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Root Penetration Is Associated with Root Diameter and Root Growth Rate in Tropical Forage Grasses

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Abstract: Soil compaction impedes root exploration by plants, which limits access to nutrients and water, ultimately compromising survival. The capability of roots to penetrate hard soils is therefore advantageous. While root penetration has been studied in various annual crops, the relationships between root growth and root penetration are poorly understood in tropical perennial grasses. This study aimed to compare root penetration capability in 10 tropical perennial forage grasses and identify relationships between root penetration, root diameter and vertical root growth. Root penetration of each species, namely *Urochloa* (syn. *Brachiaria*) *brizantha* cv. Mekong Briz, *U. decumbens* cv. Basilisk, *U. humidicola* cv. Tully, *U. hybrid* cv. Mulato II, *U. mosambicensis* cv. Nixon, *U. ruziziensis* cv. Kennedy, *Panicum coloratum* cv. Makarikariense, *Megathyrsus maximus* (syn. *Panicum maximum*) cv. Tanzânia, *Paspalum scrobiculatum* (syn. *Paspalum coloratum*) cv. BA96 10 and *Setaria sphacelata* cv. Solendar, was evaluated using wax layers of varying resistances, created from a mixture of 40% (1.39 MPa) and 60% (2.12 MPa) paraffin wax, combined with petroleum jelly. Reference root sizes were determined for the grass species by measuring root diameter and root lengths of seedlings grown in growth pouches. Vertical root growth rate for each species was measured in grasses grown in 120 cm deep rhizotrons. Species with greater root penetration at both resistances had significantly higher shoot growth rates ($r = 0.65$ at 40% wax and 0.66 at 60% wax) and greater root diameters ($r = 0.67$ at 40% wax and 0.68 at 60% wax). Root penetration was significantly higher in species with greater vertical root growth rate only in the 60% wax treatment ($r = 0.82$). Root penetration at higher resistance was correlated with the root diameter and rapid vertical root growth of the species. This may indicate a contribution of these traits to root penetration ability. The combination of greater root diameter and root vertical growth rate, as observed in *M. maximus*, may assist in the identification of perennial forage grasses suitable for agroecosystems challenged by soil compaction and rapidly drying soil surface.

Keywords: soil hardpans; soil compaction; exploitative growth; tensile strength; wax system; tropical forage grasses; root penetration; root growth rate



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1. Introduction

Compacted soil affects the growth of crops in at least 4% of global arable land [1]. It is a major agricultural constraint, with adverse effects on the establishment and productivity of annual crops [2,3] and perennial forage grass species [4]. Compacted layers in soil profiles have been observed to prevent roots of rice [2,5,6], wheat [7,8], maize [9] and other crops [3] from accessing subsoil moisture and nutrients. The capability of roots to penetrate

hard soils is therefore advantageous in areas in which hard soils are a constraint; however, root penetration through compacted soil layers is not well-described in perennial crops and pastures. Whilst the root growth rates of annual crops dramatically decrease with maturity and depth [10,11], perennial grass species have continuous growth habits, with vertical root growth at greater depths [12–14]. Therefore, the relationships between root penetration and vertical root growth rates of perennial grass species are of great interest to in the identification of forages with greater potential for survival and productivity in areas challenged by soil compaction.

Previous studies in rice [15,16], wheat [7,8], and maize [9] have demonstrated that capability for penetrating hard wax layers translates into the capacity for penetrating compacted soils in field conditions. The capability for root penetration through compacted soil layers by annual crops has been screened using wax layers to represent compacted soil layers [17]. In this method, wax layers were created by combining paraffin wax and petroleum jelly at differing ratios to test various mechanical resistances [6,17,18]. Therefore, this method can identify forage grasses with strong root penetration capabilities, enabling the selection of species suited for growth in compacted soil regions by simulating mechanical resistance conditions similar to those in the field.

Recent research has shown that certain crops, such as rice and maize, display plasticity in root traits that allow for adaptation to compacted soils, including variations in root diameter, root hair development, and the biochemical changes aiding soil penetration [19,20]. This plasticity, however, remains less understood in perennial forage crops, though studies suggest the potential for genetic selection to enhance root penetration traits [19,21]. Another study on the mechanical traits of roots in compacted soils emphasizes that root diameter, coupled with root strength, directly affects penetration and the associated stress tolerance, which could be crucial for identifying resilient forage species [22].

Positive associations between root penetration, root diameter and shoot growth have been reported in annual crop species [2]. Exertion of greater force by roots to deform hard soils is typically associated with large root diameter [3,6,23] and can occur in association with shoot growth [24]. Furthermore, recent insights into root biomechanics show that a strong relationship exists between root diameter and vascular tissue capacity, which supports effective nutrient and water transport in compacted soils [21]. Moreover, root diameter can have a positive association with shoot growth because a larger capacity of xylem and phloem transport processes in thick roots promotes root and shoot growth, even in high resistant soils [2,25]. Genetic controls influence root diameter, such that root diameter at the early growth stage can indicate the root diameter of established plants [16,26]. Therefore, this study aimed to examine variation in root penetration in forage grass species and characterise forage grass species with a high root penetration capability. The hypothesis was that positive relationships would exist between root penetration and unimpeded root diameter, shoot growth and vertical root growth rate.

2. Materials and Methods

2.1. Root Penetration

Root penetration capability of forage species was measured using the wax method [17]. Each wax system was constructed from an external PVC pipe (30 cm height $\times$ 9.5 cm diameter), an internal PVC pipe (15 cm height $\times$ 8 cm diameter), a wax layer (9 cm diameter $\times$ 0.4 cm thick) and substrate. The wax systems of this study were established using pipes filled with 2.33 kg (bulk density of 1.16 g cm⁻³) dried University of California at Davis substrate, known as UC mix (Table 1), which contained 1.02 g N, 0.40 g P and 1.31 g K per pot. In each pot, a wax layer was placed at 15 cm depth (Figure 1A). The narrower internal pipe was installed against the surface of the wax layer to prevent roots

from growing through a 2.5 mm gap between the edge of the wax layer and the external pipe. The gap was necessary to allow water to percolate to the section underneath the wax layer. The internal pipe also functioned to direct root growth towards the wax layer (Figure 1A), because the studied forage grass species have a large variation in root growth angle [14].

Table 1. Components of Cocopeat substrate and UC mix used in this study, provided by The South Australian Research and Development Institute (SARDI).

Cocopeat Substrate		UC Mix	
Ingredients	Quantity	Ingredients	Quantity
Waikerie sand	1.00 m ³	Waikerie sand	0.56 m ³
Coco peat blocks	75.00 kg	Canadian peat moss	0.44 m ³
Dolomite lime	0.90 kg	Hydrated lime	0.80 kg
Hydrated lime	0.58 kg	Agriculture lime	1.33 kg
Agriculture lime	2.50 kg	Osmocote Exact Mini 6 N + 3.5 P + 9.1 K + TE (from Fernland, Yandina, QLD 4561 Australia)	3.00 kg
Gypsum	0.90 kg		
Superphosphate	0.90 kg		
Iron sulphate	2.25 kg		
Iron chelate	0.15 kg		
Micromax Premium Trace Element Mix 0.2 B + 1.0 Cu +15 Fe + 2.5 Mn + 0.04 Mo + 1.0 Zn (from Fernland, Yandina, QLD 4561 Australia)	0.90 kg		
Calcium nitrate	2.25 kg		

The experimental design had 2 factors (10 species $\times$ 2 resistances) and 4 replications. First, seeds of *Urochloa* (syn. *Brachiaria*) *brizantha* cv. Mekong Briz, *U. decumbens* cv. Basilisk, *U. humidicola* cv. Tully, *U. hybrid* cv. Mulato II, *U. mosambicensis* cv. Nixon, *U. ruziziensis* cv. Kennedy, *Panicum coloratum* cv. Makarikariense, *Megathyrsus maximus* (syn. *Panicum maximum*) cv. Tanzânia, *Paspalum scrobiculatum* (syn. *Paspalum coloratum*) cv. BA96 10 and *Setaria sphacelata* cv. Solander, supplied by Australian Pastures Genebank at Waite, were germinated in plug trays filled with a mixture of 50% cocopeat substrate with 50% UC mix, (Table 1). At the start of the second leaf development stage, a single uniform seedling from each species was transplanted into its respective replicated wax layer system (Figure 1A).

Wax layers of consistent strength were obtained by combining melted paraffin wax and petroleum jelly (VWR International Pty Ltd., Tingalpa, Australia) in specific weight ratios [17], casting the mixture into circular moulds (9 cm diameter $\times$ 0.4 cm depth) and allowing it to solidify at room temperature. Resistances of the disks at 30 °C were measured by penetrometer as 1.39 PMa at 40% wax and 2.12 PMa at 60% wax (Figure 1B).

The systems were maintained in a growth chamber (CONVIRON, Winnipeg, Manitoba, Canada), located at The Australian Plant Phenomics Facility). Environments of the growth chamber were maintained with a constant temperature of 30 °C, 70% relative air humidity and 15 h of daylight. A continuous maximum photosynthetic photon flux density of 1000 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ was maintained for 10 h day⁻¹ in between the dawn-evening simulation (Figure 1C). Soils were irrigated to 60% water holding capacity every day. UC mix volumetric moisture content is 25.1% *v/v* at field capacity [27].

All plants were harvested 3 weeks after transplanting. Nodal roots and seminal roots at the surface of the wax layer were counted. Multiple penetrating roots were treated as one count if they shared a main nodal or seminal root. Root penetration in each experimental unit at a given resistance was evaluated by determining the ratio of nodal and seminal roots

which penetrated through a wax layer of that resistance, in proportion to total numbers of nodal and seminal roots that reached the surface of wax [17]. Shoots and roots were then oven-dried at 60 °C for 72 h to determine dry weight.

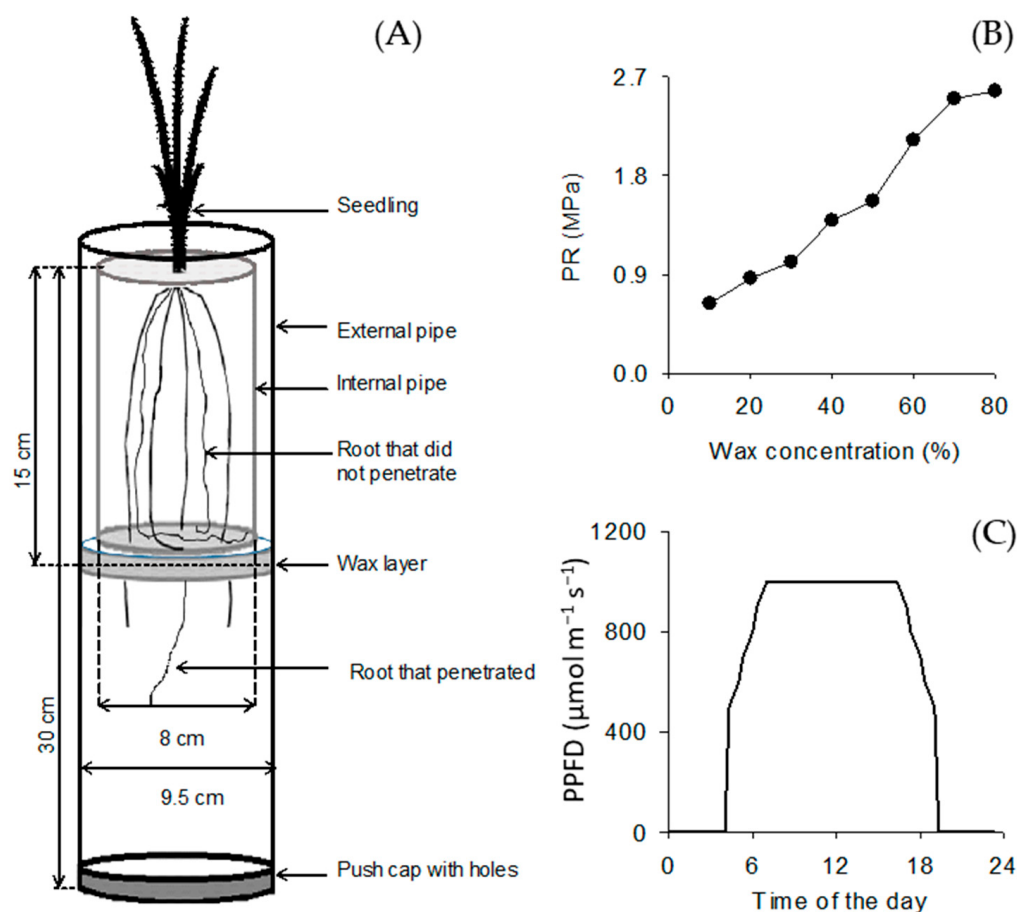


Figure 1. A wax layer system constructed by an external PCV pipe (30 × 9.5 cm) and an internal PCV pipe (15 × 8 cm), a wax disc placed underneath the internal pipe (A), penetrometer resistance (PR) of combined wax and petroleum jelly discs at various wax concentrations at 30 °C in the growth chamber (B), and photosynthetic photon flux density (PPFD) regime in the growth chamber (C).

2.2. Root Diameter

Roots of grasses grown in wax layer systems (Figure 1) grew under the resistance of the wax discs, which presented some challenges, such as roots quickly touching the bottoms of the pots, which changed the shapes of root tips. Therefore, we were not able to collect roots to measure the root diameters of each species. As root diameter is genetically determined, early measurements correlate well with mature root structure, making it a key selection criterion for breeding and crop variety selection programme [28]. Seedlings were typically grown in controlled environments, and root diameters were measured with imaging software to ensure accuracy [29]. Root diameter in forage grasses can be measured at the seedling stage to represent reference diameter and root architecture at maturity. This method aids early-stage selection in breeding programs. For the forage grasses in this study, root diameters of each species were measured using plants grown in a soil-less medium. Seedlings grown for this purpose were removed from the plug trays when the second leaf emerged, roots were gently rinsed to remove attached soil, and then each seedling was transplanted into the top compartment of a pouch bag with 13 cm width × 14 cm height dimension (Mega International, Roseville, MN, USA). The primary root of each seedling was placed through the hole into the pouch bag to ensure that roots reached the nutrient

solution and grew vertically. Each pouch bag contained 15 mL of a nutrient solution, made from a mixture of 5 mL IONI GROW hydroponic nutrient solution stock (Growth Technology, O'Connor, Western Australia, Australia) with 1.8 mS electrical conductivity and 5.8 to 6.2 pH and 1 L of distilled water. Undiluted IONI GROW stock (%w/v) contains 2.12% N (nitrate), 0.18% N (ammonium), 2.30% P, 0.33% K, 2.89% Ca, 0.95% Mg, 0.42% S, 0.11% Fe, 0.03% Mn, 0.01% B, 0.01% Zn, 0.002% Cu and 0.0005% Mo. Deionised water (5 mL) was added to pouch bags every day until root diameter measurement was undertaken. Plants of each species (5 replicates) that grew roots to 10 cm depth were selected for root diameter analyses, using the method of Watt et al. (2005). Roots were cut at the base and prepared for root diameter analysis by staining in 0.05% toluidine blue (pH 4.4) for 3 min, and washing with distilled water for 2 min. The whole roots were scanned using an EPSON Expression 10000XL scanner (EPSON Inc., Long Beach, CA, USA) at 2000 dpi. Root diameter of each plant was determined by the diameter of the root section below the root hair of the long root, which grew to 10 cm depth, using a root image analysis software WinRHIZO (Version 2024, Regent Instruments Inc., Québec, QC, Canada) Scanned images of the entire roots were used to re-analyse using WinRHIZO to obtain total root length and average root diameter, which also characterised the differences between grass species. The actual reference value for each species was the average of the 5 values.

2.3. Vertical Root Growth Rate

Reference rates of root depth development and growth of fibrous roots were obtained from a previous experiment [14], which analysed *U. brizantha* cv. Mekong Briz, *U. decumbens* cv. Basilisk, *U. humidicola* cv. Tully, *U. hybrid* cv. Mulato II, *U. mosambicensis* cv. Nixon, *M. maximus* cv. Tanzânia, *S. sphacelata* cv. Solander in rhizotrons (20 cm length × 5 cm width × 120 cm depth) in a glasshouse. Each rhizotron was filled with 11.5 L (18 kg) UC mix (Table 1) with available macronutrients of 5.52 g N, 2.16 g P and 7.06 g K. Daily temperatures ranged from 25 to 32 °C and photosynthetic photon flux density sunlight was 500 to 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The reference rate of root depth development was measured as growing degree days required for roots to reach 50 cm depth (GDD₅₀). GDD₅₀ values for *U. ruziziensis*, *P. coloratum*, and *P. scrobiculatum* were not available. Vertical root growth rate was calculated as 50 (cm)/GDD₅₀ (°Cd). The growth of fibrous roots (branch roots) was determined by the length of roots with diameters less than 0.5 mm, obtained from analysing root images using WinRHIZO.

2.4. Statistical Analyses

ANOVA of the general linear model was performed using GenStat 18th Edition (VSN International, Hemel Hempstead, England) to identify significant differences in the measured variables. Multiple mean comparisons were conducted using Fisher's Least Significant Difference (LSD) test at a 5% significance level. Additionally, the interaction between species and wax resistance was assessed. Correlation analyses were undertaken for all measured variables. This included root diameter and variables obtained from WinRHIZO analysis, such as average root diameter, total root length (RL), root length for diameters less than 0.5 mm, and root length for diameters greater than 0.5 mm (Supplementary Tables S1 and S2). Pearson's correlation coefficients (*r*) were calculated using GenStat 18th Edition.

3. Results

3.1. Root Penetration

All species had roots that penetrated through wax layers of both resistances during the three-week growth period in pots (Figure 1A). Variation between species in root penetration ratio was greater at 60% wax (0.2 to 0.9) than 40% wax (0.5 to 0.9), and there was a significant species $\times$ wax resistance interaction in root penetration ratio ($p < 0.01$) (Figure 2A,B). Grass species differed in total dry weight ($p < 0.01$, at 40% wax and $p < 0.001$, at 60% wax); however, species $\times$ wax resistance interaction in dry weight was not significant ($p > 0.05$) (Figure 2C,D).

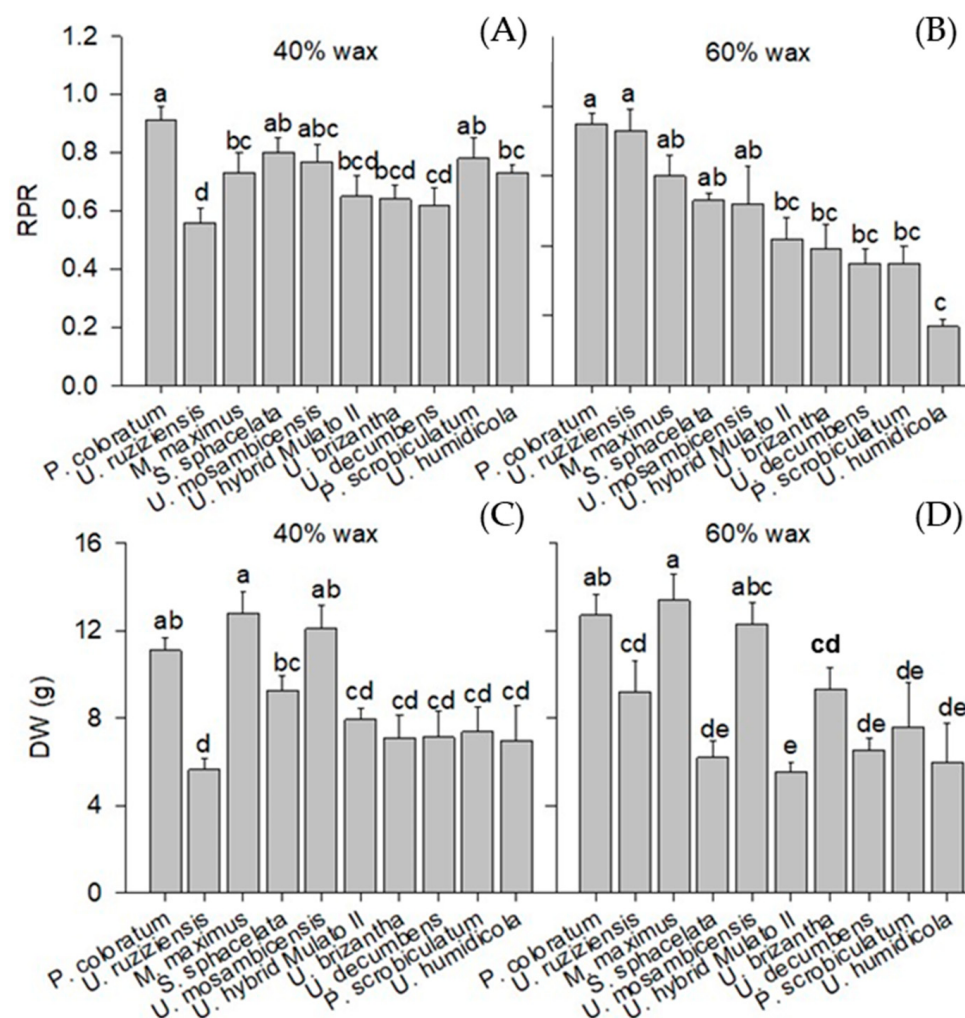


Figure 2. Grass species ranked in descending order based on root penetration at 60% wax: root penetration ratio (RPR) (A,B), total dry weight (DW) (C,D). Bars ($\pm$ SE) with different letters on top are significant differences within the wax concentration at $p < 0.05$.

3.2. Species Traits

Species had significant differences in root diameter ($p < 0.001$) and length of roots with diameters greater than 0.5 mm ($p < 0.001$) (Figure 3A,B). *M. maximus*, *S. sphacelata*, *U. mosambicensis*, *U. hybrid Mulato II*, *U. brizantha*, *U. decumbens*, and *U. humidicola* had significant differences in vertical root growth rate ($p < 0.001$) and length of fibrous roots ($p < 0.001$) (Figure 4B) after the 4-week growth in rhizotrons.

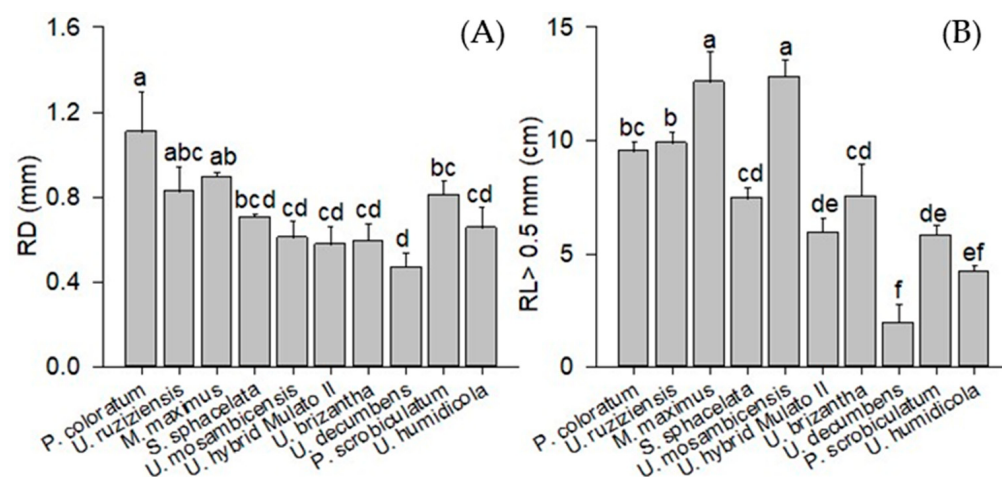


Figure 3. Root diameter (RD) (A) and root length measured in roots with diameters greater than 0.5 mm (RL > 0.5 mm) (B). Bars ($\pm$ SE) with different letters on top are significantly different means at $p < 0.05$.

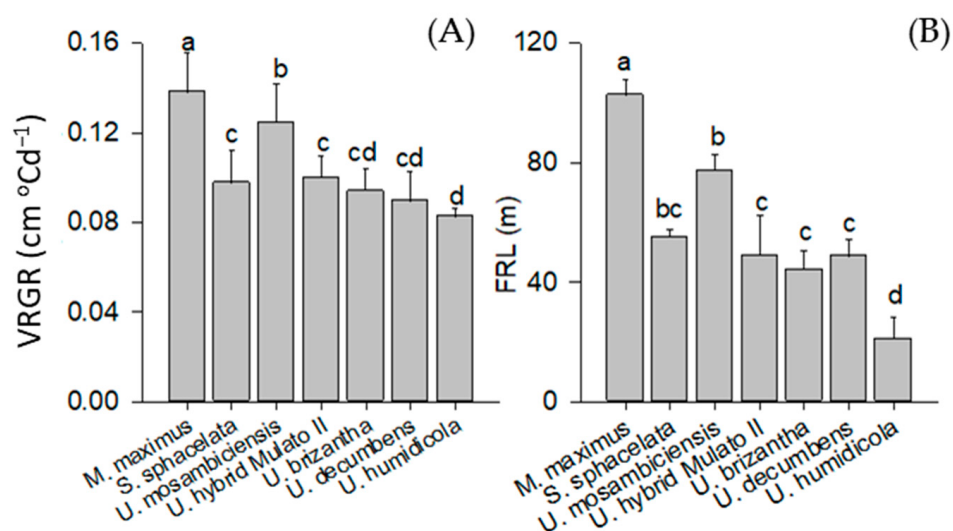


Figure 4. Root growth of grass species (without *U. ruziziensis*, *P. coloratum*, and *P. scrobiculatum*) during the four-week growth in rhizotrons: vertical root growth rate (VRGR) (A) and fibrous root length (FRL) (B). Bars ($\pm$ SE) with different letters on top are significantly different means at $p < 0.05$.

3.3. Correlations Between Species Traits and Root Penetration

At both wax concentrations, root penetration ratio significantly increased with greater root diameter ($r = 0.67$, $p < 0.05$; 40% wax and $r = 0.68$, $p < 0.05$; 60% wax) (Figure 5A,G), and greater shoot dry weight ($r = 0.65$, $p < 0.05$; 40% wax and $r = 0.66$, $p < 0.05$; 60% wax) and (Figure 5B,H). However, there was no correlation at the resistance of 40% wax concentration (Figure 5C). Only at the resistance associated with 60% wax concentration did root penetration ratio significantly increase, with increased length of roots with diameters greater than 0.5 mm ($r = 0.67$, $p < 0.05$), greater vertical root growth rate ($r = 0.82$, $p < 0.05$) and the greater length of fibrous roots ($r = 0.89$, $p < 0.01$) (Figure 5D–K). Furthermore, vertical root growth rate ($r = 0.87$, $p < 0.05$) and length of fibrous roots ($r = 0.79$, $p < 0.05$) significantly increased with the greater length of roots with diameters greater than 0.5 mm (Figure 6).

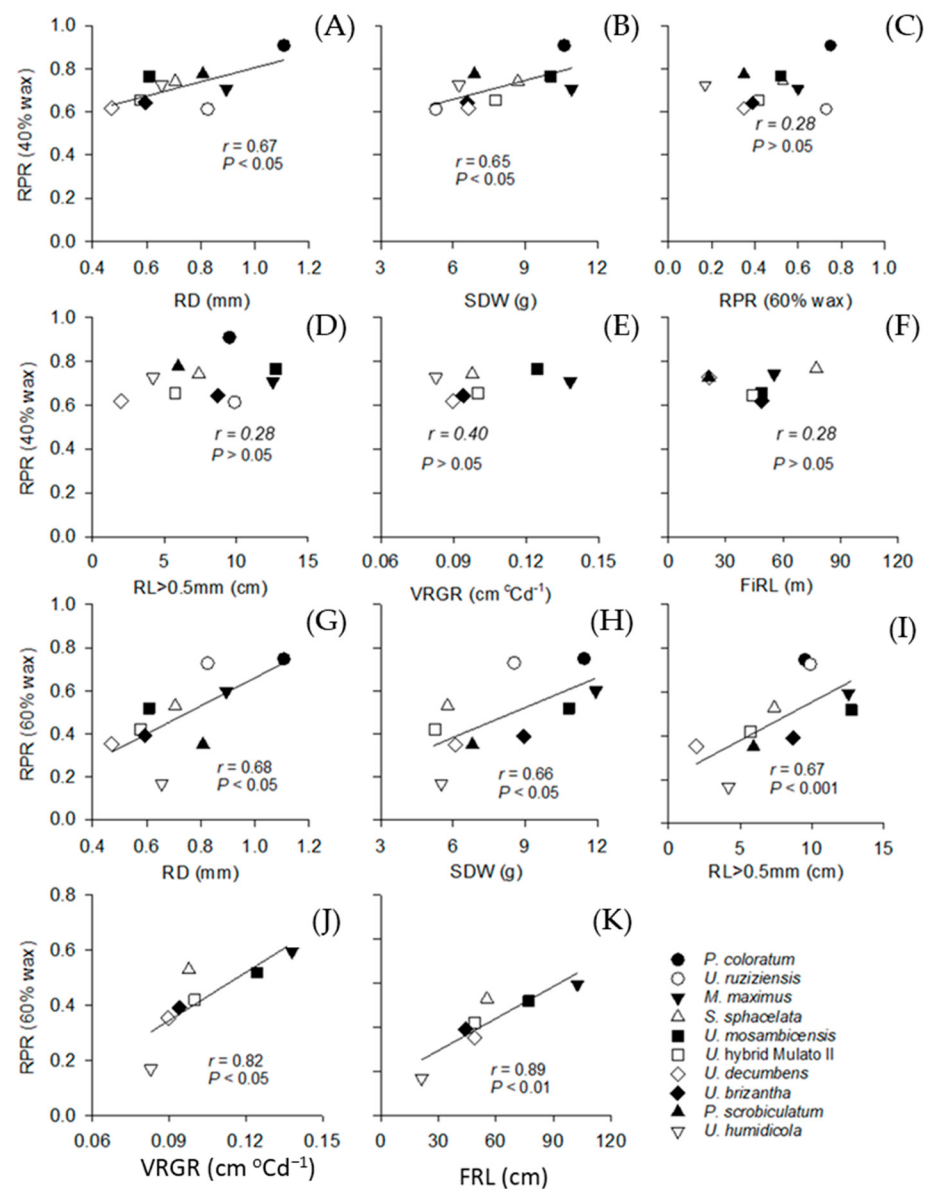


Figure 5. Relationships between root penetration ratio (RPR) and root diameter (RD) (A,G), shoot dry weight (SDW) (B,H), root penetration ratios between both wax concentrations (C), root length measured in seedling roots with diameters greater than 0.5 mm (RL > 0.5 mm) (D,I), vertical root growth rate (VRGR) (E,J), and fibrous root length (FRL) (F,K). Each point represents the mean value of each species.

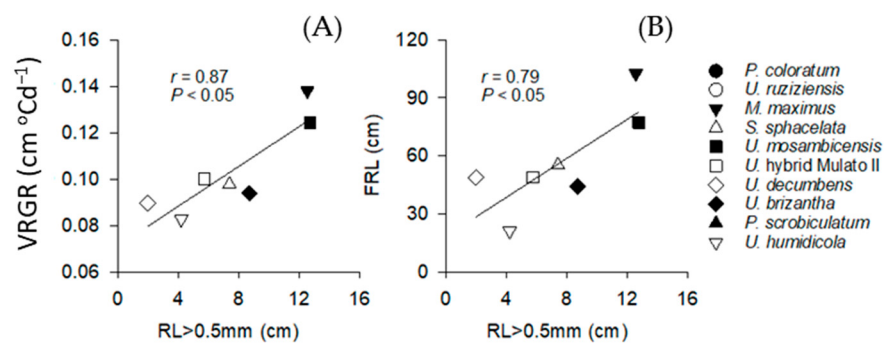


Figure 6. Relationships between root length measured in seedling roots with diameters greater than 0.5 mm (RL > 0.5 mm) and vertical root growth rate (VRGR) (A) and fibrous root length (FRL) (B) of grasses in rhizotrons. Each point represents the mean value of each species.

4. Discussion

4.1. Root Diameter

Positive correlations of root diameter and root penetration ratios at both wax concentrations may indicate that greater root diameter promoted root penetration through higher-resistance wax layers. Penetration by roots with a large diameter could be explained by thick cortical areas, greater cortical cell wall area, higher cortical cell counts and large stele diameter, as observed in maize genotypes [9]. Greater content of cell wall and greater diameter of stele in large roots reduces axial stress of root tips [23,30] and increases root rigidity and tensile strength to prevent impeded roots from bending when faced with high resistance [16,31]. This was apparent with the perennial forage grasses evaluated for root penetration capability in the present study.

4.2. Shoot Mass

The positive correlation between shoot dry weight and root penetration ratio may indicate that larger plants are more capable at penetrating layers with high resistance by forage grasses. Rapid shoot growth in forage grass species is associated with increased rates of cell division and expansion at root growing zones, leading to increased root growth that promotes root penetration [32,33]. Furthermore, the increased number of cells in cortical tissues of elongating root sections enlarges root diameters to allow root penetration into and through mechanical impedances [32,34,35].

4.3. Vertical Root Growth Rate

Despite positive correlations between shoot growth, root diameter and root penetration in both wax concentrations, vertical root growth rate of the species was correlated with root penetration only at 60% wax concentration. This is likely because 1.39 MPa did not offer sufficient resistance to expose the relative advantage of the species with a higher penetration capability. In contrast, at 2.12 MPa, fewer forage grasses were able to penetrate, with those that succeeded being species exhibiting greater vertical root growth rates. The increased vertical root growth rate of forage grasses correlated with the rapid establishment of root lengths with large diameters (greater than 5 mm) at the seedling stage (Figure 6). Thick roots developed during the early growth of perennial forage grasses in this study indicate a high capacity of root xylem transport of roots to promote shoot growth, which occurs even at high soil resistance in annual crops [2,25,36]. Thick roots also provide large phloem tissues to increase resource transport from leaves to promote root growth [9,36].

4.4. Fibrous Roots

Greater growth of fibrous roots and shoots correlated with greater capability of root penetration at 60% wax concentration (Figure 5G). Roots penetrating at high resistance layers increased with length of fibrous roots developing from nodal and seminal roots in annual crops, such as maize [9,18], barley [37] and forage radish [38]. In several of the perennial grasses used in our study, rapid growth of small roots also correlated with root vertical growth rate and shoot growth [14].

4.5. Species Comparison

Traits associated with root penetration at great mechanical impedance in forage grass species were root diameter, shoot growth and root vertical growth rate. Compared with other species, *M. maximus* had greater root penetration through both wax concentrations. *M. maximus* has rapid root establishment to depths between 50 and 120 cm compared with other forage grasses [14], and is commonly cultivated in areas with compacted subsoil layers with resistances of 2.0 to 3.5 MPa [24,39], equivalent to the range of pressures

required to penetrate wax concentrations of the current study. Given that crop species with great penetration ability in high-resistant wax layers measured in the laboratory also exhibited great root penetration and deep establishment in field environments [2,6,8], this may partially explain the success of *M. maximus* as a forage option in these environments, despite reportedly greater soil fertility requirements than other cultivars, as observed in previous studies [4,40].

4.6. Limitations of the Study

While wax layers effectively simulate compacted soil conditions and provide controlled testing environments, they may not fully replicate the complexity of natural field conditions, such as variable soil composition and environmental dynamics. Although previous studies have demonstrated the utility of wax layers in assessing root penetration potential [2,6,15,26,34], the findings may not fully translate to field performance. The study's focus on a short growth period and a limited number of species may not capture long-term adaptability or the diversity of forage grass responses. These limitations highlight the potential for complementary field trials to validate the findings and extend their applicability.

5. Conclusions

Forage grass species in this study exhibited variation in root penetration of wax with resistances of 1.39 MPa and 2.12 MPa. Variation in root penetration across grass species was greater at the higher wax resistance. The results showed a significant effect of species $\times$ wax resistance and, accordingly, there were differences in traits correlated with root penetration ratios at both wax strengths. Thicker root diameters of primary seminal roots, measured at the seedling stage, was associated with increased root penetration at both resistances. However, increased root penetration was associated with a greater length of roots with diameters greater than 0.5 mm at seedling stage and increased vertical root growth rates only at 2.12 MPa. This study suggests that combined traits of large root diameter and rapid vertical root growth rate can assist in selecting perennial forage species regions with rapidly drying soil surface and soil compaction.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/grasses4010004/s1>, Table S1: Pearson's correlation matrix of variables measured in all experiments. Values represent correlation coefficient (r). Variables include root penetration at 40% wax (PR40), root penetration at 60% wax (PR60), shoot dry weight at 40% wax (SDW40), shoot dry weight at 60% wax (SDW60), root dry weight at 40% wax (RDW40), root dry weight at 60% wax (RDW60), root diameter (RD), average root diameter (ARD), total root length (RL), root length with diameters less than 0.5 mm (RL < 0.5 mm), and root length with diameters greater than 0.5 mm (RL > 0.5 mm). Symbols indicate *, significant at $p < 0.05$, **, significant at $p < 0.01$, ***, significant at $p < 0.001$, and ns, not significant. Measurements were obtained from *U. brizantha*, *U. decumbens*, *U. humidicola*, *U. hybrid* Mulato II, *U. mosambicensis*, *U. ruziziensis*, *P. coloratum*, *M. maximus*, *P. scrobiculatum* and *S. sphacelata*.; Table S2: Pearson's correlation matrix of variables measured in all experiments. Values represent correlation coefficient (r). Variables include root penetration at 40% wax (PR40), root penetration at 60% wax (PR60), shoot dry weight at 40% wax (SDW40), shoot dry weight at 60% wax (SDW60), root dry weight at 40% wax (RDW40), root dry weight at 60% wax (RDW60), root diameter (RD), average root diameter (ARD), total root length (RL), root length with diameters less than 0.5 mm (RL < 0.5 mm), root length with diameters greater than 0.5 mm (RL > 0.5 mm), vertical root growth rate (VRGR), and fibrous root length (FRL). Symbols indicate *, significant at $p < 0.05$, **, significant at $p < 0.01$, ***, significant at $p < 0.001$, and ns, not significant. Measurements were obtained from *U. brizantha*, *U. decumbens*, *U. humidicola*, *U. hybrid* Mulato II, *U. mosambicensis*, *M. maximus*, and *S. sphacelata*.

Author Contributions: Conceptualization, C.H., J.N.M.P., Y.Z. and M.D.D.; methodology, C.H., J.N.M.P., Y.Z. and M.D.D.; software, C.H., Y.Z.; validation, C.H., J.N.M.P., Y.Z. and M.D.D.; formal analysis, C.H.; writing—original draft preparation, C.H., J.N.M.P.; writing—review and editing, C.H., J.N.M.P., Y.Z. and M.D.D.; visualization, C.H.; supervision, J.N.M.P., Y.Z. and M.D.D.; project administration, M.D.D.; funding acquisition, C.H., J.N.M.P., Y.Z. and M.D.D. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are available from the authors upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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