Root Growth and Water Extraction Response of Doubled-Haploid Rice Lines to Drought and Rewatering during the Vegetative Stage

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Abstract : Doubled haploid lines (DHLs) of rice (*Oryza sativa* L.) were used to examine responses to drought and rewatering in controlled rainfed lowland conditions, in order to determine whether confounding by unrelated traits would be less than has been reported previously for contrasting cultivars that differ in genetic background. IR62266 and four DHLs derived from the cross between IR62266 and CT9993 (DHL-32, -51, -54 and -79) were grown in pot experiments in the greenhouse during the 2000 dry and wet seasons at IRRI, Los Baños, Philippines. There were two water regimes (well-watered and drought). Estimated water extraction obtained by time domain reflectometry (TDR) was similar to cumulative transpiration estimated from pot weighing for each genotype. Genotypic variation was observed in root traits and water extraction, with extraction slower in DHL-32 and faster in DHL-79, especially in deeper soil layers. An upper bound relationship between water extraction from a soil layer and root length density (RLD) in that layer was readily apparent over DHLs and soil depths, suggesting a critical value of RLD for water extraction of 0.30 cm cm^{-3} in these conditions. Because soils in the field would not be as homogenous as the puddled soils used in these greenhouse experiments, this critical RLD for water extraction from a soil layer is a reference for ideal conditions, and requires careful validation in the field. Use of DHLs permitted comparisons with reduced confounding by genetic background, with consequent improvements in precision.

Key words : Critical RLD, Drought, Rainfed lowland, Rice, Root traits, Water extraction.

Rainfed lowland rice is grown in bunded fields, mainly is south and southeast Asia, without access to irrigation water. The area occupied by this ecosystem is 46 Mha (Huke and Huke, 1997) with an average grain yield of 2.30 t ha^{-1} (IRRI, 1997). This area accounts for about 35% of the total cultivated area for rice, and supports about 700 million people. Rice accounts for more than 40% of caloric intake in tropical Asia, reaching more than 65% in many countries and for many poor people (Dawe, 2000). Rice also accounts for 30-40% of protein consumption in Indonesia, Thailand and the Philippines, and more than 60% in Bangladesh and Myanmar. Rosegrant et al. (1995) calculated that the production of rice must be increased by 24% over current levels in order to meet growing food demands over the next 20 years. Consequently, sustainable genetic and agronomic improvements in the yield of rainfed lowland rice are required if this projected increase in productivity is to occur.

Throughout the rainfed rice ecosystem the amount and timing of water supply are considered to be the most severe constraints to productivity (Widawsky and O'Toole, 1990; Zeigler and Puckridge, 1995), with more than 50% of the rainfed lowland being drought-prone (Garrity et al., 1986). Even so, in some areas, high rainfall and poor drainage can raise water depths in paddies that submerge rice plants, causing severe yield loss. The rainfed lowlands are commonly low in soil fertility, and soil nutrient availability also depends on water availability. Therefore, improving the tolerance of rice to both drought and submergence is important, not only to increase yield stability, but also to improve the reliability of the response to improved crop management (Wade et al., 1998).

Against this background, research efforts have been directed to understanding the responses of rainfed lowland rice to drought in the greenhouse and in the field. This is important, as progress in selection of improved cultivars has been slow, due to the complexity of the target population of environments, uncertainty about which physio-morphological traits

Table 1. Sampling times (days after sowing; DAS) and the intervals (in days) between samplings for each drought period based on kg water transpired and rewatering in IR62266 and 4 DHLs.

Genotype	Sampling occasion				Intervals		
	1	2	3	4	Initial to 2 kg *Early drought*	2 to 4 kg *Late drought*	Rewater
Experiment 1							
IR62266	21	33	44	54	12	11	10
DHL-51	21	33	43	53	12	10	10
DHL-54	21	33	42	52	12	9	10
DHL-79	21	31	40	50	10	9	10
DHL-32	21	33	47	57	12	14	10
Experiment 2							
IR62266	21	33	43	53	12	10	10
DHL-51	21	33	42	52	12	9	10
DHL-54	21	33	42	52	12	9	10
DHL-79	21	34	41	51	13	7	10
DHL-32	21	34	48	58	13	14	10

should be of most benefit, and large genotype by environment interactions (Cooper et al., 1999). O'Toole (1982) emphasized the function of root-related traits such as water uptake and those of shoot-related traits such as maintenance of turgor or leaf water potential. Genotypic variation in water uptake may vary depending on the type of stress development, and expression of shoot-related traits may be confounded with the dynamics of plant water uptake (Fukai and Cooper, 1995). For this reason, it is important to quantify the relationship between plant water use (e.g. transpiration, water extraction) and the expression of traits of interest (e.g. leaf water potential, osmotic adjustment, dry matter production and grain yield) in conditions representative of the target population of environments.

Based on evidence from upland rice in continuously aerobic soils, a deep and thick root system was presumed to contribute to intermittent drought avoidance in rainfed lowland rice (O'Toole, 1982; Fukai and Cooper, 1995). However, roots of rainfed lowland rice have direct contact with soils that alternate between saturated and anaerobic, and dry and aerobic conditions. Few roots are observed in deeper soil layers in these conditions (Pantuwan et al., 1997 ; Samson et al., 2002). A number of constraints to the development of a deep and effective root system for water uptake from deeper soil layers have been considered for rainfed lowland situations, including physical and chemical constraints, oxygen supply, rate of stress onset and root signals (Wade et al., 1998). Consequently, it is important that conditions encountered by rainfed lowland rice in the field

be carefully mimicked, when experimental systems for more detailed study are designed. Difficulty in measuring roots in field experiments where site heterogeneity and associated sampling errors are large (Jongdee et al., 1997; Pantuwan et al., 1997) have forced mechanistic studies to controlled environments. A suitable experimental system that mimics conditions encountered by rainfed lowland rice in the field was reported by Wade et al. (2000).

Previous research in rainfed lowland rice has shown that rice cultivars with high seedling vigor before stress imposition and during the early drought phase produced greater root length during the following more severe drought period and had a larger green leaf biomass at the end of the drought period. In these cultivars, transpiration increased slowly and leaf area expanded rapidly, which caused superior drought recovery (Mitchell et al., 1988; Wade et al., 2000). In those cultivars, a larger proportion of assimilate was partitioned to deep roots and deep root branching increased (Azhiri-Sigari et al., 2000; Bañoc et al., 2000a, b). These observations were in accord with the conclusion that a quick root response to changing soil moisture and oxygen levels may be a desirable trait for rainfed lowland rice, because of frequent hydrological changes in rainfed lowland paddy (Ingram et al., 1994). Extraction of water from deeper soil layers in drought was then correlated with average root length density in the soil layer (Kamoshita et al., 2000), and the relationship persisted even with prolonged drought (Kamoshita et al., 2001, 2004). Although cultivars differed in osmotic adjustment as drought progressed, plant performance was more

Table 2. Weather data during the entire experimental period in experiments 1 and 2.

	Minimum temperature (°C)	Maximum temperature (°C)	Evaporation (mm d⁻¹)	Irrad* MJ m⁻² d⁻¹
Experiment 1				
Before stress	23.6	30.2	4.9	10.5
Early drought	23.7	29.5	4.9	10.0
Late drought	24.0	31.7	5.0	13.2
Rewater	24.3	33.1	5.1	13.3
Average	23.9	31.1	5.0	11.8
Experiment 2				
Before stress	24.3	29.6	4.7	6.3
Early drought	24.0	30.6	4.9	9.3
Late drought	23.4	31.4	4.8	11.5
Rewater	24.0	31.7	4.9	10.5
Average	23.9	30.8	4.9	9.4

*Calculated as 57% of actual values from IRRI wetland meteorological station about 500 m away, as defined by Kamoshita et al. (2002b).

closely associated with maintenance of leaf water potential in stress (Jongdee et al., 2002; Kamoshita et al., 2004). Patterns of adaptation of cultivars to different conditions within the rainfed lowlands could then be related to their trait combinations in studies of genotype by environment interactions (Wade et al., 1999).

Previously, the responses to drought and rewatering of cultivars differing widely in plant size, maturity and genetic background have been reported. Such background traits could be expected to confound the expression of traits more directly associated with improved adaptation to drought and recovery in rainfed lowland conditions. For example, difficulty in phenotyping is recognized as the greatest challenge in correct identification of quantitative trait loci in abiotic stress (Kamoshita et al., 2002a), where both constitutive and adaptive traits are present, and each interact with both the conditions prevailing and the genetic background (Kamoshita et al., 2002b). Consequently, the research focus is shifting to studies involving improved genetic materials such as double haploid lines (DHLs), so that traits conferring improved adaptation to drought can be studied with less confounding by genetic background, and perhaps without even the different plant sizes that alter heat load and water loss as drought progresses.

In this study, we examined responses of selected DHLs to drought and rewatering in controlled rainfed lowland conditions in the greenhouse, in order to determine whether confounding by unrelated traits would be less than in previous studies with diverse cultivars. We examined the variation in root traits, water extraction and their interrelationships, among DHLs of similar genetic background.

Materials and method

1. Cultural details

Two experiments were conducted in 2000 in the greenhouse at the International Rice Research Institute, Los Baños, Philippines (14°11´N, 121°15´E, 23 m altitude). A split-plot design with three replicates was used, with two water regimes (well-watered and drought) as main plots and five rice genotypes as subplots.

The genotypes used were IR62266-42-6-2 (IR62266) and four DHLs: IR68586-F2-CA-51 (DHL-51), IR68586-F2-CA-54 (DHL-54), IR68586-F2-CA-79 (DHL-79) and IR68586-F2-CA-32 (DHL-32) derived from a population of 220 anther-culture derived DHLs from the cross CT9993 × IR62266 developed at the International Rice Research Institute (IRRI), Los Baños. These DHLs were chosen based on research by Kamoshita et al. (2002a) in the greenhouse and Babu et al. (2003) in the field, where these DHLs differed in root traits and osmotic adjustment.

PVC pots with 20-cm internal diameter and 55-cm height were used as the experimental unit to control water stress development and allow measurement of secondary physio-morphological traits with less error. Twenty kg of sieved air-dried sandy loam soil, with pH 5.7, was placed in a plastic sleeve inside each PVC pot. Initially, about 17 kg of soil was put into the plastic bag inside the pot and about 6 kg of water was added. The whole soil layer was then stirred with a wooden stick until standing water remained. When the soil surface shrank, another 3 kg of air-dried soil was put into the pot, more water was added, and the soil was puddled again. From about 14 days after sowing, when the seedlings were at 4 to 5-leaf stage, 2 cm of ponded water was maintained. In the drought-

rewatered treatment, pots were drained at 21 DAS, and water withheld until about 4 kg of water was lost by transpiration (see section 2.2). A ponded water depth of 2 cm was maintained throughout the experiment in the well-watered treatment, and after rewatering in the drought-rewatered treatment.

The outside of the pots was covered with aluminum foil to minimize increase in soil temperatures. The soil surface was covered with small cubic polystyrene after drainage and the tops of all the pots were covered with aluminum foil to minimize evaporation, so that any changes in pot weight could be attributed to transpiration from plants and/or watering. The distance between any two neighboring pots was more than 40 cm and the effects of mutual shading were negligible. An adequate amount of fertilizer was supplied, with 2.73 g of urea for nitrogen, 1.84 g of solophos for phosphorus, and 1.04 g of muriate potash for potassium.

Four to five presoaked seeds of each of the genotypes were sown on the wet soil and thinned to one healthy seedling per pot by 10 days after sowing (DAS). The sowing dates were 28 February 2000 for experiment 1 and 24 June 2000 for experiment 2. Experiment 1 lasted 57 days while experiment 2 lasted 58 days. Green algae were removed daily from the ponded water. No disease, insect or weed damage was observed.

2. Measurements
2.1 Meteorological data
The minimum and maximum daily air temperatures were collected by a hygrothermograph and evaporation was measured with seven pan evaporimeters of 20-cm diameter randomly placed inside the greenhouse. The average daily minimum and maximum air temperatures during experiment 1 were 23.9 and 31.1 °C, respectively, and average evaporation was 5.0 mm d^{-1}. In experiment 2, the average daily minimum and maximum air temperatures were 23.9 and 30.8 °C, respectively, and average evaporation was 4.9 mm d^{-1}.
2.2 Transpiration and plant sampling
Daily transpiration was calculated from 21 DAS until the end of the experiments by measuring weight loss in the drought treatment and water added in the well-watered treatment. Cumulative transpiration after 21 DAS was calculated by the sum of daily increments in each water regime.

Plants were sampled at different intervals, according to genotype (Table 1):
1. At 21 DAS, before water was withheld from drought treatments;
2. When cumulative transpiration was about 2.0 kg, between 31 and 34 DAS;
3. When cumulative transpiration was about 4.0 kg, between 40 and 48 DAS;
4. At 10 days after sample 3, that is, between 50

and 58 DAS.

Different dates were chosen for each genotype on the second and third samplings to examine the genotypic differences when all of the genotypes used the same amount of water, thus minimizing the confounding effects of different potential growth in the well-watered treatment. In the fourth sampling, response of all the genotypes to the same duration of 10 days of rewatering was examined. To assess plant response during each stage of drought development and rewatering, we divided the growth periods during the experiment into:
1. Early drought period (between samples 1 and 2);
2. Late drought period (between samples 2 and 3); and
3. Rewatering period (between samples 3 and 4).

Transpiration rate was calculated during each drought period. Plants in both water regimes were sampled at the same time.
2.3 Root parameters
After each sampling of above-ground plant parts, and at 3 kg of transpiration (day 36-39), the soil mass within the plastic sleeve was slowly pulled from the PVC pots and divided into layers of 0-5, 5-10, 10-20, 20-30, 30-40, and 40-50 cm from the soil surface. Roots were carefully separated from the soil on a 1-mm sieve screen. Root length was measured by the COMAIR Root Length Scanner (Hawker De Havilland Victoria Limited) and the root length density (RLD) for each soil layer was calculated. Root dry matter in each soil layer was measured, and total and deep root dry matter below the 30-cm soil layer were calculated. Root to shoot ratio was estimated from total root dry matter divided by total shoot biomass. Deep root ratio was calculated as the proportion of deep root mass to total root mass (Yoshida, 1981).
2.4 Soil water content
In experiment 2, soil water status was monitored daily from 31 DAS till the end of the late drought period, using a 1502 Metallic Time Domain Reflectometer (TDR; Tektronix Inc., Wilsonville, Oregon USA). Five pairs of stainless-steel waveguides were inserted horizontally into the soil from holes drilled in the sides of pots at depths of 5, 15, 25, 35, and 45 cm from the soil surface. Installation, calibration of the TDR and conversion of readings to water content followed the protocol of Kamoshita et al. (2000, 2004). Briefly, the waveguides were connected with the TDR unit using an extension cable and electronic wavelength was recorded daily. The dielectric constant, k, was calculated from the TDR readings according to the equation of Cassel (1992) adjusted by the constant of the machine used in this experiment:

$$k = 4.08 \text{ (TDR reading)}^2$$

The third order polynomial equation between the dielectric constant and volumetric soil water content

(VWC) (Topp et al., 1980; Cassel 1992) was re-calibrated as below:

$$VWC \ (m^3 \ m^{-3}) = 0.12782 + 0.02575 \ k - 0.0018824 \ k^2 + 0.00005856 \ k^3$$

The amount of soil water extraction (WE; g) at each measured depth was calculated by multiplying the difference between VWC and soil water content at first measurement (0.38) by the dissected area of the pot in the following equation:

$$WE \ (g) = (0.38 - VWC) \times 3.14 \ x \ 10^2 \times 1$$

The amount of soil water extraction (WE10) in the 10-cm layer around each measured depth (i.e., 0-10-, 10-20-, 20-30-, 30-40- and 40-50-cm layers) was calculated according to the following equation:

$$WE10 \ (g) = WE \times 10$$

This equation converted the measured TDR values to the water content of the soil mass from 5 cm above to 5 cm below each probe. The total amount of water extracted from all the soil layers was estimated by summing the WE10 at each depth.

3. Statistical analysis

Analysis of variance was conducted for each water regime and LSD at a probability of 5% was determined using Genstat 6 (VSN International Ltd., 2000).

Results

1. Prevailing environmental conditions

Meteorological data are presented in Table 2. Differences between maximum and minimum temperature and evaporation were slight between experiments. Irradiance was higher during experiment 1 than experiment 2, especially before stress imposition at 21 DAS.

2. Transpiration

From the change in pot weights, transpiration rate was faster in experiment 1 than experiment 2 during the first 11 days of withholding water, but increased after that in experiment 2 (Fig. 1a and b). Consequently, transpiration peaked earlier in experiment 1 than experiment 2, at about 4 kg water. DHL-32 had a significantly lower transpiration rate, while DHLs 54 and 79 had higher transpiration rates, especially in the late drought period. Water loss for IR62266 was rapid during the first 7-10 days after withholding water but fell behind DHLs 54 and 79 thereafter in experiment 1. Overall, DHL-51 and IR62266 were intermediate in transpiration rate among genotypes in experiment 2. Water extraction as estimated from the TDR was closely related to cumulative transpiration by pot weighing, for the period from 31 to 42 DAS in experiment 2 (Fig. 1c).

3. Root dry weight

There were more roots in well-watered than drought treatment pots (Fig. 2), especially in shallow soil layers.
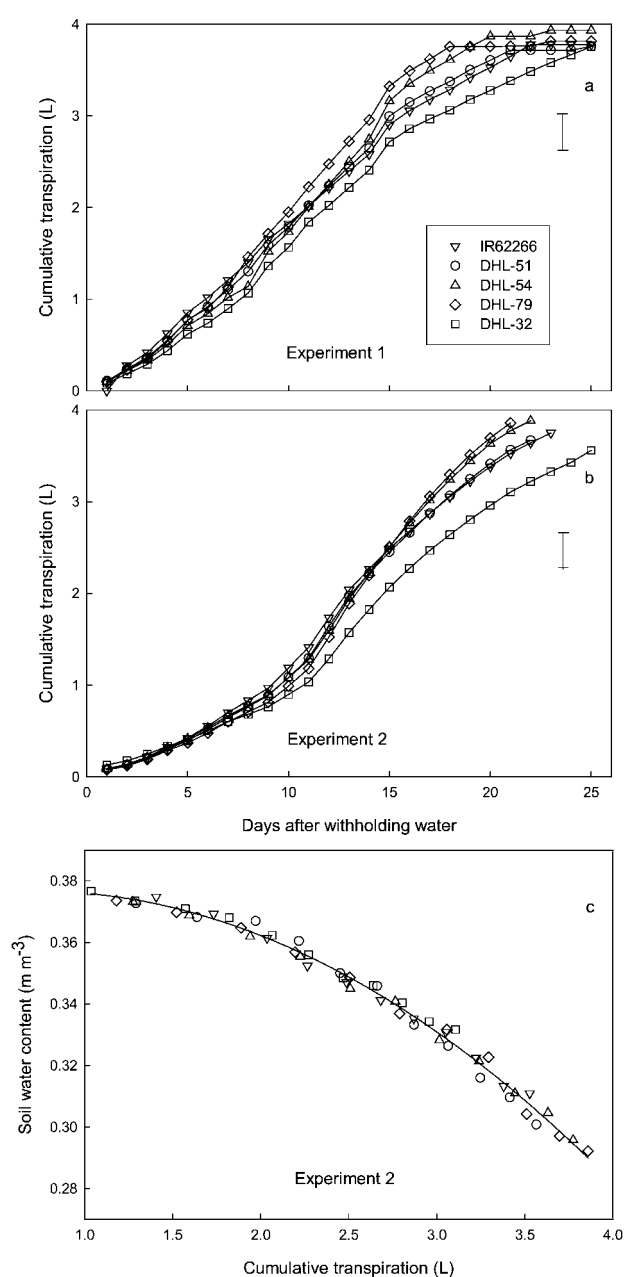

Fig. 1. Cumulative transpiration (L) measured by daily weight loss of pots of IR62266 and 4 DHL during drought in experiment 1 (a) and 2 (b), and (c) comparison of cumulative transpiration with soil water content (m^3 m^{-3}) estimated by time domain reflectometry (TDR) in experiment 2 (31-42 DAS). Nonlinear regression is shown in (c): $Y=0.0125x + 0.0088x^2$, $r^2=0.99$, P=0.01.

In well-watered, few roots were present in deeper soil layers until after about day 40. DHL-32 generally had less root dry weight than the other lines in well-watered treatments. The late increase in deep root dry weight was not consistent among the other 4 lines.

By the end of the drought/rewatering period (Fig. 2), DHLs 79, 51 and 54 generally had more root dry mass than DHL-32 and IR62266. In drought, DHL-79
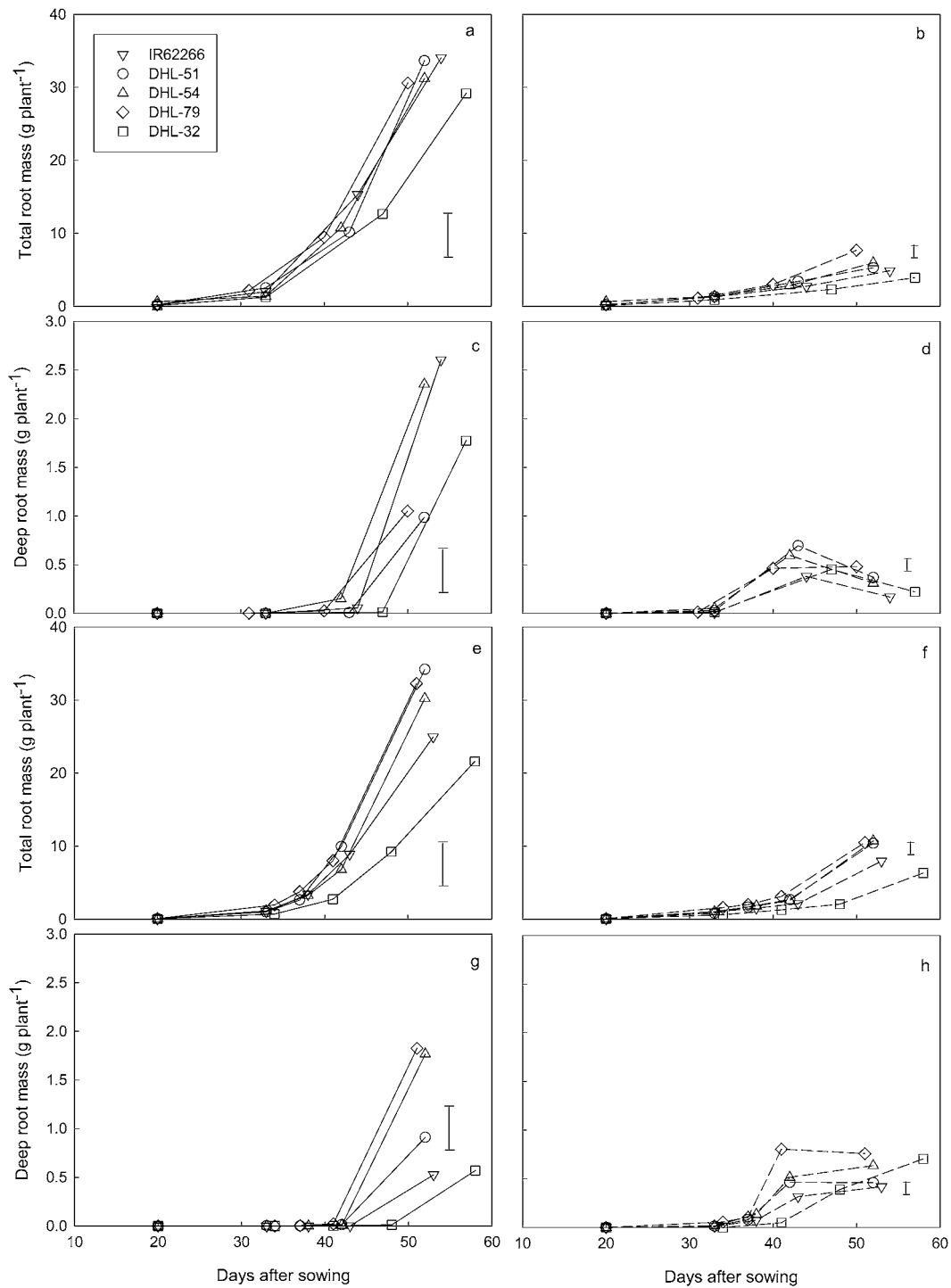
Fig. 2.   Time course of total and deep (below 30 cm) root mass (g plant^{-1}) in well-watered (a, c, e, g) and
         stress (b, d, f, h) treatments of experiments 1 (a to d) and 2 (e to h). Solid lines represent well-watered
         and dotted lines represent drought treatments. Bars represent LSD$_{0.05}$ for root mass.

generally had a higher root dry mass than other lines, especially in deeper layers in experiment 2. Likewise in experiment 1, root mass increased after rewatering in DHL-79 in both soil layers, relative to other lines. After rewatering in experiment 2, deep root mass only increased in DHL-32.

4.   Soil water extraction during drought

The TDRs failed prior to 31 DAS in experiment 2, so data on water extraction by soil layer are only available thereafter. Consequently, no TDR data are available for the first 10 days after drainage (21-31 DAS), when about 1 kg of water was lost from the pots
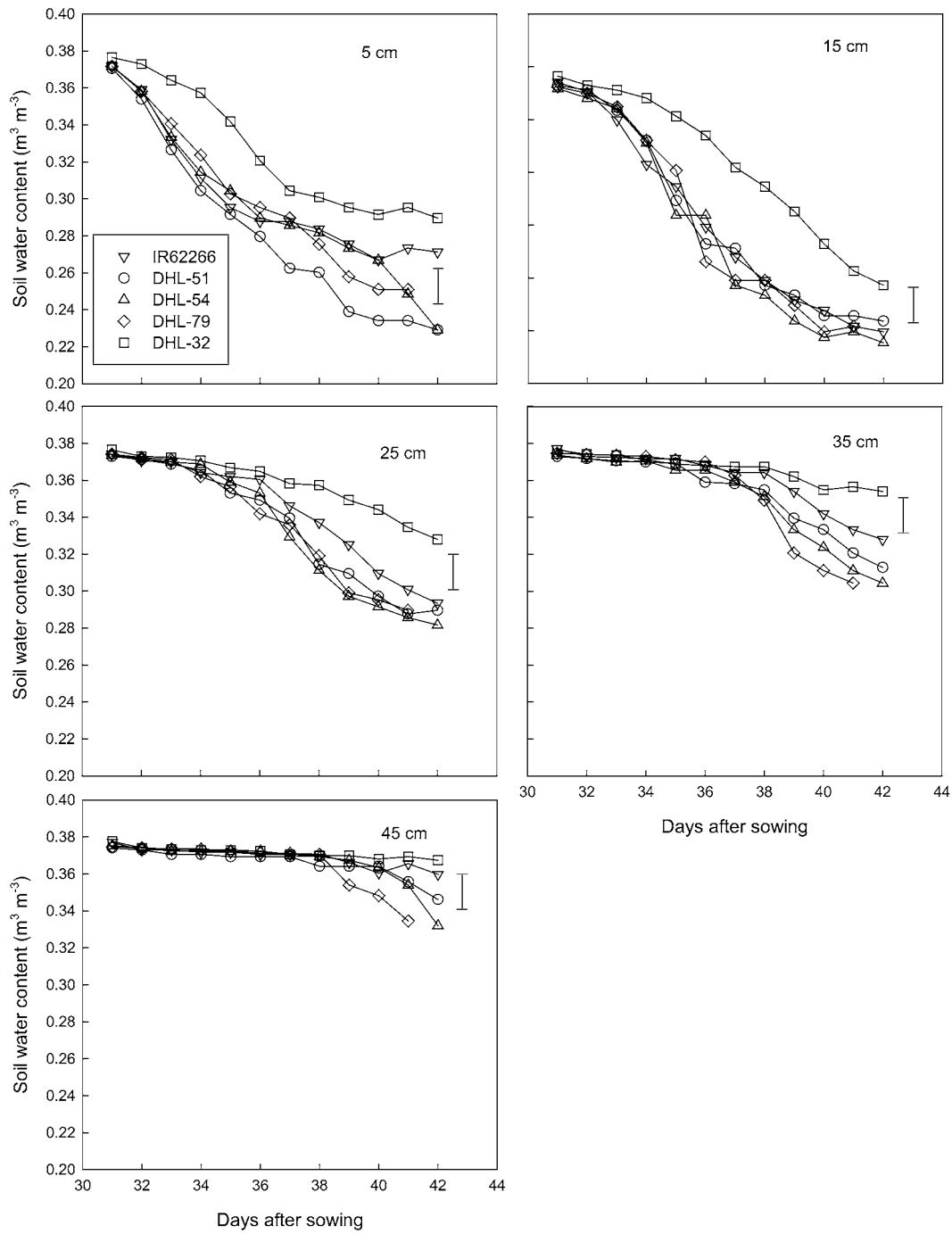
Fig. 3.   Time course of TDR-derived soil volumetric water content (VWC m^3 m^{-3}) from 31 days after sowing till the near the end of the late drought period (43 days after sowing) among IR62266 and 4 DHLs around 5-, 15-, 25-, 35- and 45-cm soil depths. Bars represent $LSD_{0.05}$ for VWC.

in experiment 2 (Fig. 1c).

The progress of soil water extraction in each soil layer from 31 to 42 DAS in Experiment 2 is shown in Fig. 3. Soil water content dropped below 0.38 at day 31 in the surface layer, but not until day 38 at 45 cm depth. Extraction was earlier and faster near the soil surface than in deeper soil layers. By day 42, most available water had been removed by DHL-51 in the surface layer, where soil water content plateaued at 0.23 from day 40. More water was extracted from surface than deeper layers, and some available water remained in deeper layers at day 42.

Lines differed in their commencement, rate and extent of water extraction in the various soil layers (Fig. 3). The progress of water extraction in DHL-32 was slower and less complete than other lines at each
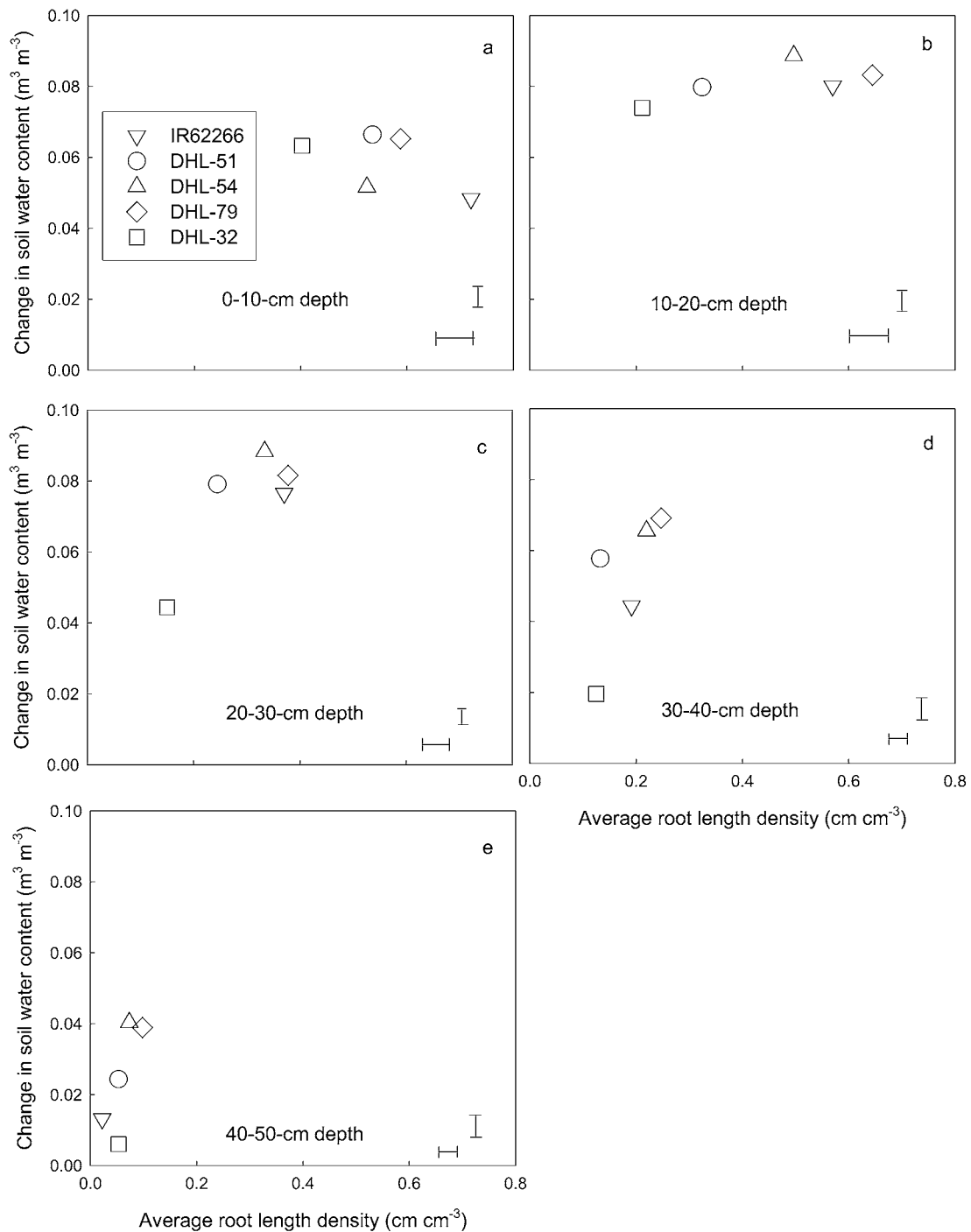
Fig. 4. Relationship between root length density (RLD cm cm^{-3}) at the third sampling (end of late drought period) and change in soil volumetric water content (VWC m^3 m^{-3}) between samples 2 and 3 (during the late drought period) in 0-10-cm (a), 10-20-cm (b), 20-30-cm (c), 30-40-cm (d) and 40-50-cm (e) soil depths. Bars represent LSD$_{0.05}$ for change in RWC (vertical) and RLD (horizontal).

soil depth. Extraction was greater for DHL-54 at 15 and 25 cm and for DHL-79 at 35 and 45 cm soil depth. IR62266 was generally intermediate in water extraction, while DHL-51 extracted water most rapidly from the surface layer.

5. Root length density and rate of water extraction

Root length density was higher in the surface layers than deeper in the soil profile (Fig. 4). RLDs of greater than 0.25 extracted similar amounts of soil water from shallower soil layers over cultivars, with slightly less water removed from 0-10 cm than 10-20 cm and 20-30
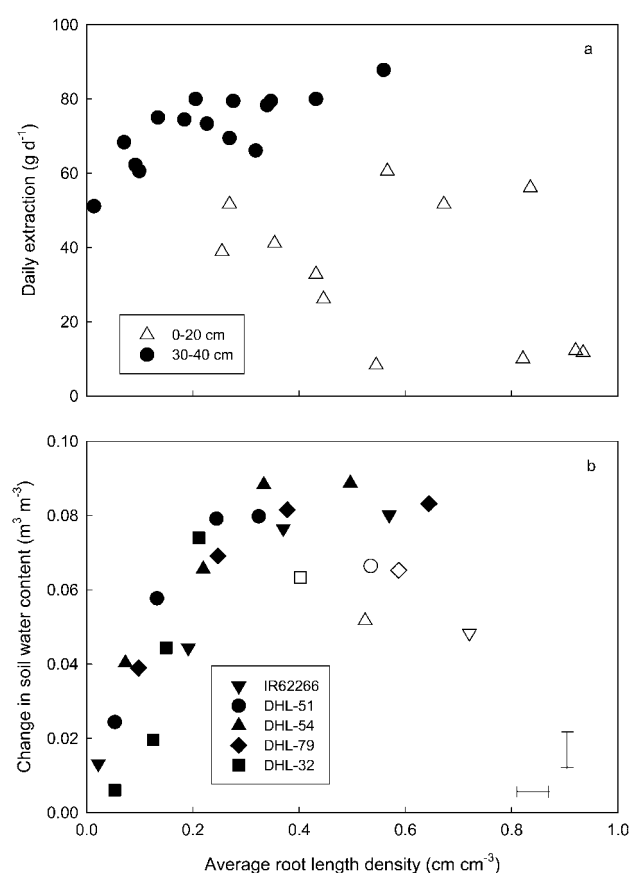
Fig. 5. Combined relationships between root length density (RLD cm cm^{-3}) at the third sampling (end of late drought period) and change in soil volumetric water content (VWC m^3 m^{-3}) in late drought over all soil layers, from Kamoshita et al. 2000 in (a) and Fig. 4 in (b). Values from the surface layer are shown with open symbols [0-20 cm in (a) and 0-10 cm in (b)], while those from deeper layers are shown with closed symbols. Bars represent LSD$_{0.05}$ for change in RWC (vertical) and RLD (horizontal).

cm layers. The only exception was for DHL-32 in 20-30 cm, where extraction was reduced with a lower RLD. In the deeper soil layers, extraction tended to be linearly related to RLD at 30-40 and 40-50 cm, with a greater RLD associated with greater extraction of soil water. In drought, RLDs were greater for DHL-79 and DHL-54, and less for DHL-32. IR62266 had a high RLD near the soil surface, while DHL-79 had a greater RLD at depth.

The overall relationship between RLD and rate of water extraction is explored in Fig. 5, where data in four soil layers from Kamoshita et al. (2000) are shown in (a), and in five soil layers from Fig. 4 of this manuscript are shown in (b). In general, extraction increased with RLD up to a maximum of about 0.08 m^3 m^{-3} change in soil volumetric water content. When data from shallow layers were excluded (5-25 cm in Fig. 5a and 0-10 cm in Fig. 5b), an upper bound relationship between water extraction and root length density was apparent, especially in Fig. 5b. Maximum rates of

extraction were attained at RLDs greater than 0.30 cm cm^{-3} (Fig. 5b).

6. Root parameters

In well-watered conditions, DHL-51 had a higher root growth rate, while IR62266 had a higher specific root length, and DHL-79 a higher root to shoot ratio (Table 3). But in drought, DHL-79 had the greatest root growth rate, while other traits were less consistent across experiments in drought. The exception was the high specific root length of IR62266, which was observed in all four environments.

In experiment 1, there was reduction in root growth rate during drought (Table 3). Likewise, root to shoot ratio and root mass per tiller also decreased under drought. Deep root mass, deep root ratio, and specific root length increased under drought conditions. The same pattern was observed in experiment 2, except DHL-32 had larger deep root mass during drought than well-watered. Root growth rate was consistently lowest for DHL-32 in both experiments in both well-watered and drought conditions. DHL-51 had highest root growth rate in well-watered conditions but DHL 79 exhibited the fastest root growth under drought conditions. Patterns of response for root to shoot ratio, deep root mass, and deep root ratio varied within experiment runs. Notably, IR62266 had the highest specific root length in all water regimes and in both experiments.

Discussion

1. Transpiration and water extraction

The progress of transpirational water loss was slower in the second experiment (Fig. 1), due to lower irradiance (Table 2). There was significant variation among DHLs in the progress of water loss (Fig. 1), which was consistent with the pattern of water extraction for the various soil layers (Fig. 3). DHLs differed in the timing, rate and extent of water extraction from different soil layers, with DHL-32 slower and DHLs 54 and 79 faster in water extraction. These results are in accord with Kamoshita et al. (2000), who also reported variation in water extraction among cultivars of rainfed lowland rice in the greenhouse, but for materials that differed widely in phenology, plant size and genetic background

2. Critical RLD for water extraction in the greenhouse

Lilley and Fukai (1994) reported a critical RLD for water extraction in upland rice of about 1.5 cm cm^{-3}, a value higher than that commonly reported for other gramineous species such as wheat and sorghum (Smit et al., 2000). Kamoshita et al. (2000) demonstrated that water extraction increased with RLD in deeper soil layers in the greenhouse, but their data were not sufficient to identify a critical RLD for water extraction

Table 3.   Average root growth rate from 21 to 43 days after sowing, root to shoot ratio, root mass per tiller, specific root length, deep root mass and deep root ratio below 30 cm from soil surface at 43 days after sowing among parent and 4 DHLs. Mean values and LSD$_{0.05}$ for genotype effect are also shown.

Genotype	Root growth rate	Root to shoot ratio	Root mass per tiller	Specific root length	Deep root mass	Deep root ratio
	(g d^{-1})	(%)	(mg)	(m g^{-1})	(g)	(%)
Experiment 1						
Well-watered						
IR62266	0.996	*35.2*	162	**100**	0.056	0.4
DHL-51	**1.045**	41.9	185	*69*	*0.007*	*0.1*
DHL-54	0.956	37.2	164	86	**0.152**	**1.4**
DHL-79	1.013	**52.3**	*152*	71	0.026	0.3
DHL-32	*0.785*	37.6	**198**	77	0.012	*0.1*
Mean	0.959	40.8	172	81	0.051	0.4
LSD$_{0.05}$	0.046	3.1	8	6	0.027	0.3
Drought						
IR62266	0.137	*14.8*	*76*	**136**	*0.379*	*13.8*
DHL-51	0.159	17.6	**158**	94	**0.695**	20.3
DHL-54	0.167	16.6	114	94	0.595	**21.1**
DHL-79	**0.249**	**21.3**	128	*91*	0.462	15.5
DHL-32	*0.103*	15.1	107	92	0.452	19.5
Mean	0.163	17.1	116	101	0.516	18.0
LSD$_{0.05}$	0.024	1.2	13	9	0.057	1.4
Experiment 2						
Well-watered						
IR62266	0.754	38.8	*262*	**88**	*0.526*	*2.1*
DHL-51	**1.068**	57.2	506	56	0.910	2.7
DHL-54	0.943	47.3	401	52	1.767	**5.8**
DHL-79	1.039	**65.6**	**535**	*48*	**1.824**	5.7
DHL-32	*0.567*	*31.8*	405	65	0.569	2.6
Mean	0.874	48.2	422	62	1.119	3.8
LSD$_{0.05}$	0.094	6.1	48	7	0.284	0.8
Drought						
IR62266	0.239	24.2	*107*	**104**	*0.418*	5.3
DHL-51	0.323	**34.8**	218	74	0.460	*4.4*
DHL-54	0.333	31.0	198	*62*	0.635	5.9
DHL-79	**0.337**	32.6	**258**	67	**0.757**	7.2
DHL-32	*0.165*	*23.0*	191	79	0.704	**11.2**
Mean	0.279	29.1	195	77	0.595	6.8
LSD$_{0.05}$	0.034	2.3	25	7	0.067	1.2

Within a column, the largest value is shown in bold, and the smallest in italics.

in rainfed lowland rice (Fig. 5a). The data from this study were consistent and not confounded by genetic background, so an upper bound relationship between extraction and RLD over DHLs and soil depths was readily apparent (Fig. 5b). This result suggests a critical value for water extraction in the greenhouse of about 0.30 cm cm^{-3}, which is similar to a report for irrigated rice of 0.1 cm cm^{-3} (Penning de Vries et al., 1989), but much less than the report of 1.5 cm cm^{-3} for upland rice (Lilley and Fukai, 1994). It is possible, however, that the RLD decreased later in the drought period with loss of fine roots, which may have reduced

our critical value of RLD.

Because the soil used in these experiments was carefully prepared by sieving and puddling in the pots, soil hydrology would be expected to be quite consistent, which is similar to conditions encountered in puddled fields in irrigated situations. Consequently, a lower critical RLD is expected here, so this report of 0.3 cm cm^{-3} is comparable with the report of 0.1 cm cm^{-3} for irrigated conditions. Our value may be slightly higher as the pots were saturated rather than flooded for the first 14 days. In contrast, when crops are direct-seeded rather than transplanted and soils

remain aerobic, soil disturbance is minimal, and roots encounter a less homogeneous environment. Presumably, roots must then find a passage between aggregates, so soil structural heterogeneity and the presence of root channels and biopores become more important (Passioura, 2002). Consequently, reports of critical values of root length density in the field tend to be larger, with values in excess of 1.5 or 1.6 seeming necessary in deeper layers (Pantuwan et al., 1997), especially if a hardpan is present (Samson et al., 2002). Nevertheless, quality data on water extraction by rainfed lowland rice in the field are scarce, and no reports specifically explore critical root length densities for water extraction in the field. Further research is needed in the field.

3.   Root parameters for greater resource capture

The greater water extraction by DHL-79 in deeper soil layers (Fig. 3) was associated with a greater root dry weight (Fig. 2), a greater root length density (Fig. 3), and a higher root growth rate below 30 cm (Table 3). The greater specific root length of IR62266 (Table 3) was of limited benefit for water extraction, though may be helpful for capture of immobile nutrients such as soil P. These results suggest that in uniform soil conditions in the greenhouse, attaining the critical root length density of 0.30 cm cm^{-3} in the various soil layers is the key parameter for water extraction. The relationship would be more complex in the field, and this conclusion requires field validation.

4.   Completeness of water extraction and resources

Despite the withholding of water for almost 36 days, by which time transpiration had peaked, especially in experiment 1 (Fig. 1), not all of the plant available water had been extracted, particularly in deeper soil layers (Fig. 3). While DHL-79, DHL-54 and DHL-51 continued to extract some water at 35 and 45 cm soil depths, substantial water remained between the apparent lower limit of plant available water content of 0.23 (Fig. 3a) and 0.33 (Fig. 3e). Had the drought been further extended and evaporative demand continued, the plants would eventually have taken up that water as well, as Kamoshita et al. (2004) demonstrated in prolonged drought.

Conclusions

We obtained a critical root length density for water extraction of 0.30 cm cm^{-3} in puddled soil conditions, which was similar to the 0.1 cm cm^{-3} reported for irrigated rice (Penning de Vries, 1989). Both values are much lower than those reported for upland rice (Lilley and Fukai, 1994) or rainfed lowland rice (Pantuwan et al., 1997; Samson et al., 2002) in the field. A higher critical value is expected in nonpuddled soils in the field, especially when root channels and biopores are important, and this requires field validation. The use

of DHLs provided a more robust basis for detailed physiological analysis and identification of traits conferring an advantage during drought and after rewatering, because of reduced confounding effects by genetic background.

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