higher  $\delta^{13}\text{C}$  values than older roots under drought.

For plants to well adapt to soil with hardpans and experiencing SMF, it is advantageous for them to develop roots in deeper soil layer well below the hardpan. In our present study therefore, we hypothesized that there should be genotypic variations in  $\delta^{13}\text{C}$  values among roots developed in different soil layers under SMF conditions, and the genotypes that are better adapted to SMF would show higher  $\delta^{13}\text{C}$  value in roots in deep layer because these roots are expected to consist of more nodal roots that newly elongated and penetrated through the hardpan during rewatering, which then branched into lateral roots during the subsequent progressive drought period as compared with those roots from other soil layers. We also assume that  $\delta^{13}\text{C}$  value can be used as an indicator for root plasticity that is expressed under SMF conditions.

In this study, we aimed to compare four rice varieties in terms of the plasticity in nodal root elongation through the hardpan during rewatering periods in SMF, subsequent deep root system development, and their functional contributions to dry matter production.

## Materials and methods

### Plant materials

We used four varieties; Sasanishiki, Habataki, Nipponbare, and Kasalath. The seeds were provided by the Rice Genome Research Center of the National Institute of Agrobiological Sciences, Japan (<http://www.rgrc.dna.affrc.go.jp/>). Nipponbare and Kasalath are the parents of a set of Chromosome Segment Substitution Lines (CSSLs). Using this set of CSSLs derived from a cross between Nipponbare and Kasalath, several studies have precisely quantified the root plasticity with minimal effects of genetic confounding (Kano-Nakata et al., 2011, 2013; Kano et al., 2011; Kano-Nakata et al., 2017; Tran et al., 2014, 2015; Suralta & Yamauchi 2008; Suralta et al., 2008, 2010), as well as the detection of QTLs associated with root plasticity (Niones et al., 2013, 2015). On the other hand, Sasanishiki and Habataki are the parents of another set of CSSLs (<http://www.rgrc.dna.affrc.go.jp/>). Katsura and Nakaide (2011) showed that Habataki had an increase in grain yield in aerobic culture relative to flooded culture while Sasanishiki had no differences between the two water treatments, indicating that Habataki may be more adaptable to water saving aerobic conditions.

### Experimental design and treatments

Two experiments were conducted in a glasshouse of Nagoya University, Japan (136°56'6"E, 35°9'5"N) during the summer (28th May to 30th July, Experiment 1) and autumn (27th August to 3rd November, Experiment 2) seasons in 2015. The monthly mean maximum and minimum temperature inside the glasshouse during the duration of the experiment was 31.8 and 23.3 °C in Experiment 1, and 24.7 and 16.4 °C in Experiment 2, respectively. These data were collected using a thermometer (T AND D Thermo Recorder TR-72Ui, T&D Corporation, Japan).

The seeds of each variety were soaked in water mixed with benomyl fungicide (Benlate; Sumitomo Chemical Garden Products Inc. Tokyo, .05% w/v) and washed thoroughly prior to incubation in a seed germinator at 30 °C for 72 h prior to sowing. Pre-germinated seeds of each variety were sown in soil-filled PVC rootbox (34.0 cm × 9.5 cm × 64.0 cm, L × W × H) previously described by Suralta et al. (in press). The experiments were arranged in a split-plot design in Randomized Complete Block Design with three replications. The soil moisture treatments were assigned as mainplot while the varieties as subplot. The seedlings were thinned to two plants per rootbox at five days after sowing (DAS).

### Soil hardpan preparation and soil moisture measurement

Air-dried sandy loam soil was sieved through a 3-mm mesh and mixed thoroughly with compound fertilizer (14-14-14) at the rate of 60 mg per kg soil. Three soil layers were set; shallow at 0–16 cm depth (above the hardpan layer) with 1.25 g cm<sup>-3</sup> bulk density, heavily compacted hardpan at 16–21 cm depth with 1.70 g cm<sup>-3</sup> bulk density, and deep layer at 21–63 cm depth (below the hardpan) with 1.25 g cm<sup>-3</sup> bulk density. The deep layer below the hardpan was first filled up with 15.55 kg soil to achieve a bulk density of 1.25 g cm<sup>-3</sup>. Afterward, the hardpan layer (5 cm thick) was filled up with 2.75 kg of dried soil containing 45% sandy loam, 45% sandy soil, premixed with 10% kaolinite. We firstly mixed well these soil components and then gradually put into a rootbox with adding a little water, and continued compacting up to 5 cm thick to achieve a bulk density of 1.70 g cm<sup>-3</sup>. Finally, the shallow soil layer was filled up with 6.46 kg soil to achieve the same bulk density (1.25 g cm<sup>-3</sup>) as that of the deep soil layer.

Soil moisture contents (% v/v, SMC) of the shallow and hardpan soil layers were measured with time domain reflectometry probe (TDR; Tektronix Inc., Wilsonville, OR, U.S.A). Two stainless steel rods (15 cm in length) were inserted into the soil at a depth of 0–12 cm leaving 3-cm length protruding above the soil surface, where TDR probes were attached to obtain SMC readings (Fujita et al., 2011). Soil penetration resistance (SPR) (MPa) was calculated based on the SMC using an equation generated by Suralta et al. (in press) as below.

1.25 g cm<sup>-3</sup> soil bulk density,  $SPR = -0.156 \ln(SMC) + 0.587$

1.70 g cm<sup>-3</sup> soil bulk density,  $SPR = -0.549 \ln(SMC) + 2.121$

### Water treatments and water use measurement

Plants were grown under two soil moisture conditions, Well-watered (WW, control) and SMF. In Experiment 1, WW was imposed by maintaining the SMC above 37% v/v through daily watering of the soil surface. On the other hand, in SMF conditions, the soil was first maintained under WW condition from 0 to 25 DAS. Thereafter, it was subjected to the first progressive drought from 26 to 45 DAS by withholding water until the target SMC reached down to 10% v/v at both shallow and hardpan soil layers. Then, plants were rewatered up to the WW conditions from 45 to 52 DAS and maintained to that condition by daily rewatering of the soil surface. Finally, the second progressive drought was imposed from 53 to 60 DAS by withholding water until SMC at shallow soil layer reached down to 10% v/v.



During the second progressive drought, water was supplied only to the deep soil layer through a small plastic tube that was installed at 55 cm below the soil surface on a side of each box. This tube served as the connector between the box and the graduated cylinder. The top end of the graduated cylinder was sealed with a rubber stopper with plastic tube inserted on it. In this setup, the water table in the deep soil layer in the rootbox could be maintained to the level of the bottom end of plastic tube in the graduated cylinder, following the principle of Mariotte bottle technique (Yamauchi et al., 1995). The amount of water reduced from the graduated cylinder was equivalent to the amount of water absorbed by the roots in the deep soil layer, which was recorded as water use. Then, the amount of water reduced inside the graduated cylinder was replenished accordingly.

In Experiment 2, the SMC was maintained above 30.9% v/v under WW by daily watering of the soil surface, whereas under SMF conditions, the soil was first maintained at WW condition from 0 to 21 DAS, and then progressively drought stressed from 22 to 43 DAS by withholding water until the SMC at shallow and hardpan soil layers reached down to 10% v/v and then rewatered back to WW condition from 43 to 50 DAS. Finally, the second progressive drought was imposed from 51 to 66 DAS by withholding again the water until the target 10% SMC v/v was reached in the shallow soil layer.

### Computation of root plasticity

At 31 cm below the open end of the box (below the hardpan), a transparent plastic tube (7 cm in diameter and 40 cm in length) was horizontally installed for monitoring root growth using a Minirhizotron camera (Regents Instruments Inc., U.S.A). The images were scanned and recorded in a computer. The number of penetrated nodal roots visually observed from each image was counted. Following the methods used by Sandhu et al. (2016) and Suralta et al. (in press), the plasticity in nodal root penetration through the hardpan, which was expressed under SMF conditions was calculated using single replicates from the SMF treatment and mean values from the control (WW) treatment as follows:

$$\text{Root plasticity} = \left[ \frac{X_{\text{SMF}} - \bar{X}_{\text{WW}}}{\bar{X}_{\text{WW}}} \right]$$

where  $X$  is the number of nodal roots that penetrated the hardpan.

### Photosynthesis and stomatal conductance measurements

Photosynthetic rate ( $P_n$ ) and stomatal conductance ( $g_s$ ) were measured at 25, 45, 54, 60 DAS in Experiment 1 and at 32, 43, 49, 56, 63, 66 DAS in Experiment 2 using a portable photosynthesis analyzer (Li-6400, Li-COR Inc., U.S.A) on the abaxial side of the topmost fully developed leaf on the main stem between 10 and 12 h under the following settings: leaf temperature, 30 °C; CO<sub>2</sub> concentration, 380 μL L<sup>-1</sup>; relative humidity, 65–75% and quantum flux density, 1200 μmol m<sup>-2</sup> s<sup>-1</sup>. We measured 3 plants (1 plant = 1 replication) for each treatment.

### Shoot and root growth measurements

Shoots were cut at the base and oven-dried at 70 °C for 3 days to record the shoot dry weight at 60 DAS in Experiment 1 and 66 DAS in Experiment 2. Roots were sampled separately from the shallow, hardpan, and deep layers, and carefully washed inside the strainer with running water. The samples were stored in a freezer at -20 °C for length measurement and δ<sup>13</sup>C analysis as described later. For total root length measurement, each root sample was cut into approximately 1-cm segments and spread on a transparent plastic sheet with minimal overlapping. Digital *tiff* files were taken using an image scanner (EPSON Expression 10000XL) with a resolution of 400 dpi. Scanned images were analyzed for root length using WinRhizo v. 2009d (Regent Instrument Inc.) (Bouma et al., 2000). A pixel threshold value was set at 175 for the root length analysis (Suralta et al., in press). The total lateral root length was estimated as the total length of roots with less than .3 mm in diameter. The total lateral root length was computed as the difference between the total root length and total nodal root length (Yamauchi et al., 1996).

The deep root length ratio was calculated as the ratio of the root length in the deep soil layer to the total root length of the whole root system.

### Carbon isotope ratio (δ<sup>13</sup>C) measurement

Harvested plants were separated into shoots and roots. The roots were also separated by soil layer from where (i.e. shallow layer, hardpan layer, and deep layer) they were sampled. Dried shoot or root samples were milled into fine powder. The δ<sup>13</sup>C values of each sample were analyzed with an isotope mass spectrometer (Sercon Ltd., Cheshire, UK) and expressed in per mil using the following equation:

$$\delta^{13}\text{C} = \left( R_{\text{sample}}/R_{\text{standard}} - 1 \right) \times 1000$$

where  $R$  refers to  $^{13}\text{C}/^{12}\text{C}$  and standard refers to the international Pee Dee Belemnite standard (a fossil Belemnite from the Pee Dee formation in South Carolina) (Farquhar et al., 1989). Measurements were performed with three repetitions for each sample.

### Statistic analysis

Data were analyzed using the generalized linear model (GLM) procedure (SAS Institute, 2003). Analysis of variance was performed separately for each experiment to test the effects of varieties, water treatments and their interaction. The varietal difference in each water treatment was analyzed using the Tukey's test at  $p < .05$ . The effect of water treatment was assessed by analysis of variance in each variety. The relationships among measured traits were analyzed using Pearson's R correlation.

## Results

### **Environmental conditions, and soil moisture and penetration resistance**

The climate data inside glasshouse were collected from 21 DAS until the end of experiment. During these periods, the average temperature in Experiment 1 (27.8 °C) was higher than in Experiment 2 (21.6 °C). The relative humidity was lower in Experiment 1 (43.0%) than in Experiment 2 (68.5%) due to the lower temperature, more rainfall and cloudy days in the latter experiment. The duration of the second progressive drought in Experiment 1 was 7 days (53–60 DAS), which was shorter than the first progressive drought in Experiment 2 (18 days from 26 to 44 DAS) due to higher temperature and expected higher evapotranspiration in Experiment 1. On the other hand, the second progressive drought in Experiment 2 lasted for 15 days (51–66 DAS), which was 8 days longer than the second progressive drought in Experiment 1 partially due to the lower temperature and higher air humidity in Experiment 2. Accordingly, plants under SMF in Experiment 2 were less affected by periods of drought than those in Experiment 1.

The SMC and the estimated soil penetration resistance at shallow and hardpan layers in Experiment 1 are shown in Figure 1. Under SMF, SMC at the shallow layer ranged from 8.4 to 42.8% and those at the hardpan ranged from 9.6 to 28.1%. The SMC values in the hardpan were lower than those in the shallow layer especially when the soil was wetter (early stage of progressive drought and rewatering period) while there was no difference between layers when the soil was already dry toward the end of progressive drought period at 45 DAS. The SMC at the shallow soil layer under WW ranged from 37.1 to 44.4% which were higher than those at the hardpan layer (27.3 to 31.2%) (Figure 1(a)).

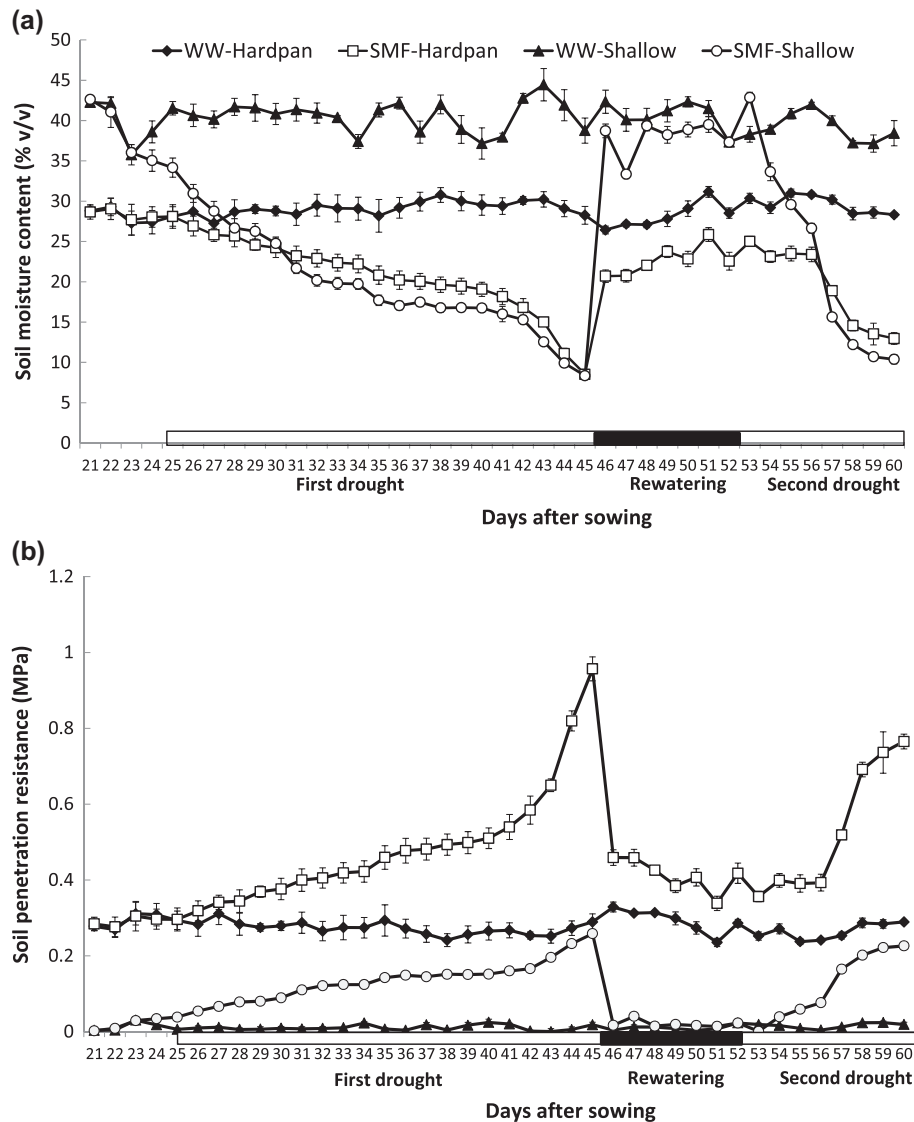
The estimated soil penetration resistance showed an inverse relationship with SMC (Figure 1(b)). Under SMF, soil penetration resistance of the shallow layer ranged from 0 to .26 MPa while those at the hardpan ranged from .30 to .95 MPa. This showed that the preparation of the heavily compacted hardpan was successful. Under WW, the estimated soil penetration resistance ranged from 0 to .02 MPa at the shallow layer which was lower than those at the hardpan which ranged from .24 to .33 MPa.

In Experiment 2, similar trends were observed although SMC tended to be higher and thus soil penetration resistance was lower than those in Experiment 1 (data not shown). The magnitude of the increase in soil penetration resistance with the decrease in SMC was greater in the hardpan than in the shallow soil layer owing to the greater bulk density of the former than the latter soil layer in both experiments.

### **Shoot and root growth**

Table 1 shows the shoot and root traits of the four varieties grown under WW and SMF treatments in Experiments 1 and 2. In both experiments, no significant genotypic differences were found in shoot dry weight (SDW) under WW conditions while under SMF, Habataki and Kasalath showed significantly higher shoot dry weight than the other two varieties in Experiment 1, and no significant genotypic differences were found in Experiment 2. For root traits, Kasalath developed significantly longer root system than Sasanishiki only under WW and Sasanishiki and Habataki under SMF in Experiment 1. In Experiment 2, only Nipponbare developed a significantly shorter root system than Habataki only under WW while no significant genotypic differences was observed under SMF. For the root length at different soil layers, these four varieties showed no significant differences under WW conditions in both experiments except for Kasalath which produced longer total root length and total nodal root (NR) length than Sasanishiki and Nipponbare but not Habataki at hardpan layer. Under SMF conditions, one distinct difference was consistently recognized, i.e. Kasalath showed the higher values than Sasanishiki in these roots traits except length of lateral roots (LR) at shallow layer in Experiment 1 but such consistent difference was not observed in Experiment 2. In the other varieties, no consistent trends were observed under SMF.

We then examined the interactions between water treatment and variety, and found out that the significant interactions were not detected for most of the traits examined in both Experiment 1 and 2 (Supplement 1) because of the limited number of replicates, but the varieties tended to show different responses to the water regimes (Table 1).



**Figure 1.** Soil moisture content (a) and estimated soil penetration resistance (b) at shallow and hardpan soil layers under well-watered (WW) and soil moisture fluctuation (SMF) conditions in Experiment 1.

Notes: Horizontal bars along x-axis of the graphs indicate the prevailing soil moisture conditions during SMF. Error bars represent the standard errors calculated from 3 replicates.

In this aspect, Habataki showed significantly greater shoot dry weight under SMF than WW in both experiments. Likewise, it produced longer roots at the deep soil layer under SMF relative to WW in Experiment 1, while root development of the four varieties tended to be decreased under SMF conditions in Experiment 2, but the reductions at the deep layer tended to be smaller in Habataki as compared with the rest of the varieties. Furthermore, the deep root length ratio of Habataki was increased by SMF relative to WW in both experiments.

#### **Plasticity in nodal root elongation through the hardpan during SMF**

In Experiment 1, Habataki expressed its plasticity in nodal root penetration through hardpan specifically during

rewatering period (Figure 2(a)) when the soil penetration resistance in the hardpan layer was low (Figure 1(b)) while the rest of the varieties showed no clear root plasticity.

In Experiment 2, Habataki also expressed its plasticity in nodal root penetration through the hardpan during rewatering period in SMF (Figure 2(b)). Kasalath also showed plasticity but in response to the first drought period but not to rewatering. Both Sasanishiki and Nipponbare did not show any root plasticity under SMF.

#### **Stomatal conductance ( $g_s$ )**

Genotypic variations in stomatal conductance among the four varieties under each water condition are shown in Figure 3. In both experiments, Habataki tended to show higher  $g_s$  than the other three varieties under WW

**Table 1.** Shoot dry weight (SDW), root length of whole root system, nodal roots (NR) and lateral roots (NR), and the total root length in each soil layer (shallow layer, hardpan, deep layer), and deep root length ratio (DRLR) of four varieties under well-watered (WW) and soil moisture fluctuation (SMF) conditions in Experiments 1 and 2.

Traits	Variety	Experiment 1			Experiment 2				
		WW	SMF		WW	SMF			
SDW (g plant <sup>-1</sup> )	Sasanishiki	17.3a	12.1a	ns	14.8a	11.7a	ns		
	Habataki	17.3a	23.5b	*	14.3a	16.0a	*		
	Nipponbare	16.8a	14.5a	ns	13.6a	13.6a	ns		
	Kasalath	25.5a	24.3b	ns	15.9a	17.2a	ns		
Root length (m plant <sup>-1</sup> )	<i>Whole root system</i>	Sasanishiki	182.5a	159.7a	ns	165.7ab	105.4a	*	
		Habataki	248.2ab	214.7a	ns	182.6b	121.4a	**	
		Nipponbare	228.9ab	231.1ab	ns	120.2a	104.3a	ns	
		Kasalath	290.2b	307.0b	ns	165.2ab	116.7a	ns	
	<i>Shallow layer</i>	Total	Sasanishiki	111.1a	96.6a	ns	74.7a	49.2a	*
			Habataki	156.3a	103.1ab	ns	91.3a	42.4a	*
			Nipponbare	162.4a	142.7b	ns	55.7a	55.5a	ns
			Kasalath	149.9a	142.1b	ns	70.6a	45.8a	ns
	NR	Sasanishiki	47.9a	39.2a	ns	31.5a	20.9a	*	
		Habataki	53.5a	48.1ab	ns	36.7a	28.2a	ns	
		Nipponbare	60.7a	56.1ab	ns	22.9a	21.7a	ns	
		Kasalath	61.3a	58.5b	ns	33.8a	22.5a	ns	
	LR	Sasanishiki	63.3a	57.4a	ns	43.2a	28.4ab	*	
		Habataki	78.9a	58.4a	ns	54.7a	14.2a	**	
		Nipponbare	101.7a	86.6b	ns	32.7a	33.8b	ns	
		Kasalath	88.7a	83.7ab	ns	36.8a	23.3ab	*	
	<i>Hardpan</i>	Total	Sasanishiki	37.1a	30.1a	ns	35.6a	20.2a	*
			Habataki	46.9ab	39.4ab	ns	42.5a	33.9a	ns
			Nipponbare	36.6a	50.7bc	*	26.0a	21.7a	ns
			Kasalath	55.5b	61.8c	ns	36.1a	23.4a	ns
	NR	Sasanishiki	12.3a	10.5a	ns	12.2a	6.2a	**	
		Habataki	16.2ab	15.9ab	ns	14.4a	11.2a	ns	
		Nipponbare	11.9a	17.6b	*	8.4a	7.2a	ns	
		Kasalath	20.6b	24.0c	ns	14.6a	9.5a	ns	
	LR	Sasanishiki	24.8a	19.6a	ns	23.4a	14.1a	*	
		Habataki	30.8a	25.3ab	ns	28.2a	22.7a	ns	
		Nipponbare	24.7a	33.0ab	ns	17.6a	14.5a	ns	
		Kasalath	34.9a	37.7b	ns	21.5a	13.9a	ns	
<i>Deep layer</i>	Total	Sasanishiki	34.2a	33.1a	ns	55.4ab	35.9ab	*	
		Habataki	44.9a	72.2ab	**	48.7ab	45.1ab	ns	
		Nipponbare	29.9a	37.7a	ns	38.5a	27.1a	ns	
		Kasalath	84.7a	103.1b	ns	58.5b	47.5b	ns	
	NR	Sasanishiki	12.0a	11.1a	ns	19.6ab	11.6ab	*	
		Habataki	16.6a	25.5ab	*	16.0ab	14.3ab	ns	
		Nipponbare	11.5a	13.1a	ns	12.1a	8.3a	ns	
		Kasalath	33.3a	40.6b	ns	22.1b	16.6b	ns	
	LR	Sasanishiki	22.2a	21.9a	ns	35.9a	24.4a	*	
		Habataki	28.3a	46.7ab	**	32.7a	30.8a	ns	
		Nipponbare	18.3a	24.6a	ns	26.4a	18.8a	ns	
		Kasalath	51.4a	62.5b	ns	36.4a	30.9a	ns	
	DRLR	Sasanishiki	.18a	.20ab	ns	.34a	.34a	ns	
		Habataki	.19a	.34c	*	.27a	.37a	***	
		Nipponbare	.12a	.16a	ns	.32a	.26a	ns	
		Kasalath	.28a	.33bc	ns	.36a	.43a	ns	

Notes: Values followed by the same letters are not significantly different among varieties within each soil moisture condition at  $p < .05$ . ns, not significant; \*, \*\*, \*\*\*, significant difference at  $p < .05$ ,  $p < .01$ ,  $p < .001$ , respectively, between two soil moisture conditions within each variety.

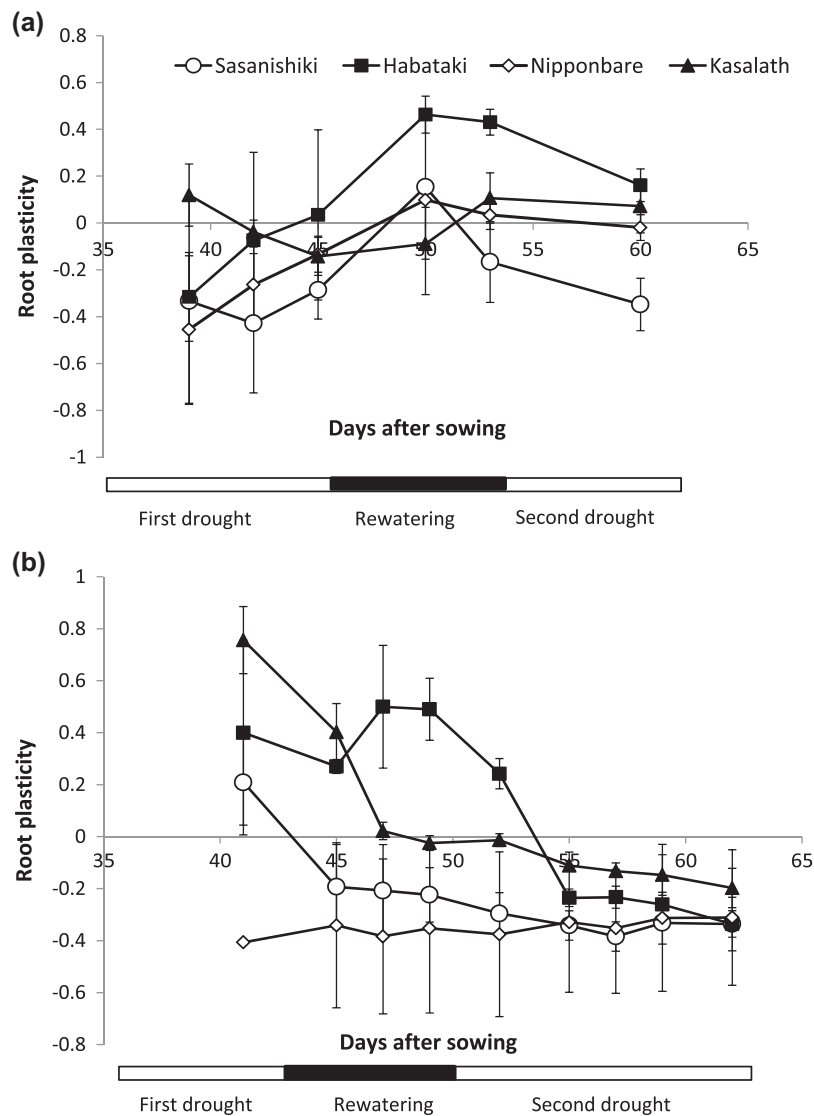
conditions. Stomatal conductance under SMF mostly increased during rewatering period in all four varieties with Habataki showing the sharpest increase by 649 and 335% in Experiment 1 and 2, respectively.

Photosynthetic rate showed no consistent trend among the four varieties under WW while similar response to rewatering was observed in Habataki with that in  $g_s$  in both experiments (Supplement 2).

### **Relationship between shoot dry weight, nodal roots that penetrated the hardpan layer, deep root development below the hardpan layer, and water use during the second progressive drought**

Table 2 shows the relationships among traits in Habataki variety whose root system tended to be more plastic than those of the other varieties. In Experiment 1,





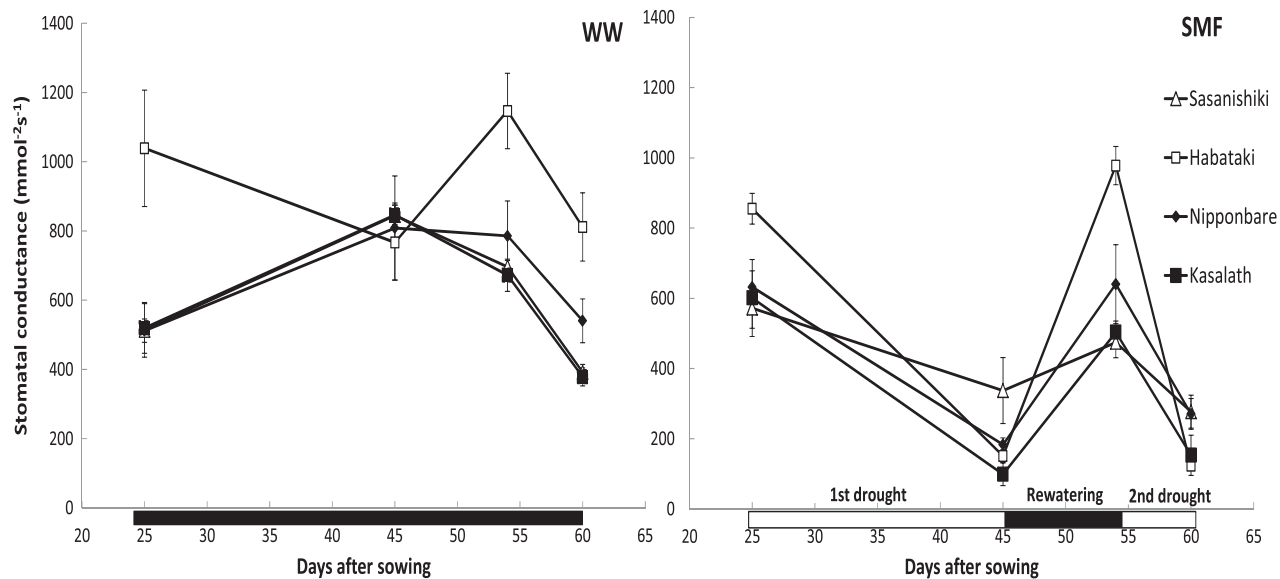
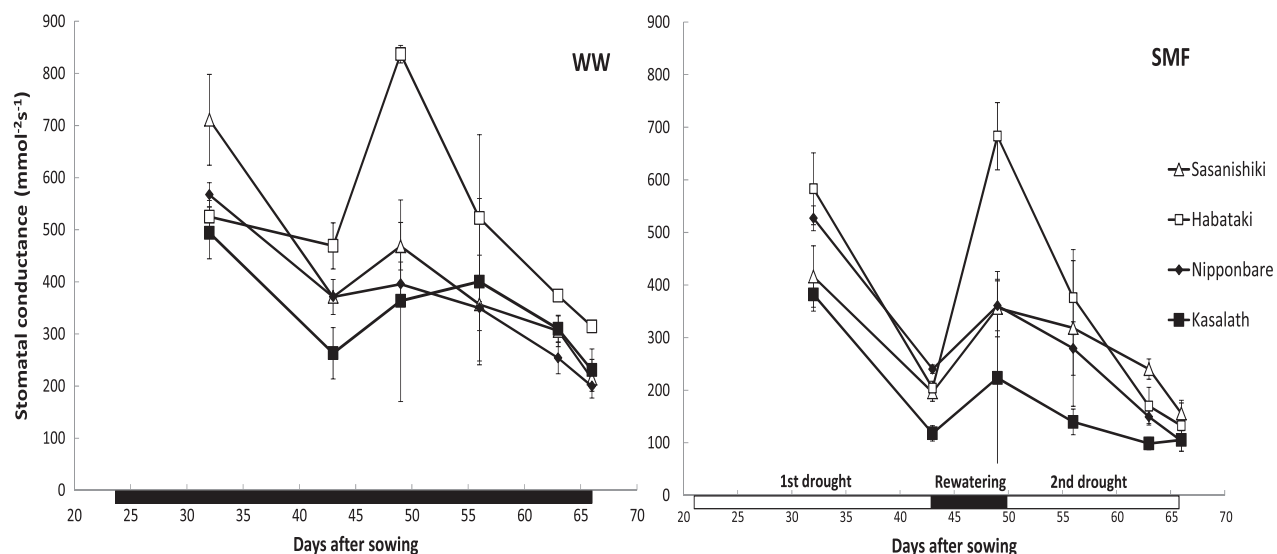
**Figure 2.** The plasticity in number of nodal roots that penetrated hardpan layer of Sasanishiki, Habataki, Nipponbare and Kasalath in Experiments 1 (a) and 2 (b).

Notes: Horizontal bars along x-axis of the graphs indicate the prevailing soil moisture conditions during SMF. Error bars represent the standard deviation calculated from 3 replicates. Root plasticity was calculated using single replicates from the SMF treatment (stress) and mean values from WW treatment (control) as follows. Root plasticity =  $\left[ \frac{X_{\text{stress}} - X_{\text{control}}}{X_{\text{control}}} \right]$  (Sandhu et al., 2016; Suralta et al., in press). Where X is the number of nodal roots that penetrated the hardpan.

positive and significant correlations were observed between shoot dry weight with water use during the second progressive drought, total root length at deep layer, deep root length ratio, number of nodal roots that penetrated hardpan layer during rewatering period, and number of nodal roots that penetrated hardpan layer at the end of experiment (second progressive drought). In addition, positive and significant correlations were observed between water use with total root length at deep layer, deep root length ratio and number of nodal roots that penetrated hardpan layer during rewatering period, total root length at deep layer with deep root length ratio (DRLR) and number of nodal roots that penetrated hardpan layer during rewatering period (NRHR), and deep root length ratio with number of nodal roots

that penetrated hardpan layer during rewatering period and number of nodal roots that penetrated hardpan layer at the end of experiment (second progressive drought).

In Experiment 2, positive and significant correlations were observed between shoot dry weight and water use, and deep-rooting-related traits such as deep root length ratio and number of nodal roots that penetrated hardpan layer during rewatering period. On the other hand, shoot dry weight did not show any significant correlation with total root length at deep layer because although Habataki was able to increase shoot dry weight in response to SMF, its total root length at the deep layer did not change under SMF relative to WW. In addition, water use had positive and significant correlation with deep root length ratio.

**Experiment 1****Experiment 2**

**Figure 3.** Stomatal conductance of Sasanishiki, Habataki, Nipponbare and Kasalath under well-watered (WW) and soil moisture fluctuation (SMF) conditions in Experiments 1 and 2.

Notes: Horizontal bars along x-axis of the graphs in SMF treatment indicate the prevailing soil moisture conditions. Error bars represent the standard deviation calculated from 3 replicates.

### $\delta^{13}\text{C}$ value of roots at different soil layers

Among the four varieties examined, we chose Sasanishiki and Habataki that showed wide genotypic differences in the plasticity in nodal root elongation through the hardpan during rewatering period as well as deep root development in Experiment 1, and measured the  $\delta^{13}\text{C}$  value of the shoots and roots grown under SMF conditions (Figure 4).  $\delta^{13}\text{C}$  in roots in the deep soil layer was significantly higher than those in the shallow and hardpan layers in Habataki only. Furthermore, the  $\delta^{13}\text{C}$  in shoots was not significantly different from those in the roots at shallow

and hardpan layers in both varieties and had significantly lower  $\delta^{13}\text{C}$  value than the roots at deep layer in Habataki.

## Discussion

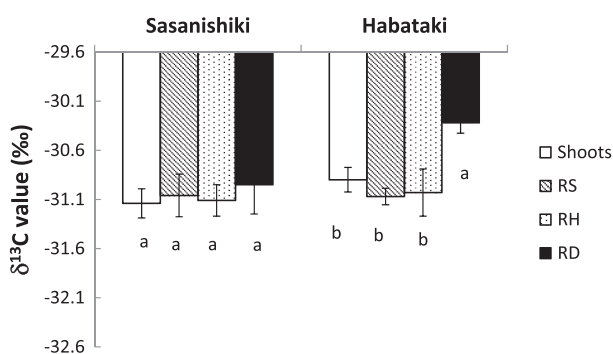
### *Root plasticity expression and soil penetration resistance dynamics*

Soil moisture and bulk density were reported to greatly influence soil penetration resistance (Cairns et al., 2011). Cairns et al. (2004) showed that there was a large increase in soil penetration resistance as drought progressed. In

**Table 2.** The relationships between shoot dry weight and root traits in Habataki variety under well-watered (WW) and soil moisture fluctuation (SMF) conditions in Experiments 1 and 2.

Trait	SDW	WU	TRLD	DRLR	NRHR	NRHD
<i>Experiment 1</i>						
SDW	1.00					
WU	.87*	1.00				
TRLD	.84*	.97**	1.00			
DRLR	.96**	.82*	.94**	1.00		
NRHR	.96**	.97**	.85*	.92**	1.00	
NRHD	.87*	ns	ns	.86*	ns	1.00
<i>Experiment 2</i>						
SDW	1.00					
WU	.82*	1.00				
TRLD	ns	ns	1.00			
DRLR	.81*	.97**	ns	1.00		
NRHR	.88*	ns	ns	ns	1.00	
NRHD	ns	ns	ns	ns	ns	1.00

Notes: ns, not significant; \*, \*\*, significant at  $p < .05$ ,  $p < .01$ , respectively. SDW, shoot dry weight; WU, water use during the second progressive drought period; TRLD, total root length at deep layer; DRLR, deep root length ratio (DRLR was calculated as the ratio of the root length in the deep layer to total root length of whole root system); NRHR, number of nodal roots that penetrated the hardpan layer during rewetting period; NRHD, number of nodal roots that penetrated hardpan layer during the second progressive drought period.



**Figure 4.**  $\delta^{13}\text{C}$  value of roots at different soil layers and shoots of Sasanishiki and Habataki under soil moisture fluctuation condition in Experiment 1.

Notes: Means followed by the same letters are not significantly different among plant parts within each variety at the 5% probability level. RS, root at shallow layer; RH, root at hardpan layer; RD, root at deep layer.

our experiments, under SMF conditions, when the SMC decreased, the soil penetration resistance increased in all soil layers (Figure 1), which were consistent with those reported by Cairns et al. (2011), Sanusan et al. (2010), Gao et al. (2016) and Suralta et al. (in press). In contrast, we found that soil penetration resistance at the hardpan layer was reduced during the rewetting period thus, nodal roots could have a chance to penetrate the hardpan layer in preparation for the subsequent drought period if the genotype confers root plasticity. Then, the promoted nodal root elongation due to plasticity triggered by SMC change from dry to wet under SMF could result in subsequent penetration through the hardpan only if the penetration resistance is reduced especially when the soil becomes wet.

Suralta et al. (in press) determined that matching of root plasticity expression and reduced soil penetration resistance due to rewetting of hardpan promoted water uptake and dry matter production using a recurrent parent IR64 and its introgression line, YTH304. Furthermore, they pointed out the need to examine genotypic variations in the functional significance of root plasticity especially such ability in growth response of the nodal roots due to plasticity could be the key trait for the adaptability in rain-fed lowland fields which are characterized by fluctuating hardpan penetration resistance. In this study, we compared four varieties, and found that Habataki tended to express sharper root plasticity in nodal root penetration through the hardpan than the other varieties, which consistently contributed to the overall growth under SMF in both experiments.

### Significance of root plasticity in deep root development, water uptake and dry matter production under SMF

SMF is one of the constraints in rice production, which could cause reduction in dry matter production (Niones et al., 2015; Siopongco et al., 2008). Katsura (2013) demonstrated that the average grain yield of the Sasanishiki/Habataki CSSLs under aerobic conditions was consistently higher than that of Sasanishiki in a two-year experiment. This implies that the Sasanishiki/Habataki CSSLs may have QTLs related to high grain yield that are inherited from Habataki. Our present study also showed that Habataki had significantly greater shoot dry weight under SMF than under WW in both experiments whereas for Sasanishiki, there was no significant difference in shoot dry weight between the two water treatments (Table 1). Thus, considering the fact that aerobic conditions inherently contains wetting and drying cycles of soil, Habataki may have a potential to produce higher shoot dry matter under SMF as compared with WW.

Root plasticity analysis can be done by comparing the roots of plants of the same genotype that are grown under different environments such as those between different water-stressed and non-stressed (control) conditions (Kameoka et al., 2015; Kano-Nakata et al., 2011; Menge et al., 2016). Under SMF conditions in this study, we found that Habataki showed consistently higher number of nodal roots that penetrated the hardpan during the rewetting period (Figure 2) as well as significant increase of total root length (Experiment 1) and maintenance of total root length (Experiment 2) at the deep layer (Table 1). This means that Habataki had the ability to penetrate the hardpan layer through root plasticity via elongation, thereby exhibiting deep root development below the hardpan layer.

In Experiment 1, under SMF, Habataki showed root plasticity at deep layer where water is available as a result of significantly higher total lateral root length as well as total nodal root length than those under WW (Table 1). Kameoka et al. (2015) suggested that the root plasticity expressed in soil portions where the soil moisture is more available increases potential adaptation of rice plants to water deficit conditions. In addition, enhanced lateral root development is known to increase root surface area and soil water extraction (Henry et al., 2011; Kameoka et al., 2015; Kato et al., 2007, 2011; Menge et al., 2016) and water use (Kobata et al., 1996; Suralta et al., 2010). Likewise, nodal root development is also important to enhance the contribution of lateral root branching to the entire root system development (Kameoka et al., 2016).

Deep root development in rice can contribute to shoot dry matter production through enhanced water uptake (Gowda et al., 2011; Uga et al., 2013). In our studies, the deep root length ratio of Habataki, which was significantly higher in both experiments, and the higher total root length at deep layer in Experiment 1 could enhance water use below the hardpan layer especially during the second progressive drought in SMF (Table 2). Such enhanced water uptake clearly resulted in sharp increases in *gs* during the rewatering periods in SMF (Figure 3) and consequently contributed to higher photosynthetic rates (Supplement 2) and shoot dry matter production in Habataki under SMF conditions (Table 1). Kato and Okami (2011) indicated that more assimilates in rice plants were allocated to the deep roots under aerobic culture than flooded conditions. Under water limited conditions, changes in assimilate partitioning tend to favor the development of deep root in rice (Gowda et al., 2011), suggesting that genetic improvement in deeper rooting would be one of the effective goals.

### **$\delta^{13}\text{C}$ value and deep root development**

We quantified  $\delta^{13}\text{C}$  value of Sasanishiki and Habataki varieties, which showed wider genotypic differences in the root plasticity in nodal root penetration through the hardpan (Figure 4). The significantly higher  $\delta^{13}\text{C}$  value of the roots observed in the deep soil layer than those at shallow and hardpan layers indicate that these may be relatively newly roots produced under drought conditions as discussed earlier. Specifically, we may speculate that these roots mostly would be the ones that expressed plasticity in response to rewatering, elongated through the hardpan and subsequently branched at the deep soil layer during the second drought period. This subsequently contributed to the increase in water uptake below the hardpan layer and thus increased dry matter production. The genotypes that show higher  $\delta^{13}\text{C}$  value in roots at deep layer may be more adapted to SMF than the ones with lower value because Habataki showed such responses in this study.

In this aspect, the future study is needed to examine whether the  $\delta^{13}\text{C}$  can be an indicator for root plasticity that is expressed under SMF stress conditions.

### **Conclusion**

Among four varieties (Sasanishiki, Habataki, Nipponbare, and Kasalath) examined in two experiments, Habataki tended to exhibit higher plasticity in nodal root elongation, which resulted in hardpan penetration, and consequently deep root development than the other varieties under SMF conditions. We showed that such plasticity in nodal root elongation through the hardpan occurred during the rewatering period in SMF, when the soil moisture increased and penetration resistance decreased. This root plasticity then contributed to greater shoot dry matter production through enhanced water uptake in deep soil layer below the hardpan where moisture was relatively higher than those in the shallow and hardpan layers. The roots that penetrated through the hardpan as a consequence of the plasticity in nodal root elongation were assumed to be comprised with relatively new roots as prominent sink for newly photo-assimilated carbon acquired during the subsequent progressive drought period because these roots showed higher  $\delta^{13}\text{C}$  values than the roots in and above the hardpan layers as well as those in shoots. We need further study if the  $\delta^{13}\text{C}$  value could be used as an indicator for root plasticity under SMF. Further studies using the CSSLs (Sasanishiki/Habataki) are ongoing to identify QTLs associated with root plasticity traits in hardpan penetration during SMF.

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### **Disclosure statement**

No potential conflict of interest was reported by the authors.

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