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Functional Roles of the Plasticity in Deep Root System Development in Soil Water Uptake and Dry Matter Production of Doubled Haploid Lines of Rice under Upland Drought Condition

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Abstract Drought limits production of upland rice. Deep and thick roots are key traits for adaptation to upland drought. However, deep root system development (DRSD) may not only a function of maximum nodal rooting depth (MNRD) but also in combination with the intensity of lateral root development and its branching to maximize the access of soil water from the deep and consequently, maintain greater dry matter production (DMP) under drought. This study aimed to quantify the DRSD in rice and its role in maintaining high water use in deeper soil, and DMP under upland drought using CT9993/IR62266 doubled-haploid lines (DHLs) relative to the recurrent parent IR62226 and identify DHLs with functional DRSD. In the first experiment, 88 DHLs were evaluated in mylar tubes with soil and subjected to progressive drought to identify lines with greater MNRD than IR62266. In the second experiment, the selected DHLs were further evaluated under progressive drought in mylar tubes embedded with 5-cm gravel layer at 30 cm below the soil surface. In the first experiment, 10 DHLs were selected based on their greater MNRD than IR62266 and further evaluated in the second experiment. In the second experiment, the use of 5-cm thick gravel layer blocked the capillary rise of water resulting in two distinct soil layers during drought: dry (4% soil moisture content, SMC) above and wet (>15% SMC) layer below the gravel. Among the selected DHLs, only DHL57 showed a significantly 34% greater shoot DMP than IR62266. Relative to IR62266, DHL57 had greater ability to maintain nodal root production (28%) and greater DRSD based on total root length (1545%) due to the integrated response of greater MNRD (38%), greater deep root (604%) and lateral root length (680%) ratios, and deep root branching index (233%). These integrated root responses contributed to the 25% greater water use from the deeper soil and consequently, 28% increase in shoot DMP under upland drought.

Keywords: Deep root system development, dry matter production, rice, upland drought, water use

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Introduction

Drought is one of the most serious abiotic stresses that limit crop production in rainfed rice ecosystems. There are about 100 million ha of rice areas in the world and 89% of them are in Asia. Of the total rice area in Asia, 45% are in rainfed areas of which 25% is never flooded and thus classified as upland condition (Serraj *et al.,* 2009). The rainfed upland field has poor accumulation of water due to uneven upper toposequence, absence of bunds, and lower water-holding capacity of the soil (Bernier *et al*. 2008). Under upland conditions, it is generally thought that deep and thick roots are the key traits for adaptation in rice (O'Toole and Bland, 1987) particularly roots below 30 cm (Yoshida and Hasegawa, 1982), which is greatly influenced by both genotypic variations and intensity of drought stress (Lilley and Fukai, 1994; Nguyen *et al*., 1997; Price *et al.,* 2002; Kato *et al*., 2006, 2007b; Uga *et al*., 2011).

Deep rooting can be influenced by the combined effects of the root angle and root length in seminal and nodal roots in cereals (Araki *et al.,* 2002; Kato *et al*., 2006; Uga *et al*., 2009, 2010). While deep rooting is the focus of improving drought resistance in upland conditions, understanding the contribution of other component root traits at deeper soil layer to deep root system development is equally important. For instance, promoted lateral root production increases the size of contact with soil for greater water (Bañoc *et al*., 2000; Wang and Yamauchi, 2006) and nutrient uptake (Suralta, 2010) and thus when combined with maximum nodal rooting depth, it may maximize drought dehydration avoidance ability and consequently, maintain dry matter production and yield. In this study, we hypothesized that maximum nodal rooting depth alone may not be sufficient to increase the dehydration avoidance ability of rice under drought but should be in consonance with the promotion of other component root traits such as the degree of lateral root development in terms of number and length. Therefore, the integrated response in deep root system development to drought can be more functional in maintaining greater water uptake from the deeper soil layer and consequently maintained of greater dry matter production.

To prove the above hypothesis, the "raisedbed" method in determining the function of deep rooting (Kato *et al.* 2007a) was used. This method raised the bed to 30 cm above the ground level by adding a 25-cm-thick layer of topsoil above a 5-cm-thick gravel layer that let the surface soil dry more easily and frequently and blocked the potential capillary rise of water. Thus, this method can quantify the advantage of known deep rooting genotypes than the shallow rooting ones in accessing soil water from the deep (Kato *et al.,* 2007a). However, the Kato *et al.* (2007a) study did not quantify and validate actual deep root system development.

In the present study, we have modified the raised bed system of Kato

et al. (2007a). Instead of soil bed, we used mylar plastic tubes filled with soil and with gravel layer similarly embedded at 30 cm soil depth below soil surface. The modification was intended to easily and accurately quantify the root system development below the gravel layer and precisely relate to soil water uptake at deeper soil layer. In order to reduce effects of genetic confounding, we used homogeneous genetic materials specifically DHLs from a cross between CT9993 and IR62266. This DH population has wide genotypic variations in yield, osmotic adjustment (OA), and root system development such as deep and thick rooting under drought (Zhang *et al.*, 2001; Kamoshita *et al.*, 2002; Babu *et al.*, 2003; Siopongco *et al.*, 2009), L-type LR plasticity, transpiration, and water use efficiency (Suralta *et al.*, 2012).

This study aimed to evaluate deep root system development in terms of deep nodal rooting and branching of lateral roots under upland drought condition and quantify their contribution to water uptake at deeper soil, and dry matter production, and to identify DHLs with functional DRSD, which can be used as one of the potential parents in upland rice breeding program.

Materials and methods

Time and place of study

Two experiments were successively conducted in a greenhouse between January to April 2013 at the Philippine Rice Research Institute-Central Experiment Station (PhilRice-CES), Muñoz, Nueva Ecija, Philippines (15^0 40' N, 120^0 53' E, 57.6 masl).

Plant materials

Eighty-eight DHLs from a cross between CT9993 and IR62266 were used in this study. CT9993 is an upland *japonica* type with specific adaptation to drought conditions while IR62266 is an *indica* type with general drought adaptation across environments with stable yields. The DHLs were jointly developed at Centro International de Agricultura Tropical (CIAT), Columbia, and the International Rice Research Institute (IRRI), Philippines.

Experiment 1. Evaluation of CT9993/IR62266 DHLs under drought condition

Garden soil (sandy loam: 72% sand, 21% silt, 7% clay; pH 5.5 and a field capacity of 32% w/w) was thoroughly dried and sieved to remove mixtures. The mylar tubes (5.5 cm in diameter and 1.0 m in height) similar to that used by Kano-Nakata *et al.* (2013) were filled with 4.8 kg dried soil

pre-mixed with fertilizer at the rate of 120-60-60 kg NPK ha^{-1}. Each tube contained pre-mixed fertilizers of 0.61 g of urea (46-0-0), 0.78 g solophos (0-18-0) and 0.23 g of muriate of potash (0-0-60).

Plant management and imposition of drought condition

Three pre-germinated seeds from each genotype were grown in plastic tube. The seedlings were later thinned to one seedling per tube at 3 days after sowing (DAS). The soil in each tube was initially saturated with water (25% soil moisture content, SMC). Then water was added at 3 DAS to adjust to field capacity (30% SMC) and maintained at the same level SMC from the day of sowing up to 21 DAS. Thereafter, watering was withheld and the soil was allowed to dry down to 15% and maintained to that level of SMC until 35 DAS, the day the experiment was terminated. The decrease in soil moisture of each tube was monitored daily to record the amount of evapotranspiration. Tubes were weighed daily using a digital balance to record the wet mass of the soil. The SMC (% by mass) in each tube was calculated as the ratio between water mass (difference between the wet mass of the soil excluding the tube on a given day and the dry mass (4.8 kg) of the soil) and the dry mass (4.8 kg) of soil. Once the target 15% SMC was reached, watering was done to replace the amount of water lost and maintain the 15% SMC. A few tubes (n = 3) not planted and were used to measure the amount of water lost through evaporation so that the amount of water lost through whole plant transpiration alone could be estimated. The total water use per tube was calculated as the accumulated daily water use throughout the duration of the progressive drought condition (21-35 DAS).

Plant samplings and measurements

Plant sampling was done at 35 DAS. The shoots were cut and oven-dried at 70 ^{0}C for 48 h before weighing. Root sampling was done by slicing vertically the mylar tubes with blade to expose the whole root system. Thereafter, the maximum nodal rooting depth (MNRD) was traced and measured by a meter stick. The roots were carefully washed and preserved in 95% ethyl alcohol for further measurements.

The total nodal root length (TNRL) was measured with a meter ruler as the sum length of each nodal root. Thereafter, the whole root system was scanned at 600 dpi (EPSON 4990) and analyzed for total root length (TRL) using WinRhizo v. 2007d (Régent Instruments, Québec, Canada). A pixel threshold value of 175 was set for the root length analysis. After analysis, the roots were oven-dried at 70 ^{0}C for 48 h prior to weighing of the root dry weight (RDW). The total lateral root length (TLRL) was computed as the difference between TRL and TNRL.

Experiment 2. Shoot growth, deep root system development and water use of selected DHLs under drought condition

Based from the result in Experiment 1, the top ten selected DHLs with maximum rooting depth such as DHL40, DHL44, DHL50, DHL57, DHL61, DHL70, DHL103, DHL113, DHL138, and DHL142 were used together with the recurrent parent IR62266 (Table 2).

This experiment also utilized mylar plastic tubes as described in Experiment 1 with some modifications. The garden soil used was similar to that described in Experiment 1. A 5-cm thick gravel layer (5-10 mm in diameter) was embedded at 30 cm below the soil surface. The mylar tubes were first filled up with 3,134 g of garden soil to a height of 65 cm from the bottom, followed by 5-cm layer of 220 g of gravel and finally 1,446 grams of garden soil above the gravel layer to a height of 30 cm.

The rates of fertilizers applied and plant management were similar to those used in Experiment 1. Imposition of drought was done by withholding water from 25 DAS until the termination of the experiment (45 DAS).

Plant and soil samplings, and measurements

Sampling was done after initial well-watered conditions (30% SMC) prior to the imposition of drought (25 DAS) and after imposition of drought (26-45 DAS) conditions. The shoots were cut and oven-dried at 70 ^{0}C for 48 h before weighing the dry weights. Each tube was sliced vertically with a blade to expose the whole root system. Thereafter, the maximum nodal rooting depth or the length of deepest nodal root, was traced and measured by a meter stick. The soil profile was divided into six portions corresponding to six soil depths: 0-15 cm, 15-30 cm, 30-45 cm, 45-60 cm, 60-75 cm and 75-90 cm below the soil surface. The soil portion at 90-100 cm soil depth was not samples since the roots did not reach at this portion regardless of genotypes. Soil samples were taken from each portion prior to washing of roots. The soil from each portion was weighed for fresh weight and then oven-dried at 105^{0}C for 48h for determining the dry weight. The fresh and dry weights of sampled soil from each portion were used for the calculation of SMC expressed as % w/w. Meanwhile, the roots from each portion were extracted, carefully washed with tap water and preserved in 95% ethyl alcohol for further measurements.

For component root trait measurements below the gravel layer (>30 cm), the number and length of nodal roots (NR) was manually counted and measured using a metric ruler, respectively. Thereafter, the root samples from each portion were scanned and analyzed for root length similar to those in Experiment 1. After analysis, the roots were oven-dried at 70 ^{0}C for 48 h prior to weighing the RDW. The LRL was computed as the difference between TRL and NRL.

The deep NRL ratio, deep root length ratio, deep LRL ratio and deep RDW ratio were calculated as the ratio between the values of each trait at soil portion below the 5-cm thick gravel layer and their total values from the whole soil profile. The deep root branching index was calculated as the ratio between the lateral root length and nodal root length below the 30-cm gravel layer.

Statistical Analysis

The experiment 1 was laid out in a completely randomized design while the experiment 2 was laid out in randomized complete block design, both with four replications. In experiment 2, the ANOVA and calculation of means were done for all traits measured using IRRISTAT program (version 4.1). Means were compared using Tukey's HSD at 5% level of significance.

Results and discussion

Performance of DHLs and their parents under upland drought condition

There was significant genotypic variations in shoot dry weight, root growth and water use among DHLs under upland drought conditions (Table 1). The results also showed that root system developmental responses to drought between parents and among DHLs were wide and significant (Table 1). Although shoot dry weight (SDW) was similar between DH parents, component root traits such as MNRD, lateral root development based on branching ability, TRL, TLRL and RDW were generally greater in CT9993 than in IR62266 (Table 1). On the other hand, the number of nodal roots per plant and TNRL were smaller in CT9993 than in IR62266. The mean SDW in DHLs under drought as well as most of the component root traits except MNRD, were greater than either both of the parents or least compared to IR62266 (Table 1). The mean MNRD of DH population was comparable to that of IR62266. The results indicate that transgressive segregants or those DHLs that have better performance than both of the parents are present. This also indicates the suitability of CT9993/IR62266 DH population in quantifying deep root system development and its functional roles for soil water uptake at deeper soil layers and dry matter production under upland drought.

Table 1. Mean values and range of shoot and root growth, and water use ± standard deviation at 35 days after sowing (DAS) of CT9993/IR62266 doubled haploid lines (DHLs) under upland drought condition in Experiment 1.

Traits	CT9993	IR62266	DHL Population		
			Mean	Range	ANOVA
Shoot Dry Weight (g plant^{-1})	0.25 ± 0.03	0.24 ± 0.10	0.27 ± 0.1	0.14 ± 0.04 – 0.55 ± 0.06	**
Maximum Nodal Rooting Depth (cm)	68.9 ± 2.4	49.5 ± 7.5	58.3 ± 7.0	31.8 ± 8.6 – 80.0 ± 7.0	**
Number of Nodal Roots per Plant	5.0 ± 0.6	10.3 ± 1.7	9.8 ± 2.5	2.7 ± 2.2 – 22.3 ± 4.9	**
Total Nodal Root Length (cm plant^{-1})	1.83 ± 0.33	3.45 ± 0.13	3.09 ± 0.66	1.58 ± 0.16 – 8.85 ± 1.58	**
Total Root Length (m plant^{-1})	22.59 ± 7.34	18.17 ± 3.18	20.77 ± 4.29	9.57 ± 1.54 – 35.53 ± 12.50	**
Total Lateral Root Length (m plant^{-1})	20.76 ± 8.22	14.72 ± 3.60	17.88 ± 3.98	6.90 ± 1.89 – 31.62 ± 11.92	**
Root Dry Weight (g plant^{-1})	0.17 ± 0.04	0.13 ± 0.03	0.16 ± 0.04	0.07 ± 0.02 – 0.33 ± 0.11	**
Branching ability (cm LRL cm^{-1} NRL)	11.5 ± 3.8	4.2 ± 0.9	7.0 ± 2.2	1.7 ± 0.6 - 24.2 ± 14.4	**
Water use (cc plant^{-1})	236.7 ± 20.0	254.3 ± 5.8	276.4 ± 21.5	192.6 ± 44.0 – 338.4 ± 37.0	**

** significant at P0.01

The top DHLs based on the ability to produce greater MNRD have been identified (Table 2) for further quantitative analysis in Experiment 2. These DHLs had a range of MNRD from 61.0 to 79.8 cm, which were greater than that of IR62266 (49.5 cm). These selected DHLs had greater SDW and water use than IR62266 (Table 2). In terms of other component root traits, not all had shown significantly greater values than IR62266. For instance, compared to IR62266, only DHL61 had significantly greater TNRL while 4 DHLs (DHL40, DHL57 and DHL138) had significantly greater values for TRL and TLRL (Table 2). Root dry weight was significantly greater in most of the selected DHLs than in IR62266 while only 3 DHLs (DHL57, DHL138 and DHL44) showed a significantly greater value of their water use than that of IR62266 (Table 2).

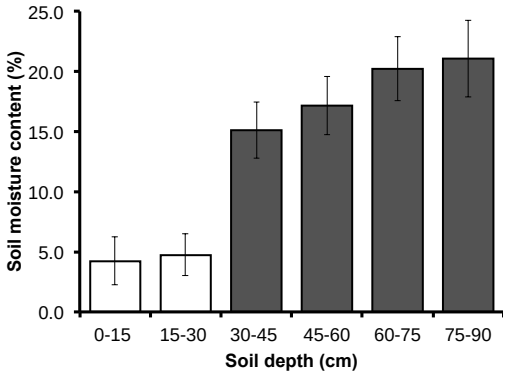
Table 2. Mean root trait parameters and shoot dry weight ± standard errors of selected CT9993/IR62266 doubled haploid lines (DHLs) based on maximum nodal rooting depth at 35 DAS under upland drought condition in Experiment 1.

Genotype	Maximum nodal rooting depth (cm)	Shoot dry weight (g plant^{-1})	Nodal Roots (no. plant^{-1})	Total nodal root length (m plant^{-1})	Total root length (m plant^{-1})	Total lateral root length (m plant^{-1})	Root dry weight (g plant^{-1})	Branching ability (cm LRL cm^{-1} NRL)	Water use (cc)
DHL50	79.7±7.3	0.27±0.09	11.7±0.9	4.01±0.73	11.78±4.68	7.77±3.95	0.140±0.04	1.7±0.6	267.8±74.0
DHL40	79.0±4.7	0.25±0.05	11.0±2.1	4.21±0.72	26.18±1.13	21.97±0.65	0.128±0.04	5.5±0.9	300.0±43.8
DHL57	76.8±11.5	0.33±0.02	10.3±0.7	3.52±0.77	26.54±0.42	23.01±1.09	0.163±0.05	7.4±1.9	259.3±10.1
DHL138	75.2±4.6	0.27±0.08	11.0±3.5	3.91±1.05	35.50±12.51	31.62±11.93	0.216±0.06	8.2±2.2	322.8±33.3
DHL44	74.2±14.7	0.30±0.10	12.3±4.8	3.09±0.76	23.86±5.11	20.77±5.83	0.114±0.03	8.3±3.2	306.7±25.0
DHL70	67.3±6.4	0.35±0.03	9.0± 0.6	4.08±0.55	22.22±2.41	18.14±1.89	0.223±0.06	4.5±0.3	291.9±12.8
DHL61	65.0±10.2	0.37±0.04	18.3±5.8	5.49±1.30	29.20±10.51	23.71±9.21	0.226±0.06	3.9±1.0	300.8±10.7
DHL142	63.2±12.7	0.32±0.08	11.0±2.0	3.58±0.54	23.86±5.11	20.85±8.22	0.220±0.06	4.5±0.3	291.9±12.8
DHL113	62.3±4.5	0.37±0.05	14.0±5.3	4.02±0.94	24.68±7.84	20.67±7.67	0.193±0.05	5.6±1.8	254.5±27.2
DHL103	61.0±8.7	0.38±0.02	7.7± 0.3	2.89±0.21	18.81±1.58	15.92±1.69	0.141±0.04	5.6±0.9	283.7±13.1
IR62266	49.5±7.5	0.24±0.10	10.3±1.7	3.45±0.13	18.17±3.18	14.72±3.60	0.129±0.03	4.2±0.9	254.3±5.8

Soil moisture dynamics at different soil depths with 5-cm gravel layer blocking the capillary rise of soil water from deeper soil layers during progressive drought stress

The mean SMC above (0-15 and 15-30 cm soil depths) and below (>30 cm soil depth) the gravel embedded at 30 cm soil depth were 4 and 15-21%, respectively (Figure 1A). These two soil layers with distinct differences in SMC indicate that the capillary rise of water in soil from deeper to shallow layer was successfully controlled by the embedded gravel layer (Figure 1B) similar to those shown by Kato *et al.* (2007b) in their raised bed experiment. This condition facilitated an accurate way of quantifying the role of the deep root system development to deep soil water uptake and dry matter production.

A



B

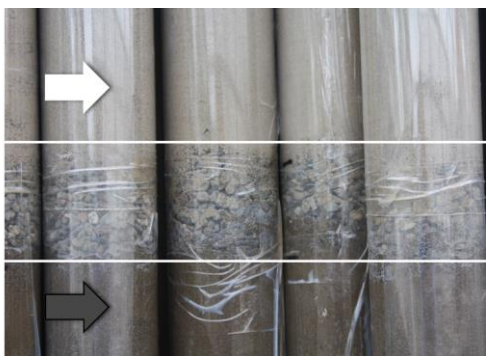


Figure 1. (A) Mean soil moisture contents above (0-15 and 15-30 cm depths) and below (>30 cm depth) the gravel layer at the end of drought stress in Experiment 2. Data are means of 4 replications ± standard errors. (B) Closer photo of soil within the mylar tubes with embedded gravel layer at 30 cm below the soil surface. Note the difference in visual soil moisture conditions which is dry above (white arrow) the gravel layer but relatively wet below (gray arrow) the gravel layer.

Deep root system development, water use and dry matter production of selected DHLs under upland drought condition

Generally, there were inconsistencies in trends in maximum nodal rooting depth and SDW of selected DHLs, relative to IR62266 between the two experiments (Tables 2 and 3). In Experiment 1, the selected DHLs had greater maximum nodal rooting depth and SDW than the IR62266 parent (Table 2) while in Experiment 2, only few DHLs had greater maximum nodal rooting depth and SDW than IR62266 (Table 3). This was partially attributed to the differences in drought treatments between experiments which led to their differences in the availability of soil moisture and soil moisture gradients. In Experiment 1, the soil was progressively dried down and maintained to 15% SMC by daily rewatering and thus, SMC was uniform throughout the soil profile. An expected capillary rise of soil water from the deep during soil drying in between rewatering helped replenish the water lost at the surface due to evapotranspiration. This provided the opportunity for the whole root system to take up the available water at all soil depths rather than relying solely on deep root system ability in response to drought treatment. Thus, the greater SDW shown by selected DHLs, relative to IR62266, were directly related to the greater whole root system development in response to drought stress. On the other hand, in Experiment 2, drought was imposed by continuous progressive soil drying without maintaining the SMC. Also, the embedded gravel layer at 30 cm below the soil surface disrupted the capillary rise of water preventing replenishment of the water lost at the soil surface due to evapotranspiration. This resulted SMC that was more available in the depth and thus root water uptake rely more on the degree of root system development at the deep (*see previous section*). And thus, genotypic performance among selected DHLs from Experiment 1 expectedly varied in Experiment 2.

At the end of well-watered conditions prior to imposition of drought stress treatment (25 DAS), the SDW and the number of nodal roots per plant of selected DHLs were generally less than that of IR62266 (Table 4). All of the other component root traits below the gravel layer was not significantly different among DHLs, and between DHLs and IR62266 (Table 4). These results indicate that the selected DHLs have the same constitutive root system development with IR62266 especially at deeper soil layer prior to the imposition of progressive drought. Thus, the expected differences among DHLs and between DHLs and IR62266 after the imposition of drought stress were mostly attributed to the root system development in response to progressive drought rather than due to the differences in constitutive root system development.

Table 3. Shoot dry weight and component root traits below (>30 cm) the gravel layer of selected CT9993/IR62266 doubled-haploid lines (DHLs) at 25 days after sowing prior to imposition of drought stress treatment in Experiment 2.

Genotypes	Shoot dry weight (g plant^{-1})	Maximum nodal rooting depth (cm)	Nodal roots (no. plant^{-1})	Penetrated nodal roots (no. below the gravel layer)	NRL below the gravel layer (cm)	Total root length below the gravel layer (cm)	LRL below the gravel layer (cm)	RDW below gravel layer (g)
DHL40	0.31 cde	34.8 a	37.0 bcde	1.00 a	8.5 a	28.47 a	19.97 a	0.002 b
DHL44	0.36 bc	29.7 a	43.7 ab	0.33 a	1.0 a	**1.12** a	0.09 a	0.001 b
DHL50	0.26def	35.8 a	26.5 de	1.00 a	5.7 a	24.58 a	18.88 a	0.001 b
DHL57	0.43 b	36.8 a	41.5 abc	4.50 a	8.8 a	23.42 a	14.67 a	0.002 b
DHL61	0.36 bcd	30.3 a	38.5 abcd	3.00 a	12.8 a	24.99 a	12.19 a	0.001 b
DHL70	0.43 b	36.6 a	35.0 bcde	2.00 a	11.4 a	24.09 a	12.69 a	0.008 a
DHL103	0.22 ef	25.1 a	31.0 cde	0 a	0 a	0 a	0 a	0 b
DHL113	0.28 cde	33.6 a	33.0 bcde	2.50 a	11.6 a	0 a	0 a	0.002 b
DHL138	0.16 f	22.1 a	25.5 e	0 a	0 a	0 a	0 a	0.001 b
DHL142	0.35 bcd	29.4 a	32.7 bcde	1.33 a	7.4 a	19.12 a	11.69 a	0.002 b
IR62266	0.67 a	35.0 a	50.0 a	1.50 a	10.0 a	48.03 a	38.03 a	0.001 b

In a column, means followed by the same letters are not significantly different at 5% level of Tukey's HSD

Table 4. Shoot dry weight and component root traits below (>30 cm) the gravel layer of selected CT9993/IR62266 doubled haploid lines (DHLs) after drought stress treatment at 45 DAS in Experiment 2.

Genotypes	Shoot dry weight (g plant^{-1})	Maximum nodal rooting depth (cm)	Nodal roots (no. plant^{-1})	Penetrated nodal roots (no. below the gravel layer)	NRL below the gravel layer (cm)	Total root length below the gravel layer (cm)	LRL below the gravel layer (cm)	RDW below gravel layer (g)
DHL40	0.47 ef	41.3 abcd	40.0 b	3.0 ab	27.0 abc	367.6 bc	340.6 bc	0.013 bc
DHL44	0.65 cd	35.2 cd	42.5 ab	7.0 ab	28.6 abc	429.8 bc	401.2 bc	0.017 bc
DHL50	0.76 bc	51.3 a	34.0 b	9.0 a	83.2 ab	1012.5 ab	929.4 ab	0.071 a
DHL57	0.94 a	51.1 a	52.5 a	9.0 a	89.9 a	1790.0 a	1700.1 a	0.027 bc
DHL61	0.69 bcd	39.8 abcd	45.5 ab	2.5 ab	18.0 abc	259.2 bc	241.2 bc	0.004 c
DHL70	0.75 bc	42.6 abcd	43.5 ab	9.5 a	63.7 abc	517.7 bc	454.0 bc	0.047 ab
DHL103	0.59 de	44.0 abc	38.5 b	7.0 ab	71.3 abc	810.6 abc	739.3 abc	0.020 bc
DHL113	0.67 cd	50.6 ab	40.0 b	5.5 ab	45.2 abc	861.6 abc	816.5 abc	0.032 bc
DHL138	0.47 ef	34.6 cd	41.0 b	1.0 b	7.9 bc	31.2 c	23.3 c	0.002 c
DHL142	0.81 b	42.6 abcd	39.5 a	9.5 a	63.0 abc	505.7 bc	442.7 bc	0.009 c
IR62266	0.70 bcd	37.0 bcd	41.0 b	2.5 ab	15.8 abc	108.8 bc	93.0 d	0.007 c

In a column, means followed by the same letters are not significantly different at 5% level of Tukey's HSD

Table 5. Deep nodal root length ratio, deep root length ratio, deep lateral root length ratio, deep root dry weight ratio and deep root branching index of selected CT9993/IR62266 doubled haploid lines (DHLs) after drought stress treatment at 45 DAS in Experiment 2.

Genotypes	Deep nodal root length ratio (%)	Deep root length ratio (%)	Deep lateral root length ratio (%)	Deep root dry weight ratio (%)	Deep root branching index (cm LRL cm^{-1} NRL)
DHL40	5.7 abc	5.5 bc	5.5 bcd	4.9 bc	16.5 ab
DHL44	6.5 abc	6.7 bc	6.7 bcd	6.1 bc	16.0 ab
DHL50	11.5 a	16.1 a	16.8 a	15.3 a	11.5 b
DHL57	9.7 ab	15.8 a	16.4 a	4.8 bc	19.0 a
DHL61	3.1 bc	4.2 bc	4.3 bcd	0.9 c	16.0 ab
DHL70	8.7 abc	9.0 abc	9.0 abcd	8.6 ab	6.7 b
DHL103	8.7 abc	9.0 abc	9.0 abcd	4.8 bc	8.5 b
DHL113	7.1 abc	12.5 ab	13.1 ab	9.0 ab	21.5 a
DHL138	1.9 bc	0.7 c	0.6 d	0.9 c	2.0 c
DHL142	8.5 abc	9.6 abc	9.7 abc	2.7 bc	7.5 b
IR62266	2.8 bc	2.2 c	2.1 cd	2.1 bc	5.7 bc

In a column, means followed by the same letters are not significantly different at 5% level of Tukey's HSD

After the imposition of progressive drought stress (45 DAS), only DHL57 showed a significantly greater SDW by 34% compared with IR62266 (Table 3). In terms of deep root system developmental responses to progressive drought, most of the selected DHLs had greater MNRD than IR62266 although only 2 DHLs (DHL50 and DHL57) had significantly greater values than IR62266 (Table 3). In order to assess the better performance of DHL57 than IR62266 in terms of SDW, we compared the deep root system development of this DHL with other DHLs using IR62266 as a reference. The DHL57 produced more number of NR per plant than IR62266 while DHL50 had similar values with IR62266. Both DHLs had no significant differences in the component root traits below the gravel layer such as number of penetrated NR, as well as deep NRL ratio, deep root length ratio and deep LRL ratio. The NRL, TRL, and TLRL at the deeper soil layer were also similar between the DHL57 and DHL50 although the values in the latter DHL for these traits were intermediate not significantly different from those of IR62266. The main difference between the two DHLs was in terms of the deep root branching ability (Table 5), which was significantly higher in DHL57 than in DHL50. The branching ability of DHL50 was similar to that of IR62266. Furthermore, DHL113 is also deep rooting (Table 3) with high deep root branching index (Table 5). However, this DHL has less number of penetrated NR, NRL, TRL and TLRL below

the gravel layer similar to those of IR62266 and thus, low water uptake ability at deeper soil layer (Figure 2) and consequently produced less dry matter production under drought. The results indicate that deep root system development under upland drought condition is an integrated response of component root traits such as deep rooting, more number of penetrated nodal roots and lateral root development at deeper soil layer.

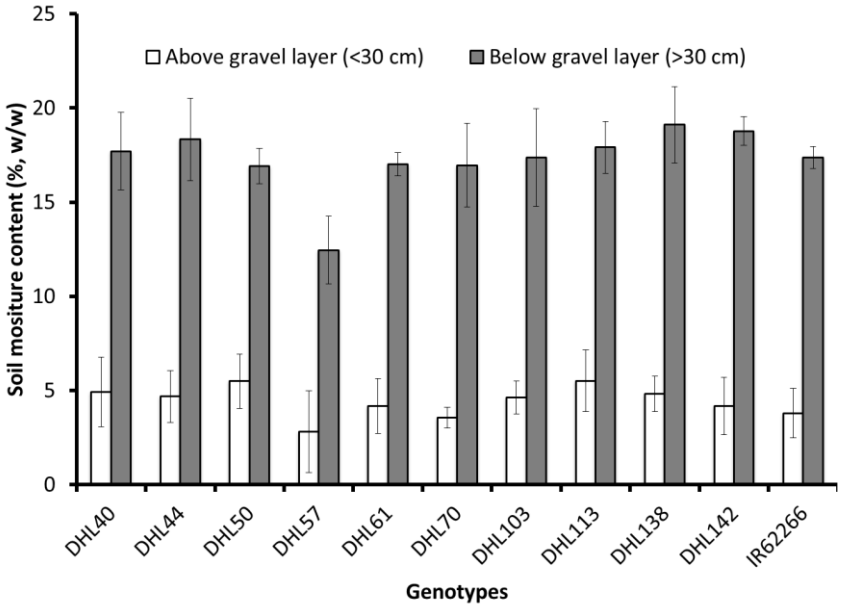


Figure 2. Soil moisture dynamics above (<30 cm) and below (>30 cm) the gravel layer as affected by different CT9993/IR62266 doubled haploid lines (DHLs) in Experiment 2. Data are means of 4 replications ± standard errors.

Upland rice ecosystem generally lacks hardpan or compacted subsoils normally found in rainfed lowland rice ecosystem (Cairns *et al*., 2011). The presence of hardpan can inhibit root penetration in the deeper soil layer especially during the period of progressive soil drying due to its higher magnitude of increase in penetration resistance in response to the decrease in soil moisture (Kato *et al.,* 2013). Under upland soil condition without a hardpan, root penetration at depths may be possible but dependent on the magnitude of soil mechanical impedance (Cairns *et al.*, 2004). Thus, the vertical distribution of roots through increased nodal root elongation (Kameoka *et al.*, 2015; Menge *et al.*, 2016) is important to increase efficiency of accessing the available soil moisture at the deeper soil layer. Simultaneously, the horizontal distribution of roots through the plasticity in lateral root development (Bañoc *et al.*, 2000; Suralta *et al.*, 2008, 2010; Kano *et al.*, 2011; Kano-Nakata *et al.*, 2011; Niones *et al.*, 2015; Kameoka *et al.* 2015; Menge *et al.*, 2016) increased water uptake from the drying soil. Since lateral roots mainly comprised the whole root system in terms of

number and length, its response to the changes in soil moisture directly dictates whole root system development (Yamauchi *et al.,* 1996). Thus, DHL57 which showed a deep root system developmental response brought about by the integrated responses in greater number of penetrated nodal roots at deep, maximum nodal rooting deep and lateral root development to progressive drought has the advantage over the other DHLs and IR62266 which had poor response in at least one of the root developmental traits.

The SMC at the shallow soil surface was around 4.5%, which was already low and unavailable for both the DHLs and IR62266 (Figure 1A). On the other hand, the SMCs in the soil below the gravel layer (30-60 cm depths) were high and available to the rice plants (Figure 1A). The dynamics in soil moisture under progressive soil drying conditions can be attributed to the genotypic differences in the ability to take up available soil water. Kato *et al.* (2007b) used the soil moisture status as an indicator of genotypic differences in soil water uptake ability. In the present study, only DHL57 had the lowest SMC at the deeper soil layer (Figure 2), which indicates that this DHL was able to extract more water at deeper soil layer than the rest of the genotypes due to its greater root system development than IR62266 (Tables 2 and 3).

The greater soil water uptake from deeper soil was highly associated with most of the component root traits below the gravel layer except maximum nodal rooting depth and root dry weight (Table 6). Among component root traits below the gravel layer, which had significant contributions to greater water uptake at deeper soil layer, the lateral root length had greater contribution than nodal root length for water uptake (Table 6). Thus, water uptake from deeper soil layer was greatly associated with the total root length (nodal root length + lateral root length), which mainly comprised with the lateral roots in terms of length and number (Yamauchi *et al.,* 1996). The above results also indicate that vertical distribution of roots (i.e. maximum nodal rooting depth and total nodal root length) through the soil profile is ineffective for water uptake without effective horizontal distribution of roots (i.e. lateral roots).

Eventually, this greater soil water uptake by DHL57 from the deeper soil layer contributed to its greater dry matter production under drought as indicated by negative and significant relationship with SMC below the gravel layer and SDW under drought stress (Figure 3). The ability to maintain greater transpiration under drought is directly related to high water extraction by roots in the drying soils which translates to the maintenance of greater stomatal conductance and photosynthetic rate and thus contributes to the ability of rice to produce greater extent of dry matter at greater production (Suralta *et al.,* 2010).

Table 6. The relationship between component root traits and soil moisture content below (>30 cm) the gravel layer.

Parameters	Soil moisture content below the gravel layer at 30-60 cm depths (%)
Number of nodal roots per plant	-0.47 **
Maximum nodal rooting depth (cm plant^{-1})	-0.32 ^{ns}
Total root length (cm plant^{-1})	-0.63 ***
Nodal root length (cm plant^{-1})	-0.47 **
Lateral root length (cm plant^{-1})	-0.64 ***
Root dry weight (g plant^{-1})	-0.14 ^{ns}

ns, not significant; *, ** and ***, significant at P<0.05, 0.01 and 0.001, respectively

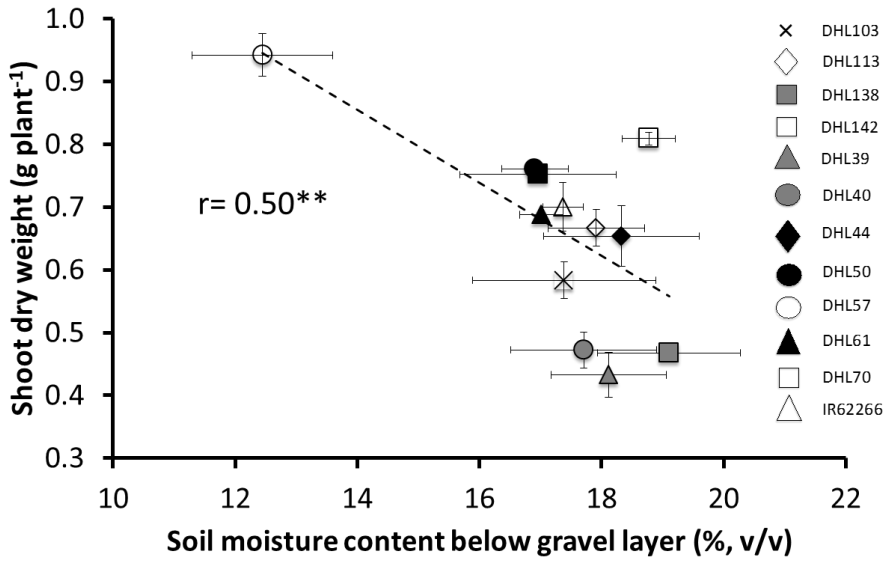


Figure 3. The relationship between soil moisture content below the gravel layer (30-60 cm depth) and shoot dry matter production at the end of drought stress at 45 DAS in Experiment 2. Values are means of 4 replications ± standard errors.

Conclusion

This study showed that under upland drought conditions, functional deep root system development is an integrated response in component root traits such as deep nodal rooting ability, greater nodal root length ratio and greater lateral root development as indicated by branching ability index. This consequently maximizes greater water uptake at deeper soil layer

during progressive drought and thus, contributes to the maintenance in greater dry matter production. The inability of deep root system development to simultaneously increase its deep rooting, deep nodal root development and the plasticity in lateral root development as indicated by greater branching ability will result in failure to adapt to upland drought conditions.

The DHL57 showed a functional deep root system development and thus it can be used as potential parent in improving the dehydration avoidance ability of available high yielding varieties to make them adapted to drought prone upland rice ecosystem. Since the genotypic and phenotypic information for this DHL is already available, future work may also focus on the genetic control of the plasticity in deep root system development under upland rice conditions.

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