

Assessing root growth and water extraction for rainfed rice

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Preamble

In drought-prone rainfed lowlands, rice encounters fluctuating water conditions in the same season, ranging from anaerobic soils with ponded water to aerobic soils with water deficit (Wade et al 1998, 1999a, b). Other rainfed environments encounter varying combinations of stresses over a related range of hydrology and oxygen status, from submergence to saturated soils to severe water deficit. Whichever ecosystem is the target, rice fields are variable—especially those that are rainfed—and field measurements of root systems are difficult. Controlled environments offer the opportunity to provide repeatable conditions that mimic those likely to be encountered in the field, but in circumstances in which reliable measurements on root growth and water extraction can be obtained. Water uptake can readily be monitored by weighing the pots in which plants are growing. For a more complete understanding, water extraction by soil layer can be measured using time-domain reflectometry. Field validation is essential for correct results.

Materials used

- PVC pipes, 0.20 m in diameter with lids; silicone sealant; and platform balance with 35-kg capacity
- Comair Root Length Scanner (Hawker De Havilland Victoria Limited, Australia)
- 1502 Metallic Time Domain Reflectometer (Tektronix Inc., Wilsonville, Oregon, USA)
- Ocular micrometer for root thickness measurements

Methods adopted

A simple experimental protocol was developed to mimic the fluctuating hydrology of rainfed lowland fields, using soil columns in pots containing a single rice plant (Wade et al 2000, Azhiri-Sigari et al 2000, Kamoshita et al 2000, 2004). Each pot is an experimental unit to control water deficit and allow measurement with less error.

Cultural details

Cylindrical columns are made from polyvinyl chloride (PVC) of 0.20-m internal diameter and 0.55-m height, with lids fitted to the bases with epoxy to seal the pots. Twenty-one kg of sieved air-dried Maahas clay soil (28% clay, 44% silt, 28% sand; pH 5.2; Wopereis 1993) are placed in a plastic sleeve inside each column, with each 5-cm

increment firmed to a consistent bulk density in turn. Holes for drainage or water entry are drilled just above the base of the pot and covered with a rubber stopper for the period during which ponded water is required (before imposition of stress). Four presoaked seeds are sown on the wet soil and thinned to one healthy seedling per pot by 10 days after sowing (DAS). The top of each column is then covered with aluminum foil around the base of the plant to minimize soil evaporation. The sides of the pots are also covered with aluminum foil to minimize the increase in soil temperature. The distance between any two neighboring pots is usually more than 0.40 m, to keep the effects of mutual shading negligible. A split-plot design is used, with two watering regimes (well-watered and drought followed by re-watering) as main plots and genotypes as subplots, with five replicates. Ponded water of 0.02-m depth is maintained in the well-watered treatment. At the beginning of the water-deficit treatment, water is drained from the bottom of the columns, and no further water is added until the end of the drought treatment, when water loss is replenished and then water is added daily to keep the level of ponded water similar to that of the well-watered treatment.

Measurements

The minimum and maximum daily air temperatures are collected by a thermohydrograph, and evaporation measured with seven pan evaporimeters randomly placed inside the greenhouse. Daily transpiration or plant water uptake is measured by weight loss using a platform balance of 35-kg capacity in the drought treatment and the amount of water added is measured in the well-watered treatment. Cumulative transpiration is calculated as the sum of daily increments. Plants are harvested before stress imposition, at the end of the drought period, and the end of the

re-watering period. After sampling of above-ground parts, the soil mass within the plastic sleeve is slowly pulled from the PVC columns, and divided into layers of 0–5, 5–10, 10–20, 20–30, 30–40, and 40–50 cm from the soil surface. Gravimetric water content and bulk density are taken from soil samples of known volume from each soil layer. Roots are carefully separated from the soil on a 1-mm sieve screen. Root length is determined with the Comair Root Length Scanner for each soil layer, and root dry weight is determined. Root thickness is measured with an ocular micrometer for major seminal and nodal root axes at a distance of 1 cm from the crown of the plant. Root length density, root dry mass per tiller, root to shoot ratio, deep root to shoot ratio, and specific root length are calculated.

Time Domain Reflectometer

The water status of each soil layer can be monitored daily during the drought period using a Time Domain Reflectometer. Five pairs of stainless-steel waveguides are 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 (Fig. 1). The waveguides are connected to the TDR unit using an extension cable, and electronic wavelength is recorded daily. The dielectric constant, k , is calculated from the TDR readings according to the equation of Cassel (1992) adjusted by the constant of the machine used in the experiment, in our case:

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

The three-degree polynomial equation between the dielectric constant and volumetric soil water content (VWC) (Topp et al 1980, Cassel 1992) is re-calibrated as below:

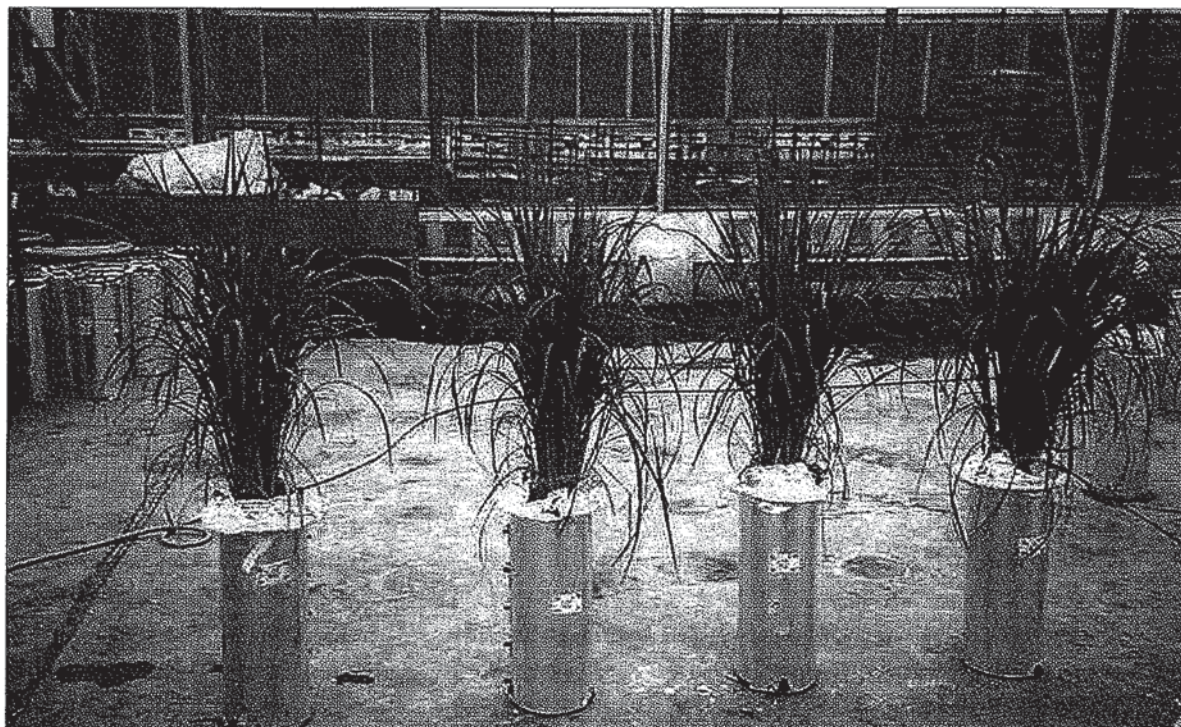


Fig. 1. Plants growing in PVC cylinders with holes drilled in the side for insertion of TDR waveguides to monitor soil water uptake.

$$\text{VWC (m}^3 \text{ m}^{-3}\text{)} = 0.080280 + 0.036329 k - 0.002816 k^2 + 0.00008416 k^3$$

The amount of soil water extraction (WE; g) at each measured depth is calculated by multiplying the difference between VWC and soil water content just after drainage by the dissected area of the pot in the following equation:

$$\text{WE (g)} = (0.50 - \text{VWC}) \times 3.14 \times 10^2$$

Since the TDR may not measure high VWC well, the estimated value of VWC just after drainage can be calculated from the gravimetric water content of the soil ((pot weight after draining – weight of PVC column)/mass of dry soil that was filled into the PVC column) times the bulk density of the soil (mass of dry soil that was filled into the PVC column/volume of the PVC column that is occupied by the soil).

Soil water extraction (WE10) in the 10-cm layer around each measured depth (i.e., 5–15-, 15–25-, 25–35-, and 35–45-cm layers) is calculated according to the following equation:

$$\text{WE10 (g)} = \text{WE} \times 10$$

This equation converts the measured TDR values to the water content of the soil mass from 5 cm above and below each probe. The total amount of water extracted from the 5–45-cm soil layer can be estimated by simply summing the WE10 at each depth. The amounts of soil water extracted from the 0–5- and 45–50-cm layers are not included in the TDR estimation because measurements may become invalid if all of the volume scanned by the waveguide is not soil.

Traits

- Leaf stage, tiller number, and leaf area
- Progress of water use by weighing of soil columns on platform balance
- Number of seminal and nodal roots
- Root length of the main seminal and nodal root axes
- Gravimetric soil water content and bulk density
- Root and shoot dry mass

Precautions

Care is needed to ensure consistency in packing of soil into the columns so that bulk density is consistent. Soil should be added to the columns in 5-cm-depth increments, which should be pressed into place with a flat plunger before the next increment is added. Using a consistent mass of soil for each pot is essential to ensure that a consistent volume of plant-available water is available to each genotype.

Case study

The experimental regime described above was implemented by Siopongco et al (2005, 2006), with drought pots drained at 21 DAS and water withheld until about 4 kg of water was lost by transpiration, as estimated by pot weighing. Soil water content in each soil layer was also evaluated nondestructively during the drought period using TDR, from 31 DAS (10 days after drought imposition, during the late drought period).

Results

There was close agreement between estimates of soil water content from pot weight and TDR measurements. Genotypes differed significantly in root parameters and in patterns of water

extraction over soil depth. The greater water extraction by DHL-79 in deeper soil layers was associated with a greater root dry weight, a greater root length density, and a higher root growth rate below 30 cm (Table 1). The system provided robust measurements of root growth and water extraction, for quantitative assessment of rice response to water deficit under rainfed lowland conditions. These methods can be adapted to other target ecosystems for study of root growth and water extraction. In addition, the methods could be adapted for study of nutrient uptake dynamics in relation to patterns of root growth in controlled conditions. The use of undisturbed soil cores may provide additional merit for nutrient studies in nonpuddled soils.

Common mistakes

The most common mistake is to inadequately simulate the complex hydrology of rainfed lowland fields, where plants are subjected to fluctuating water conditions, from anaerobic soils with ponded water to aerobic soils with water deficit, during the same growing season. Simplifying the water regime, by using saturated soils from aerobic culture, or well-watered soils as in a favorable upland environment, alters the pretreatment condition before the onset of water deficit, so the plants encounter water deficit from a different initial phenotype. Second, it is essential that the duration of the drought period match the expected duration and intensity of water deficit in the field. If plants are commonly subjected to drought of 28 days' duration in the field, it is essential that the drought period be 28 days in the soil columns, with the intensity of water deficit (i.e., the extent of water extraction) being similar to the field (i.e., similar soil water potential or similar percent of plant-available volumetric

Table 1. 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 four DHLs. Mean values and $LSD_{0.05}$ for genotype effect are also shown.

Genotype	Root growth rate ^a (g d ⁻¹)	Root to shoot ratio (%)	Root mass per tiller (mg)	Specific root length (m g ⁻¹)	Deep root mass (g)	Deep root ratio (%)
Experiment 1						
<i>Well-watered</i>						
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
<i>Drought</i>						
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						
<i>Well-watered</i>						
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
<i>Drought</i>						
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

^aWithin a column, the largest value is shown in bold, and the smallest in italics.
Source: Siopongco et al (2005).

water remaining). For valid outcomes in any target environment (e.g., irrigated lowland, rainfed lowland, flood prone, upland, aerobic, alternate wetting and drying, etc.), it is essential that conditions in soil columns mimic those in the field. Hydrologic sequence has strong implications for patterns of $G \times E$ and adaptation (Wade et al 1999c, Samson et al 2002, Acuna et al 2008). Consequently, field validation is essential for controlled-environment studies to ensure that results are correct.

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Notes

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