Assessing root penetration ability and resource capture from deeper soil layers

Len J. Wade, Rolando T. Cruz, Joel Siopongco, Sergio Moroni,
Benjamin K. Samson, and Tina Acuna

Preamble

Rice crops commonly encounter zones of restricted root access, which can greatly reduce uptake of resources from deeper soil layers. As a result, yield can decrease with greater vulnerability to fluctuating weather conditions, especially under rainfed systems (Samson et al 2002). Root access can be restricted by hardpan formation during cultivation, smearing during puddling, or sudden changes in soil texture with depth. These zones of higher soil strength and increased impedance to root elongation are not uniform across the field, so repeatable screens involving the placement of a paraffin wax/petroleum jelly layer in a soil column have been used to identify promising lines (Yu et al 1995, Ray et al 1996, Babu et al 2001, Clark et al 2000, 2002). Field validation is still essential (Samson et al 2002).

Materials used

- Paraffin wax pellets
- Petroleum jelly (Vaseline, Sigma-Aldrich)
- PVC pipes, 0.20 m in diameter

Methods adopted

Wax layers

Wax layers were prepared by melting together paraffin wax pellets with petroleum jelly in the required quantities to produce wax layers (WV) 0.20 m in diameter and 0.03 or 0.05 m thick of 3:97, 20:80, 40:60, 60:40, and 80:20 paraffin wax:petroleum jelly (Acuna and Wade 2005, Acuna et al 2007), following the techniques of Yu et al (1995) and Clark et al (2000). The mixture was poured into molds of 0.20 m in diameter to match the internal diameter of the polyvinyl chloride (PVC) columns. Yu et al (1995) reported that these wax layers were equivalent to strengths of 0.1, 0.2, 0.5, 1.5, and 3.0 MPa, respectively, at 20 °C. With a hand-held pressure gauge, our values were 0.01, 0.03, 1.00, 1.70, and 2.50 MPa.

Cultural details

Cylindrical columns made from PVC of 0.20-m internal diameter and 0.55-m height were split in half length-ways for easy access to roots. Columns were partially filled with air-dried Mahaas clay soil (28% clay, 44% silt, 28% sand; pH 5.2, Wopereis 1993) to a depth of 0.20 m below the intended soil surface. The wax layer was placed on top and sealed to the column wall with silicone to prevent roots from escaping. The column above the wax layer was then filled with Mahaas clay soil, so the wax layer was at 0.20-m depth. Holes for drainage or water entry were drilled in pots just above the depth of the wax layer, and just above the base of the column, which was sealed to the column with epoxy (Siopongco et al 2008, 2009). Depending on the treatment, these drainage holes could be sealed with a rubber stopper to prevent water entry or loss, or the stopper could be removed to allow drainage or water entry. Columns were watered from the soil surface and/or by placing the column in a shallow tray containing 0.10 mm of water, according to the treatment.

Treatments

Four contrasting water regimes were applied: well-watered above and below the wax layer (WW), water deficit above and well-watered below (WD/WW), well-watered above and water deficit below (WW/WD), and water deficit above and below the wax layer (WD/WD). For WW, the water regime depended upon the target environment. For rainfed lowland, a flooding depth of 0.02 m was maintained, while for aerobic or upland conditions, soil was watered to saturation without ponding of surface water. For WD, columns were drained and watering withheld for the period of drought stress. If re-watering was intended after the period of drought stress, conditions reverted to the WW treatment imposed earlier.

Variations

This experimental system allows water availability to be varied independently of wax layer strength, allowing exploration of how an entry may be successful in penetrating a zone of higher impedance (Acuna and Wade 2005, Acuna et al 2007). Likewise, a series of wax layers may be placed within the same soil column, with successive wax layers being higher in impedance (i.e., higher wax content), in order to efficiently screen for root penetration ability (Kubo et al 2004). Alternative methods can also be evaluated, such as weighted soils, as it is important to recognize that all artificial systems involve assumptions about root behavior (Clark et al 2002). A root finds a passage between soil particles, rather than penetrating per se. The wax layer itself is anaerobic and uniform in texture, so it is recommended that the wax layer be kept thin. Although agreement in penetration ability is reported between wax layers and the field (Clark et al 2002, Samson et al 2002), we believe it is essential to validate results in the field.

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 above and below the wax layer
- Root length of the main seminal and nodal root axes below the wax layer
- Gravimetric soil water content and bulk density above and below the wax layer
- Root and shoot dry mass
- Proportions of root number and of root dry weight below the wax layer

Precautions

To ensure consistent resistance to root penetration, the paraffin wax/vaseline mixture should be poured into the mold on an electronic balance so that consistent mass and thickness are attained (Acuna and Wade 2005, Acuna et al 2007). Allow the mixture to cool and harden before placing it in the soil columns. Use a consistent mass of soil below and above the wax layer in each column. Add soil to the column in 0.05-m increments, pressing each layer into place firmly with a flat plunger to ensure that a consistent bulk density is attained. It is essential that the wax layer be placed gently but firmly onto the leveled and tamped soil surface in the column, prior to sealing the wax layer to the column wall with silicone. Soil above the wax layer is then added and firmed in 0.05-m increments until the desired depth to wax layer is achieved.

Case study

A modified system of sealing the wax layer in place, so there was no prospect of root escape through cracks or past the wax layer, was devised by Cruz et al (n.d.), as described below.

Methods

PVC cylinders were cut horizontally to create an upper 20-cm and a lower 30-cm layer (Fig. 1A, B). The upper and lower halves of the cylinders were attached by clamps and silicone was applied on the cut surfaces. The bottom of the lower half was capped and silicone was applied on the edges to prevent water seepage. After increments of soil were added to form the lower layer, clamps were removed, and a thin-walled PVC cylinder (0.3 cm thick, 30 cm long) with a 30-cm slit tightly enclosed the upper portion of the lower cylinder. The wax layer was placed on the surface of the PVC and soil (i.e., it extended to the outside edge of the inner cylinder). Then, the upper 20-cm PVC cylinder was slid inside the jacket until it tightly compressed the wax layer, and the clamp was tightened around the jacket over the wax layer zone, rendering it rigid. Increments of soil were then loaded and pressed into the upper cylinder. Compression of the wax layer by the PVC cylinders prevented root escape (Fig. 1 C, D).

Two wax strengths were used: (a) 35 wax:65 vaseline, 4 mm thick, giving a wax strength of 0.05 MPa, and (b) 70 wax:30 vaseline, 8 mm thick, giving a wax strength of 0.40 MPa. Pots were well watered initially, with a ponded water depth of 1 cm being maintained. From 26 DAS (days after sowing), water was withheld in the drought treatment for 28 days.

Results

In the well-watered treatment, the number of nodal roots that penetrated the wax layer increased linearly, and root penetration was higher with 0.05 MPa than with 0.40 MPa wax strength (Table 1). Under water deficit, the number of penetrated roots declined significantly, and the reduction was greater with 0.40 MPa wax strength. Percent of total root dry weight below the wax layer was higher in the stress than in the control. In stress and control, the percent of total root dry weight below the wax layer was greater with 0.05 MPa wax strength.

Root dry weight in the control increased linearly during the treatment period. The increase was greater with 0.05 MPa wax strength than with 0.40 MPa wax strength. Under water deficit, root dry weight decreased significantly, and reached a plateau at 21 DAWW (days after withholding water). Although root dry weight was lower under water deficit, the plateau was higher for 0.05 MPa wax strength than for 0.40 MPa wax strength. Shoot dry weight increased exponentially in well-watered at both wax strengths. Under water deficit, shoot dry weight was lower than the control, but still increased linearly with time.

This system, with the wax layer carefully clamped in place, allowed robust data to be collected on root penetration and root growth below the wax layer, without any root escape.

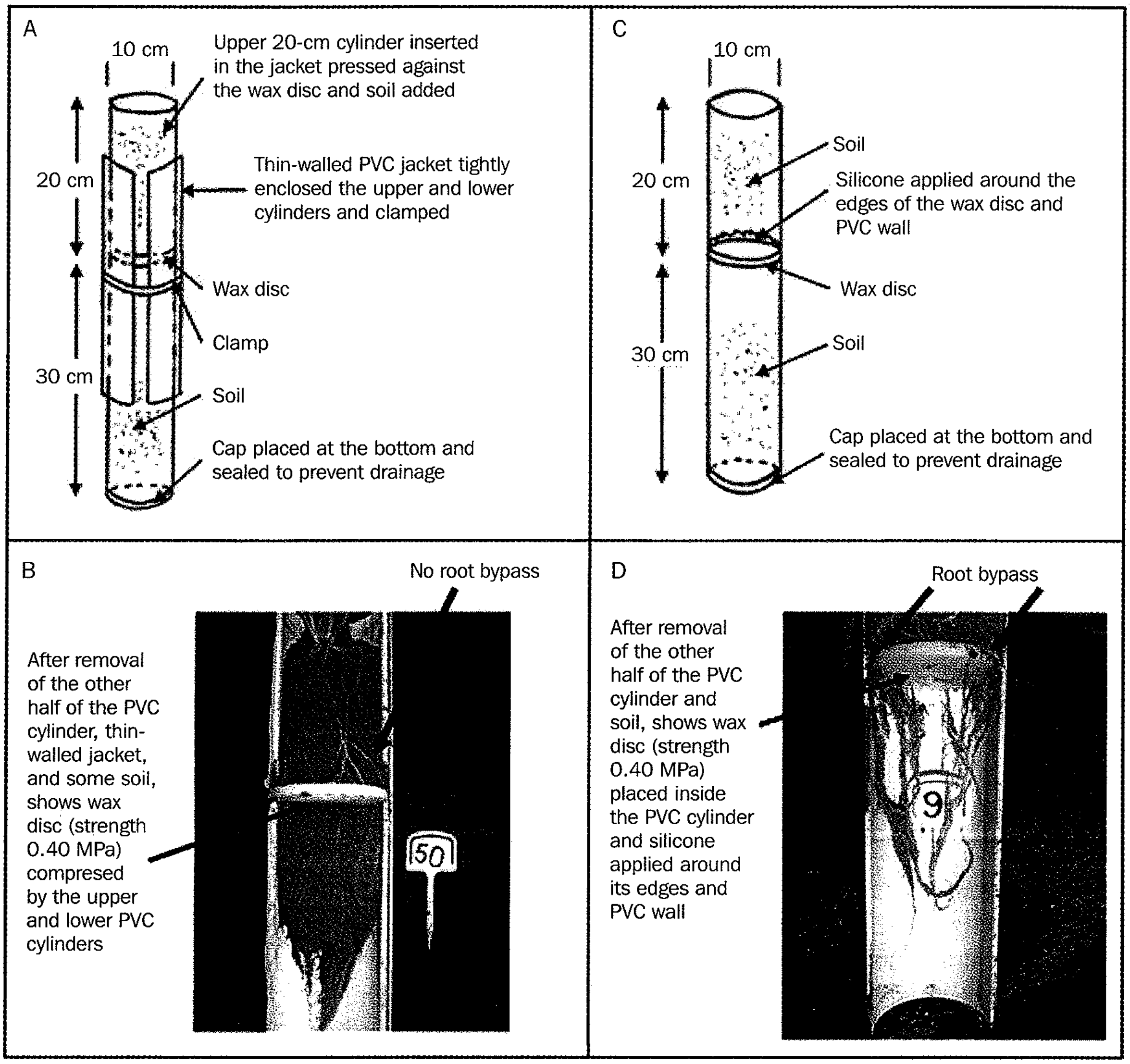

Fig. 1. (A) Schematic of the PVC cylinder/pot–wax disc/layer system used in the current study. After placing the wax disc on the surface of the soil and lower 30-cm PVC cylinder, the upper 20-cm PVC cylinder was inserted in the thin-walled PVC jacket until it compressed the wax disc. Then, soil, NPK fertilizers, and water were added. (B) Compressing the wax disc (8 mm thick, 0.40 MPa wax strength) prevented root bypass as shown here. A similar response was observed with wax disc 4 mm thick and 0.05 MPa wax strength (photo not shown). Hence, the technique of compressing the wax disc enabled better assessment of root penetration through wax layers of various strengths. (C) Schematic of the PVC cylinder–wax disc system used in an earlier trial. After inserting the wax disc and laying it on the surface of the soil at 20-cm depth, silicone was applied around the edges of the wax disc and wall of the cylinder. (D) Application of silicone around the edges of the wax disc and PVC wall did not completely seal the edges of the wax disc and PVC wall and this resulted in 30–40% of the roots bypassing the wax disc or roots passing through spaces between the silicone and wax disc and between the silicone and PVC wall.

Table 1. Number of roots that penetrated the wax layer and % of total root dry weight below the wax layer for CT and IR at 28 DAWW. Root dry weight (above + below) and shoot dry weight of CT and IR at 28 DAWW. WW = well-watered, WD = water deficit, DAWW = days after withholding water.

Treatment	No. of roots that penetrated the wax layer	% of total root dry weight below the wax layer	Root dry weight (g/plant)	Shoot dry weight (g/plant)
0.05 MPa wax				
WW-CT	80.0	24.9	3.5	11.9
WW-IR	64.4	20.0	2.3	8.7
WD-CT	20.7	57.3	0.8	4.5
WD-IR	19.3	60.3	0.7	4.7
0.40 MPa wax				
WW-CT	63.3	12.7	2.9	12.0
WW-IR	46.3	12.0	2.2	8.4
WD-CT	9.0	27.6	0.6	5.2
WD-IR	7.0	27.0	0.4	4.4

Common mistakes

The most common mistake is to break the wax layer or to separate it from the column wall, allowing roots to escape past the impeding wax layer. Most commonly, this occurs if pots are handled carelessly during pot weighing for cumulative water use, or during measurements and re-watering. The case study above outlines a system for eliminating root bypass, by robustly securing the wax layer in place and preventing column flexing by enclosing the column inside a clamped jacket, so the column and wax layer remain rigid. In addition, artificial methods include assumptions about root behavior, so it is essential that results from controlled screens be tested and validated in the field (Clark et al 2002, Samson et al 2002).

References

Acuna TLB, Pasuquin E, Wade LJ. 2007. Genotypic differences in root penetration ability of wheat through thin wax layers in contrasting water regimes and in the field. Plant Soil 301:135-149.

Acuna TLB, Wade LJ. 2005. Root penetration ability of wheat through thin wax-layers under drought and well-watered conditions. Aust. J. Agric. Res. 56:1235-1244.

Babu RC, Shashidhar HE, Lilley JM, Thanh ND, Ray JD, Sadasivam S, Sarkarung S, O'Toole JC, Nguyen HT. 2001. Variation in root penetration ability, osmotic adjustment and dehydration tolerance among accessions of rice adapted to rainfed lowland and upland ecosystems. Plant Breed. 120:233-238.

Clark LJ, Aphale SL, Barraclough PB. 2000. Screening the ability of rice roots to overcome the mechanical impedance of wax layers: importance of test conditions and measurement criteria. Plant Soil 219:187-196.

Clark LJ, Cope RE, Whalley WR, Barraclough PB, Wade LJ. 2002. Root penetration of strong soil in rainfed lowland rice: comparison of laboratory screens with field performance. Field Crops Res. 76:189-198.

Cruz RT, Moroni S, Wade LJ. n.d. Leaf and root osmotic adjustment and dry matter in rice in response to water deficit and mechanical impedance to root growth (in preparation).

Kubo K, Jitsuyama Y, Iwama K, Hasegawa T, Watanabe N. 2004. Genotypic difference in root penetration ability by durum wheat (*Triticum turgidum* L. var. *durum*) evaluated by a pot with paraffin-Vaseline discs. Plant Soil 262:169-177.

Ray JD, Yu L, McCouch SR, Champoux MC, Wang G, Nguyen HT. 1996. Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L.). Theor. Appl. Genet. 42:627-636.

Samson BK, Hasan M, Wade LJ. 2002. Penetration of hardpans by rice lines in the rainfed lowlands. Field Crops Res. 76:175-188.

Siopongco J, Sekiya K, Yamauchi A, Egdane J, Ismail AM, Wade LJ. 2008. Stomatal responses in rainfed lowland rice to partial soil drying: evidence for root signals. Plant Prod. Sci. 11:28-41.

Siopongco J, Sekiya K, Yamauchi A, Egdane J, Ismail AM, Wade LJ. 2009. Stomatal responses in rainfed lowland rice to partial soil drying: comparison of two lines. Plant Prod. Sci. 12:17-28.

Wopereis MCS. 1993. Quantifying the impact of soil and climate variability on rainfed rice production. PhD thesis. Wageningen (Netherlands): Wageningen Agricultural University. 188 p.

Yu LX, Ray JD, O'Toole JC, Nguyen HT. 1995. Use of wax-petrolatum layers for screening rice root penetration. Crop Sci. 35:684-687.

Notes

Authors' addresses: L.J. Wade and S. Moroni, Charles Sturt University, EH Graham Centre, Wagga Wagga NSW 2678, Australia; R.T. Cruz, Philippine Rice Research Institute, Science City of Muñoz, Nueva Ecija 3119, Philippines; J. Siopongco, National Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8515, Japan; B.K. Samson, IRRI-Lao Office, NAFRI Compound, Xaythani District, Vientiane Capital, Lao PDR; T. Acuna, University of Tasmania, Tasmanian Institute of Agriculture, Hobart TAS 7001, Australia.