Dry Matter Production of Two Rice Cultivars with Contrasting Root Plasticity Expression Under Different Topographic Conditions Subjected to Soil Moisture Fluctuation

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In rainfed lowland rice fields characterized by sloping terrains and the presence of a hardpan in a flat topography, plants are often exposed to soil moisture fluctuation (SMF) stress due to erratic rainfall patterns. Root plasticity is one of the key traits that play important roles in plant adaptation under such conditions. In this study, two contrasting genotypes, KDML105 and IRAT109, were examined to quantify the expression of plasticity in root branching at different positions in the toposequence (TP) and in a flat topography with a hardpan, both without a groundwater table, and subjected to SMF. Results showed that KDML105 exhibited improved adaptation to SMF conditions due to its greater root system because of the promoted nodal root production and development of lateral roots in the upper soil layer (0 – 20 cm soil depth) along the TP and above the hardpan in a flat topography, which led to the maintenance of its stomatal conductance and dry matter production. IRAT109, on the other hand, did not express root plasticity in deep-rooting due to the absence of groundwater table, particularly in the upper TP, as well as branching ability above the hardpan in a flat topography, which resulted in less water uptake and reduced dry matter production under SMF. Overall, the results indicated that root plasticity in the upper soil layer could be an important trait for the adaptation of rice, both in a sloping TP without a hardpan or in a flat topography with a hardpan, experiencing SMF.

Keywords: hardpan, rainfed lowland, rice, root plasticity, toposequence

Abbreviations: DAT—days after transplanting, Dro1—deeper root 1, LRL—lateral root length, QTL—quantitative trait locus/loci, RLD—root length density, SDW—shoot dry weight, SMC—soil moisture content, SMF—soil moisture fluctuation, TP—toposequence, TRL—total root length

INTRODUCTION

Rainfed lowland rice is defined as having a level to slightly sloping bunded fields with non-continuous flooding of variable depth and duration (Zeigler and Puckridge 1995). The uneven soil moisture supply could be a major limiting factor for rainfed lowland rice production (Sujariya et al. 2019) and generally varies greatly among the location of the fields along the toposequence (TP). The upper TP usually experiences early drought stress (Fukai and Ouk 2012) even when the lower TP still has standing water (Boling et al. 2008). This is because paddies in a higher TP lose a large amount of water readily, particularly after heavy rainfall through surface run-off and seepage, while those in the lower TP may intercept the water that flows in from the paddies at the upper TP (Wade et al. 1999). Also, fields along the toposequence differed in their proximity to the groundwater table (Fukai and Ouk 2012) and thus,
experienced different magnitudes of drought stress (Jongdee et al. 2006; Sujariya et al. 2019). Under these conditions, rice adaptability may rely on the penetration ability of its roots and deep root system development to access the available moisture at the level of the lowermost TP (Price et al. 2002; Kameoka et al. 2015).

Additionally, a typical soil moisture fluctuation (SMF) stress (Suralta et al. 2008) in rainfed lowland rice fields brought about by erratic rainfall is also common and negatively affects rice yield (Niones et al. 2012; Owusu-Nketia et al. 2018). Root plasticity, which is expressed in response to changing environmental conditions (O'Toole and Bland 1987), is a key trait for crop adaptation and maintenance of productivity (Suralta et al. 2018; Schneider and Lynch 2020). In rice, several studies have shown the contribution of root plasticity, such as promoted lateral root development in response to varying intensities of drought stress (Kano et al. 2011; Kano-Nakata et al. 2011, 2013; Tran et al. 2014; Kameoka et al. 2015; Menge et al. 2016), continuous cycles of alternating waterlogged and drought stress (Niones et al. 2012; Owusu-Nketia et al. 2018; Niones et al. 2019; Nguyen et al. 2020), re-watering after drought (Baño et al. 2000b; Wade et al. 2000; Siopongco et al. 2005; Sandhu et al. 2016), and transient drought preceded by waterlogged conditions and vice versa (Suralta et al. 2008, 2010, 2015) to the maintenance of dry matter production and yield in growing conditions with limited soil depths. Deep rooting is also an adaptive drought avoidance response that enable plants to penetrate their root to a deeper soil layers to access available soil moisture, although it can be impeded during the decrease of moisture supply due to an increase in soil hardness (Cairns et al. 2011) or the presence of hardpan (Suralta et al. 2018).

Recently, quantitative trait locus (QTL) for deep rooting has been identified (Uga et al. 2011). The deeper root 1 (Dro1) gene is functionally expressed by altering the root angle to re-orient rooting depth without causing a negative effect on the overall root and shoot growth (Uga et al. 2013). This deep rooting ability brought about by the Dro1 gene however, is constitutive since it is expressed regardless of the prevailing soil moisture supply. Nevertheless, root system evaluation under different positions in TP along a sloping field is necessary to precisely quantify the root plasticity responses including deep rooting (Kameoka et al. 2015).

A study on the plasticity in deep-rooting using IRAT109, an upland rice-adapted japonica, and KDML105, a lowland-adapted indica, was done in a simulated sloping field with a groundwater table (Kameoka et al. 2015). IRAT109 maintained its shoot dry matter production across the TP due to its ability to increase its root length density at the deeper soil layer (40 – 60 cm) where water is available. On the other hand, KDML105 had reduced dry matter production as the TP went higher since it was only expressing root plasticity in the shallow soil layer (0 – 20 cm) which did not match with the available water located at the deep soil layer, especially in the higher TP (Kameoka et al. 2015). In another study, the New Rice for Africa (NERICA) 1 and NERICA 4 varieties grown in a sloping TP showed contrasting plastic responses to imposed drought conditions (Menge et al. 2016). Without a hardpan layer, NERICA 4 exhibited higher dry matter production than NERICA 1 under moderate drought because of its greater ability to express plasticity in deep root development. However, when the hardpan layer was present, NERICA 1 exhibited greater shoot dry matter production than NERICA 4 because of its greater ability to express plasticity in lateral root development in the shallow layer (Menge et al. 2016), which is important for higher growth during the re-watering period.

The above study (Kameoka et al. 2015) however, focused on differences in soil depth with the source of water coming from the groundwater table simulated by the water stored in the pond. Thus, root system plasticity and growth performance of rice along the toposequence with no groundwater table and subjected to SMF still needs to be done. In this study, it was hypothesized that in rainfed lowland rice, ecosystems located in different toposequence subjected to SMF, plasticity in root branching at the shallow soil layer is more significant than deep rooting plasticity in maintaining dry matter production under conditions similar to a flat TP but with restrictive hardpan. Thus, this research aimed to determine the root plasticity expression and its contribution to dry matter production of two contrasting rice cultivars under simulated rainfed lowland conditions (sloping and experimental flatbed with hardpan) with no prominent groundwater table subjected to SMF.

**MATERIALS AND METHODS**

Two field experiments were conducted under a rain-out shelter in a sloping bed system without a hardpan and groundwater table (Experiment 1), and a shallow watertight experimental flatbed (30 cm depth) with a hardpan (Experiment 2) under SMF conditions at Togo Field Science Center, Graduate School of Bioagricultural Sciences, Nagoya University, Togo-cho, Aichi, Japan (3506’42”N, 13704’57”E) in 2016 and 2017, respectively. The soil used for both experiments was sandy loam.
Two contrasting rice genotypes namely, IRAT109 (upland-adapted japonica) and KDML105 (lowland-adapted indica), based on their pattern of root plasticity expression in response to drought stress (Kameoka et al. 2015), were used. KDML105 has greater root plasticity particularly at the upper soil layer (Bañoc et al. 2000a; Kano-Nakata et al. 2013; Kameoka et al. 2015) while IRAT 109 has a greater deep root ratio (Kato et al. 2006, 2007; Kameoka et al. 2015).

**Experiment 1. Evaluation of the Expression of Root Plasticity Along the TP**

To evaluate the expression of root plasticity of IRAT109 and KDML105 along the TP, an experimental sloping bed system (Kameoka et al. 2015; Menge et al. 2016) was used with slight modifications. In this study, there was no groundwater table, and genotypes were evaluated under SMF conditions along the TP. Thus, the sloping bed system was established using a removable metallic bars (200 cm in length), with heights ranging from 30 cm (lower TP) to 80 cm (upper TP) and without a water-tight pool and underlying impermeable plastic sheet.

The seeds of each genotype were soaked in water containing benomyl fungicide (Benlate; Sumitomo Chemical Garden Products Inc. Tokyo, 0.15 % w/v) and then incubated in a seed germinator at 28°C for 72 h before sowing. The pre-germinated seeds of each genotype were sown in black plastic trays with the soil fully saturated with water (i.e., field capacity). Twenty-one-day-old seedlings from each genotype, with intact root systems, were transplanted at one seedling per hill (50 cm between rows and 28 cm between hills) in a row with a length of 2.0 m. Thus, each row contained seven plants of each genotype grown on the soil with increasing heights (32, 37, 45, 50, 57, 60, and 65 cm) from the bottom to the top (Kameoka et al. 2015). The plants grown at soil heights of 32 and 37 cm were classified as lower position in the TP (lower TP); 45, 50, and 57 cm as middle position in the TP (mid TP); and 60 and 65 cm as upper position in the TP (upper TP).

The experiment was laid out in a Completely Randomized Design (CRD) with three replications (1 row = replication). Genotypes were randomly assigned to each row. Compound chemical fertilizer was applied at a rate of 120 kg N ha⁻¹, 120 kg P ha⁻¹, and 120 kg K ha⁻¹ after transplanting. The transplanted seedlings were kept under well-watered conditions for 14 d to achieve recovery from transplanting shock before SMF treatments were imposed.

Six soil tensiometers (Daiki soil and moisture, Daiki Rika Kogyo Co., Japan) were installed at 20 cm soil depth at the lower TP and upper TP to monitor the soil water potential. Eighteen soil moisture sensors (EC-5 Decagon, Utah, USA) were also installed at three soil depths at 0 – 20 cm (upper soil layer), 20 – 40 cm (middle soil layer), and 40 – 60 cm (deeper soil layer) to record the soil moisture content (SMC).

**Soil Moisture Fluctuation (SMF) Treatment**

At 14 d after transplanting (DAT), watering was withheld until soil water potential in the upper TP reached down to -30 kPa, which was considered as mild drought. Afterwards, re-watering was done to bring the soil water potential back to 0 kPa and maintained to that level of soil water potential for 10 d. This cycle of SMF treatment was repeatedly done until 78 DAT. The plants at the mid and upper TPs were exposed to SMF treatment while those at the lower TP were kept under well-watered conditions. At the end of each drought and re-watering cycle, the number of tillers per plant was recorded. The experiment was terminated at 78 DAT.

**Stomatal Conductance Measurements**

The stomatal conductance of the second youngest fully expanded leaf of the main stem was measured with a leaf porometer (Decagon, Utah, USA) between 10 – 14 h at the end of each drought and re-watering period.

**Shoot and Root Sampling, and Measurements**

Shoot and root samples were collected at 78 DAT. Root sampling at different soil depths were done following the methods of Kameoka et al. (2015) and Menge et al. (2016) using a monolith stainless cylinder (15 cm diameter × 20 cm height). At the lower TP, root sampling was done at 0 – 20 cm soil depth. At the mid TP, root sampling was done at 0 – 20 and 20 – 40 cm. Root sampling in the upper TP was done at 0 – 20, 20 – 40, and 40 – 60 cm depths.

Shoot and root measurements were done according to Owusu-Nketia et al. (2018). The root length density was calculated as the total root length per unit volume of soil from which roots were extracted.

**Experiment 2. Evaluation of The Expression of Root Plasticity Above The Hardpan of A Flat Topography in Response to SMF**

To evaluate the expression of root plasticity of KDML105 and IRAT109 above the hardpan, a water-tight experimental bed of size 3.6 × 8.4 m per treatment plot with 30 cm depth of sandy loam soil was used. A hardpan layer was imposed by laying an impermeable

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plastic sheet beneath the experimental flatbed at a depth of 20 cm from the soil surface. Seed sowing, seeding, and fertilizer management were done as in Experiment 1. Transplanted the 21 d old seedlings of each variety at a rate of one seedling per hill, at 20 cm between hills and 25 cm between rows.

The experiment was laid out as a Completely Randomized Design (CRD) with three replications. The transplanted seedlings were allowed to recover from transplanting shock for 14 d prior to the imposition of SMF treatment. Plants were grown until 70 DAT.

Soil Moisture Treatments
The plants were exposed to two conditions of soil moisture namely, continuously well-watered (WW; control) and SMF stress treatments. Under WW, the soil moisture was maintained at 0 kPa until the termination of the experiment. Under SMF treatment, the soil moisture was first kept under WW for 14 d, after which watering was withheld until soil water potential dropped down to -30 kPa. Thereafter, rewatering was done to bring the level of soil moisture condition back to that of WW control (Niones et al. 2012; Owusu-Nketia et al. 2018). This cycle was done repeatedly until 70 DAT. Stomatal conductance was measured similar to that in Experiment 1.

Shoot and Root Sampling, and Measurements
Shoot and roots were sampled at the end of every drought and rewatering period. Root sampling was done as described by Niones et al. (2012). The shoot and root measurements were done following those of Owusu-Nketia et al. (2018).

Statistical Analysis
Treatment means comparison was determined by Student’s t-test. The relationships between root traits and shoot traits were determined using regression analysis.

RESULTS

Experiment 1. Soil Moisture Dynamics, and Shoot and Root Development along the Sloping Topography
Prior to the imposition of SMF at 15 DAT, the SMC regardless of the positions in the TP and soil depths was above 30% — a typical well-watered condition (Fig. 1). During SMF, the SMC decreased at the upper, middle, and deeper soil layers in the mid and upper TP during progressive drought while the SMC at the lower TP remained at the level of well-watered conditions. During re-watering periods, SMC at the mid and upper TP recovered back to the level of well-watered conditions. On the other hand, the SMC at the middle soil layer (20 – 40 cm) of mid TP, and the middle and deeper soil layers (20 – 40 and 40 – 60 cm) of upper TP continued to decrease even during rewatering periods at 35 DAT. The mean SMC at shallow (0 – 20 cm) soil layer in mid TP was lower than the upper TP.

The shoot dry weight (SDW) of KDML105 was consistently higher than that of IRAT109 along the TP except at 50 and 57 cm soil heights near the mid TP. The SDW of both genotypes generally decreased with an increase in soil height. Higher decreases in SDW relative to the lower TP were observed from 37 – 60 cm (Fig. 2a).

The stomatal conductance was not significantly different between KDML105 and IRAT109 at the lower TP. However, KDML105 had significantly higher stomatal conductance than IRAT109 at the mid and upper TP.

Tillering and nodal root production were generally higher in KDML105 than in IRAT109 except at the soil heights of 50 cm (number of tillers per plant) and 57 cm (number of nodal roots per plant) which were similar for both genotypes.

The number of tillers per plant in both genotypes was also reduced at 37 – 50 cm soil height and thereafter increased at the upper TP (Supplementary Fig. 1a). KDML105 had a generally higher number of nodal roots than IRAT109 along the TP except at the soil height of 45 cm near the mid TP. The number of nodal roots also decreased in both genotypes at mid TP but increased at the upper TP, relative to the lowermost TP. The number of nodal roots was also reduced at 32 – 37 cm soil height in the lower TP (Supplementary Fig. 1b).

Fig. 1. Soil moisture content (% v/v) at lower, middle and deeper soil layers along the toposequence in a sloping bed subjected to soil moisture fluctuation (SMF) condition in Experiment 1. Data are means of 3 replications ± standard errors.
The root length density (RLD) of KDML105 was significantly greater than IRAT 109 at the shallow (0 – 20 cm depth) soil layer in both the lower and upper TP. At the middle (20 – 40 cm depth) and deeper (40 – 60 cm depth) soil layers, the RLD was not significantly different between genotypes. The RLD at the shallow (0 – 20 cm depth) soil layer generally decreased in both genotypes as the soil height increased from 37 – 60 cm. On the other hand, RLD in both genotypes at the upper TP was like the lowermost TP (Fig. 3).

Significant and positive relationships between stomatal conductance and SDW (Fig. 4a), RLD and stomatal conductance (Fig. 4b), RLD and SDW (Supplementary Fig. 2a), and the number of tillers and number of nodal roots (Supplementary Fig. 2b) along the TP were observed in KDML105 only.

Experiment 2. Shoot and Root Responses to SMF Above The Hardpan in a Flat Topography

The SDW of KDML105 was not affected by SMF conditions from 30 – 70 DAT (Fig. 5a), relative to its WW counterparts. On the other hand, the SDW of IRAT109 was significantly lower in SMF than in WW at 40 and 70 DAT. The stomatal conductance of KDML 105 was reduced significantly during drought periods of the SMF. However, during the rewatering period, the stomatal conductance of KDML105 recovered back to the level of WW control (Fig. 5b). In IRAT109, on the other hand, the stomatal conductance was reduced by drought periods during SMF, and it did not recover to the level of its WW control during rewatering, especially at 60 DAT.

The number of tillers per plant in KDML105 was not significantly different between the water treatments (Supplementary Fig. 3a). On the other hand, the number of tillers per plant in IRAT109 was significantly lower in SMF than in WW conditions, except at 60 DAT (Supplementary Fig. 3a).

The number of nodal roots was significantly lower in SMF than in WW from 30 – 70 DAT in IRAT109 (Supplementary Fig. 3a). On the other hand, the number of nodal roots per plant in KDML105 was similar between water treatments at 30 and 40 DAT, and significantly higher under SMF than under WW at 40, 50, and 70 DAT (Supplementary Fig. 3b).

The total root length (TRL) and lateral root length (LRL) of KDML105 significantly increased in SMF relative to WW conditions at 50 – 70 DAT (Fig. 6). On the other hand, the TRL and LRL of IRAT109 significantly decreased under SMF relative to WW conditions, especially at 50 – 70 DAT.
A significant and positive relationship between TRL and SDW (Fig. 7a), TRL and LRL (Fig. 7b), TRL and number of nodal roots per plant (Supplement 4a), and number of tillers per plant and number of nodal roots per plant (Supplement 4b) was observed in KDML105 under SMF only. In contrast, the relationships of the above-mentioned traits were positive and significant in IRAT109 under WW only, while the relationship between TRL and LRL of IRAT109 was positive and significant under both water treatments (Fig. 7a and b).

DISCUSSION

Results from the experiments showed that regardless of the topography of the rainfed lowland rice ecosystem — whether in a sloping TP without a hardpan or a flat TP with a restrictive hardpan and without a groundwater table — the plasticity in lateral root development was the key root trait for maintaining higher shoot dry matter production in rice subjected to SMF conditions.

In a sloping topography, it was observed that the SMC at the mid TP was lower compared to those in the upper and lower TPs, especially at the latter stage of the experiment (35 – 78 DAT). This was probably due to high surface run-off (Tsubo et al. 2006) brought about by slightly compacted soil which prevented less downward movement of water through the soil. The soil compaction itself is already a cause for the decrease in SMC (Rataj et al. 2016) in the middle and deeper soil layers of the upper TP. Since the water was applied at the upper TP during rewatering, the SMC at the shallow soil layer (0 – 20 cm) was relatively higher in upper TP than that of the mid TP (Fig. 1). On the other hand, in a flat topography with restrictive hardpan, the only source of soil moisture during progressive soil drying is the shallow soil layer above the hardpan (Kameoka et al. 2015). Thus, in the absence of a groundwater table in the sloping topography or in the presence of a restrictive hardpan,
the plasticity in lateral root development at the upper soil layer was key for adaptation under soil moisture fluctuations. This has been exemplified by the KDML105, which showed higher RLD at the upper soil layer at the upper and lower TPs in a sloping bed (Fig. 3) as well as total lateral root length in the flat experimental bed with restrictive hardpan (Fig. 6). KDML has promoted lateral root elongation (Fig. 6b) and nodal root production (Supplementary Fig. 3b) due to high tillering (Supplementary Fig. 6) under SMF which increased TRL (Fig. 6a). Thus, KDML105 can express the plasticity of its lateral root development in the upper soil layer (0 – 20 cm) with or without a hardpan under SMF stress to match with the moisture availability in this layer (Shen et al. 2020) which supported its higher stomatal conductance than IRAT109.

The ability of KDML105 to maintain greater root length can compensate for the limited water availability in soil under progressive drought periods during soil moisture fluctuation by expanding the root surface area and increasing water extraction (Siopongco et al. 2005, 2006; Suralta et al. 2010; Henry et al. 2011; Kato et al. 2007, 2011; Jia et al. 2018). Thus, faster recovery of stomatal conductance brought about by greater root water uptake ability (Matsuo et al. 2010; Kano et al. 2011) of KDML105 during the rewatering period after drought (Fig. 5b) (Suralta et al. 2018; Tran et al. 2019; Yu et al. 2019) in SMF could contribute to faster root growth recovery (Kondo et al. 2003; Kano-Nakata et al. 2013). Hence, the promoted lateral root production in the upper soil layer in response to soil moisture fluctuations played key roles in the adaptation of rice plants to the condition (Niones et al. 2012, 2019; Menge et al. 2016; Owusu-Nketa et al. 2018). Consequently, these differences in the root system developmental responses in upper soil layer of the sloping bed or above the hardpan have explained the greater dry matter production in KDML105 than IRAT109 (Fig. 2 and 7a) due to its promoted root growth (Fig. 6a and 7b).

Furthermore, in the mid TP, the RLD values of both KDML105 and IRAT109 were similar, but lower than those in the upper soil layers for both upper and lower TPs due to the lower SMC in the upper and middle soil layers in the former, brought about by high surface runoff (Fig. 1). In the absence of the groundwater table in the sloping bed, the deep rooting ability, as well as the lateral root production of IRAT109, was inhibited. Also, in the experimental bed with restrictive hardpan, IRAT109 cannot express its deep rooting ability. In those conditions, IRAT109 did not take advantage of expressing the root plasticity in the upper soil layers where the soil moisture was available, either in the upper TP (Fig. 1) or above the hardpan, and thus was unable to efficiently collect soil moisture available at the upper soil layer during SMF.

**CONCLUSION**

In this study, two genotypes were tested (KDML105 and IRAT109) with contrasting root plasticity expressions to drought stress by growing them at different positions in TP and in a flat topography with a hardpan. Both without a groundwater table and subjected to SMF. The KDML105 exhibited improved adaptation to SMF conditions due to its greater root system because of promoted nodal root production and development of lateral roots in the upper soil layer (0 – 20 cm) along the TP and above the hardpan in a flat topography, which led to the maintenance of its stomatal conductance and dry matter production. On the other hand, the IRAT109 did not express the plasticity in deep-rooting ability due to the absence of groundwater table, particularly in the upper TP, as well as promoted branching ability above the hardpan in a flat topography, which resulted in less water uptake and reduced dry matter production under SMF. The results implied that genotypes have better adaptation to rainfed lowland ecosystems with or without a hardpan, and with no prominent groundwater table were those that can express plasticity in lateral root branching ability in the upper soil layer in response to soil moisture fluctuations. This further implied that a careful characterization of the target water-stressed crop production environments was important to design and breed an ideal root system custom-tailored to the characterized environments.
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Supplementary Fig. 1. Number of tillers per plant (a) and number nodal roots per plant (b) of IRAT109 and KDML105 under soil moisture fluctuation (SMF) along the toposequence in Experiment 1. * and **, significant at $p < 0.05$ and 0.01, respectively. Data are means of 3 replications ± standard errors.

Supplementary Fig. 2. Relationship between root length density and shoot dry weight (a), and number of tillers per plant and number of nodal roots per plant (b) of IRAT109 and KDML105 under soil moisture fluctuation (SMF) along the slope of the toposequence Experiment 1. ns, not significant; ** significant at $p < 0.01$. 
Supplementary Fig. 3. Number of tillers per plant (a) and number of nodal roots per plant (b) of KDML105 and IRAT109 under well-watered (WW) and soil moisture fluctuation (SMF) conditions in Experiment 2. White bars indicate progressive drought while black bars indicate re-watering period in SMF. * and **, significant at $p < 0.05$ and 0.01, respectively. Data are means of 3 replications.

Supplementary Fig. 4. Relationship between the number of nodal roots per plant and total root length roots (a), and number of tillers per plant and number of nodal roots per plant (b) of KDML105 and IRAT109 under well-watered (WW) and soil moisture fluctuation (SMF) conditions in Experiment 2. * and **, significant at $p < 0.05$ and 0.01, respectively. Data are means of 3 replications.