The Adaptation of Tropical Perennial Grasses to Abiotic Constraints in Sandy Soils

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Chapter 5	Submitted to Crop & Pasture Huot C, Philp JNM, Zhou Y, Denton MD (submitted) Root penetration is associated with root diameter and root growth rate in tropical forage grasses. *Crop & Pasture Science*
Chapter 6	Manuscript to be submitted Huot C, Zhou Y, Philp JNM, Denton MD (submitted) Rhizome growth of C4 grass species in arid regions is related to aridity index, rainfall and evapotranspiration.

ABSTRACT

The inclusion of perennial forage grasses in agricultural systems has the potential to improve livestock production, ecosystem health and climate resilience. However, the establishment of forage grasses in many ecosystems is challenged by abiotic stresses. Perennial forage grass species are often grown in environments with limited water availability following establishment and rely on accessing water deep in the soil profile to survive. In sandy soils with rapid surface drying and hardpan soils, root growth to greater soil depths enables forage grass species to survive soil surface drying following establishment and is assisted by increased root growth rate and greater ability to penetrate compacted soils. In addition, the presence of large rhizomes in rhizomatous grasses promotes post-harvest regrowth rate and post-establishment survival or drought resistance without relying on root access to water at depth. Therefore, characterisation of these traits in perennial forages grass species will provide an objective means for species selection, based on the local abiotic constraints. Five glasshouse experiments were conducted to identify the mechanisms of perennial grass adaptation to abiotic constraints in ecosystems with rapid surface-drying soils and hardpan soils.

The first experiment assessed variations in vertical root growth rates between tropical perennial forage grass species, and characterised traits associated with higher vertical root growth rates. Tropical forage grasses, namely *Urochloa* (basionym: *Brachiaria*) *brizantha* Mekong Briz, *Urochloa decumbens* cv. Basilisk, *Urochloa humidicola* cv. Tully, *Urochloa* hybrid cv. Mulato II, *Urochloa mosambicensis* cv. Nixon, *Megathyrsus maximus* (basionym: *Panicum maximum*) cv. Tanzânia, and *Setaria sphacelata* cv. Solander, were established in large rhizotrons that facilitated measurement of the rate of root depth development, the rate of root length development, photosynthesis and morphological traits. Rapid vertical root growth with narrow root angle, high photosynthetic rate, high ratio of root length to leaf area and high percentage of fibrous roots were apparent mechanisms that enabled *M. maximus* and *U. mosambicensis* to establish deep roots faster than other forage grass species. *M. maximus* and *U. mosambicensis* were identified as species with exploitative growth strategy. Wide root angles and a higher proportion of shallow root distribution to 10 cm depth were associated with decreased vertical root growth rate. The exponential rate of root depth development per growing degree day increased with average root diameter in *U. humidicola* and *Urochloa* hybrid Mulato II, indicating a conservative growth strategy.

The second and third experiments investigated traits correlated with vertical root growth rates, the stability of variation between grasses in root and shoot growth in summer and winter, and relationships between vertical root growth rates and post-establishment drought resistance, using 12 bermudagrass ecotypes (*Cynodon* spp.) from varied Australian climatic zones. Previous field experiments using these ecotypes found that drought resistance was promoted by water extraction; however, relationships between vertical root growth rates and drought resistance are poorly understood. The 12 ecotypes were established in large rhizotrons during experiments in mild winter (17 to 24 °C mean temperature) and summer (19 to 38 °C mean temperature). A proportion of root length became inactive due to seasonal root death in winter conditions, and vertical root growth rate during winter significantly decreased as this proportion increased. During summer, vertical root growth rate significantly increased with a greater tiller appearance rate but significantly decreased with increased root distribution to 10 cm depth. Despite the inconsistency of variation between ecotypes in shoot growth, the genotypic rank of root length, root dry weight, vertical root growth rate and leaf area were consistent in both seasons. Positive correlations between vertical root growth rate measured in both seasons and drought resistance were found, suggesting that increased vertical root growth rate promotes active roots for extracting water at the greater depth of soil profiles in association with post-establishment drought resistance.

The fourth experiment was conducted to examine variations in root penetration in forage grass species and characterise forage grass species with a high root penetration capability using wax layers to measure root penetration. *U. brizantha*, *U. decumbens*, *U. humidicola*, *U.* hybrid cv. Mulato II, *U. mosambicensis*, *U. ruziziensis*, *M. maximus*, and *S. sphacelata, Panicum coloratum* cv. Makarikariense, *Paspalum scrobiculatum* cv. BA96 10 were evaluated. Root diameters were determined for each species from seedlings grown in growth pouches. Increased root penetration at high resistance was associated with larger root diameter and increased vertical root growth rate. The results indicate that *M. maximus* can avoid water stress during soil surface drying better than other forage species by accessing profile moisture due to its greater vertical root growth rate and capability of root penetration.

The fifth experiment analysed variation in rhizome growth and correlated traits to examine relationships between rhizome growth and aridity index, rainfall and evapotranspiration, using bermudagrass ecotypes collected from environments with varying aridity indices. A total of 142 ecotypes collected from regions in Australia with varied aridity indices were grown in pots for 14 weeks during the summer in South Australia to measure rhizomes and plant traits. Rhizome growth between ecotypes from arid (aridity indices less than 0.65) and non-arid environments was not significantly different. Bermudagrasses with the largest rhizomes were amongst those ecotypes that originated from arid environments. Moreover, rhizome growth of bermudagrass ecotypes from arid environments had a positive response to environments with more humid climate conditions in winter, while the rest of the year is dry. Amongst bermudagrass ecotypes from both regions, increased rhizome growth during establishment was associated with greater leaf width and decreased internode length.

Through the combined results of the five experiments presented within this thesis, several mechanisms that enable perennial grass adaptation to abiotic constraints in ecosystems with rapid surface-drying soils and hardpan soils were identified. Increased vertical root growth rate was associated with narrow root angle, greater leaf area and greater shoot growth. Increased root penetration at high resistance was associated with large root diameter and increased vertical growth rate. Post-establishment drought resistance is promoted by greater vertical root growth rate. Bermudagrass ecotypes with the greatest rhizome growth originated from arid regions. In relation to plant traits, greater leaf width and decreased internode length were characteristics of bermudagrasses with large rhizomes.

This research provides new insights on the beneficial characteristics of vertical root growth in perennial forage grasses that can be well-adapted to sandy soils with rapid surface drying and hardpan soils. For field application, *M. maximus* appears to be well-adapted to sandy soils because it had rapid vertical root growth and great capability of root penetration that can be associated with drought resistance and high yield. Caution is advised when recommending *M. maximus* because it expressed an exploitative growth strategy that is associated with high nutrient requirement to sustain production. Furthermore, results from bermudagrass ecotypes showed that drought-resistant perennial grasses did not have specific traits that differed from drought-susceptible grasses. These findings indicate that drought resistance in perennial grasses may involve complex trait interplay. Therefore, the drought resistance and high yield of forage species such as *U. humidicola* and *U.* hybrid cv. Mulato II may be associated with other characteristics, but not the vertical root growth rate. These species that expressed a conservative growth strategy may be well-adapted to a wide range of agronomic conditions.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Chanthy Huot 29/10/2021

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

Perennial C4 forage grass species have great potential to contribute to livestock production in warm regions because they have the high efficiency of resource mobilisation and persistent growth in limited-water conditions and temperatures (Ghimire *et al.* 2015; Mathews *et al.* 2004; Peters *et al.* 2013). In comparison with C3 plant species, the photosynthetic pathway of C4 grasses has a greater ability to fix carbon under high radiation (Edwards *et al.* 2010), and C4 grasses from both temperate or tropical origins usually exhibit a greater rate of assimilation (Crush and Rowarth 2007; Ludlow 1985). The majority of C4 forage grasses that originate from African regions are well-adapted to highly variable rainfall (Sarmiento 1992).

Forage C4 grasses are difficult to rotate with annual crops such rice due their high competition (Crusciol *et al.* 2021). Rhizomatous grass species can spread rapidly to become potential weeds for other crops (Cook *et al.* 2005; Muoni *et al.* 2013). However, with a good practice of crop management, integrated farming systems of some forage grasses and other crops is a prominent strategy for intensification of sustainable food production (Martin-Guay *et al.* 2018; Tan *et al.* 2020). Integration of forage grasses with maize or sorghum shows viability of agriculture production and increase in land productivity per annuum (Borghi *et al.* 2013; Mateus *et al.* 2020). Furthermore, intercropping tropical forage non-rhizomatous grasses such as *Megathyrsus maximus* and *Urochloa brizantha* with upland rice increases biomass production (Crusciol *et al.* 2021).

Integration of improved forage C4 grass species into farming systems, especially in developing countries, promotes livelihoods of small-scale livestock holders and ecosystem services and enhances the quality of animal production potential (Ghimire *et al.* 2015; Hasnah *et al.* 2016; Rao *et al.* 2015). Perennial forage grasses cultivated in sandy-soil ecosystems such as the lower

Mekong regions, including Laos, Thailand, Cambodia and Vietnam, can be established successfully in existing cropped lands such as rice-growing regions where farmers adopt the cut-and-carry forages to supply feed to their animals (Nguyen *et al.* 2013; Philp *et al.* 2019). Accordingly, farmers tend to adopt integrated farming systems of forage grasses and food crops (Bush *et al.* 2014).

Some potential perennial grasses have been already introduced into sandy-soil ecosystems in the lower Mekong regions (Philp *et al.* 2019). However, sandy soil profiles with rapid surface-drying and hardpan soils challenge establishment by preventing the roots some forage grass species from accessing available water at greater depths (Bell *et al.* 2007; Bell *et al.* 2021; Jin *et al.* 2015). The annual fluctuation of water significantly reduce productivity in annual crops in these challenging profiles (Bodner *et al.* 2015; Sasidharan *et al.* 2017) and can also limit the establishment of forage grasses. Therefore, there is a need to investigate the mechanisms in perennial forage grass species for adaptation to abiotic constraints in ecosystems with rapid surface-drying soils and hardpan soils.

1.2 Objective

This thesis aimed to characterise traits of perennial forage grasses that are potentially suitable for regions rapid-surface drying and hardpan soils. Research objectives addressed in Chapters 2 to 6 yielded specific evidence for the attainment of this aim (Fig. 1.1):

1. Chapter 2 reviews the abiotic constraints in sandy soils that negatively affect the root and shoot growth of perennial forage grass species, adaptations that may enable forage grasses to overcome them, and knowledge gaps that set the research agenda for subsequent chapters.
2. Chapter 3 analyses variations in rates of root depth development between tropical perennial forage grass species, and characterises traits associated with rates of root

depth development that are possible the adaptive mechanisms of forage grass species to survive in rapid soil-surface drying ecosystems after establishment.

3. Chapter 4 investigates intraspecific variations in perennial grass roots and shoot growth in response to seasonal change. Rates of root depth development and the stability of variation between grasses in root and shoot growth during establishment under different seasonal conditions is analysed in C4 perennial grass ecotypes, and relationships between the rates of root depth development and post-establishment drought resistance of those grasses are determined.
4. Chapter 5 analyses variations in root penetration in forage grass species and characterises forage grass species with a high root penetration capability.
5. Chapter 6 analyses variation in rhizome growth and mechanisms for rhizome growth of perennial C4 grasses originating from various weather conditions.

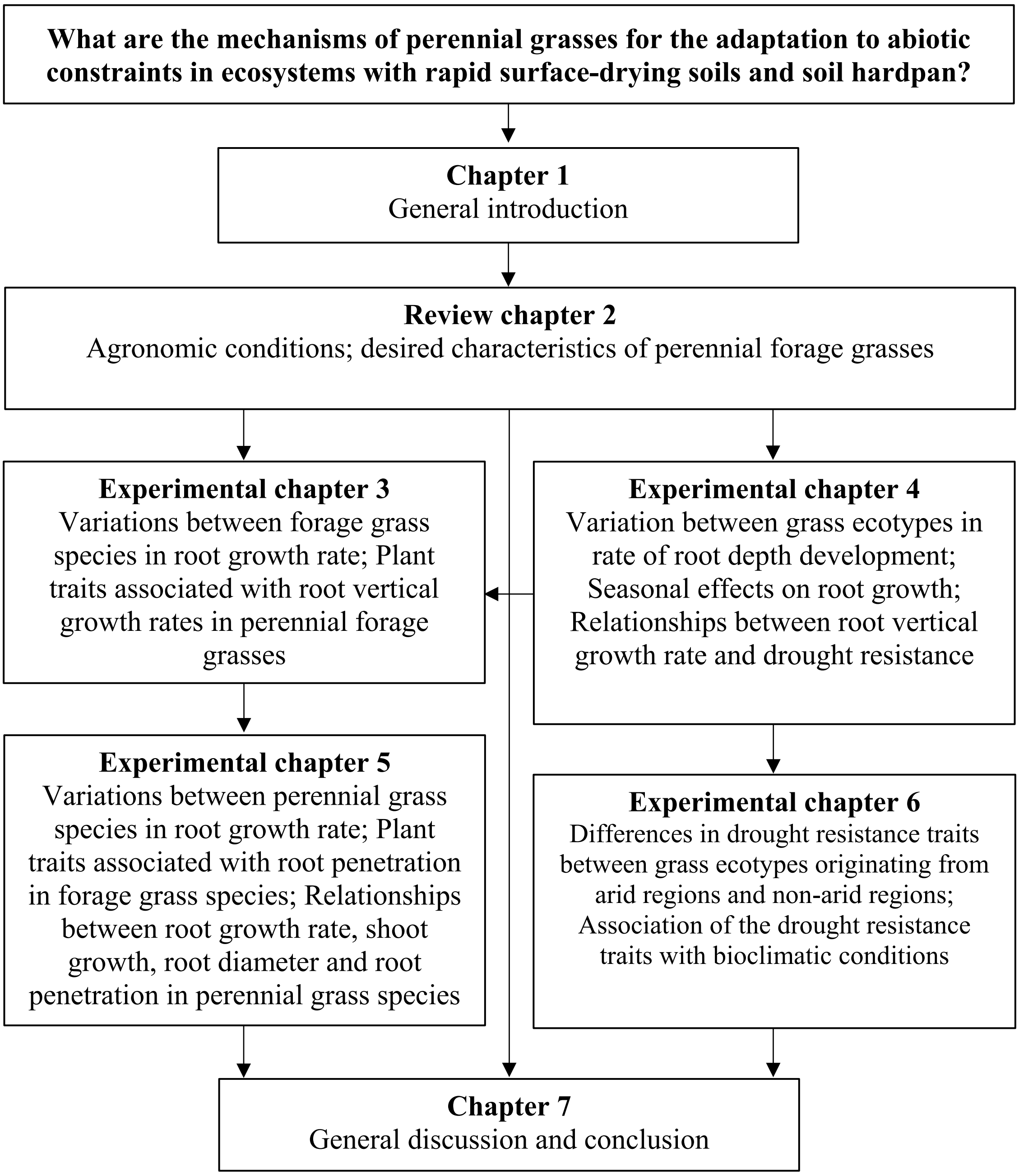

Fig. 1.1 Thesis structure demonstrating the relationships between chapters

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

C4 perennial forage grasses have the potential to contribute to livestock production systems in sandy soils in the lower Mekong regions. However rapid surface-drying and hardpan soils in sandy soil profiles (Bell *et al.* 2007) challenge establishment by preventing the roots some forage grass species from accessing available water at greater depths. Therefore, there is a need to investigate the mechanisms in perennial forage grass species for adaptation to abiotic constraints in ecosystems with rapid surface-drying soils and hardpan soils. The aims of this chapter were 1) to explore the available forage genetics that are reported to be adapted to constraints of roots accessing water in sandy soil profiles and 2) to identify knowledge gaps for further investigation.

2.2 Abiotic constrains of sandy soils in the lower Mekong regions

2.2.1 Rapid surface drying

Sandy soils with rapid surface-drying occupy approximately 40% of rice-based cropping regions in the lower Mekong countries (Bell and Seng 2003; Bell *et al.* 2007; Gaiser *et al.* 2000; Trisurat *et al.* 2018; White *et al.* 1997). The majority of areas are shallow sandy-over-clay soil type, whereas the deep sandy soil type covers about 1.6 % of central lowland rice areas in Cambodia and 10% in Southern Laos (Fig.2.1a) (Bell *et al.* 2007; Linquist 1998).

Surface drying conditions in sandy soils occur rapidly without frequent rainfall, irrigation or a persistent shallow water table (Bell *et al.* 2021; Fukai *et al.* 2000). Sandy soils are subject to rapid surface evaporation (Allen *et al.* 1998) and limit the uptake of water by plants because they inherently have low water holding capacity as observed in the lower Mekong regions (Fig. 2.1a) (Bodner *et al.* 2015; Seng *et al.* 2005). In the rainy season, water loss from infiltration and runoff combined accounts for 40 to 50 % (Liu *et al.* 2002) and soil evaporation up to 35% of water loss from sandy soils (Debaeke and Aboudrare 2004). The water storage in sandy soils with less than 7% clay or loamy sand is 4 to 5 mm per 10 cm soil depth (Inthavong *et al.* 2011). Plant available water content in sandy soils is limited by high infiltration rates that vary between 1 to 6 mm day^{-1} as observed in Northeast Thailand, Cambodia and Central Laos (Fukai *et al.* 2000; Inthavong *et al.* 2011; White *et al.* 1997). In such soils, the plant available water content can supply for 2 days growth in 0 to 20 cm depth and 8 to 11 days growth in 0 to 100 cm depth at a crop water use rate of 5 mm day^{-1} (Bell *et al.* 2021). The limitation of soil water for forage establishment is also affected by unreliability of sufficient rainfall, variation in start and end date of the wet season, and high variability of average annual rainfall with potential drought (Fig. 2.1b) (Bell *et al.* 2021; Thoeun 2015). The upper layers of soil profiles are often prone to drought, even in the wet season (Bell *et al.* 2021).

Conversely, plant available water after wet season varies with landform elements of lower, middle or upper positions in the lower Mekong regions (Vial *et al.* 2020). Fields of the lower terraces receive subsurface lateral water movement and deep drainage from the upper terraces (Fukai *et al.* 2000). Degrees of variation in average annual rainfall in the regions (Fig. 2.1b) are unlikely a high risk of crop failure during pre- and post-monsoon periods because plant available water exist in deep soil profiles in the lower positions (Bell *et al.* 2021). Crop species with rapid root establishment to depth below 75 cm can be suitable for the lower Mekong regions (Vial *et al.* 2020). Furthermore, forage grasses can access to plant available water for the growth of most crops when their roots have established to soil horizons between 100 and 120 cm in rice-producing regions (Bell *et al.* 2006; Bell *et al.* 2021; Cornish *et al.* 2018; Lodge and Murphy 2006; Seng *et al.* 2007). Those areas are potential for alternative use for forage grasses, especially deep root grass species (Philp *et al.* 2019).

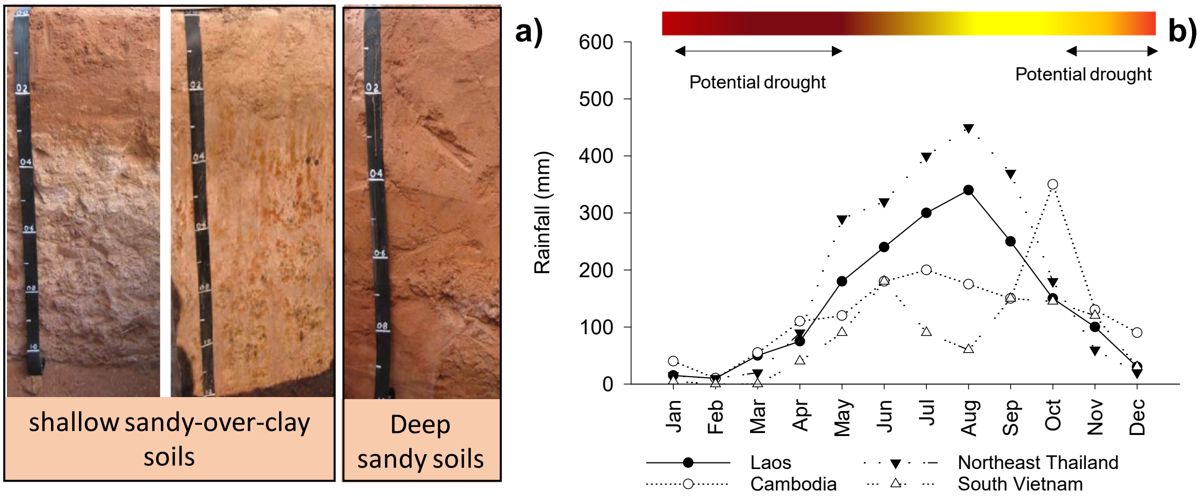

Fig. 2.1 Abiotic constraints in soil profiles in rice-based cropping systems with potential underneath hardpan soils (Bell *et al.* 2007) (a). Annual rainfall pattern and the occurrence of potential drought conditions in the lower Mekong regions such as Cambodia (adapted from Thoeun 2015) and Laos, Northeast Thailand and South Vietnam (adapted from Wolf *et al.* 2020).The bar is unscaled and indicates levels of water availability for plant growth, the variation from very dry (dark red) to over-saturated (light yellow). The colour presentation of potential drought is unscaled.

2.2.2 Hardpan soils

In sandy soil profiles (Fig. 2.1a) of the lower Mekong regions, layers underneath topsoils are hardpan soils that occur naturally during water deficit or result from farming practices (Bell *et al.* 2006; Bell *et al.* 2007; Krümmelbein 2011). Hardpan soils are the compacted layers underneath the uppermost topsoil layer and restrict root penetration and water movement, and its strength or penetration resistance is measured by penetrometer (Busscher 2011; Jin *et al.* 2015). Hardpan soils occur undernearth the sandy-surface soil type and shallow sandy-over-clay soil type in rice-based cropping systems (Bell and Seng 2003; Sharma *et al.* 1994) and have low permeability that may deliberately increase runoff of water from rice paddies in the lower Mekong regions (Fukai *et al.* 2000; Inthavong *et al.* 2011).

2.2.3 Impacts of rapid surface drying and hardpan soils on forage production

Potential drought caused by rapid surface drying leads to unpredictable loss in crop production in rainfed systems (Serraj *et al.* 2003; Wolf *et al.* 2020). The rate of drying in rainfed sandy soils of Southeast Asia is such that water stress has been observed in rice within one week of rainfall ceasing (Sharma *et al.* 1995). As drying occurs rapidly in the upper soil layers in particular at depth above 20 cm, crops with intense roots in the topsoil are vulnerable due to incapability of roots accessing water at the greater depth of soil profiles (Allen *et al.* 1998; Bodner *et al.* 2015). Hence, the rapid surface drying may affect the establishment of forage grasses, especially species with intense roots at the topsoils.

Root distribution to soil depth is impeded by hardpan soils, preventing access to subsoil moisture and increasing vulnerability to surface drying (Clark *et al.* 2008; Jin *et al.* 2015). Worldwide, resistances of subsoils between 0 and 60 cm depth varying between 2.0 and 3.5 MPa in most cropping systems adversely affect the establishment and productivity of various crop species (Kuhwald *et al.* 2020; Sharma *et al.* 1994; Whalley *et al.* 2008), and most likely some forage grasse species (Philp *et al.* 2019). In most crop species, soil resistance between 2 and 3 MPa in sandy loam soils prevent 75 to 90 % of total root growth into soil profiles (Fig. 2.1c) (Nawaz *et al.* 2013).

Therefore, combination of rapid surface-drying and hardpan soils in sandy soil profiles challenges the establishment by preventing the roots some forage grass species from accessing available water at greater depths.

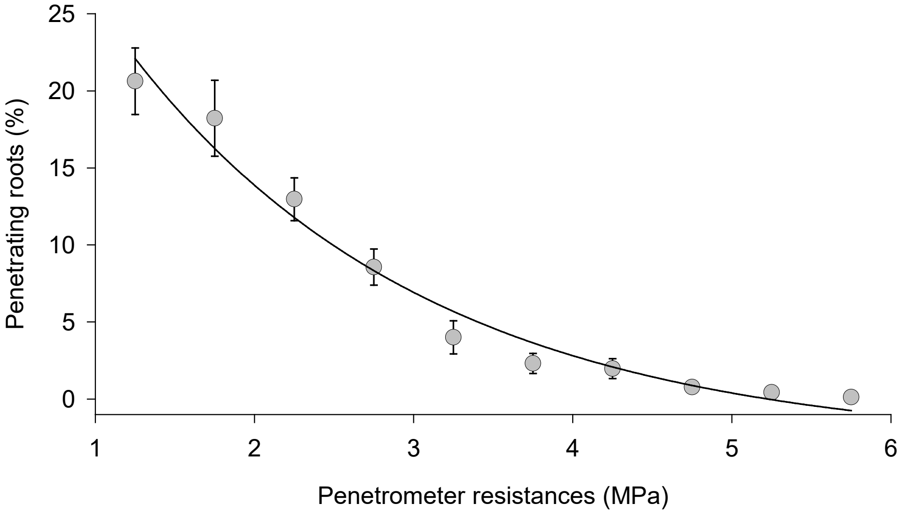

Fig. 2.2 Effects of soil resistances on root penetration in plant species (adapted from Nawaz *et al.* 2013).

2.3 Selection of forage grasses in sandy soils with rapid surface drying and hardpan soils

2.3.1 Drought resistance of perennial forage grass species after establishment

Edaphic factors including rapid surface drying, low water holding capacity and hardpan layers in sandy soils cause water deficit, especially in the topsoils. Forage grass species are established under well-watered conditions and often cultivated with limited irrigation after the establishment in ecosystems with sandy soils. Root systems of some grass species are well developed in soil depth prior to arriving drought stress after establishment. Efficient deep rooting in the soil profiles can be a mechanism for drought avoidance and the key for both survival and high crop production (Bodner *et al.* 2015; Thorup-Kristensen *et al.* 2020). Drought avoidance is the capacity of a grass species to survive during a dry period by retaining high water status in plants (Levitt 1980). Therefore, the survival of desired perennial forage grasses species may rely on rooting depth, the species adaptive traits that correspond to drought resistance strategies (Fig. 2.3).

Different from drought resistance strategies of most annual crops (Fig. 2.3), some perennial grass species may have evolved to use other strategies to survive a prolonged drought and recover immediately after watering (Munns *et al.* 2010). Without relying on root water extraction to avoid drought, drought resistance in established grasses of some forage species such as bermudagrasses (*Cynodon* spp.) entirely depends on the survival of rhizomes or underground stolons that can go dormant under drought for up to 7 months, and afterwards, the drought dormant rhizomes can rapidly regrow under minimal watered conditions (Cook *et al.* 2005; Pandey and Ahirwal 2020).

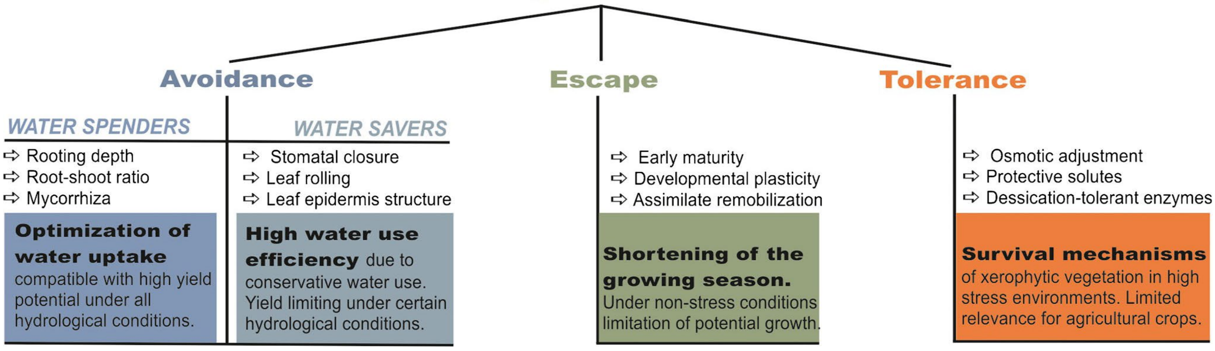

Fig. 2.3 Drought resistance strategies and corresponding adaptive traits of crop species after establishment (Bodner *et al.* 2015).

2.3.2 Deep rooting forage grass species

Forage production on sandy soils with rapid surface drying requires plants to be able to access water from deeper in the soil profile. Species with greater effective roots at soil depth can ensure the survival after establishment in sandy soil with rapid surface drying (Kulmatiski and Beard 2013; Singh and Chahal 2020). In C4 forage grass species, active roots that extract water at the greater depths of soil profiles promote drought avoidance and optimise the growth and harvest (Bodner *et al.* 2015; Cardoso *et al.* 2015; Kulmatiski and Beard 2013; Zhou *et al.* 2014). Deep roots of forage grass species also increase the uptake of nitrate, a mobile nutrient that can easily leach to the greater depths in sandy soil profiles (Acuña *et al.* 2010; Louvieaux *et al.*

2020; Philp *et al.* 2021). When drying occurs, perennial grasses tend to survive and recover if they have already established a deep root system to access moist soil before the onset of drying (Bodner *et al.* 2015; Rao *et al.* 2015), but are vulnerable if the drying occurs before this. However, there are potential hardpan layers in the soil profiles. Therefore, forage grass species with fast-growing roots that have a high capacity of to penetrate hardpan soils will therefore have a shorter period of vulnerability to surface water deficit after establishment.

2.3.3 Highly rhizomatous forage grass species

Rhizomes of especially rhizomatous clonal grasses such bermudagrasses (*Cynodon* spp.) play an important role in storing energy for post-drought recovery, nutrient and water that regulate growth (Bai *et al.* 2010) and promotes drought resistance (Marshall *et al.* 2001; Wang *et al.* 2008; Zhou *et al.* 2014). An extensive rhizome growth of bermudagrasses during the establishment also promotes post-harvest recovery (Zhou *et al.* 2014; 2015), even in the mild temperate climates (Van Tran *et al.* 2017). These characteristics indicate that highly rhizomatous perennial grasses have the potential for forage productions and pastureland restoration.

Forage bermudagrass cultivars have been increasingly used in hay production and pastures (Gomes *et al.* 2019; Hill *et al.* 2001; Pandey and Ahirwal 2020; Rao *et al.* 2015; Zhang *et al.* 2020), whereas *Panicum*, *Paspalum, Pennisetum* and *Urochloa* (syn. *Brachiaria*) grasses are used in grazing, cut-and-curry and processing (Cook *et al.* 2005; da Silva *et al.* 2015; Rao *et al.* 2015). Genetic diversity and broad adaptation of bermudagrass ecotypes across a wide range of climates enable this forage grass species to maintain high forage production from wet tropical and arid temperate regions (Anderson *et al.* 2009; Cook *et al.* 2005; Taliaferro *et al.* 2004; Zhang *et al.* 2019). Bermudagrass ecotypes that naturally occur in latitudes between 45 °S and 53 °N (Harlan and De Wet 1969) allow forage breeders to select the most suitable genotypes for a specific region. Therefore, bermudagrass ecotypes that have high drought resistance, deep-rooting growth habits and rapid growth of extensive rhizome systems can have the potential for forage production and pasturelands in sandy soils and drought-prone regions.

2.4 Knowledge gaps

In summary, perennial forage grasses that can be well-adapted to rapid surface drying and hardpan soils in sandy soil regions possess rapid vertical root growth and enhanced root penetration or large rhizomes. However, there is a lack of comparative studies on traits associated with great vertical root growth rate, great root penetration and extensive rhizome growth.

2.4.1 Vertical root growth rate

Vertical root growth is defined as the development of root distribution including the development of root depth and root length in soil profiles (Hauggaard-Nielsen *et al.* 2000; Lecompte *et al.* 2003). In a study on 48 root architectures of crop species in relation to drought, rapid root depth development is a key trait for accessing plant available water in depth of soil profiles during drought, whereas root length density is a relevant trait for exploring soil moisture when plant water supply is obtained from rainfall or irrigation (Tron *et al.* 2015). Rate of root depth development was used to define the vertical root growth rates in previous studies on perennial grasses (Acuña *et al.* 2010; Boeri *et al.* 2020; Christensen *et al.* 2017; Fuentealba *et al.* 2015). Due to difficulties of investigating root development under field conditions, large rhizotrons or rhizo-boxes (up to 5 m depth) have been used for measuring the vertical root growth of various field crops (Thorup-Kristensen *et al.* 2020). Plants grown in rhizotrons allow the periodic measurements of root depth and vertical root distribution of various field crop and grass species (Acuña *et al.* 2010; Aziz *et al.* 2017; Christensen *et al.* 2017; Wagner *et al.* 2020).

However, traits associated with vertical root growth rate have not been characterised in the previous studies. Forage grass species, such as grasses in Table 2.1 from different genera, possess different morphological traits and growth habits (Cook *et al.* 2005). Previous studies on maize, wheat, and rice found that there were negative relationships between vertical root growth and root angles (Hammer *et al.* 2009; Kato *et al.* 2006; Oyanagi *et al.* 1993; Uga *et al.* 2015). Furthermore, species functional traits are associated with plant growth strategies (Reich 2014; Valverde-Barrantes *et al.* 2017; Wright *et al.* 2004). Therefore, it was hypothesised that forage grass species in Table 2.1 would have genetic variation in vertical root growth rate, and root angle and their plant growth strategies would be associated with vertical root growth rate (Chapter 3).

Table 2.1 Drought resistance and forage yield of perennial forage species that have been grown in sandy soils, semi-arid tropical savannah climates of the lower Mekong regions. Colours indicate high (green cells), moderate (yellow cells), and poor (red cells) (Philp *et al.* 2019)

Grass species[1]	Adaptation	
	Drought resistance	Forage yield
Andropogon gayanus	🟩	🟩
Cynodon dactylon	🟩	🟩
Megathyrsus maximus	🟩	🟩
Paspalum scrobiculatum	🟨	🟨
Pennisetum hybrid	🟩	🟩
Pennisetum purpureum	🟨	🟩
Setaria sphacelata	🟨	🟩
Urochloa brizantha	🟩	🟩
Urochloa decumbens	🟩	🟩
Urochloa humidicola	🟩	🟩
Urochloa hybrid	🟩	🟩
Urochloa hybrid	🟩	🟩
Urochloa mosambicensis	🟨	🟨
Urochloa mutica	🟥	🟩
Urochloa ruziziensis	🟥	🟩

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[1] Names of grass species have been updated: *Brachiaria* spp. to *Urochloa* spp., *Panicum maximum* to *Megathyrsus maximus,* and *Paspalum atratum* to *Paspalum scrobiculatum*.

2.4.2 Relationships between vertical root growth rate and drought resistance

Forage species suitably adapted to sandy soils in the lower Mekong regions were reviewed, and the drought resistance of grass species has been defined by the ability to retain green leaf and to persist during the dry season (Table 2.1) (Philp *et al.* 2019). Of perennial grasses, the drought resistance associated with green cover during drought is promoted by water extraction at the greater depth of soil profiles (Zhou *et al.* 2014) that may also be associated with increased vertical root growth rate and active root length to those depths. Hence, the high adaption of perennial grass species to sandy soils with rapid surface drying may be associated with a rapid vertical root growth rate to avoid the drought.

Relationships between water extraction, drought resistance and vertical root growth rate of perennial grasses have not been examined in previous studies. Large variations in drought resistance and water extraction at greater soil depths have been found in previous studies on bermudagrasses collected throughout Australia (Table 2.2) (Zhou *et al.* 2014), suggesting that bermudagrasses may have a large variation in vertical root growth rates. In addition, there is a large genetic diversity in bermudagrasses and even among bermudagrass ecotypes in Australia (Jewell *et al.* 2012a; Jewell *et al.* 2012b; Lambrides *et al.* 2013; Zhang *et al.* 2019). Therefore, using bermudagrass ecotypes with established drought resistance in previous field experiments (Zhou *et al.* 2014) to study root depth development may provide a deep understanding of vertical root growth rate in relation to drought resistance and shoot growth in perennial grass species. It was hypothesised that variation between the shoot and root growth of bermudagrass ecotypes from differing climatic zones in Australia would be unstable across seasons (Chapter 4). A previous study reported that the drought resistance of bermudagrasses was positively correlated with water extraction at the greater depth of soil profiles but not correlated with root length density (Zhou *et al.* 2014). Water extraction during drought may be performed by active roots associated with vertical root growth rate. Accordingly, it was hypothesised that there would be a positive relationship between vertical root growth rate and drought resistance (Chapter 4).

Table 2.2 Drought resistance perennial grass ecotypes representing a diversity of bermudagrasses in Australia. Drought resistance is the number of days required for green cover to fall to 50% (GC50) without irrigation under field conditions. Bioclimatic data include annual average temperature (AT), annual rainfall (WRF), annual evapotranspiration (AET_o) and aridity index (AI). Wintergreen (WG) is a commercial cultivar.

Ecotypes	Species GPS	GC_{50} (days)	AT (°C)	ARF (mm)	AET_o (mm)	AI
MED1	-33.311790, 117.339770	277	23	843	975	0.86
MED3	-31.996687, 115.751509	251	24	702	932	0.75
40	-26.196127, 152.680493	244	27	1272	1254	1.01
MED2	-32.853120, 115.924160	243	22	843	1025	0.82
394	-19.260880, 146.783586	234	29	1132	1093	1.04
573	-35.082453, 139.285123	231	23	390	1048	0.37
55	-24.831166, 152.466283	224	27	1017	1031	0.99
698	-37.970428, 145.031045	216	25	731	788	0.93
WG	-27.502668, 153.016821	210	27	979	914	1.07
25a1	-26.404041, 153.073875	195	26	1554	905	1.72
659	-35.741564, 143.945841	193	23	359	1108	0.32

2.4.3 Relationships between vertical root growth rate and root penetration

Capability for root penetration of crop species has been tested using wax layers to construct artificial hardpan soils due to the reliability and convenience of physical and chemical wax properties that do not influence plants growth (Taylor and Gardner 1960; Yu *et al.* 1995). The stable rigidity state of wax is not affected by soil moisture, which provides a reliable condition to examine root penetrability of crops, while the hardness of hardpan soils in fields may change according to soil water content (Yu *et al.* 1995; Zhou *et al.* 2017a). Wax methods have been used to screen major crop species such as rice varieties (Babu *et al.* 2001; Colmer and Voesenek 2009; Yu *et al.* 1995), durum wheat ecotypes (Kubo *et al.* 2004) and maize genotypes (Chimungu *et al.* 2015).

Relationships between vertical root growth rate and capacity of root penetration is poorly documented in perennial grass species that possess continuous growth. The latest review on root penetration has demonstrated positive associations between root penetration, root diameter and shoot growth in annual crops (Jin *et al.* 2015). Rapid shoot growth in perennial grasses can be positively associated with faster vertical root growth (Acuña *et al.* 2010; Kong *et al.* 2019; Reich 2014; Valverde-Barrantes *et al.* 2017). Vertical root growth rates of annual crops may dramatically decrease with maturity and soil depth (Liu *et al.* 2011; Perkons *et al.* 2014), but perennial grass species with continuous growth habits may have a constant vertical root growth rate in their growth. Therefore, it was hypothesised that root penetration in forage grass species would be positively related to root growth rate, shoot growth and root diameter (Chapter 5).

2.4.4 Variation in rhizomatous grasses across climates

Compared with agricultural forage cultivars, crop species from highly varied environments in their habitats can grow roots deeper (Thorup-Kristensen *et al.* 2020). Extensive rhizomes and deep rooting are desirable characteristics for drought resistance of bermudagrasses (Zhou *et al.* 2014) and high forage production under highly variable water conditions (Acuña *et al.* 2010; Bodner *et al.* 2015; Christensen *et al.* 2017). Approximately 1000 bermudagrass genotypes were identified across wild habitats throughout all climatic zones of Australia (Kearns *et al.* 2009). Some bermudagrass genotypes were evaluated for the same potential for herbage production as commercial forage grass cultivars (Hacker *et al.* 2013) and already studied drought-resistance in glasshouse and field trials (Zhou *et al.* 2013a; 2014; 2015; Zhou *et al.* 2013b). The potential forage bermudagrasses with extensive rhizomes originate from wild habitats of dry regions with sandy soils (Zhou *et al.* 2014; 2015). The increase in rhizome growth seems to be a revolutionary adaption of bermudagrass ecotypes to arid conditions (Marcora *et al.* 2017; Paula and Pausas 2006; Zhou *et al.* 2014). Therefore, it was hypothesised that rhizome growth would be greater in bermudagrass ecotypes from arid environments than in non-arid environments (Chapter 6).

CHAPTER 3: ROOT DEPTH DEVELOPMENT IN TROPICAL PERENNIAL FORAGE GRASSES IS ASSOCIATED WITH ROOT ANGLE, ROOT DIAMETER AND LEAF AREA

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Contribution to the Paper	Planned and conducted the experiments, analysed and interpreted the data, and wrote the manuscript
Overall percentage (%)	85%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is

	not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	29/9/2021

Co-Author

By signing the Statement of Authorship, each author certifies that:
i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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REGULAR ARTICLE

Root depth development in tropical perennial forage grasses is related to root angle, root diameter and leaf area

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Abstract

Aims Tropical perennial grasses that can rapidly establish deep roots have a greater potential to survive soil surface drying sooner after establishment. This research aimed to identify species that establish deep roots quickly and to investigate the mechanisms that drove rapid vertical root development.

Methods Perennial grass species *Brachiaria brizantha*, *Brachiaria decumbens*, *Brachiaria humidicola*, *Brachiaria* hybrid Mulato II, *Brachiaria mosambicensis*, *Panicum maximum*, *Setaria sphacelata* and *Cynodon dactylon* were grown in rhizotrons that facilitated measurement of the rate of root depth development, the rate of root length development, photosynthesis and morphological traits. Principal component analysis was employed to separate grass species according to functional traits of vertical root development and their growth strategies.

Results *P. maximum* established roots more rapidly to 50 cm depth than the other species examined and accumulated biomass and leaf area faster. It was differentiated from other species by a greater percentage of fine root length (< 0.2 mm diameter), a higher photosynthetic rate and ratio of root length to leaf area according to the principal component analysis. The growing degree days required for root establishment to 50 cm depth was negatively correlated with the percentage of fine root length, and positively correlated with root angle. The exponential rate of root depth development per growing degree day increased with average root diameter in *B. humidicola* and *B.* hybrid Mulato II, indicating a conservative growth strategy.

Conclusion Rapid vertical root development with a narrow root angle, higher photosynthetic rate, higher ratio of root length to leaf area and higher percentage of fine roots were apparent mechanisms that enabled *P. maximum* to establish deep roots faster than other species. *P. maximum* appears to be better able to avoid water stress during surface drying by accessing profile moisture. Greater average root diameter and percentage of root length with higher diameters was related to increased exponential rate of root depth development and wide root angle.

Keywords *Brachiaria* · Growth strategies · *Panicum* · Root growth · *Setaria* · *Urochloa*

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Keywords *Brachiaria*, Growth strategies, *Panicum*, Root growth, *Setaria*, *Urochloa*

3.2 Introduction

The establishment of perennial forage grasses to support the livestock sector in tropical rice-producing ecosystems is challenged by increasingly variable and changing climate conditions (Cornish *et al.* 2018; Sloat *et al.* 2018), including shorter wet seasons and an increasing incidence of severe drought (Chadwick *et al.* 2016). Under these circumstances, surface soil drying occurs more frequently and for longer throughout the year. When drying occurs, perennial grasses tend to survive and recover if they have already established a deep root system to access moist soil before the onset of drying (Bodner *et al.* 2015; Rao *et al.* 2015), but are vulnerable if the drying occurs before this. Soil horizons between 100 and 120 cm generally provide sufficient moisture for the growth of most crops in rice-producing regions (Bell *et al.* 2006; Cornish *et al.* 2018; Lodge and Murphy 2006; Seng *et al.* 2007). Species with fast-growing roots will therefore have a shorter period of vulnerability to surface water deficit after establishment, making it useful to identify these species and any associated traits.

Faster root depth development is associated with vertical root distribution (Fuentealba *et al.* 2015). Previous research has found that root distribution down the soil profile was negatively correlated with root angle in rice, maize and wheat (Hammer *et al.* 2009; Kato *et al.* 2006; Oyanagi *et al.* 1993; Uga *et al.* 2015). Faster root depth development is also associated with below- and above-ground accumulation biomass in *Paspalum* spp. (Acuña *et al.* 2010). The promotion of below-ground biomass could indicate a rapid rate of total root length development that may be associated with increased shoot growth of grasses. Therefore, these findings suggest that there could be relationships between root angle, vertical root distribution and plant growth. This has not been investigated in perennial forage species, which can vary greatly in root distribution (Guenni *et al.* 2002).

The rate of vertical root development may vary among forage grass species due to their distinct morphologies and associated light interception and assimilate partitioning (Fustec *et al.* 2005). Perennial forage grass genera such as *Brachiaria* (syn. *Urochloa*), *Cynodon, Panicum and Pennisetum* can be caespitose, stoloniferous or rhizomatous (da Silva *et al.* 2015; Ghimire *et al.* 2015). This diversity indicates a large variation in shoot and root traits, with implications for root growth. For example, stoloniferous grasses allocate less dry matter towards root development because the development of stolons requires more fixed carbon than the development of leaf area (Irving 2015; Poorter *et al.* 2012). Furthermore, photosynthesis of perennial forage grasses depends on canopy structure (ranging from erect or prostrate structures), as demonstrated in *Brachiaria* spp. (Dias-Filho 2002) and on leaf traits, such as specific leaf area (Carlen *et al.* 1999). This suggests that root development rates are dependent on the trade-off between below- and above-ground biomass allocations, in association with species functional traits such as total root length, leaf area and total dry weight, which may differ among perennial grass genera.

Covariations between species functional traits result in the optimisation of the whole plant growth in specific environments (Reich 2014; Valverde-Barrantes *et al.* 2017; Wright *et al.* 2004). In perennial grass ecosystems, the growth rates vary largely between species that express exploitative or conservative growth strategies (da Pontes *et al.* 2015). The exploitative species produce more aboveground biomass and at a faster rate than the conservative species (Duchini *et al.* 2018). The ability to grow rapidly is due to the resource accumulation of the exploitative growth strategy, with larger specific leaf area, higher leaf N content and photosynthesis and a lower leaf lifespan; a conservative growth strategy is the opposite (Reich 2014; Wright *et al.* 2004). Furthermore, the exploitative perennial grass species are highly capable of resource capture due to greater specific root length that increases root N uptake but reduces root lifespan (Kong *et al.* 2019; Weemstra *et al.* 2016). Despite the slow growth, conservative grass species have the potential for greater survival in variable conditions due to the resource conservation with low specific root length, high root tissue density, thick root diameter and longer root lifespan (Reich 2014). The coordination between root and shoot traits is a means for plant species to balance resource acquisition differently between roots and shoots among contrasting growth strategies (Freschet *et al.* 2015). Positive associations are expected between specific root length, root N content, specific leaf area and leaf N content in exploitative species, and negative associations are expected between root tissue density, root diameter, specific leaf area and leaf N (Kong *et al.* 2019; Reich *et al.* 2008; Valverde-Barrantes *et al.* 2017). Leaf photosynthesis can accelerate assimilation rates in tropical perennial grasses (Ghannoum *et al.* 2005; Yamori *et al.* 2010). Therefore, we hypothesised that vertical root development would have positive relationships with specific root length, root length density and leaf photosynthesis in perennial forage grass species. We analysed variations in rates of vertical root development in tropical perennial forage grass species, to assess covariations in rates of vertical root development, photosynthesis and morphological traits, to identify the possible mechanisms for root depth development as an adaptive trait for surviving soil surface drying after establishment.

3.3 Materials and methods

3.3.1 Experiments and plant materials

A glasshouse experiment was conducted at the Waite Precinct, The University of Adelaide from February to March 2018. Forage grass seeds from each species in Table 1 were germinated in plug trays with cell dimensions of 3.5 × 2.2 × 5.0 cm LWH. One vigorous seedling per cell was grown for 6 weeks before transplanting, except for seedlings of *C. dactylon* which were grown from one cutting per cell. Each cell was filled to a depth of 4.0 cm with air-dried UC mix produced by SARDI Plant Research Centre, Waite, Australia. A 1 m^3 of UC mix is composed of 0.56 m^3 of Waikerie sand, 0.44 m^3 of Canadian peat moss, 0.80 kg of hydrated lime, 1.33 kg of agriculture lime and 3 kg of Osmocote Exact Mini (16–3.5-9.1 + TE) from Fernland (Yandina, QLD 4561 Australia). During the growth in the plug trays, seedlings were pruned regularly to maintain uniformity. During transplanting, seedlings were removed from the trays with the whole root mass and attached UC mix intact. All tillers except the youngest with two leaves on each plant were removed at the base. The seedlings were then transplanted into a rhizotron. Four to six seedlings of *C. dactylon* were planted per rhizotron so that fresh weight that was equal to the fresh weight of the other species. Plants growing during the experiment were irrigated daily at 0930 h by bringing the moisture close to the water holding capacity. As the UC mix was saturated at transplanting, each rhizotron was irrigated with 0.2 L day^{-1} in week 1, which increased to 0.3 L day^{-1} in week 2 and 3 after transplanting and then with 0.5 L day^{-1} until the end of the experiment.

Large rhizotrons of a similar design to Aziz *et al.* (2017) were used in the experiments. The rhizotrons were made of folded galvanised steel, 120 × 20 × 5 cm in dimension, with the open vertical dimension of the rhizotron fitted with a removable clear polycarbonate sheet for root growth observation. Each rhizotron was filled with 11.5 L (18 kg) of air-dried UC mix. The available nutrients in the substrate were 5.52 g N, 2.16 g P and 7.06 g K per rhizotron. Uniform bulk density was achieved by air-drying the UC mix before the filling process, which involved laying the rhizotron flat and spreading a known weight of UC mix evenly across the full length of the rhizotron 3 times before securing it in place by mounting the clear polycarbonate sheet. The rhizotron and the polycarbonate sheet were fixed together by 5 nuts and bolts and sealed with silicone in each side to provide a firm structure. Once the silicone set, the rhizotrons were stood up and slowly watered to field capacity. The bottom of the rhizotrons had drainage slits permitting the movement of water but not soil. All rhizotrons were securely positioned at a 45° angle against benches with the transparent side facing downwards. The transparent side was covered by a black PVC sheet during the experiments. The rhizotrons in both experiments were arranged around benches (80 cm) in the centre of the glasshouse as a completely randomised block design with three replications. The block was organised to account for the effects of morning and afternoon sunlight.

3.3.2 Root development rates

Vertical root development was defined by the rate of root depth development (RRDD) and rate of root length development (RRLD). RRDD was calculated from weekly measurements of root depth, determined by the single deepest visible nodal root from a marked point where the bottom of the seedling was placed during transplanting, to the deepest root tip. The maximum root depth was limited to 115 cm by the height of the rhizotrons. RRLD was analysed from the measurement of total root length. Weekly total root length was derived from the weekly root tracking of nodal roots visible along the transparent sheet, using the technique described in Aziz *et al.* (2017). The black PVC sheet on the transparent side of each rhizotron was replaced with transparent plastic films and the visible nodal roots growing from the base of the plants were traced on the films with a permanent black pen. Then, the films were scanned using an EPSON Expression 10000XL scanner (EPSON Inc., Long Beach, CA 90806, USA) at 400 dpi resolution. The images were analysed by root image analysis software WinRHIZO V.2009a (Regent Instruments Inc., Quebec, Canada). The weekly total root length at time *t* (RL_t) was estimated by the relationship between the last measurement of root tracking (RT_r) and the actual total root length obtained from root scanning at the harvest. The empirical model was

$RL_t = -114{,}422 + 15{,}637 \times Exp\ (0.0006 \times RTr_t)$ (Supplementary Fig. 3.S1)[2]. The final destructive measurement of all samples occurred at the first instance of a 115 cm root depth in any species.

RRDD and RRLD were derived from the empirical relationships between weekly root depth and total root length as a function of thermal time or growing degree days (GDD). GDD was measured as the sum of the daily temperature above a base temperature of 10 °C, suitable for the subtropical grass species used (Moreno *et al.* 2014). Temperature and light intensity were measured inside the glasshouse using Hobo Pendant Temp-Light Data Loggers (OneTemp Pty Ltd., Bourne, MA 02532, USA) (Fig. 3.1). The record in the glasshouse showed that the daily temperatures and even minimum temperatures never reached the base temperature set. Curve-fitting was performed using an exponential growth function (single, three parameters) according to previous studies (Atkin *et al.* 2000; Evers 2016; Reynolds and Thornley 1982). Given that there can be a large variation in the delay of early growth in tropical grasses Philp *et al.* (2019), an exponential growth function was appropriate for parameterisation of the growth rates, as described in (Thornley and Johnson 1990). Therefore, root depth at GDD was $f(GDD) = y_0 + a \times exp(b \times GDD)$, where b is the slope of the curve, the growth of root depth and root length, respectively equal to RRDD and RRLD. y_o and a are the constants of the model and species-specific parameters.

The exponential growth function was used to predict continuous development; however the maximum root depth used to parameterise the model was 120 cm. Therefore, $f(GDD) = y_0 + a \times exp(b \times GDD)$ was used to predict the thermal time or growing degree days requirement for each species to grow roots to the depths of 50, 100 and 120 cm, defined as GDD_{50}, GDD_{100}

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[2] Supplementary material is available at *Plant and Soil* volume 456, pages 145–158 https://doi.org/10.1007/s11104-020-04701-2

and GDD_{120}, respectively. The values of the growing degrees predicted explained the rates of root depth development to the field depth between 50 and 120 cm.

3.3.3 Root measurements

Root angle was measured for each plant as the angle created by the two outermost primary roots growing from the base of the shoot (Mace *et al.* 2012). The substrate in each rhizotron was sectioned into 0 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50, 50 to 60 and 60 to 115 cm to analyse vertical root distribution, defined as RL0–10, RL10–20, RL20–30, RL30–40, RL40–50, RL50–60 and RL60–115, respectively. The depths from 60 to 115 cm were treated as one section because the roots of most species concentrated in the sections between 0 and 60 cm depth. The roots in each section were separated from the UC mix and washed with running water on a laboratory test sieve with an aperture of 500 µm (Endecotts Ltd., London, UK). The washed root samples were preserved in 50% ethanol and stored at 4 °C until scanning. The procedures for the root scanning and the analyses of the images for the total root length were as described above. After scanning, the root samples from all sections were combined to determine the dry weight (DW) after drying at 60 °C for 72 h. This method for DW analysis was also used for other plant components sampled in this study. The vertical root distribution was defined as root length density (RLD) and percentage of root length (%RL) for each section. RLD in respective depths was calculated as root length divided by the volume of soil in each section. RLD in the root zone was calculated as total root length divided by the volume from the base to the deepest root tip. The analysis using WinRHIZO provided average root diameter, root volume, root surface area, and root length for different root diameters: < 0.2 mm (RL < 0.2 mm), 0.2–0.5 mm (RL0.2–0.5), 0.5–1 mm (0.5-1 mm), 1–2 mm (RL1-2 mm) and > 2 mm (RL > 2 mm). For species functional trait analyses, root tissue density was calculated as root DW divided by root volume, specific root surface area was calculated as root surface area by root DW, specific root length was calculated as RL length by root DW, and root length to leaf area ratio was calculated as root length by leaf area. The root length in each root diameter group was calculated as the percentage to total root length as %RL < 0.2 mm, %RL0.2–0.5 mm, %RL0.5-1 mm, %RL1-2 mm and %RL > 2 mm, respectively.

3.3.4 Shoot measurements

Shoot traits and variables were measured at harvest, except for rate of tiller number development (RTND), that was calculated from the weekly tiller counts using $f(GDD) = y_0 + a \times exp(b \times GDD)$. Leaf area of plants was estimated by leaf scanning (Rose *et al.* 2018; Zhou *et al.* 2017b; Zhou *et al.* 2017c). The leaf blades were detached from the sheaths and scanned immediately (CS9000FMKII, Canon, Tokyo, Japan) at 400 dpi. The images were then used to measure the total area using ImageJ software (Window version 1.8.0, Wayne Rasband, Maryland, USA). Specific leaf area was determined as leaf area divided by leaf DW, leaf sheaths excluded. The assimilate allocation to each plant component was defined by dry weight ratio and dry weight fraction measured in roots, culms and leaf blades. Culm DW was determined as the sum of sheath and tiller DW. Root to shoot ratio was determined as root DW divided by shoot DW (i.e., the sum of culm DW and leaf DW), and culm to leaf blade ratio was determined as culm DW divided by leaf blade DW. Mass fraction was defined as the percentage of DW allocated to roots, shoot, culms and leaves.

3.3.5 Photosynthesis

Gas exchange measurements were taken twice for each plant, 3 and 4 weeks after transplanting, using a Li-Cor 6400 (Portable Photosynthesis Systems, Lincoln, NE, USA). The device was integrated with a leaf chamber fluorometer with a diameter of 1 cm (Li-6400-40 LF, Portable Photosynthesis Systems) using procedures of measurement adapted from Zhou *et al.* (2017b).

The measurement was taken between 1100 and 1230 h. The flow rate in the chamber and the rate of the reference CO_2 concentration were both set at 400 µmol mol^{-1}. The extra CO_2 source was supplied by the CO_2 mixer. The photosynthetic photon flux density was obtained from the external quantum sensor at the optimal waveband of 1500 µmol m^{-2} s^{-1}, red/blue with 10% blue (Dias-Filho 2002; Gómez *et al.* 2013).

3.3.6 Statistical analysis

Mean comparisons were performed using GenStat 18th Edition (VSN International Ltd., Hemel Hempstead, UK). A general linear model was performed for the analysis of variance (ANOVA) in randomised blocks to assess the significant differences of the variables among species. Two-way ANOVA in randomised blocks was used to evaluate the species × section and species × depth interactions for the root distributions in each section and GGD requirement for depths of 50, 100 and 120 cm. The significant differences of means were distinguished by Fisher's least significant difference (LSD) at $P = 0.05$. Curve fittings were performed using SigmaPlot 14.0 (Systat Software Inc., San Jose CA, USA). The significance of relationships between all measured variables was evaluated by Pearson correlation analysis, two-sided tests against zero at $P = 0.05$, using GenStat 18th Edition (Supplementary Table 3.S2). Principal component analysis (PCA) was used to assess co-variations of species functional traits using the statistical analysis package of SigmaPlot 14.0. To fulfil the requirement of PCA analysis, some variables were selected and linearised by ln-transformation (Supplementary Table 3.S3). For example, plant size parameters such as leaf area, DW and total root length were excluded from the PCA analysis to reduce the size effects. The percentage of root length in the 30–115 cm was summed to make a new variable as the zero value was not valid for ln-transformation.

3.4 Results

3.4.1 Vertical root development

The experiment was concluded for the destructive final measurements at 28 days after transplanting (583 °Cd) when *P. maximum* had root tips that reached the bottom of rhizotron (Fig. 3.2a). The second deepest root at the time of harvest was observed in *B. mosambicensis* at 85 cm depth (Fig. 3.2a), while *B.* hybrid Mulato II and other species had root depths that measured approximately 60 cm at the harvest (Fig. 3.2b). In a system with an unconstrained growing depth, all species were predicted to grow with significantly varied RRDD ($P < 0.001$, LSD = 17.50, Fig. 3.2c). All species required significantly different thermal time to grow roots to a depth of 50 cm ($P < 0.001$, LSD = 80), 100 cm ($P < 0.05$, LSD = 114) and 120 cm ($P < 0.05$, LSD = 128), (Fig. 3.2d). Despite small RRDD, *P. maximum* and *B. mosambicensis* had a high root depth development at 100 °Cd after transplanting, relative to other species. *B. humidicola* grew root depth slowly until 300 °Cd before exhibiting a rapid increase in root depth that resulted in the greatest RRDD. *B. decumbens* and *S. sphacelata* required a longer time than other species to establish root depth to 50, 100 and 120 cm.

In relation to the development of the entire root system, delayed or slower growth patterns of root length development were evident until 300 °Cd (Fig. 3.2e, f). Species varied in RRLD ($P < 0.001$, LSD = 8.29, Fig. 3.2g). For a long term-growth prediction, *P. maximum* showed the largest total root length at harvest but had similar RRLD as *B. humidicola*, *B.* hybrid Mulato II and *B. mosambicensis. B. brizantha* started to develop root length after 100 °Cd and had a smaller RRLD over the long term. Despite the slow initial growth, *B. decumbens* and *S. sphacelata* had the greatest estimated RRLD. During the experiment, *C. dactylon* grew slowly and did not produce root depth and length with sufficient data points for analysis of RRDD and RRLD.

All grass species significantly differed in the vertical root growth as measured in root length density and the percentage of root length in each section between 0 and 60 cm depths (Fig. 3.4a, b). Only *P. maximum* and *B. mosambicensis* grew roots into the 60–115 cm depth section, with 7% and 5% of roots, respectively, in this lower section. The interaction of species × depth was non-significant in root length density ($P > 0.05$ and LSD = 1.50, Fig. 3.3a), but significant in the percentage of root length distribution ($P < 0.01$ and LSD = 12.20, Fig. 3.3b). Approximately 20% and 10% of *P. maximum* and *B. mosambicensis* roots were below 50 cm depth, respectively, but only 1% to 3% of root length in other species were observed at these depths. *C. dactylon* did not have roots distributed below 20 cm.

The percentage of root length with diameter of <0.2 mm, 0.2–0.5 mm, 0.5–1 mm, 1–2 mm and > 2 mm significantly differed among species (Fig. 3.3c). The interaction of species × root diameter was significant ($P < 0.001$, LSD = 5.23). The fine root length with <0.2 mm and 0.2–0.5 mm each varied between 30 and 40%, the 0.5-1 mm group between 15 and 20%. A large variation was found in root diameter of 1–2 mm and > 2 mm, which accounted for small proportions of roots.

Differences in plant biomass parameters were significant for dry weight ($P < 0.001$, LSD = 2.05) (Fig. 3.4a), and root fraction ($P < 0.001$, LSD = 4.45) (Fig. 3.4b). Over a four-week growth period, the fastest growth was observed in *P. maximum*, with an average of 13 g plant^{-1}. An increase between 4 and 7 g plant^{-1} was observed in other *Brachiaria* spp. and *S. sphacelata*, while the least growth was observed in *C. dactylon* at 1.15 g plant^{-1}. *P. maximum* also had a large root fraction that was similar to *B. mosambicensis*, *B.* hybrid Mulato II and *B. decumbens*.

3.4.2 Possible mechanisms for vertical root development

Correlations between all measured variables are provided as Supplementary Table 3.S2. The forage species were differentiated by PCA according to the observed variables that defined the growth strategies (Fig. 3.5). There were 10 PC with eigenvalues >1. PC 1 explained 40% of the total variation and *P. maximum* was distinguished from other species by increasing root length density in the 0–50 cm section, percentage of fine root length (<0.2 mm diameter) and photosynthesis. The correlation analysis revealed that GDD_{50} decreased with increasing percentage of root length < 0.2 mm ($r = -0.57$, $P < 0.01$). Photosynthetic rate was greater with increasing root length to leaf area ratio ($r = 0.85$, $P < 0.01$) and root length density in the 0–50 cm section ($r = 0.45$, $P < 0.05$). PC 2 (13%) separated species with high values for RRDD, average root diameter, percentage of root length (0.5–1 mm diameter) such as *B. humidicola* and *B.* hybrid Mulato II; the factors of RRLD, GDD_{50}, specific leaf area and RTND were influential in *B. decumbens* and *S. sphacelata*. *B. mosambicensis* and *B. brizantha* seemed to be the intermediate species and *B. mosambicensis* with a small root angle. RRDD increased with greater average root diameter ($r = 0.54$, $P < 0.5$), but decreased the higher percentage of root length < 0.2 mm diameter ($r = -0.58$, $P < 0.01$). Average root diameter was greater with increasing percentage of length of larger roots with 1–2 mm diameter ($r = 0.72$, $P < 0.01$) and root angle ($r = 0.62$, $P < 0.01$), but was not correlated with percentage of root length < 0.2 mm ($r = -0.42$, $P > 0.05$). Unexpectedly, RRDD was not correlated with RRLD ($r = 0.11$, $P > 0.05$). Furthermore, the variables correlated with RRDD were not correlated with RRLD. RRLD increased with increasing root to shoot ratio ($r = 0.61$, $P < 0.01$), specific leaf area ($r = 0.56$, $P < 0.05$) and RTND ($r = 0.71$, $P < 0.01$), none of which were correlated with RRDD.

The vertical root development increased with the greater leaf area that contributed the rapid development of fine roots (< 0.2 mm) and the increased photosynthetic rate (Fig. 3.6a, f). Root development to 50 cm was slower in plants with wide root angles, which also had an increased percentage of roots in the 0–10 cm section, reduced root length density in the 0–50 cm section, and lower photosynthetic rate (Fig. 3.6b-e). The species that grew roots first to 50 cm also tended to rapidly reach 120 cm. GDD_{120} was correlated with GDD_{50} ($r = 0.61$, $P < 0.01$) despite no correlation with fine roots of <1–2 mm diameter ($r = 0.04$, $P > 0.05$).

3.5 Discussion

3.5.1 Relationship between root angle and root depth development

Species with large root angles tended to grow root systems horizontally rather than vertically, leading to shallow root depth at harvest and greater thermal time required to reach 50 cm depth. *B. brizantha*, *B. decumbens* and *S. sphacelata* had greater root distribution in the 0–30 cm sections and fewer roots below 50 cm. Similarly, Guenni *et al.* (2002) also found that *B. brizantha* and *B. decumbens* had shallow root distribution, possibly associated with large root angle as observed in the present study, and other studies of wheat (Oyanagi *et al.* 1993), rice (Kato *et al.* 2006) and maize (Hammer *et al.* 2009). Wide root angle species may be compromised in their ability to rapidly growing roots to depth. In contrast, perennial grasses with narrow root angles viz. *P. maximum* and *B. mosambicensis*, tended to grow roots downwards and had more roots in the 60–115 cm section. As a result, these species also required less growing degree days to develop roots to reach depths of at least 120 cm. These findings indicate that small root angle could lead to deep root distribution and rapid root establishment to the depth between 50 and 120 cm. Therefore, root angle could be one of the selection criteria to increase adaptation of perennial forage grass species grown in soils that are prone to surface drying.

3.5.2 Relationship between root diameter and depth development

P. maximum was differentiated from other species in PC 1 by specific plant growth strategies that are categorised in the plant economics spectrum (da Pontes *et al.* 2015; Valverde-Barrantes *et al.* 2017; Wright *et al.* 2004). The rapid root development to 50 cm depth (smaller GDD_{50}, Figs. 3.2d and 5a) was associated with larger values of root length to leaf area ratio, photosynthetic rate and the proportion of fine roots with <0.2 mm diameter that could indicate an exploitative growth strategy of *P. maximum*. The rapid establishment of the fine roots likely promoted the growth of root length density in the 0–50 cm section and could lead to increased root uptake to boost plant growth, as observed in other perennial grasses (Busso *et al.* 2001). However, fine roots have shorter lifespans that may influence the outcome over the longer term (Kong *et al.* 2019; Reich 2014; Weemstra *et al.* 2016). This could be a reason that fine roots were negatively correlated with rate of root depth development, as observed in this study.

The greater percentage of the fine roots (< 0.2 mm diameter) was a possible mechanism enabling rapid vertical root development to 50 cm depth. As a result, fine roots could also contribute to the increased overall root tissue density in the exploitative growth strategies. Root tissue density was positively correlated with root length density in the 0–30 cm section ($r = 0.45$, $P < 0.05$) and the percentage of root length below 60 cm ($r = 0.57$, $P < 0.01$). These results indicate that the fast-growing roots of *P. maximum* with the greater root tissue density could result from the rapid development of thick lignified stele and high cell wall contents (PC 1, Fig. 3.5a; Wahl and Ryser 2000). In contrast, *B. humidicola* and *B.* hybrid Mulato II appeared to have conservative growth strategies because their higher rate of root depth development was associated with greater average root diameter and a decreased percentage of fine roots (< 0.2 mm diameter). The increased average root diameter in the conservative species was associated with a greater percentage of the length of roots with 1 to 2 mm diameter. This relationship indicates that high average root diameter could be a possible mechanism related to conserving resources (da Silva *et al.* 2015; Duchini *et al.* 2018; Roumet *et al.* 2016; Wahl and Ryser 2000).

3.5.3 Relationship between leaf area and root depth development

The exploitative growth strategies were attributed to greater root length to leaf area ratio in *P. maximum* in this study, which may be associated with leaf traits associated with fast-growth species (Reich 2014; Valverde-Barrantes *et al.* 2017). *P. maximum* had the highest photosynthetic rates at elevated root length to leaf area ratio that may indicate a trade-off in the leaf-root relationship (Reich 2014; Wright *et al.* 2004). A possible mechanism could be a functional balance between leaf and root activities in plant growth regulation, whereby leaves transfer a proportion of the photosynthates to allow for root expansion to optimise water uptake to maintain the high productivity in fast-growing perennial grass species (da Pontes *et al.* 2015; Roumet *et al.* 2016). Previous studies on perennial forage grasses demonstrated that rapid growth of leaves promoted the expansion of root systems in association with increased leaf photosynthesis (Acuña *et al.* 2010; Crous *et al.* 2010; Guyonnet *et al.* 2018; Thompson *et al.* 2017). In this study, species with high tiller development showed rapid expansion of the entire root system. This may be related to leaf area development, which, in *Cynodon* spp. (also observed in this study) and *Zoysia* spp., influenced root growth rates (Fuentealba *et al.* 2015; Steinke *et al.* 2013). The current findings suggest that the increase in tiller production may increase the rate of leaf area expansion or leaf dry weight fraction that could lead to the increased root dry weight fraction, as observed in *P. maximum*. This relationship could be explained through two possible mechanisms. Firstly, root growth rates depend on a carbon flux to the roots supplied by the leaves (Poorter *et al.* 2012) which was indicated in the current research through the positive correlation between rate of root length development and root to shoot ratio, but not in root fraction, as was expected. Secondly, net assimilation is also increased by leaf area in perennial grasses (Greco and Cavagnaro 2003; Jones *et al.* 2015), and could be accelerated by high root length to leaf area ratio, as observed in this study. Accordingly, the species with large leaf areas had larger root lengths, and higher total and root dry weights, that could result in the exploitative growth strategy and a deep root system to 120 cm, as observed in *P. maximum*.

3.5.4 Increased rate of root depth development as a survival strategy

The lack of correlation between the rates of root depth and root length development suggests that the increased rate of root depth development could be an evolutionary survival strategy rather than a resource exploitation strategy. The results of this research demonstrate that the rate of root depth development increased with increasing average root diameter and percentage of root length with 0.5–1 mm diameter, as found in conservative species such as *B. humidicola* and *B.* hybrid Mulato II (Fig. 3. 5a). This association suggests that the greater root diameter, as defined by average root diameter and root length with 0.5–1 mm diameter in this study, could contribute to the survival strategy of conservative species, which supports previous research (Baruch and Merida 1995; Cardoso *et al.* 2013; Cardoso *et al.* 2014; Cardoso *et al.* 2015).

Rate of root depth development was not correlated with root fraction ($r = 0.25$, $P > 0.05$) even though it appeared to promote the rapid root development to 50 cm depth. Root fraction was correlated with GDD_{50} ($r = 0.45$, $P < 0.05$) and with specific root length ($r = 0.57$, $P < 0.01$). Greater specific root length indicates the potential for resource exploitation from the soil and the significant association between specific root length, root diameter and root tissue density are mechanisms for the *trade-off* between *conservation* and *exploitation* strategies (Reich 2014; Roumet *et al.* 2016). Furthermore, root diameter and root tissue density are major traits in the plant economics spectrum that promote root lifespan but reduce the rate of nutrient acquisition, especially in conservative species (Liu *et al.* 2016; Reich *et al.* 2008; Weemstra *et al.* 2016).

However, these relationships vary according to plants species and the ecoclimates of species origins and adaptation (Kong *et al.* 2019; Valverde-Barrantes *et al.* 2017; Weemstra *et al.* 2016). Although root nutrient content was not included in the current analyses, root diameter and root tissue density also did not appear to be part of the total root length development but did contribute to the root depth development in tropical forage grass species in this study. The positive correlations between average root diameter and rate of root depth development were observed as expected in the conservative species *B. humidicola* and *B.* hybrid Mulato II (Baruch and Merida 1995; Cardoso *et al.* 2013; Cardoso *et al.* 2014; Cardoso *et al.* 2015).

3.5.5 Implications for field application

The lower thermal time requirement for *P. maximum* and *B. mosambicensis* to develop roots up to 120 cm depth after transplanting compared with other species in this study indicates that they have will have a shorter period of vulnerability to surface water deficit after establishment. For example, perennial grass species with a root depth of 120 cm were found to better tolerate prolonged drought and recover afterwards in various pastureland ecosystems, for example, semiarid upland pastures in France (Zwicke *et al.* 2015), dry grazing pasturelands in Australia (Lodge and Murphy 2006) and in tropical regions of South America (Fisher *et al.* 1994). Caution is advised when recommending *P. maximum* because it expressed an exploitative growth strategy that is associated with high nutrient requirements to sustain production (Cook *et al.* 2005; Rao *et al.* 2015), and there will be complex trade-offs that require consideration in the selection of perennial grasses for particular agroecosystems (Philp *et al.* 2019).

3.6 Conclusion

Forage species varied in rates of vertical root developments and their dependency on root depth and root length development. Rapid establishment of roots to depths between 50 and 120 cm was not associated with the exponential rate of root development (unlimited root depth) or its correlated traits. Faster root depth development was associated with narrow root angle, fine roots (< 0.2 mm diameter) and higher root length to leaf area ratio, as part of an exploitative growth strategy. One exploitative species, *P. maximum*, showed great potential for adaptation to tropical climates with seasonal moisture deficits through the rapid establishment of root length and leaf area after transplanting. In contrast, *B. humidicola* and *B.* hybrid Mulato II had root development traits that were consistent with more conservative growth strategies, perceived to be indicators of persistence in challenging ecosystems in the long-term, following slower initial establishment.

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3.7 Tables and Figures

Table 3. 1 Growth habits of species assessed (Cook et al. 2005)

Genus/taxon	Short description of growth habits
***Brachiaria* (syn. *Urochloa*)**	
B. brizantha cv. Mekong Briz	Erect or slightly decumbent with short rhizomes
B. decumbens cv. Basilisk	Erect or decumbent, rhizomatous and stoloniferous
B. humidicola cv. Tully	Strongly stoloniferous and rhizomatous with dense stolon ground colonisation
B. hybrid cv. Mulato II	Semi-erect, root growth by spreading from lower culm nodes
B. mosambicensis cv. Nixon	Loose tufted, roots and branches developing from lower nodes
Panicum	
P. maximum (syn. *Megathyrsus maximus)* cv. Tanzânia	Clump forming, loosely or densely tufted with short rhizomes
Setaria	
S. sphacelata cv. Solander	Tussock with short rhizomes
Cynodon	
C. dactylon cv. Wintergreen	Robustly stoloniferous with rhizomes

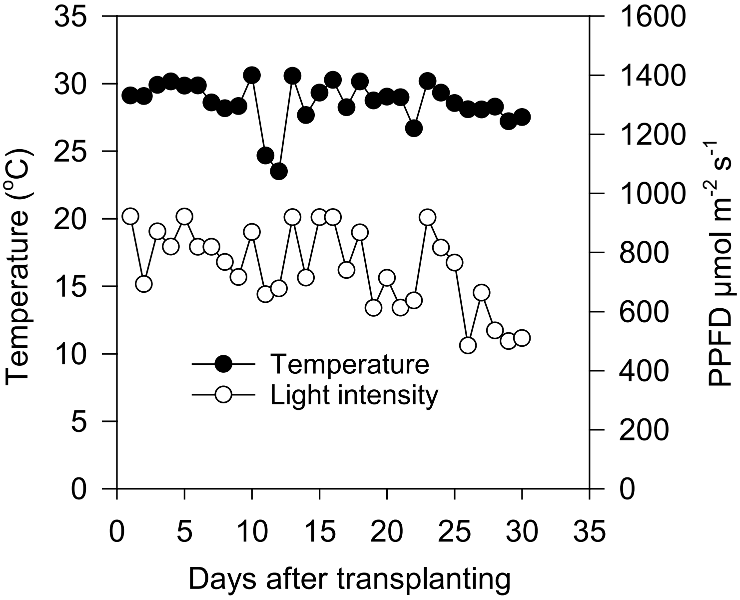

Fig. 3.1 Daily temperature and photosynthetic photon flux density (PPFD) inside the glasshouse during the experiment from 23 February to 4 April 2018.

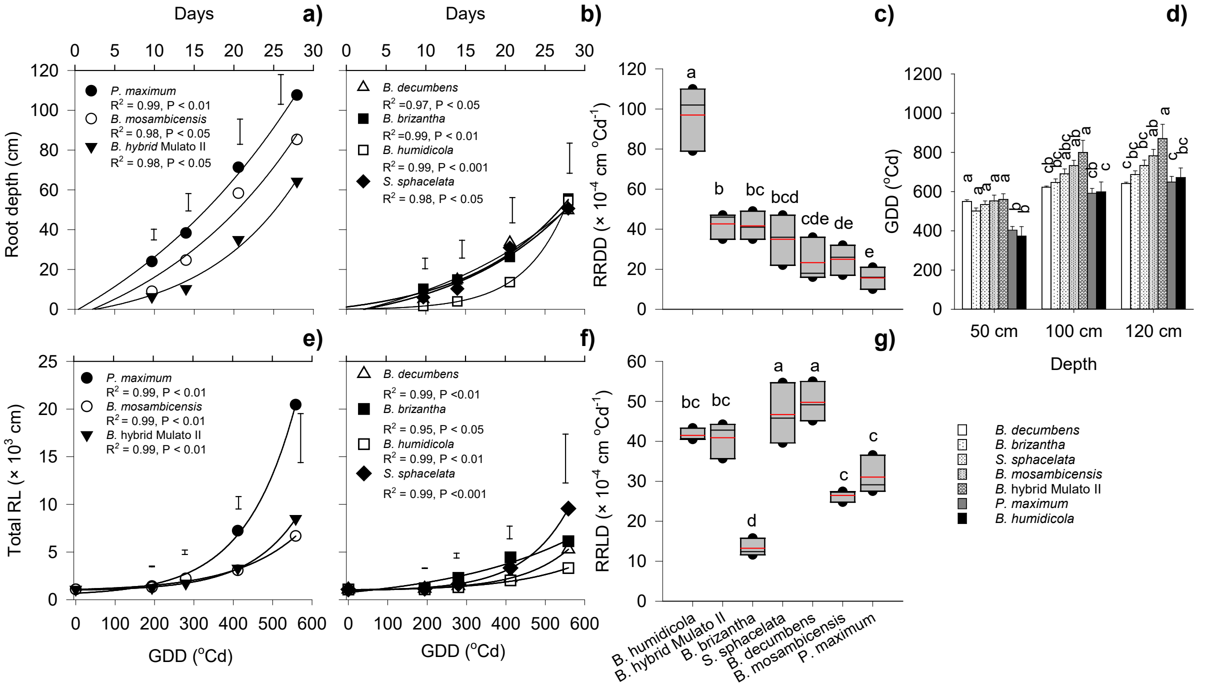

Fig. 3.2 Vertical root development: growing degree days related to root depth (a & b) and total root length (RL) (e & f). The exponential function (single, three parameters) regressions used to predict c) rate of root depth development (RRDD) and e) rate of total root length development (RRLD) in an unlimited limited depth. The models in a & b used to compare time required for each species to grow roots to 50 cm (GDD$_{50}$), 100 cm (GDD$_{100}$) and 120 cm (GDD$_{120}$). Vertical bars represent Fisher's LSD$_{.05}$ and lines, regressions (the constants of the models in Supplementary Table 3.S1). In c and g, symbols indicated red lines as mean values and the dots as 95% confident interval. In d, bars represent mean ± SE. Letters indicate the significant differences between species. Data obtained from *C. dactylon* was not included because it was insufficient for valid regression analyses.

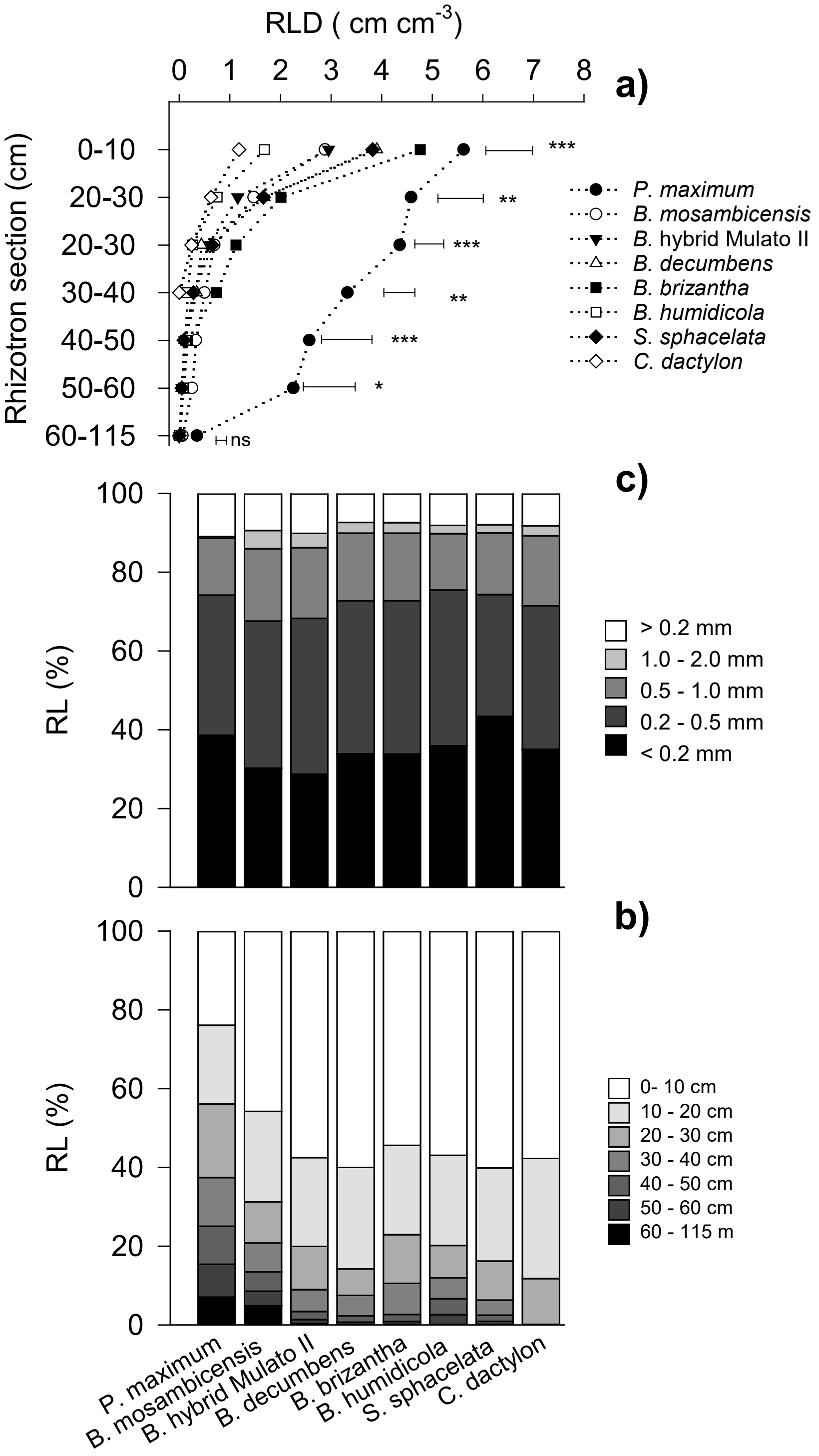

Fig. 3.3 Root length density (RLD) (a), percentage of total root length (RL) at differing depths (b) and percentage of RL in different diameter ranges (c), at harvest. Horizontal bars represent Fisher's LSD$_{.05}$ of differences in each section. Significant, * at P <0.05, ** at P <0.01, *** at P <0.001.

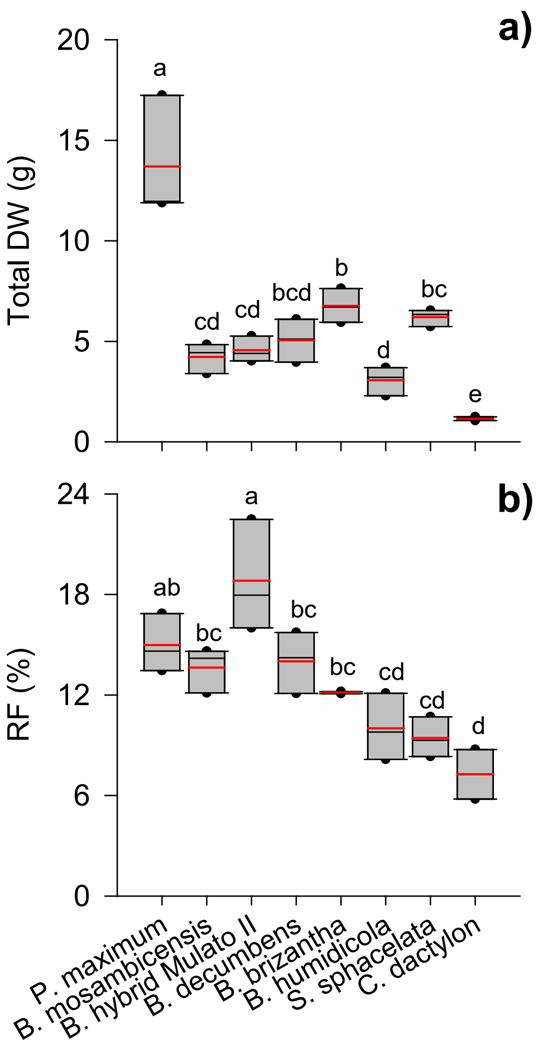

Fig. 3.4 Plant growth parameters during the experiment, total dry weight (DW) (a) and root fraction (RF) (b). Red lines indicate means and dots indicate 95% confident interval. Letters shared between groups indicate no significant difference between those groups.

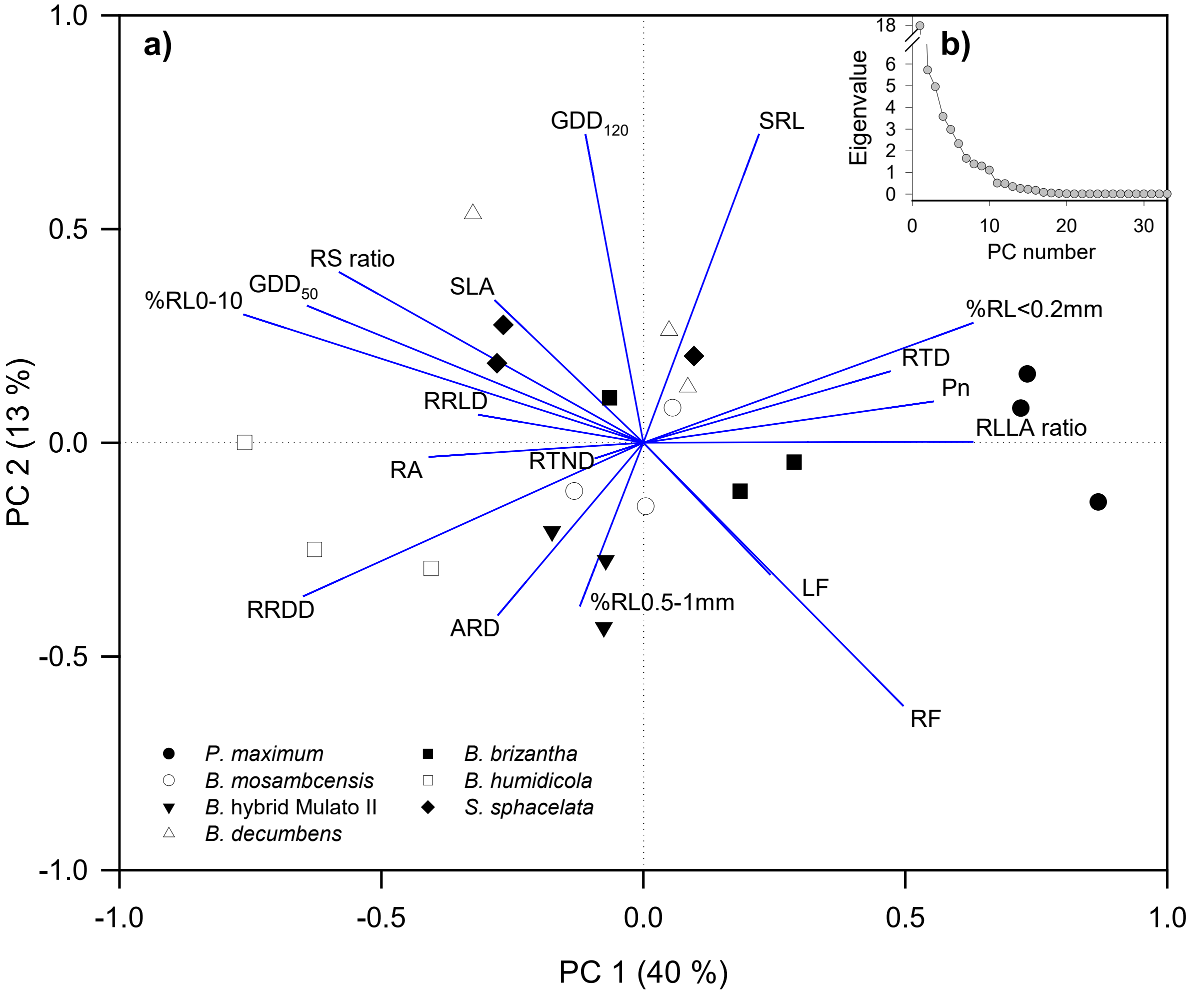

Fig. 3.5 PCA biplot combining main observed variables that contributed to vertical root development and plant growth strategies, component loadings (all loading vectors in Supplementary Table 3.S3) and components scores of grass species (a) and 10 principal components (PC) with eigenvalues >1 (b). Variables are rate of root depth development (RRDD), rate of root length development (RRLD), rate of tiller number development (RTND), growing degree days required for depth of 50 cm (GDD_{50}) and 120 mm (GDD_{120}); photosynthetic rate (Pn), root angle (RA), percentage of root length in the 0-10 cm section (%RL0-10), root to shoot (RS) ratio, specific leaf area (SLA), root length to leaf area (RLLA) ratio, root fraction (RF), leaf fraction (LF), specific root length (SRL), average root diameter (ARD), root tissue density (RTD), percentage of root length with < 0.2 mm diameter (%RL<0.2mm) and 0.5-1 mm diameter (%RL0.5-1mm).

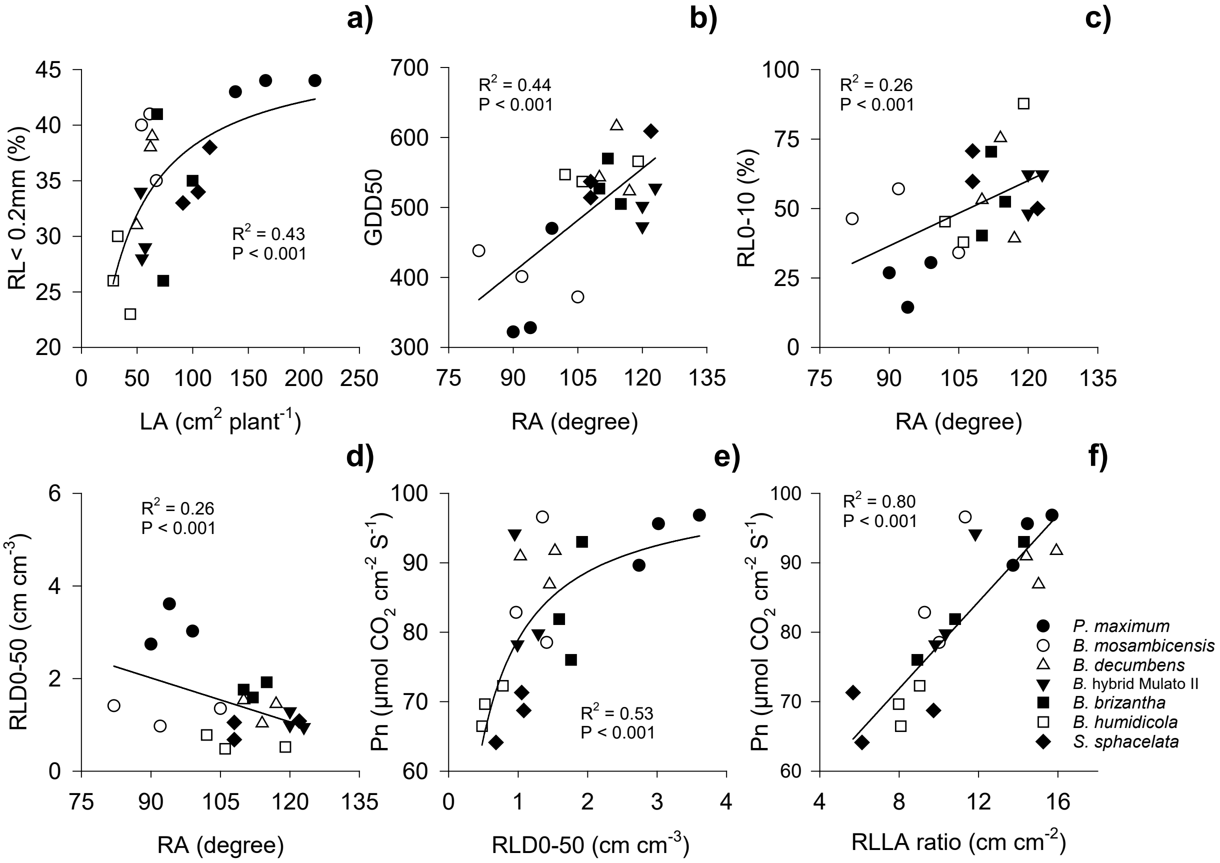

Fig. 3.6 Relationships of leaf area (LA) and root angle (RA) with plant variables that contributed to the vertical root development; LA with root length of diameter < 0.2 mm (RL<0.2mm) (a); root angle with growing degree day requirement for 50 cm depth (GDD_{50}) (b), root length in the 0-10 cm section (RL0-10) (c) and root length density in the 0-50 cm section (RLD0-50) (d); photosynthetic rate (Pn) and RLD0-50 (e), and root length/leaf area (RLLA) ratio (f). Non-linear regressions in a and e are hyperbola function, $y = a \times x/(b+x)$.

CHAPTER 4: GENOTYPIC AND SEASONAL VARIATION IN ROOT DEPTH DEVELOPMENT DURING ESTABLISHMENT OF C4 PERENNIAL GRASS ECOTYPES

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

Signature		Date	29/9/2021

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ii. permission is granted for the candidate in include the publication in the thesis; and

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Genotypic and seasonal variation in root depth development during establishment of C_4 perennial grass ecotypes

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ABSTRACT

Context. Perennial forage grass species are often grown with limited water following establishment and rely on accessing water deep in the soil profile to survive. **Aim.** This study aimed to characterise bermudagrass (*Cynodon* spp.) genotypes with rapid vertical root growth associated with post-establishment survival. **Methods.** Twelve bermudagrasses representing genotypes from diverse climate zones in Australia were established in rhizotrons to analyse the stability in genotypic variation in root and shoot growth in winter and summer experiments. Genotypic rank of root length, leaf area, and root dry weight were consistent in both seasons. **Key results.** Bermudagrass genotypes exhibited different traits correlated with root vertical growth rate and inconsistency of genotypic rank of shoot growth. During winter establishment, the rate of root depth development (RRDD) ($r = -0.64$) was correlated with the proportion of root length that became inactive, that was likely due to seasonal root death in winter conditions; during summer establishment, RRDD was correlated with tiller appearance rate ($r = 0.45$) and root distribution to 10 cm depth ($r = -0.62$). Shoot dry weight was correlated with photosynthesis ($r = 0.85$) and transpiration ($r = 0.79$) in summer, but not in winter. RRDD ($r = 0.75$, winter and $r = 0.77$, summer) was correlated with drought resistance index, previously analysed under field conditions. **Conclusions and implications.** Genotypes from the Mediterranean climates in Australia showed rapid growth of roots and shoots in both seasons and have the greatest potential for broader application for forage production in variable environments.

Keywords: arid pastures, Bermuda grass, drought resistance, forage management, root depth development, seasonal growth, turfgrass, winter dormancy.

4.1 Abstract

Aim Perennial forage grass species are often grown with limited water following establishment and rely on accessing water deep in the soil profile to survive. This study aimed to characterise bermudagrass (*Cynodon* spp.) genotypes with rapid vertical root growth associated with post-establishment survival.

Methods Twelve bermudagrasses representing genotypes from diverse climate zones in Australia were established in rhizotrons to analyse the stability in genotypic variation in root and shoot growth in winter and summer experiments. Genotypic rank of root length, leaf area, and root dry weight were consistent in both seasons.

Results Bermudagrass genotypes exhibited different traits correlated with root vertical growth rate and inconsistency of genotypic rank of shoot growth. During winter establishment, the rate of root depth development (RRDD) ($r = -0.64$) was correlated with the proportion of root length that became inactive, that was likely due to seasonal root death in winter conditions; during summer establishment, RRDD was correlated with tiller appearance rate ($r = 0.45$) and root distribution to 10 cm depth ($r = -0.62$). Shoot dry weight was correlated with photosynthesis ($r = 0.85$) and transpiration ($r = 0.79$) in summer, but not in winter. RRDD ($r = 0.75$, winter and $r = 0.77$, summer) was correlated with drought resistance index, previously analysed under field conditions.

Conclusions Genotypes from the Mediterranean climates in Australia showed rapid growth of roots and shoots in both seasons and have the greatest potential for broader application for forage production in variable environments.

Keywords: arid pastures, Bermuda grass, drought resistance, forage management, root depth development, seasonal growth, turfgrass, winter dormancy.

4.2 Introduction

Bermudagrasses (*Cynodon* spp.) are increasingly used in hay production and pastures due to their high productivity, even when grown on marginal land (Gomes *et al.* 2019; Hacker *et al.* 2013; Hill *et al.* 2001). Herbage from rapid shoot regrowth after defoliation often contains a high concentration of crude protein that enhances bermudagrass forage value (Gomes *et al.* 2019; Zhang *et al.* 2020). Forage bermudagrass has been cultivated as a pasture crop on approximately 9 million hectares in the USA since the 1930s (Taliaferro *et al.* 2004). Recently, the Tifton 85 cultivar has been grown in intensive livestock systems in the USA (Baseggio *et al.* 2015; Burton *et al.* 1993), in Latin America (Pequeno *et al.* 2015) and in China (Zhang *et al.* 2020). Studies on a large number of bermudagrasses collected from Australia found that genotypes originating from warm and arid regions have great potential as forage (Hacker *et al.* 2013; Zhou *et al.* 2015).

Bermudagrass has evolved to adapt to specific edaphic and climatic habits that vary between wet tropical and arid temperate regions (Paula and Pausas 2006; Taliaferro *et al.* 2004; Zhang *et al.* 2019). Bermudagrass stolons spread rapidly aboveground while rhizomes spread belowground (Cook *et al.* 2005; da Silva *et al.* 2015). Reproduction in bermudagrass occurs both sexually via seeds and asexually by propagation from stolons, crown buds and rhizomes (Ahring *et al.* 1982; Stier *et al.* 2013; Taliaferro *et al.* 2004). Rhizomes are a crucial reproductive organ for propagation in diverse ecosystems such as those exposed to bushfires and prolonged periods of flooding (up to 7 months) and drought (Pandeya and Ahirwalb 2020). Both asexual and sexual reproduction provides strong adaptability (Zhang *et al.* 2019), such that genotypes of bermudagrass are widely distributed and well adapted to latitudes between 45 °S and 53 °N (Harlan and De Wet 1969). In Australia, approximately 1000 bermudagrass genotypes have been collected across climatic zones that range widely in temperatures (Kearns *et al.* 2009); these genotypes vary in their adaptation to the climatic zones from which they originate (Jewell *et al.* 2012a; Paula and Pausas 2006; Van Tran *et al.* 2017). The broad environmental adaptation allows for the establishment of suitable genotypes for specific local environments within that range (Conaghan *et al.* 2008; da Silva *et al.* 2015; Robins *et al.* 2020; Vogel *et al.* 1993).

Instability of variation in root and shoot growth according to climatic variation usually occurs across perennial forage grasses either within the same or different perennial grass species, such that experiments conducted in different seasons provide an opportunity to identify regionally-adapted genotypes to meet specific climatic conditions (Carnevalli *et al.* 2021; Conaghan *et al.* 2008; da Silva *et al.* 2021; Robins *et al.* 2020; Vogel *et al.* 1993). The growth response of grass genotypes to seasonal changes can vary marginally or substantially, depending on genotype (Crossa 2012).

Root growth of perennial grasses appears to be regulated by temperature, even in mild temperate regions (Côté *et al.* 1998; Esmaili and Salehi 2012; Reasor *et al.* 2018). In low-temperature conditions, a decrease in photosynthesis in bermudagrass leads to a reduction of shoot growth and shoot carbohydrate supply directed to root growth (Esmaili and Salehi 2012; White and Schmidt 1990). The reduced availability of carbohydrates for root growth can lead to roots becoming inactive (Kaufmann 1994). Low-temperature environments also induce internal root genetic programming that causes the death of root tissues (Joslin *et al.* 2001; Woo *et al.* 2013). Some bermudagrass genotypes collected in Australia might become dormant at temperatures below 20 °C (Esmaili and Salehi 2012; Wu 2011). Dormancy induces a continuous reduction in metabolic activity that leads to cell death in perennial grass species (Comas *et al.* 2000; Razar and Missaoui 2018; Wells and Eissenstat 2001). The combination of insufficient carbohydrates and programmed cell death can cause the death of some root tissues (Ott and Hartnett 2012; West *et al.* 2003), which can be recognised by colour changes from active white roots to inactive brown roots (Peek 2007). Roots of perennial grasses also become inactive through aging processes (Wahl and Ryser 2000).

A greater proportion of inactive roots in the soil profile may reduce water extraction at depth and thereby negatively affect post-establishment drought resistance in bermudagrass. Despite a positive association between root length density and water extraction reported in studies of different perennial species, drought resistance in bermudagrass genotypes collected in Australia was not correlated with root length density (Zhou *et al.* 2014), potentially because individual roots may vary in their water extraction activity independent of root length density (Kulmatiski and Beard 2013).

The rapid development of deep roots is considered an advantageous trait that may assist the establishment of bermudagrasses as a forage crop when surface moisture is limited. Rapid root distribution by perennial grass species deep in the soil profile is also associated with greater shoot growth and greater vertical root growth rates (Acuña *et al.* 2010; Fuentealba *et al.* 2015; Guenni *et al.* 2002; Huot *et al.* 2020). The rate of total root length development and the rate of root depth development have been reported to be uncorrelated in C4 grasses (Huot *et al.* 2020), although the rate of root depth development has also been used as an indicative trait for vertical root growth rates in perennial grasses (Acuña *et al.* 2010; Boeri *et al.* 2020; Christensen *et al.* 2017; Fuentealba *et al.* 2015). The aims of this research were: 1) to characterise bermudagrasses that represent genotypes originating from multiple climatic zones in Australia by analysing rates of root depth development and evaluating the stability of variation between genotypes in root and shoot growth during establishment under different climatic conditions, and 2) to investigate the relationships between early rate of root depth development of bermudagrass genotypes, measured in this study, and post-establishment drought resistance, as analysed in a previous study (Zhou *et al.* 2014). This present study hypothesised that variation between the shoot and root growth of bermudagrass genotypes from differing climatic zones in Australia would be unstable across seasons.

4.3 Materials and methods

4.3.1 Grass genotypes and glasshouse experiments

A commercial cultivar Wintergreen (WG), and 11 genotypes from major Australian climatic zones, namely MED1, MED2, MED3, 40, 698, 55, 1189, 573, 659, and 394 (geographical distribution of genotypes in Zhou *et al.* 2014) were selected to represent a diversity of bermudagrasses in Australia for this study because they have morphological and genetic differences (Jewell *et al.* 2012b) and large variation in drought resistance (Zhou *et al.* 2014). Although some bermudagrass genotypes are self-compatible (Hacker *et al.* 2013; Taliaferro *et al.* 2004; Zhang *et al.* 2019), bermudagrass genotypes used in this study are self-incompatible (Lambrides *et al.* 2013). In self-incompatible bermudagrass genotypes, the production of seeds is achieved by cross-pollination of two self-incompatible plants grown from vegetative parts in alternative rows (Ahring *et al.* 1982; Tan *et al.* 2014); therefore seedlings obtained from seed germination have different genetic materials from the parent plants. To ensure that experimental plants were genetically identical to parent plants, cuttings were rooted in University of California at Davis soil mix (UC mix) and grown in tapered plug cells (35 mm length × 40 mm width × 50 mm depth) for four weeks before transplanting. Ingredients of 1 m^3 UC mix, produced by the South Australian Research and Development Institute, include 0.44 m^3 of Canadian peat moss, 0.56 m^3 of Waikerie sand, 1.33 kg of agriculture lime, 0.80 kg of hydrated lime, and 3 kg of 16-3.5-9.1+TE Osmocote Exact Mini, a controlled release fertiliser (Fernland, Yandina, QLD 4561 Australia). In the final two weeks, propagules in the plug trays were pruned by cutting stolons at 1 cm above the cell base to ensure uniformity at the time of transplantation. An individual propagule from each plug cell was transplanted into a rhizotron (20 cm length × 5 cm width × 120 cm depth), containing 11.5 L UC mix which formed a single experimental unit. Each rhizotron had the available macronutrients: 5.52 g N, 2.16 g P and 7.06 g K; no fertiliser application after transplanting was required (Zhou *et al.* 2013a). Three replicate rhizotrons per genotype were positioned at 45 ° against benches (0.8 m high) in the centre of a glasshouse.

The experiments were carried out in a glasshouse at the University of Adelaide for 107 days from 23 April to 8 August 2018 (winter experiment) and for 85 days from 4 December 2018 to 25 February 2019 (summer experiment). Grasses were monitored daily to ensure that UC mix water content from the top to the bottom column of rhizotrons was maintained at around 60% water holding capacity. The volumetric moisture content of UC mix at field capacity is 25.1 v/v % (Zhou *et al.* 2013a). When the surface started to become dry, the grasses were irrigated by slowly adding 0.40 L water, equal to 60% of UC mix water holding capacity in the top 20 cm section. In winter, grasses were manually irrigated every four days in the first two weeks and then every two days until the end of the experiment. In summer, grasses were watered by a drip irrigation system twice per day during the first two weeks and then four times per day during the highest temperatures of the summer period (Supplementary Fig. S1)[3]. As the frequency of irrigation was increased, the volume of each watering was set to 0.2 L to reduce the rapid percolation and to ensure that the upper section of the rhizotron was well-watered. The bottom of each rhizotron had openings to enable drainage to avoid waterlogged conditions at the bottom.

4.3.2 Root measurements

Root depth was measured weekly until harvest. Root length and root dry weight were measured at harvest. The root column in each rhizotron was separated into 10 cm layers from 0 to 60 cm

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[3] Supplementary material is at Crop & Pasture Science at https://doi.org/10.1071/CP21258

depth and a final layer from 60 to 100 cm. Root samples of each section were washed, then scanned at 400 dpi resolution using an EPSON Expression 10000XL scanner (EPSON Inc., Long Beach, CA 90806, 124 USA) to produce images that were then analysed using WinRHIZO (Regent Instruments Inc. 2019). During the harvesting of the winter experiment, active roots and inactive roots were separated according to root colour. The total root biomass in each rhizotron was dried at 60 °C for 72 h to determine root dry weight.

4.3.3 Photosynthesis

For plants in each rhizotron, the two youngest fully-expanded leaves from different stolons were used to measure leaf gas exchange between 1100 h and 1230 h prior to harvest, using a Li-Cor 6400 (Portable Photosynthesis Systems, Lincoln, NE, USA). The Li-Cor 6400 was connected to a leaf chamber supplied with 400 μmol mol^{-1} CO_2 and 1500 μmol m^{-2} s^{-1} photosynthetic photon flux density.

4.3.4 Shoot measurements

Tillers were counted weekly during the experiment. At harvest, shoot dry weight, root dry weight, and leaf area were determined. Shoot dry weight was obtained from oven-drying shoot samples at 60 °C for 72 h. Leaf area was analysed by scanning all leaf blades of the entire plants in each rhizotron, using a CS9000FMKII Canon scanner (Tokyo, Japan) at 400 dpi. Scanned leaf images were used to analyse leaf area using ImageJ software (Rasband 1997). All variables sampled in both rhizotron experiments are listed in Supplementary Tables 4.S1 & 2.

4.3.5 Field experiments for drought resistance

Previously, the drought resistance of WG, MED1, MED2, MED3, 40, 698, 55, 573, 659, and 394 was assessed in a previous field experiment conducted in the Gatton research station at the University of Queensland (Zhou *et al.* 2014). Grass genotypes were transplanted into plots on

28 November 2010 and grown under automatic rainout shelters. Irrigation of 40 mm week^{-1} was applied until 20 March 2011 and followed by cessation of irrigation to assess drought resistance in each genotype. The drought resistance was determined by the number of days required for green cover to fall to 50% (GC_{50}) without irrigation under field conditions. After irrigation ceased, the grasses continued to grow for 266 days until the percentage of green leaves in each plot was less than 15%. During the drought period, two photos were taken in each plot to analyse the green leaf ratio. This study tested the relationships between the rate of root depth development and drought resistance of WG, MED1, MED2, MED3, 40, 698, 55, 573, 659, and 394 determined in the field. To reduce environmental effects of the field experiment, the drought resistance of each genotype was normalised to a drought resistance index by dividing GC_{50} of each genotype with the average GC_{50} of all genotypes in this study.

4.3.6 Statistical analyses

The weekly root depth increase tended to increase as the experiment progressed (Fig. 4.1). Therefore, an exponential curve was fitted to obtain rate of root depth development. Empirical relationships of root depth with growing degree days (GGD) had an exponential growth function applied (single, three parameters) $y = y_0 + ae^{(bx)}$ using the command of regression wizard on Sigmaplot 14.0 (Systat Software Inc. 2017). y is root depth (RD) at the time of measurement; y_o and a are the constants; b is the slope of the model, indicating rate of root depth development (RRDD in cm °Cd^{-1}); and x is GDD. Similarly to root depth, weekly tiller counts during the experiments were used to analyse tiller appearance rate by curve-fitting using an exponential growth function $y = y_0 + ae^{(bx)}$, where y is tiller numbers at the time of counting; y_o and a are the constants; b is the slope of the model, tiller appearance rate (TAR in tiller °Cd^{-1}); and x is GDD. RRDD and TAR indicated the rates of each genotype established under conditions of unlimited depth beyond the 100 cm limit. GDD was calculated from the daily temperatures recorded during the experiments (Supplementary Fig. 4.S1) using a base temperature of 10 °C (Esmaili and Salehi 2012; Reasor *et al.* 2018).

The stability of variation between genotypes in root and shoot growth across seasons were evaluated using the consistency of rank change across grass genotypes established in winter and summer experiments. GenStat 20th Edition was also used to calculate Pearson's correlation coefficients (r) (Supplementary Tables 4.S1-3). Correlation analysis was performed: first, on the variables to examine relationships between variables measured in each season of rhizotron experiments to identify the possible mechanism for shoot growth and root depth development of grass genotypes established in each season; and second, to test relationships between RRDD in the rhizotron experiments and drought resistance index in the previous field experiment. Principal components analysis (PCA) using the multivariate statistics package in Minitab 15 (Minitab Inc. 2010) was performed to investigate the pattern of variability between genotypes in the growth of roots and shoots according to seasons. The size of the input matrices was 31 rows (sampled plants) by 12 columns (measured variables) in winter, and 27 rows by 12 columns (variables) for the summer experiment. The 12 variables selected for PCA analysis (Supplementary Tables 4.S4 & 5) were selected according to two criteria: 1) typical traits representing the growth, e.g. root length, dry weight and leaf areas and 2) if a group of traits (Supplementary Tables 4.S1 & 2) were correlated with each other, one of the traits was chosen.

In each experiment RRDD, the percentage of root distribution in each section of rhizotron, inactive root ratio, PC scores, and drought resistance index were analysed for significant differences by ANOVA using GenStat 20th Edition (VSN International 2019). Mean comparison was performed according to Fisher's least significant difference (LSD) at $P < 0.05$.

4.4 Results

4.4.1 Vertical root development

Root depth of each genotype increased exponentially over time (Fig. 4.1). During winter with 17 to 24 °C daily temperatures and 50 to 760 μmol m^{-2} s^{-1} photosynthetic photon flux intensity (Supplementary Fig. 4.S1a & c), results of the exponential model (Fig. 4.1) showed that grasses had significantly different RRDD between genotypes ($P < 0.01$, Fig. 4.2a). When the grasses were grown under summer conditions of 19 to 38 °C daily temperature and 400 to 1580 μmol m^{-2} s^{-1} photosynthetic photon flux intensity (Supplementary 4.Fig. S1b & d), RRDD showed significant differences between genotypes ($P < 0.05$, Fig. 4.2b).

In both seasons, RRDD of MED1 and MED2 were similar and greater than the RRDD of most other genotypes, except for MED3. Genotype MED3 exhibited a remarkably lower RRDD in winter (Fig. 4.2a) despite exhibiting the greatest RRDD in summer (Fig. 4.2b). Other genotypes had similar RRDD values that tended to be consistently lower in winter than summer.

Grass genotypes had 20 to 50% of total root length (Supplementary Table 4.S6) distributed above 10 cm depth in winter (Fig. 4.2c), whereas 40 to 80% of roots were distributed above this depth in summer (Fig. 4.2d). Variation in root length ratio distributed in the top 10 cm depth was significant ($P < 0.01$, winter and $P < 0.01$, summer).

During winter, in which a visual distinction between active and inactive roots at harvest was observed (Fig. 4.2e), there was a significant difference between genotypes in inactive root fractions at harvest ($P < 0.01$, Fig 2f). Approximately 80% of MED3, 55, 1189, WG, 25a1 and 55 roots were inactive. The inactive roots accounted for 50 to 60% of roots in other genotypes, except for MED1 (40%).

4.4.2 Consistency of genotypic rank of root and shoot growth

Between seasons, RRDD measurements were relatively consistent and positively correlated ($r = 0.76$, $P < 0.01$; Fig. 4.3a), with the exception for MED3, which had the greatest difference (Fig. 4.2a, 2b & 3a). However, traits correlated with root depth development differed between seasons. When bermudagrass genotypes were established in winter, RRDD significantly decreased with increasing inactive root ratio ($r = -0.61$, $P < 0.01$; Fig. 4.3b). Inactive root length significantly increased with increasing active root length ($r = 0.51$, $P < 0.01$; Fig. 4.3c), but active root length was not correlated with RRDD ($r = 0.13$, $P > 0.05$; Supplementary Table 4.S1). In summer, RRDD significantly increased with greater tiller appearance rate ($r = 0.59$, $P < 0.01$; Fig. 4.3d), but significantly decreased with increasing percentage of roots distributed in the surface 10 cm of soil ($r = -0.62$, $P < 0.001$; Fig. 4.3e).

Variation in the growth of total root length ($r = 0.70$, $P < 0.05$), leaf area ($r = 0.76$, $P < 0.001$), and root dry weight ($r = 0.65$, $P < 0.01$), (Fig. 4.4a-c) were consistent across bermudagrass genotypes between the measurements in winter and summer. However, the variation in shoot dry weight among the genotypes was inconsistent (Fig. 4.4d). In both seasons, shoot dry weight significantly increased with the greater root length ($r = 0.83$, $P <0.001$; winter and $r = 0.88$, $P < 0.001$; summer) and the greater leaf area ($r = 0.79$, $P < 0.001$; winter and $r = 0.81$, $P < 0.001$; summer) (Fig. 4.4e-h). In summer, there were positive correlations between shoot dry weight and photosynthetic rate ($r = 0.64$, $P < 0.001$), shoot dry weight and transpiration rate ($r = 0.48$, $P < 0.05$) (Fig. 4.4i & j), total root length and photosynthetic rate ($r = 0.66$, $P < 0.001$), and total root length and transpiration rate ($r = 0.48$, $P < 0.05$) (Fig. 4.4k & l), whereas in winter no relationships were observed (Supplementary Table 4.S1).

Results of PCA analysis showed that scores of genotypes were significantly different in PC 1 ($P < 0.01$, winter and $P < 0.05$, summer), but PC 2 scores were not significantly different (P

<0.05, both seasons) (Fig. 4.5a & b). PC scores demonstrated that genotypes 40 and 573 had high shoot dry weight, leaf areas, total root length and root depth at harvest in winter (Fig. 4.5a & c), and shoot dry weight, total root length and rhizome dry weight in summer (Fig. 4.5b & d). Genotype MED1 showed a high RRDD in both seasons (Fig. 4.5).

4.4.3 Relationships between root development and drought resistance index

Drought resistance index of bermudagrass genotypes ranged from 0.8 to 1.2 (Fig. 4.6a), with MED1 being the highest. Other genotypes with drought resistance index above 1 were MED1, MED3, 573, 394, and 40. Drought resistance index significantly increased with increasing RRDD values obtained from both rhizotron experiments (r = 0.75, $P < 0.05$; winter, and r = 0.77, $P < 0.01$; summer) (Fig. 4.6c & d).

4.5 Discussion

Analyses of rank change in root and shoot growth across bermudagrass genotypes were carried out in two different seasons where daily temperatures and photosynthetic photon flux intensities during the establishment ranged 17 to 24 °C and 50 to 760 μmol m^{-2} s^{-1} in winter, and 19 to 38 °C and 400 to 1580 μmol m^{-2} s^{-1} in summer. Between seasons, differences in daily temperature and sunlight contributed to the inconsistency of variation between genotypes in shoot growth and the differences in traits correlated with rates of root depth development across bermudagrass genotypes collected from diverse climatic zones of Australia.

4.5.1 Genetic variation in rate of root depth development

The rhizotron experiments revealed a relative consistency of variation in rate of root depth development between experiments. However, MED3 collected from the Mediterranean climatic zone of Australia, the same as MED1 and MED2, appeared to be sensitive to low temperatures and low sunlight in winter during our rhizotron experiments. MED3 had the greatest difference in rate of root depth development between seasons (Fig. 4.2a & b). It is uncertain if this genotype belongs to the Australian Mediterranean native group (Zhou *et al.* 2014) or to the halophyte group that can grow roots well in all seasons (Jewell *et al.* 2012a; Van Tran *et al.* 2018; Van Tran *et al.* 2017). Importantly, MED3 exhibited the highest rate of root depth development in the summer experiment and had greater water extraction than genotypes from other climatic zones of Australia in the field experiment (Zhou *et al.* 2014). MED3 may be one of the bermudagrass genotypes that may have existed in Australia for 500 years (Jewell *et al.* 2012a) that have been well-adapted to drought and high temperatures (Paula and Pausas 2006)

4.5.2 Bermudagrass genotypes for forage cultivation

The genotypes MED1, MED2 and MED3, collected from the Mediterranean climatic zone, exhibited a rapid rate of root depth development in this study, and were previously shown to have high salt tolerance (Van Tran *et al.* 2018), high sprouting rates from nodes in all seasons (Van Tran *et al.* 2017), fast shoot regrowth rates (Zhou *et al.* 2015), and a large capacity for water extraction from the depth of the soil profile without irrigation supplied after establishment (Zhou *et al.* 2014). Rapid post-harvest shoot regrowth of the bermudagrass genotypes in this study is a favourable trait for bermudagrass cultivars intended for forage (Anderson *et al.* 2009; da Silva *et al.* 2015; Gomes *et al.* 2019; Zhang *et al.* 2020). Most importantly, genotype MED1, which had rapid vertical root growth in both seasons in this study and a highly rhizomatous root system (Zhou *et al.* 2015), could have favourable herbage production in marginal lands affected by drought, salinity and poor soil fertility (Jones *et al.* 2015). Rhizomatous bermudagrass genotypes MED1, MED2, 573 and 40 also have potential utility as forage because they rapidly developed extensive root systems (Fig. 4.5) that could enable them to access subsoil moisture sooner than other genotypes, shortening their dependency on surface moisture. The high shoot production of 573 and 40 (Fig. 4.4d & Fig.

4.5) in both seasons indicated adaptation to a wide range of temperatures that may be favourable in variable climates. According to previous studies and the present study on bermudagrass genotypes collected in Australia, genotypes MED1, MED2, and MED3 collected from the Mediterranean climatic zone have demonstrated combined traits that may have the potential for broader application in livestock production (Gomes *et al.* 2019; Hill *et al.* 2001).

4.5.3 Mechanisms for rate of root depth development

During the winter establishment, an increased proportion of inactive roots appeared to have a negative influence on rate of root depth development, even though the inactive root length significantly increased with increasing active root length. Growth of new roots in bermudagrasses is sustained by photosynthesis in green leaves, even in low temperatures that ranged close to the 10 °C base temperature (Esmaili and Salehi 2012; Hanna *et al.* 2013; Reasor *et al.* 2018). Leaves of bermudagrass genotypes in winter remained green and were not damaged as the temperatures remained above the base temperature of 10 °C (Supplementary Fig. 4.S1a). Nonetheless, new active roots (Fig. 4.2e & f) of bermudagrass genotypes were unlikely to grow into the deeper sections of the rhizotrons by harvest, such that active roots were not correlated with the rate of root depth development during winter. The development of inactive roots in winter could indicate the seasonal death of root tissues, as observed in perennial grass species in natural grasslands at low temperatures (Ott and Hartnett 2012; West *et al.* 2003).

During the establishment in summer, the positive correlation of tiller appearance rate with rate of root depth development indicates that rapid shoot growth was a possible mechanism promoting vertical root growth rate. In summer, vigorous growth of shoots and roots were promoted by high photosynthetic and transpiration rates (Fig. 4.4i & l). Shoot growth of bermudagrass is increased by lateral growth of meristems triggered by high temperatures

(Fagerness *et al.* 2002). The rapid shoot growth and leaf area expansion, as indicated by tiller appearance rate in this study, could lead to greater net photosynthesis that promotes root growth (Guyonnet *et al.* 2018; Johnson and Matchett 2001). Therefore, increased root distribution in rhizotron sections below 10 cm depth was associated with a greater rate of root depth development (r = 0.46 to ~0.60, $P < 0.05$) and with greater shoot dry weight (r = 0.6 to ~0.80, $P < 0.01$) (Supplementary Table 4.S2). Similarly, rapid shoot growth promotes the vertical growth of root systems of African bermudagrass and zoysiagrass (Christensen *et al.* 2017; Fuentealba *et al.* 2015), bahiagrass hybrids (Acuña *et al.* 2010; Boeri *et al.*), and other forage grass species (Guenni *et al.* 2002; Huot *et al.* 2020). Negative correlations between the proportion of root distribution above 10 cm depth, and the rate of root depth development (Fig. 4.3e), leaf area ($r = -0.40$, $P < 0.05$) and photosynthetic rate ($r = -0.46$, $P < 0.05$) in the summer experiment (Supplementary Table 4.S2) indicate that shallow rooting habits are disadvantageous. The disadvantageous characteristics of bermudagrasses in this study are in contrast with the fast-growing perennial grass species that have a rapid growth of leaf area and high photosynthetic rate in association with great ability of establishing deep roots (da Pontes *et al.* 2015; Reich 2014; Wright *et al.* 2004). Similarly, previous studies on perennial forage species revealed that fast-growing species such as *Megathyrsus maximus* cv Tanzânia (syn. *Panicum maximum*) had rapid vertical root growth rate, high photosynthesis and rapid growth of leaf areas, as observed in rhizotron experiments (Huot *et al.* 2020). These characteristics are considered advantageous ecosystems with fast-drying, sandy soil profiles (Philp *et al.* 2021; Philp *et al.* 2019). Therefore, in this present study, rapid vertical root growth rate, high shoot growth and great photosynthesis rate can be considered as advantageous traits to select bermudagrass genotypes, especially for forage production in dry regions.

4.5.4 Seasonality differences in variation for shoot growth

The lack of consistency in shoot dry weight rankings (Fig. 4.4d) across genotypes between winter and summer experiments in this study indicates the instability of variation in shoot growth in perennial grasses throughout the year (Carnevalli *et al.* 2021; da Silva *et al.* 2021; Robins *et al.* 2020). Variation in shoot dry weight of perennial grass species, as observed in bermudagrasses in this study, and in ryegrasses (Conaghan *et al.* 2008) and wheatgrasses (Vogel *et al.* 1993), can increase or decrease because of the differential genotypic responses to seasonal changes of temperatures and sunlight intensity over the year. At the higher temperatures and sunlight intensities in summer, shoot growth of bermudagrass genotypes was promoted by photosynthesis and transpiration (Fig. 4.4i & j). In winter with lower temperatures and sunlight, bermudagrass genotypes had similar and lower photosynthetic rates (around 270 μmol CO_2 cm^{-2} s^{-1}) and transpiration rates (13 mmol H_2O cm^{-2} s^{-1}) (Supplementary Table 4.S6). Reduction of photosynthesis in bermudagrasses could lead to insufficient carbohydrate for axillary bud production to accelerate shoot growth, as observed in a previous study on these same bermudagrass genotypes (Van Tran *et al.* 2017). This could provide a reason why shoot dry weight was not correlated with photosynthesis nor transpiration during winter establishment (Supplementary Table 4.S1). Inconsistency in variation between genotypes in shoot growth can be used to determine the level of adaption of bermudagrasses and to select widely-adapted genotypes across seasons. Genotypes 40 and 573 possessed stability and high productivity of shoot growth regardless growing seasons, while other genotypes showed the lack of adaptation in shoot growth to winter condition in this study.

4.5.5 Seasonality differences in variation for vertical root growth rate

Genotypic variation in vertical root growth rate of bermudagrass genotypes was apparently unaffected by variation of seasonal conditions in this study. Despite the temperature and sunlight differences between summer and winter experiments, most bermudagrass genotypes maintained consistency of genotypic ranks in the rate of root depth development (Fig. 4.3a), root length, leaf area and root dry weight (Fig. 4.4a-c). In each season, increased root growth was associated with greater shoot growth and greater leaf area expansion (Fig. 4.4e-h). Low photosynthesis in winter likely supported the growth of new roots to maintain green leaves and shoot growth, but did not appear to accelerate shoot growth (Fagerness *et al.* 2002; Hanna *et al.* 2013). While some roots became inactive in winter, bermudagrass genotypes also had the capacity for establishing new roots and maintaining shoot growth. These results indicate the great adaption of bermudagrass genotypes in this study for survival during extreme weather among their habitats in Australia (Jewell *et al.* 2012a; Paula and Pausas 2006).

5.5.6 Rapid vertical root growth in the early stage associated with increased performance in post-establishment drought

Rapid vertical root growth could be a mechanism for promoting drought resistance after establishment in bermudagrass genotypes as indicated by positive correlations between rate of root depth development and drought resistance index (Fig. 4.5b & c). Rapid vertical root growth can accelerate the growth of active roots into deep horizons of soil profiles, as observed in other perennial grasses (Bodner *et al.* 2015; Boeri *et al.* ; Kulmatiski and Beard 2013). Increased water extraction to promote drought resistance among bermudagrass genotypes in the previous field experiment (Zhou *et al.* 2014) may be associated with the greater active root length in deep horizons of soil profiles. However, there was no clear differentiation in PCA scores of drought resistant genotypes such as MED1, MED2 and MED3 and 40 (Fig. 4.5a & b). This finding indicated that drought-resistant bermudagrass genotypes did not have specific traits (Fig. 4.5c & d) that differed from drought-susceptible bermudagrass genotypes, and accordingly their drought resistance may involve complex trait interplay.

4.6 Conclusion

Rapid vertical root growth rates, as measured in winter and summer as rate of root depth development in the early stages using rhizotrons, can be used as a trait for the performance in post-establishment drought under field conditions. Greater vertical root growth rate was associated with rapid shoot growth, both of which are desired characteristics of forage grass species. Genotypes 573 and 40 showed stability and high productivity of shoot growth, regardless of the season. In the experiment winter, an increased inactive root length ratio negatively influenced root depth development. Genotypes MED1 and MED2 maintained high vertical root growth rates in both seasons. Overall, genotypes MED1, MED2, 573 and 40 demonstrated broad adaptation to wide temperature ranges and to drought and appear to have greater potential for broad application in providing for forage production.

Supplementary materials

Supplementary materials are available online. Supplementary materials are supplied as extra files. The details include temperature and light conditions inside of the glasshouses, root depth measurements and all measured variables with the results of statistical analyses. The harvest measurements in supplementary materials (Table S6) include total root length, root angle, average root diameter, root tissue density, root dry weight, shoot dry weight, leaf area, tiller appearance rate, photosynthetic rate and transpiration rate. The materials can be made available online when this manuscript has been accepted.

Data availability

The data that support this study are available in the article and accompanying online supplementary material. Dr Yi Zhou and the co-authors in http://dx.doi.org/10.1071/FP13249 supplied data from a field experiment that include the time required to fall to 50% green cover (GC_{50}), root length density from 0 to 30 cm depth, root length density from 0 to 200 cm depth, and rhizome dry weight. In the present study, those data were used to correlate with rate of root depth development of the rhizotron experiments (Table S3).

Conflicts of interest

The co-author Matthew D Denton is the associate editor of Crop and Pasture Science.

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4.7 Figures

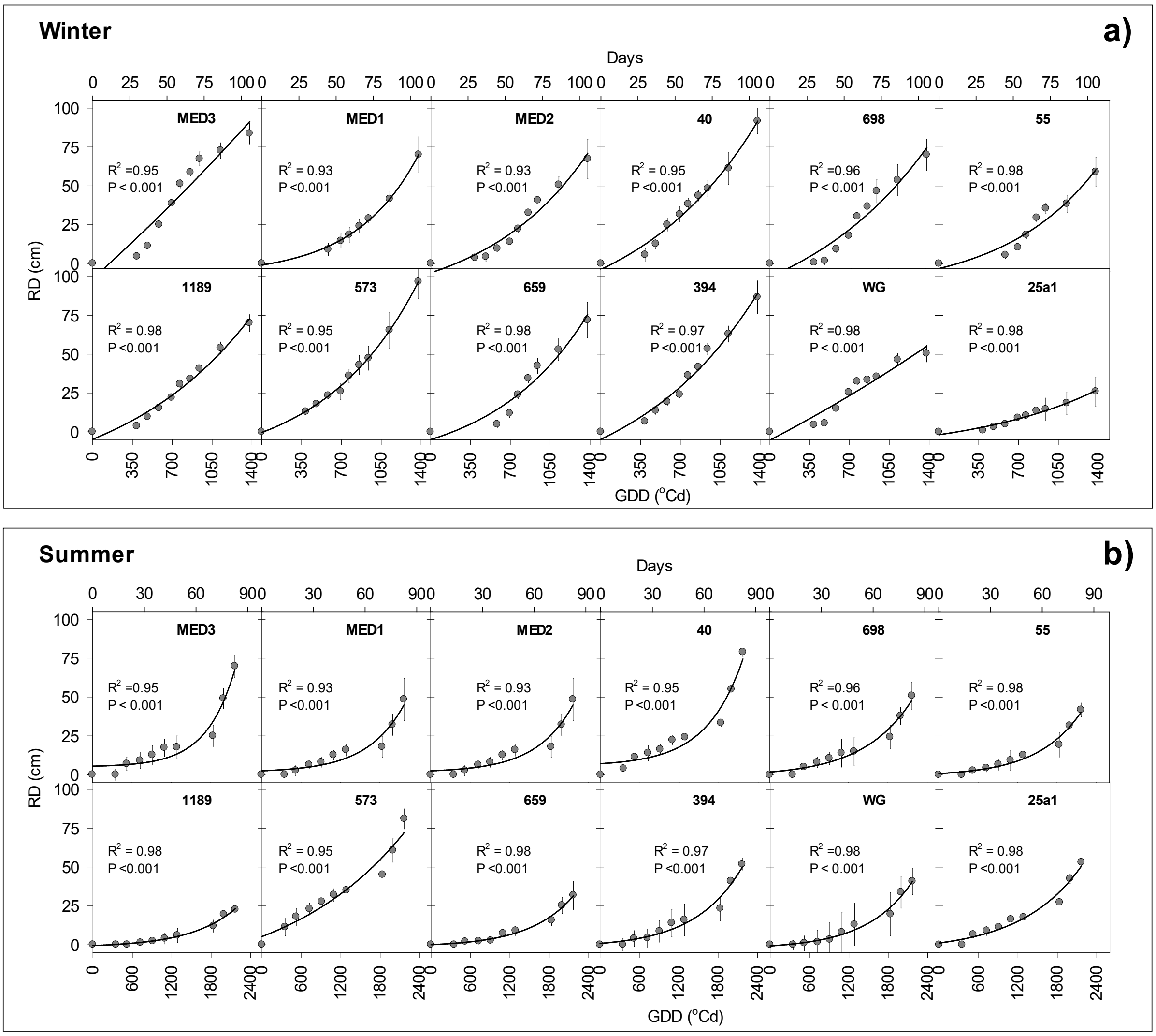

Fig. 4.1 Root depth was related to growing degree days (GDD) in winter experiment (a) and summer experiment (b). Regression was fit by exponential growth function, $y = y_0 + ae^{(bx)}$. Each point of measurement represents mean ± standard error.

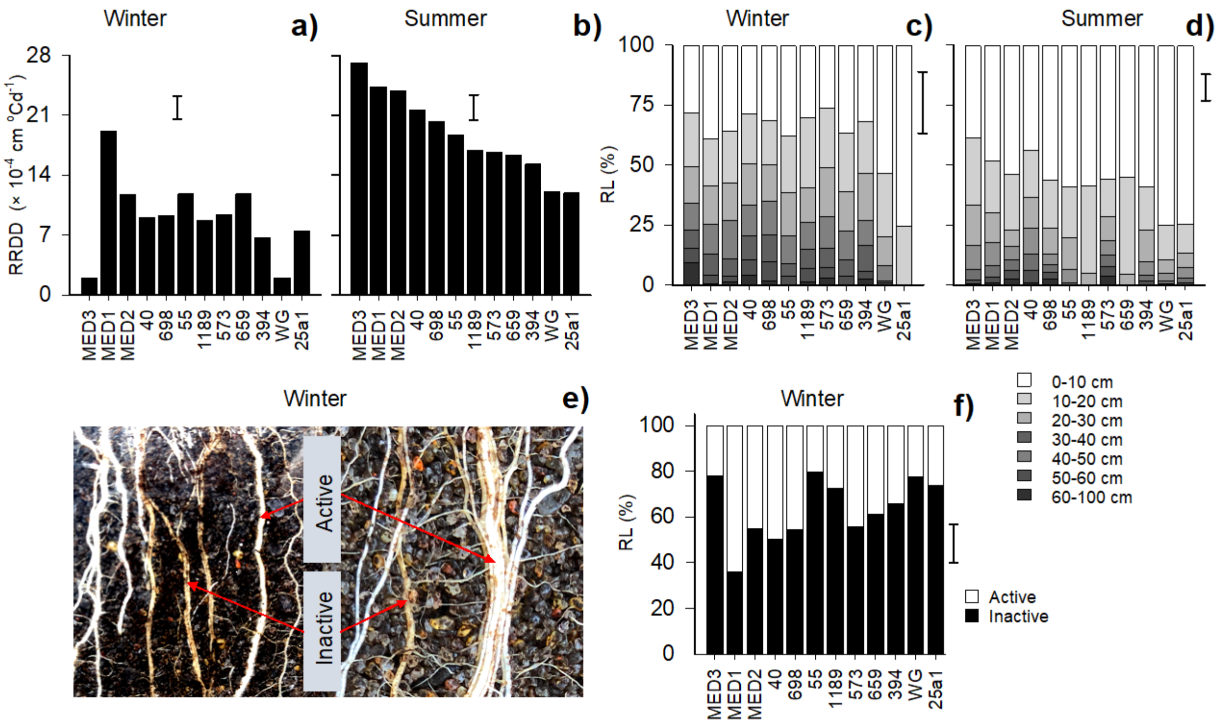

Fig. 4.2 Vertical root development during establishment in rhizotron experiments: rate of root depth development (RRDD) (a & b), vertical root distribution (c & d), active roots and inactive roots at harvest (e) and active-inactive root ratio (f). Vertical bars indicate LSD at *P* = 0.05. LSD bars in c and d indicate the differences in the percentage of roots distributed in the top rhizotron section between 0 and 10 cm. LSD bar in e indicates inactive root ratio in winter.

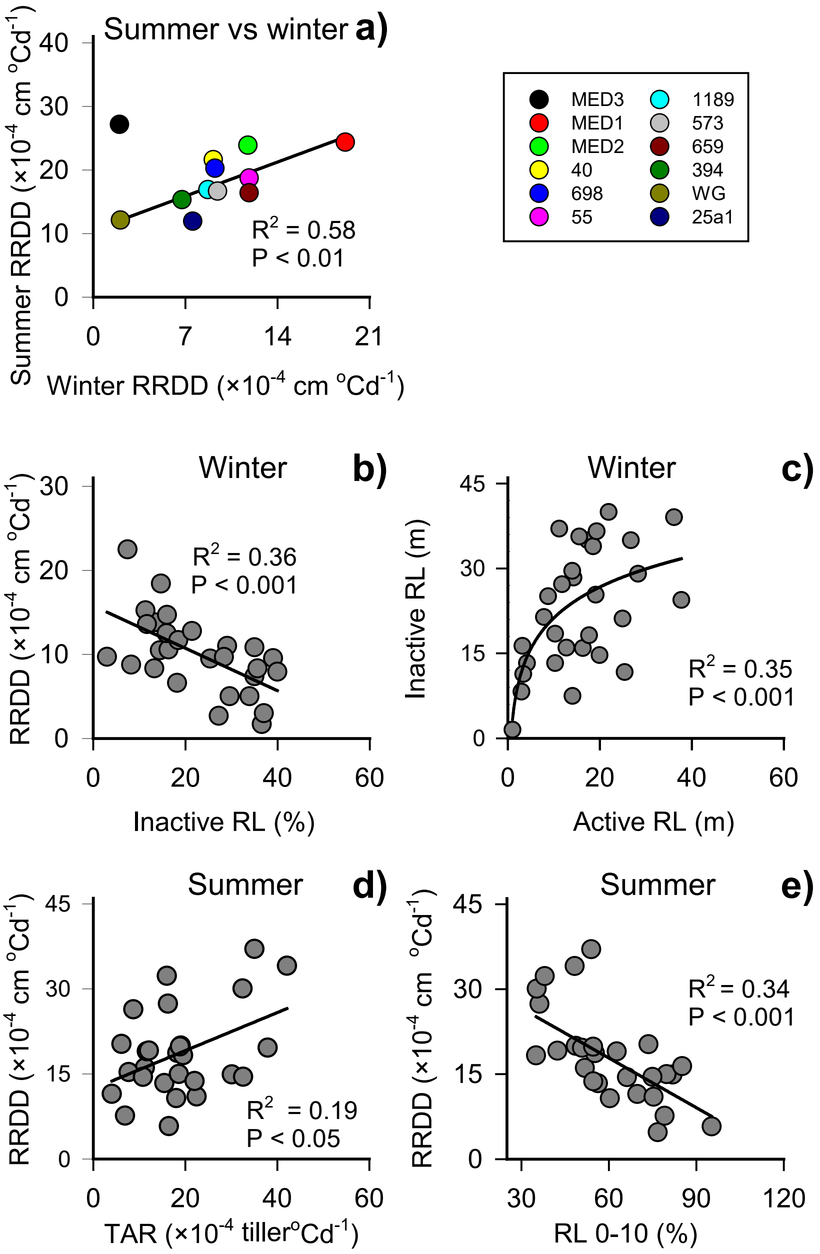

Fig. 4.3 Relationships of root depth development. Rate of root depth development (RRDD) values of both seasons were related (a). In winter, relationships were between rate of root depth development (RRDD) and inactive root length (RL) (b) and inactive RL and active RL (c). In summer, RRDD was related to tiller appearance rate (TAR) (d), and root length in the top rhizotron section between 0 and 10 cm (RL0-10) (e). Each point represents the mean value in a and each measurement in b-e.

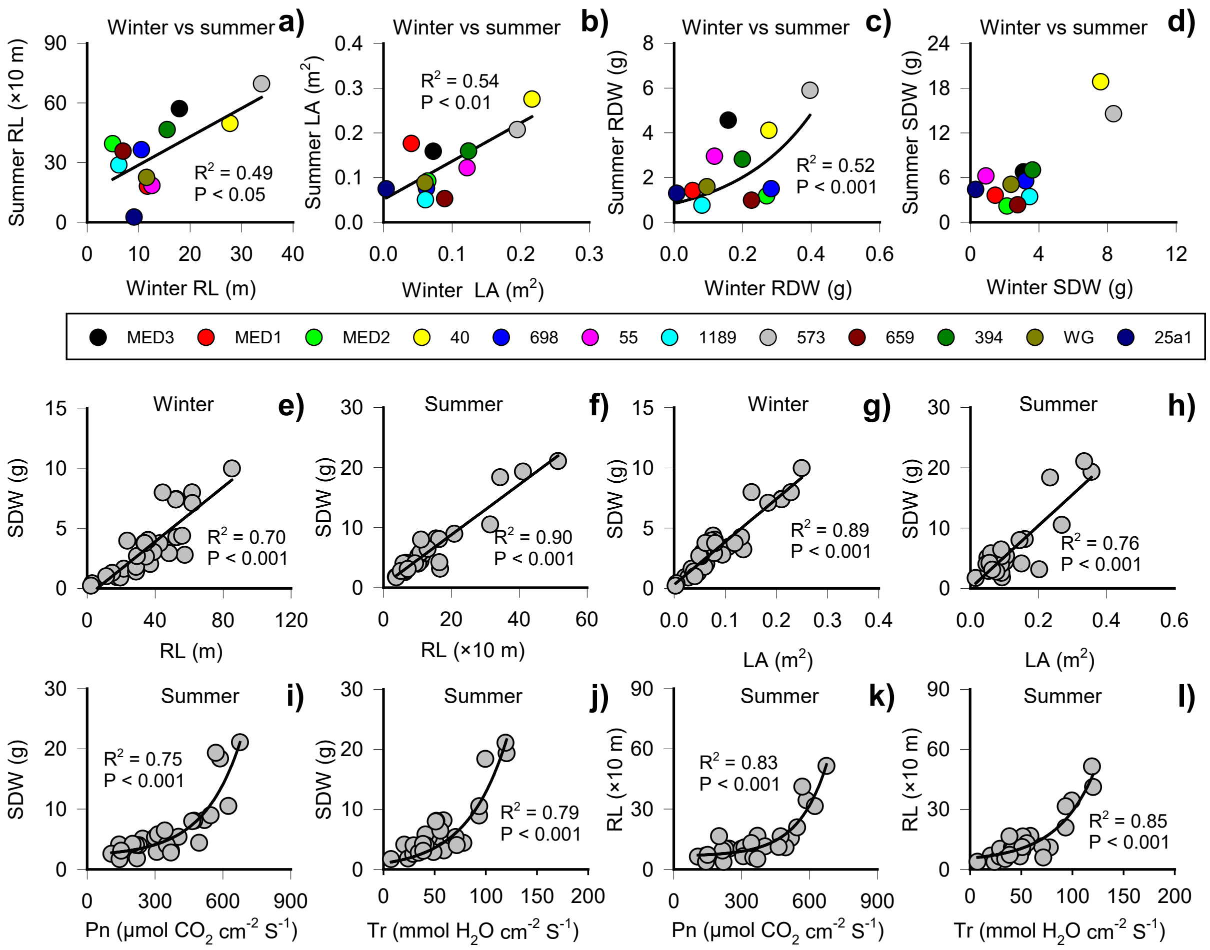

Fig. 4.4 Relationships of root and shoot growth in rhizotron experiments. Between seasons: relationships were total root length (RL) (a), leaf area (LA) (b), root dry weight (RDW) (c), and shoot dry weight (SDW) (d). In each season, shoot dry weight (SDW) was related to total root length (RL) (e & f), leaf area (LA) (g & h). In summer, shoot dry weight (SDW) was related to photosynthetic rate (Pn) (i) and transpiration rate (Tr) (j); and total root length was related to photosynthetic rate (Pn) (k), and transpiration rate (Tr) (l). Points represent mean values (a-d) and each measurement (e-j). Measurements of each genotype are provided in Supplementary Table S6.

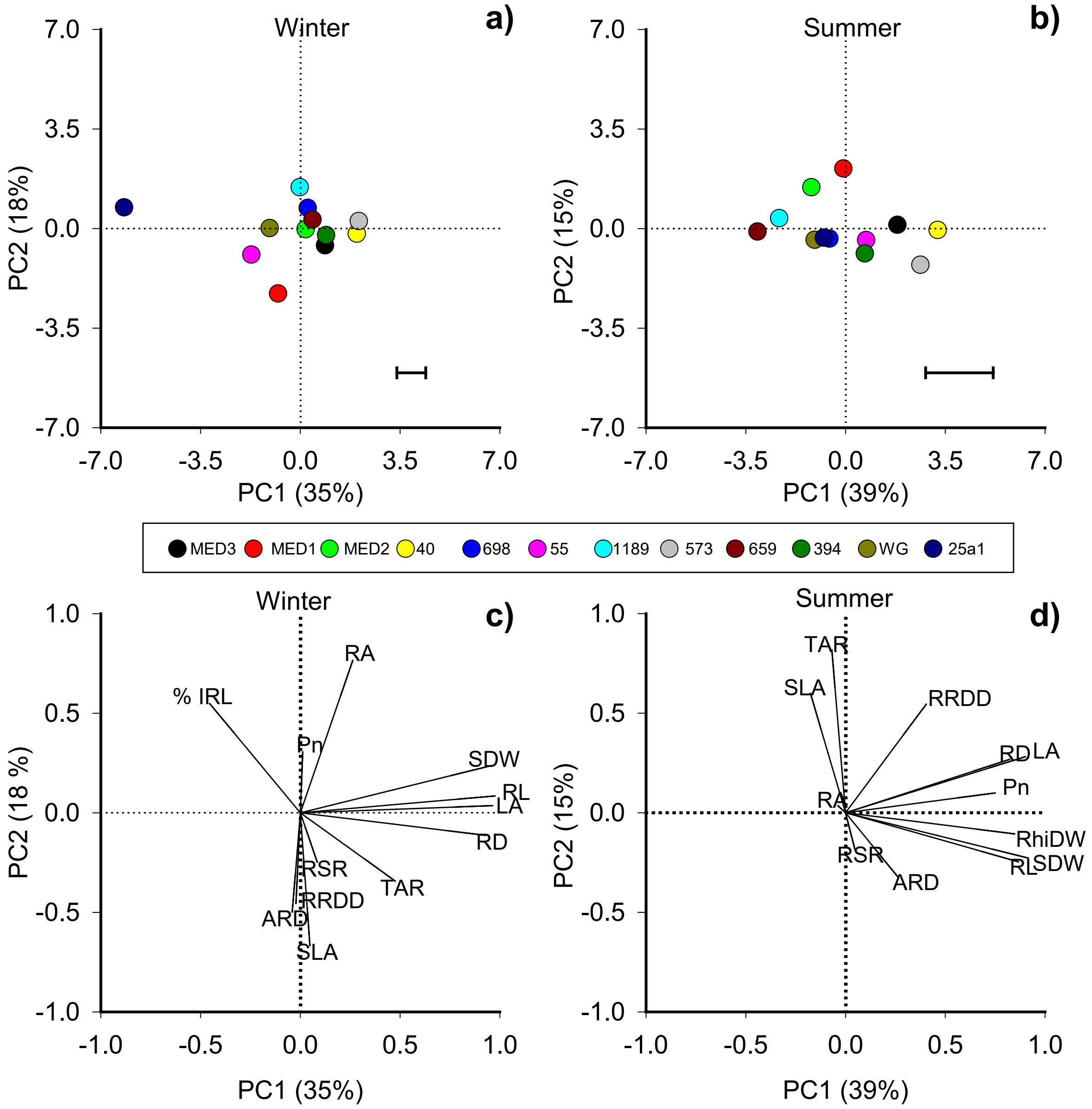

Fig. 4.5 Biplots from PCA analyses of 12 bermudagrass genotypes established in winter (a) and summer (b). Variables are rate of root depth development (RRDD), root depth at harvest (RD), root angle (RA), average root diameter (ARD), tiller appearance rate (TAR), shoot dry weight (SDW), rhizome dry weight (RhiDW), root to shoot ratio (RSR), leaf area (LA), specific leaf area (SLA), total root length (RL), percentage of inactive root length (%IRL), photosynthetic rate (Pn) and transpiration rate (Tr). Vertical bars in a & b indicate LSD at $P = 0.05$ for comparison of PC 1 scores. PC 2 scores were not significant.

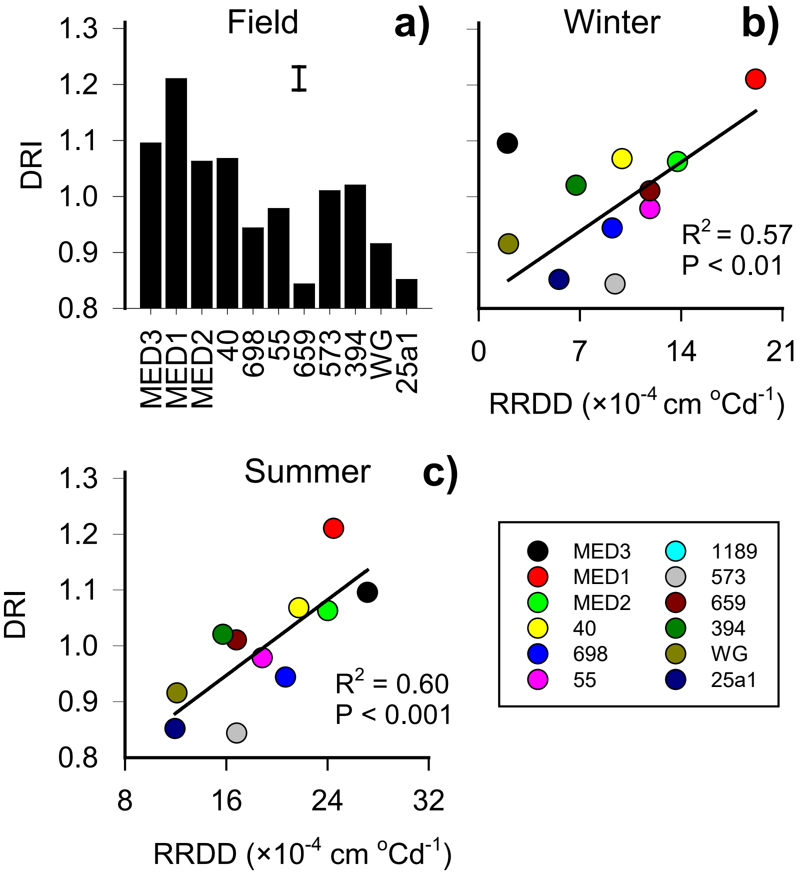

Fig. 4.6 Relationships between drought resistance and root development. Drought resistance indices (DRI) (a). DRI related to rate of root depth development (RRDD) in rhizotrons during winter (b) and RRDD in rhizotron in summer (c). The bar in a indicates LSD at *P* = 0.05

CHAPTER 5: ROOT PENETRATION IS ASSOCIATED WITH ROOT DIAMETER AND ROOT GROWTH RATE IN TROPICAL FORAGE GRASSES

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Principal Author

Name of Principal Author (Candidate)	Chanthy Huot		
Contribution to the Paper	Planned and conducted the experiments, analysed and interpreted the data, and wrote the manuscript		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	29/10/2021

Co-Author

By signing the Statement of Authorship, each author certifies that:

i. the candidate’s stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate’s stated contribution.

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5.1 Abstract

Aims Soil compaction limits root exploration by forage grasses, which impedes uptake to nutrient and root water from deep soils, and compromises competition and, ultimately, survival. While root penetration has been studied in many annual crops, the relationships between root growth and root penetration are poorly understood in grasses that have perennial growth habits. This study investigated relationships between root penetration, root diameter and vertical root growth in tropical perennial forage grasses.

Methods Root penetration 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 ten grass species by measuring root diameter and root lengths in seedlings grown in growth pouches. Vertical root growth rate for each species was measured in grasses grown in 120 cm deep rhizotrons.

Results Root penetration in both wax concentrations increased significantly with rapid shoot growth ($r = 0.65$ at 40% wax and 0.66 at 60% wax) and larger root diameter ($r = 0.67$ at 40% wax and 0.68 at 60% wax). However, the root penetration significantly increased with the greater vertical root growth rate only in the 60% wax treatment ($r = 0.82$).

Conclusions Results demonstrated that root penetration at higher resistance was promoted by root diameter and rapid vertical root growth. The combination of root diameter and root vertical growth rate, as observed in *Megathyrsus maximus*, can assist in the identification of perennial forage grasses suitable for agroecosystems challenged by soil compaction and rapidly drying soil surface.

Keywords hardpan soils, soil compaction, exploitative growth, tensile strength, wax system, tropical forage grasses, root penetration, root growth rate

5.2 Introduction

Compacted soil affects the growth of crops in at least 4% of global arable land (Food and Agriculture Organization 2015). A compacted soil layer with physical characteristics limiting root penetration and restricting water movement is called hardpan soils (Busscher 2011). Hardpan soils can result from farming practices or occur naturally during water deficit (Bell *et al.* 2006; Bell *et al.* 2007; Krümmelbein 2011). Soil compaction is a major agricultural constraint with adverse effects on the establishment and productivity of annual crops (Jin *et al.* 2015; Materechera *et al.* 1992). Low permeability of hardpan soils may deliberately lead to reduced vertical loss of water in sandy soils of rice-based cropping systems in the lower Mekong regions (Fukai *et al.* 2000; Inthavong *et al.* 2011). These constraints may affect the perennial forage grass species that are established in rice-based cropping ecosystems of the lower Mekong regions (Philp *et al.* 2019).

Compacted layers in soil profiles have been observed to prevent roots of annual crop species such as rice (Clark *et al.* 2008; Jin *et al.* 2015; Kato *et al.* 2006), wheat (Botwright Acuña and Wade 2012; Kubo *et al.* 2004), maize (Chimungu *et al.* 2015) and other crops (Materechera *et al.* 1992) from accessing subsoil moisture and nutrients. However, root penetration through compacted soil layers is poorly understood in perennial crops and pastures. Whilst the root growth rates of annual crops dramatically decrease with maturity and depth (Liu *et al.* 2011; Perkons *et al.* 2014), tropical perennial grass species have continuous growth habits with a vertical root growth in soil profiles (Acuña *et al.* 2010; Fuentealba *et al.* 2015; Huot *et al.* 2020; Nie *et al.* 2008). Therefore, the relationships between root penetration and vertical root growth rates of perennial grass species are of great interest to enhance forage survival and productivity.

The capability for root penetration by annual crops has most often been screened using wax to represent compacted soil layers (Yu *et al.* 1995). In this method, wax layers are constructed by combining paraffin wax and petroleum jelly at differing ratios to test various mechanical resistances (Botwright Acuña *et al.* 2007; Clark *et al.* 2008; Zhou *et al.* 2017b). Previous studies in rice (Babu *et al.* 2001; Clark *et al.* 2008), wheat (Botwright Acuña *et al.* 2007; Kubo *et al.* 2004), and maize (Chimungu *et al.* 2015) have demonstrated that capability for penetrating hard wax layers translates into the capacity for penetrating compacted soils in field conditions.

Positive associations between root penetration, root diameter and shoot growth have been reported in annual crop species (Jin *et al.* 2015). Exertion of greater force by roots to deform hard soils is typically associated with large root diameter (Clark *et al.* 2008; Kirby and Bengough 2002; Materechera *et al.* 1992) and can occur in association with shoot growth (Whalley *et al.* 1998). Moreover, root diameter can have a positive association with shoot growth because a great capacity of xylem and phloem transport processes of thick roots promotes root and shoot growth, even under high resistant soils (Dodd 2005; Schachtman and Goodger 2008). 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. Therefore, in this research we hypothesised that root penetration was positively related with root diameter, shoot growth and vertical root growth rate.

5.3 Methodology

5.3.1 Evaluation of root penetration

Root penetration capability of forage species was measured using the wax method (Yu *et al.* 1995). 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 (Glen Osmond SA 5064, Australia), were germinated in plug trays filled with a mixture of 50% cocopeat substrate with 50% University of California at Davis soil mix (UC mix, Table 1). One uniform seedling of each species (4 replicates) at the start of developing the second leaf was transplanted into each wax layer system (Fig. 5.1a). Pots were arranged in completely randomized designs.

Each wax layer system was constructed from an external PVC pipe (30 cm height × 9.5 cm diameter), an internal PVC pipe (15 cm height × 8 cm diameter), a wax layer (9 cm diameter × 0.4 cm thick) and substrate. Wax layers of consistent strength were obtained by melting a mixture of paraffin wax and petroleum jelly (VWR International Pty Ltd, Tingalpa, Australia) in specific weight ratios (Zhou *et al.* 2017b), casting the mixture into circular moulds (9 cm diameter × 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 (Fig. 5.1b). Each pot was filled with 2.33 kg (bulk density of 1.16 g $\mathrm{cm^{-3}}$) dried UC mix (Table 5.1) that contained 1.02 g N, 0.40 g P and 1.31 g K, with a wax layer placed at 15 cm depth.

An 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 (Fig. 5.1a) because the studied forage grass species have a large variation in root growth angle (Huot *et al.* 2020).

The systems were maintained in a growth chamber with a constant temperature of 30 °C, 70% relative air humidity and 15 hours of daylight. A continuous maximum photosynthetic photon flux density of 1000 μmol photon m^{-2} s^{-1} was maintained for 10 hours day $^{-1}$ in between the dawn-evening simulation (Fig. 5.1c). Soils were irrigated to 60% water holding capacity every day. UC mix volumetric moisture content is 25.1% v/v at field capacity (Zhou *et al.* 2013a).

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 combined 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 that penetrated through a wax layer of that resistance, in proportion to total numbers of nodal and seminal roots that reached the surface of wax (Yu *et al.* 1995). Shoots and roots were then oven-dried at 60 °C for 72 hours to determine dry weight.

5.3.2 Measurement of root diameter

Root diameters of each species were measured using plants grown in a soil-less medium. When the second leaf emerged, the seedlings were removed from the plug trays, and roots were gently rinsed to remove attached soil, and each seedling was transplanted into the top compartment of a pouch bag with 13 cm width × 14 cm height dimension (Mega International, Minnesota, 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, 6163 Western 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 analysis was undertaken. Plants of each species (5 replications) that grew roots to 10 cm depth were selected for root diameter analyses, using the method in 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 minutes, washing with distilled water for 2 minutes. The whole roots were scanned using an EPSON Expression 10000XL scanner (EPSON Inc., Long Beach, CA 90806, 124 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 (Regent Instruments Inc. 2019). Scanned images of the entire roots were used to re-analyse using WinRHIZO to obtain total root length and average root diameter that also characterised the differences between grass species.

5.3.3 Measurement of vertical root growth rate

Reference rates of root depth development and growth of fibrous roots were obtained from a previous experiment (Huot *et al.* 2020) that 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 μmol m^{-2} s^{-1}. The reference rate of root depth development was measured as growing degree days required for roots to reach 50 cm depth (GDD_{50}). GDD_{50} values for *U. ruziziensis*, *P. coloratum,* and *P. scrobiculatum* were not available. Vertical root growth rate was calculated as 50 (cm) / GDD_{50} (°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 (Regent Instruments Inc. 2019).

5.3.4 Statistical analyses

ANOVA of the general linear model on GenStat 18th Edition (VSN International 2019) was used to identify significant differences in measured variables. Multiple mean comparisons were performed using Fisher's least significant difference (LDS) at 5% probability. Correlation analysis using all measured variables were performed to identify possible mechanisms for root penetration. For traits of each species analysed using seedlings, root diameter and variables obtained from WinRHIZO analysis such as average root diameter, total root length (RL), root length with diameters less than 0.5 mm, and root length with diameters greater than 0.5 mm included in the correlation analysis (Supplementary Table S5.1 & 2). Pearson's correlation coefficients (r) were computed using GenStat 18th Edition.

5.4 Results

5.4.1 Variation in root penetration, root diameter and vertical root growth rate

All species had roots that penetrated through wax layers of both resistances during the three-week growth period in pots (Fig. 5.1a). 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 × wax resistance interaction in root penetration ratio ($P < 0.01$) (Fig. 5.2a & b). Grass species differed in total dry weight ($P < 0.01$, at 40 % wax and $P < 0.001$, at 60 % wax); however species × wax resistance interaction in dry weight was not significant ($P > 0.05$) (Fig. 5.2c & d). In pouch bags, species had significant differences in root diameter ($P < 0.\ 001$) and length of roots with diameters greater than 0.5 mm ($P < 0.\ 001$) (Fig. 5.3a & 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 ($P < 0.\ 001$) (Fig. 5.4) after the 4-week growth in rhizotrons.

5.4.2 Relationships between plant 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) (Fig. 5.5a & g), and greater shoot dry weight ($r = 0.65$, $P < 0.05$; 40 % wax and $r = 0.66$, $P < 0.05$; 60 % wax) and (Fig. 5.5b & h). However, there was no correlation at the resistance of 40 % wax concentration (Fig. 5.5c). Only at the resistance associated with 60 % wax concentration, root penetration ratio significantly increased 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 ($r = 0.89$, $P < 0.01$) (Fig. 5.5d-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 (Fig. 5.6).

5.5 Discussion

Positive associations of root diameter and root penetration ratio at both wax concentrations indicated that larger root diameter promoted root penetration through higher-resistance wax discs (Fig. 5.6). Penetration by roots with a large dimeter could be assisted by thick cortical areas, greater cortical cell wall area, higher cortical cell counts and large stele diameter, as observed in maize genotypes (Chimungu *et al.* 2015). Greater content of cell wall and larger diameter of stele in large roots reduces axial stress of root tips (Hettiaratchi 1990; Kirby and Bengough 2002) and increases root rigidity and tensile strength to prevent impeded roots from bending when faced with high resistance (Clark *et al.* 2008; Loades *et al.* 2013). This was apparent with the perennial forage grasses evaluated for root penetration capability in the present study. Root diameter is determined by genetic controls, such that root diameter at the early growth stage can indicate root diameter of established plants (Clark *et al.* 2008; Price *et al.* 2002).

The positive relationship between root penetration ratio and shoot dry weight indicated that shoot growth was associated with root penetration of 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 (Croser *et al.* 2000; Ubeda-Tomás *et al.* 2012). 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 (Clark *et al.* 1996; Croser *et al.* 2000; Whitmore and Whalley 2009).

Despite positive correlations between shoot growth, root penetration and root diameter in both wax concentrations, the increased root penetration was associated with the greater vertical root growth rate only for the 60 % wax concentration. The increased vertical root growth rate of forage grasses was associated with the rapid establishment of lengths of large diameters (greater than 5 mm) at the seedling stage (Fig. 5.6). Thick roots developed during the early growth of perennial forage grasses in this study indicates a high capacity of root xylem transport of roots to promote shoot growth, which occurs even at high soil resistance in annual crops (Dodd *et al.* 2010; Jin *et al.* 2015; Schachtman and Goodger 2008). Thick roots also provide large phloem tissues to increase resource transport from leaves to promote root growth (Chimungu *et al.* 2015; Dodd 2005; Jin *et al.* 2015; Schachtman and Goodger 2008).

Furthermore, increased growth of fibrous roots and shoots were associated with a greater capability of root penetration at the higher resistance of 60% wax concentration (Fig. 5.5g). The greater length of fibrous roots developing from nodal and seminal roots in annual crops such as maize (Bengough *et al.* 2016; Chimungu *et al.* 2015), barley (Haling *et al.* 2013) and forage radish (Chen and Weil 2010), increased the stability of nodal or seminal roots penetrating at high resistance layers. In several of the perennial grasses used in this present study, rapid growth of small roots also promoted root vertical growth rate and shoot growth (Huot *et al.* 2020).

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. Previously, *M. maximus* had rapid root establishment to depths between 50 and 120 cm compared with other forage grasses (Huot *et al.* 2020), and is commonly cultivated in areas with compacted subsoil layers with resistances of 2.0 to 3.5 MPa (Sharma *et al.* 1994; Whalley *et al.* 2008), 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 root establishment in field environments (Botwright Acuña *et al.* 2007; Clark *et al.* 2002; Jin *et al.* 2015), 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 (Philp *et al.* 2021; Philp *et al.* 2019).

5.6 Conclusion

Forage grass species in this study exhibited variation in root penetration of wax with resistance of 1.39 MPa and 2.12 MPa. Variation in root penetration across grass species was larger at the higher wax resistance. The results showed a significant effect of species × wax resistance, and accordingly, there were differences in traits correlated with root penetration ratio at both wax strengths. Thicker root diameter 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 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.

Conflicts of interest

The co-author Matthew D Denton is an associate editor of *Crop & Pasture Science*, and he took no part in the review and acceptance of this manuscript, in line with the publishing policy.

Declaration of funding

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5.7 Tables and Figures

Table 5.1 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	
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 + 2.5Mn + 0.04 Mo + 1.0 Zn (from Fernland, Yandina, QLD 4561 Australia)	0.90 kg		
Calcium nitrate	2.25 kg		

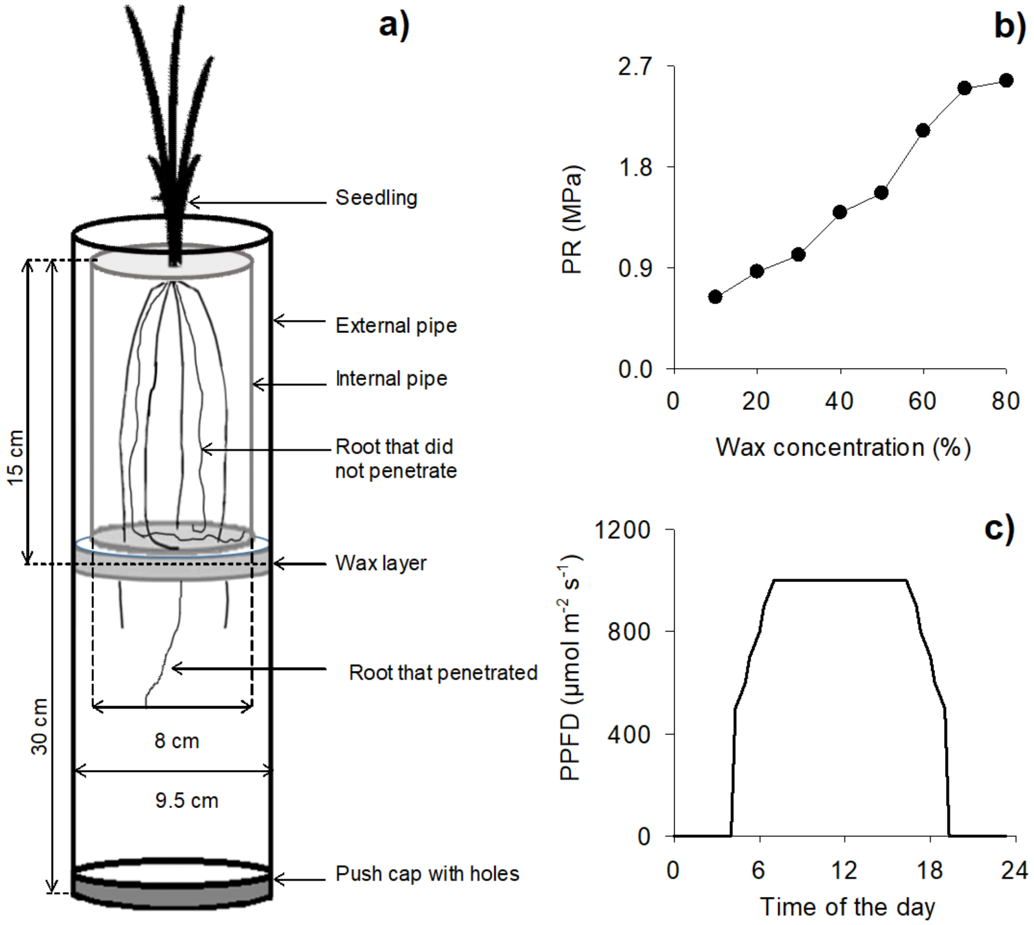

Fig. 5.1 A wax layer system constructed by an external PCV pipe (30 × 9.5 cm) and an internal PCV pipe (15 × 8 cm), a wax disc placed underneath the internal pipe (a), penetrometer resistance (PR) of combined wax and petroleum jelly discs at various wax concentrations at 30 °C in the growth chamber (b), and photosynthetic photon flux density (PPFD) regime in the growth chamber (c).

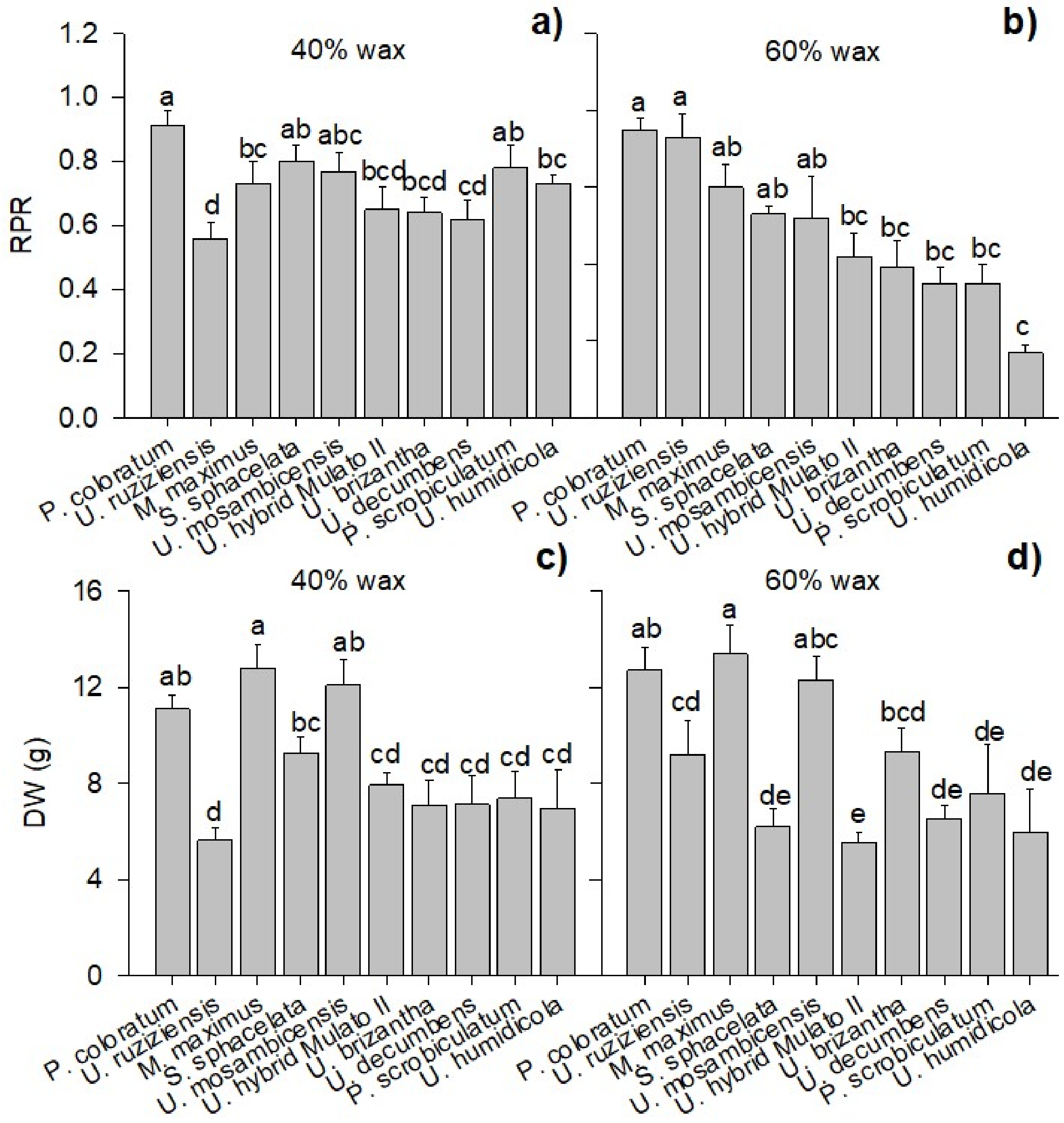

Fig. 5.2 Grass species ranked in descending order based on root penetration at 60% wax: root penetration ratio (RPR) (a & b), total dry weight (DW) (c &d). Bars (± SE) with different letters on top are significant differences within the wax concentration at $P < 0.05$

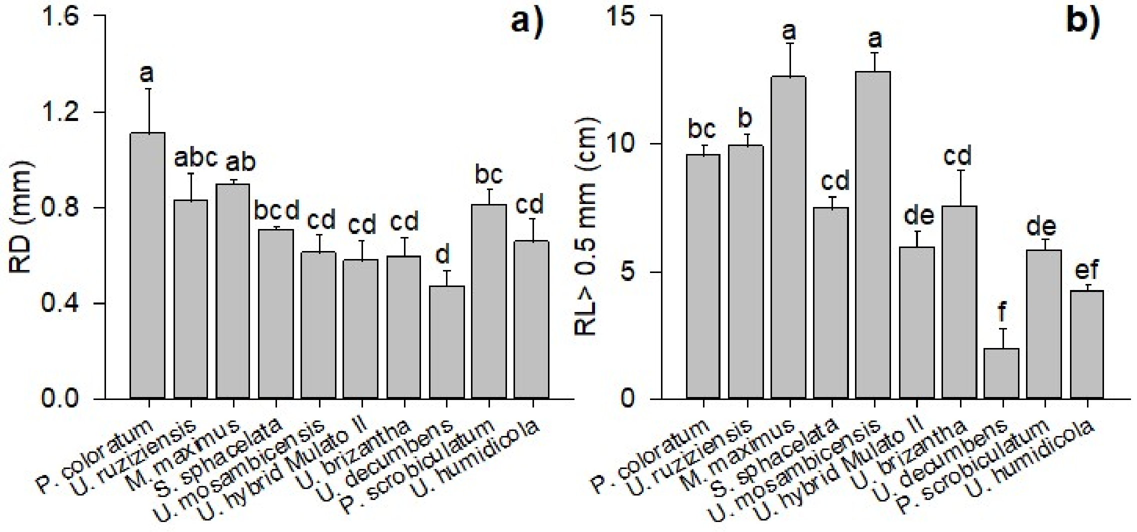

Fig. 5.3 Reference root size: root diameter (RD) (a) and root length measured in roots with diameters greater than 0.5 mm (RL>0.5mm) (d). Bars (± SE) with different letters on top are significantly different means at $P < 0.05$

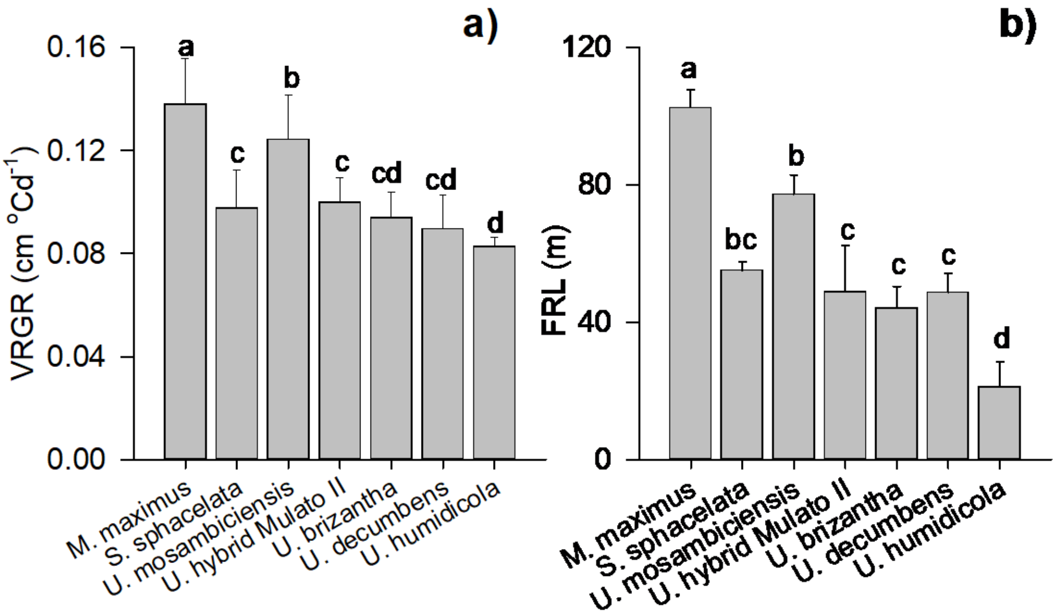

Fig. 5.4 Root growth of grass species (without *U. ruziziensis*, *P. coloratum*, and *P. scrobiculatum*) during the four-week growth in rhizotrons: vertical root growth rate (VRGR) (a) and fibrous root length (FRL) (b). Bars (± SE) with different letters on top are significantly different means at $P < 0.05$.

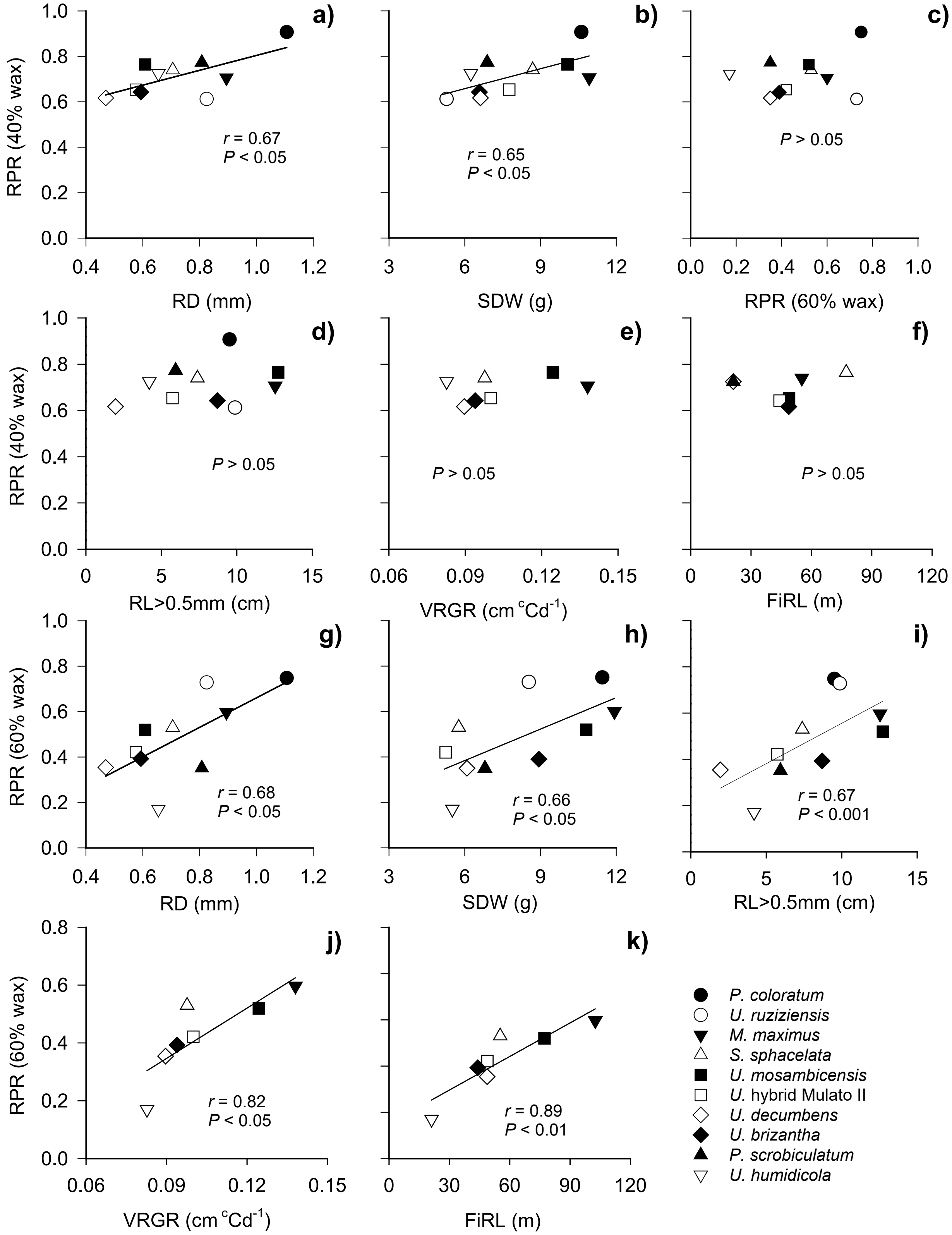

Fig. 5.5 Relationships between root penetration ratio (RPR) and root diameter (RD) (a & g), shoot dry weight (SDW) (b & h), root penetration ratios between both wax concentrations (c),

root length measured in seedling roots with diameters greater than 0.5mm (RL>0.5mm) (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. *U. ruziziensis*, *P. coloratum*, and *P. scrobiculatum* were not measured in rhizotrons during the four-week growth in rhizotrons.

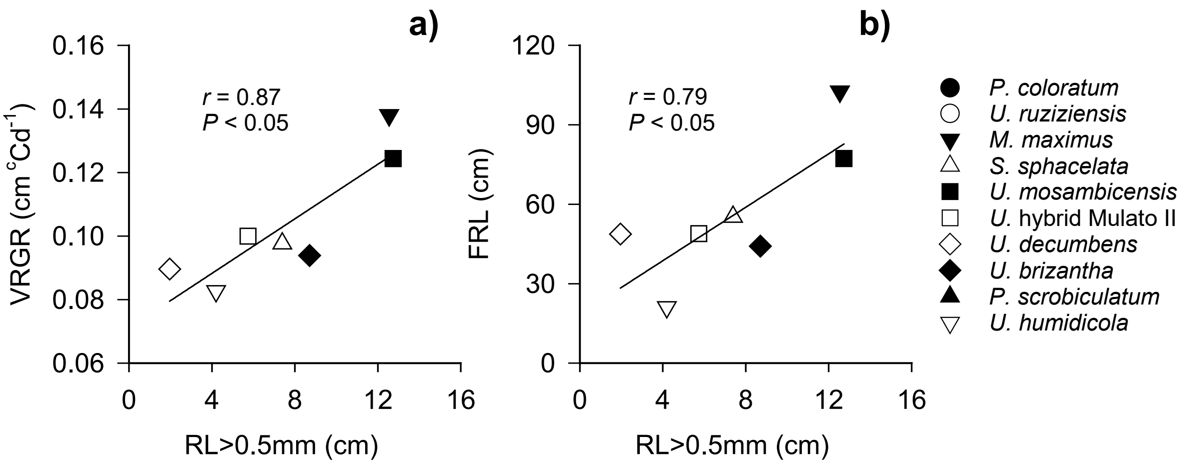

Fig. 5.6 Relationships between root length measured in seedling roots with diameters greater than 0.5 mm (RL>0.5mm) and vertical root growth rate (VRGR) (a) and fibrous root length (FRL) (b) of grasses in rhizotrons. Each point represents the mean value of each species. *U. ruziziensis*, *P. coloratum*, and *P. scrobiculatum* were not measured in rhizotrons during the four-week growth in rhizotrons.

5.8 Supplementary data

Table S5.1 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< 0.5mm), and root length with diameters greater than 0.5 mm (RL> 0.5mm). 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*.

Variable	PR40	PR60	SDW40	SDW60	RDW40	RDW60	RD	ARD	RL
Wax									
PR40	-								
PR60	0.28ns	-							
SDW40	0.65*	0.46ns	-						
SDW60	0.41ns	0.66*	0.67*	-					
RDW40	0.26ns	0.08ns	0.56ns	0.62ns	-				
RDW60	0.48ns	0.42ns	0.54ns	0.78**	0.85**	-			
Seedlings									
RD	0.67*	0.68*	0.47ns	0.6ns	0.12ns	0.5ns	-		
ARD	-0.21ns	0.21ns	-0.36ns	0.03ns	-0.55ns	-0.33ns	0.13ns	-	
RL	-0.37ns	-0.34ns	-0.32ns	-0.18ns	-0.21ns	-0.28ns	-0.52ns	0.44ns	-
RL<0.5mm	-0.38ns	-0.54ns	-0.50ns	-0.52ns	-0.41ns	-0.49ns	-0.58ns	0.37ns	0.92ns
RL>0.5mm	0.28ns	0.68*	0.61ns	0.85**	0.57ns	0.65*	0.49ns	-0.15ns	-0.54ns

Table S5.2 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< 0.5mm), root length with diameters greater than 0.5 mm (RL> 0.5mm), vertical root growth rate (RVGR), and fibrous root length (FiRL). 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*.

Variables	Wax					
	PR40	PR60	SDW40	SDW60	RDW40	RDW60
Rhizotron						
RVGR	0.4ns	0.82*	0.95***	0.86*	0.68ns	0.7ns
FiRL	0.28ns	0.89**	0.94**	0.82*	0.64ns	0.67ns
	Pouch					
	RD	ARD	RL	RL<0.5mm	RL>0.5mm	
Rhizotron						
RVGR	0.66ns	-0.43ns	-0.48ns	-0.71ns	0.87*	
FiRL	0.62ns	-0.49ns	-0.39ns	-0.61ns	0.79*	

CHAPTER 6: RHIZOME GROWTH OF C4 GRASS SPECIES IN ARID REGIONS IS RELATED TO ARIDITY INDEX, RAINFALL AND EVAPOTRANSPIRATION

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Publication details	

Principal Author

Name of Principal Author (Candidate)	Chanthy Huot		
Contribution to the Paper	Planned and conducted the experiments, analysed and interpreted the data, and wrote the manuscript		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	29/10/2021

Co-Author

By signing the Statement of Authorship, each author certifies that:
i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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6.1 Abstract

A large rhizome system enables bermudagrasses to survive in arid regions and promotes post-harvest regrowth and capacity to recover from severe drought. Therefore, the collection of bermudagrass genetic resources from arid ecosystems can provide value for the breeding of improved bermudagrasses with greater drought resilience. This study examined relationships between rhizome growth in bermudagrass ecotypes and the aridity of the environment from which they were originally collected. Bermudagrass ecotypes (n = 96 arid; 46 non-arid) were collected from temperate regions of Australia, where environments at the origins of bermudagrass ecotypes possess a slight variation in mean temperature but vary markedly in rainfall. A pot experiment was conducted for 14 weeks during summer to measure rhizome dry weight and shoot morphological traits. There was a non-significant difference in rhizome dry weight between ecotypes in arid and non-arid regions. The highest rhizome dry weight was found amongst ecotypes from arid environments. In ecotypes from arid environments only, rhizome growth showed a positive response to a more humid climate condition in winter, and leaf width was positively correlated with annual evapotranspiration. In ecotypes from both regions, rhizome growth was positively correlated with belowground to aboveground biomass ratio and leaf width, and belowground to aboveground biomass ratio was negatively correlated with internode length. These findings suggest that wider leaves optimise rhizome growth of bermudagrass ecotypes in arid areas with the majority of rainfall during winter. The ecotypes found to have the greatest rhizome dry weight may assist in meeting production niches in drought-prone regions.

Keywords: arid ecosystems, adaptive evolution, drought resistance, forage development

6.2 Introduction

Bermudagrasses (*Cynodon* spp.) have been widely cultivated as pastures and turfgrass in many parts of the world (da Silva *et al.* 2015; Hill *et al.* 2001; Pandeya and Ahirwalb 2020). The most desirable trait in the section of bermudagrasses is the extensive rhizome system (Casler and Duncan 2003; Zhou *et al.* 2015). Rhizomes serve as storage organs for meristems and resources that allow them to regrow after disturbances (Bai *et al.* 2010; Casler and Duncan 2003; Cook *et al.* 2005; Dong and de Kroon 1994), and can survive in bushfires or flooding conditions for several weeks whilst other reproductive organs such as seeds and aboveground stolons are vulnerable (Cook *et al.* 2005; D'Antonio and Vitousek 1992). Furthermore, rhizomes can become drought-dormant for 7 months in prolonged drought and then still have a great capacity of growing roots rapidly to produce stolons under the least favourable watered conditions (Chai *et al.* 2010; Cook *et al.* 2005; Pandeya and Ahirwalb 2020). In the bermudagrasses after establishment, an extensive rhizome system promotes drought resistance and post-harvest regrowth rate (Zhou *et al.* 2014; 2015), even in mild temperate conditions (Van Tran *et al.* 2017).

Growing an extensive rhizome system of bermudagrass ecotypes seems to be an adaptive trait to cope with arid climates (Paula and Pausas 2006; Pignatti *et al.* 2002). An increase in carbon supply to rhizomes in a short growth period due to a short rainy season and low annual rainfall in arid regions may be associated with shoot traits that have evolved as an adaptive divergence among ecotypes of a crop species after existing in a specific environment for an extended period (Berger *et al.* 2017; Lowry *et al.* 2014). Approximately 1000 bermudagrass ecotypes have been identified in Australia (Kearns *et al.* 2009). Some of those grasses may have evolved to adapt according to the diverse habitats in Australia, where there are large variations in aridity indices (Casadebaig *et al.* 2016) and annual rainfall (Bureau of Meteorology 2020). Therefore, analysing adaptive responses of rhizome growth to local bioclimatic variation can identify bermudagrass ecotypes cultivated to meet production niches in specific environments such as drought-prone regions.

A previous study using bermudagrasses originating in Australia reported that the ecotypes with a larger rhizome production during the establishment had a higher fraction of biomass allocated from the shoots (Zhou *et al.* 2015). Variations in shoot traits between grass ecotypes originating from varied environments are strongly associated with functional trade-offs for the growth of plant organs (Cavender-Bares *et al.* 2016; Pérez-Harguindeguy *et al.* 2013; Wright *et al.* 2004). For example, the delayed development of leaf clusters along spreading stolons is associated with the length of internodes and the growth of stolons (da Silva *et al.* 2015; Matthew *et al.* 2001; Sbrissia *et al.* 2003). These characteristics may adversely affect the biomass allocation for the growth of rhizomes during the establishment.

Previous research on bermudagrass ecotypes in Australia revealed a consistent ecotypic ranking of rhizome growth between bermudagrass ecotypes in field experiments under subtropical and Mediterranean environments (Zhou *et al.* 2014), indicating that environmental plasticity in rhizome size is low. In addition, plasticity in the rhizome and belowground growth is low regardless of factors such as nutrient variations (Dong and de Kroon 1994) or seasonal variations in temperatures and sunlight (Huot *et al.* 2021). These findings suggest that that screening bermudagrass ecotypes according to rhizomes are not affected by experimental conditions and can be achieved by a multi-species trial.

The objectives of this study were to analyse variation in rhizome growth of bermudagrass ecotypes collected from areas with various aridity indices to examine relationships between rhizome growth and aridity index, rainfall and evapotranspiration, and to characterise traits correlated with rapid rhizome growth. This study hypothesised that during establishment, rhizome growth of bermudagrass ecotypes from arid environments would be more rapid than bermudagrass ecotypes from non-arid environments.

6.3 Materials and Methodology

6.3.1 Bermudagrass ecotypes

A total of 142 bermudagrass ecotypes originating from temperate regions between 29 °S and 37 °S latitudes of Australia (Fig. 6.1a, Supplementary Table S6.1) were studied. In these systems, rainfall predominantly falls during the winter and varies in these regions (Bureau of Meteorology 2020). Moreover, there is a large variation in aridity indices (Fig. 6.1a) (Casadebaig *et al.* 2016). The climatic conditions are 20 to 25 °C annual mean temperatures, 0.10 to 2.50 aridity indices, 100 to 1500 mm year^{-1} rainfall, and 590 to 1340 mm year^{-1} reference evapotranspiration. Five ecotypes of those grasses, namely MED1, MED2, MED3, 573, and 659, were used in previous studies finding that their rhizome dry weight production during establishment was a mechanism for drought resistance of established grasses (Zhou *et al.* 2014; 2015). Therefore, these ecotypes were used as references in this study.

6.3.2 Experiments

A pot experiment was conducted during summer from 1 December 2019 to 16 March 2020 (photosynthetic photon flux density and daily temperature in Fig. 6.1b & c). Randomized complete block design was used for the experiment. Multiple cuttings of each genotype were transplanted into each pot (10 cm width × 10 cm length × 14 cm depth) to ensure successful rooting; then, rooted cuttings were reduced to 2 plants pot^{-1} (3 pots genotype^{-1}) 2 weeks after transplanting. Pots were arranged outdoors on glasshouse benches (0.8 m height), and filled to 1.47 kg l^{-1} density with the University of California at Davis mix (UC mix), produced by the South Australian Research and Development Institute at Waite. One cubic meter of UC mix consisted of 0.56 m^3 Waikerie sand, 0.44 m^3 Canadian peat moss, 0.80 kg hydrated lime, 1.33 kg agriculture lime, 3.00 kg Osmocote Exact Mini (6 N + 3.5 P + 9.1 K + TE) (from Fernland, Yandina, QLD 4561 Australia). The UC mix contained 0.48 g l^{-1} N, 0.19 g l^{-1} P and 0.61 g l^{-1} K (Huot *et al.* 2020), and 25.1 v/v % volumetric water content at field capacity (Zhou *et al.* 2013a). During the experiment, grasses were irrigated to saturation twice per day.

6.3.3 Data collection

Aboveground shoots of all grass ecotypes were cut at 1 cm above the ground 6 weeks after transplanting. The two longest stolons of cut shoots from the outdoor experiments were selected to measure stolon length, length of the first two internodes, leaf length and leaf width of the first leaves from the first and second nodes. After the analyses, shoot samples were oven-dried for shoot dry weight. Destructive harvest was conducted on 16 March 2020 to measure the dry weight of rhizomes, roots and shoots.

Rainfall, reference evapotranspiration and temperatures at the origin for each ecotype were obtained from analysing data of 20-to-50 year records by weather stations nearest to their collection sites (Fig. 6.1a) (Bureau of Meteorology 2020). The aridity index was calculated as the ratio of rainfall over reference evapotranspiration. Habitats with aridity indices less than 0.65 were classified as arid, based on the FAO aridity index and Köppen–Geiger classification (Spinoni *et al.* 2015). Accordingly, 96 ecotypes of bermudagrasses were categorised as being from arid environments, and 46 ecotypes were from non-arid environments (Fig. 6.1a).

6.3.4 Statistical analyses

Rhizome dry weight was analysed for significant differences between ecotypes, using GenStat 20th Edition (VSN International 2019). Mean comparison for rhizome dry weight was performed according to Fisher's least significant difference (LSD) at $P < 0.05$. Pearson correlation coefficient (*r*) was calculated using GenStat 20th Edition (VSN International 2019) to examine relationships between rhizome dry weight, plant variables and bioclimatic variables such as aridity index, annual rainfall and reference evapotranspiration. Principal component analysis (PCA) using Sigmaplot 14.0 (Systat Software Inc. 2017) was performed to differentiate bermudagrass ecotypes from arid and non-arid regions.

6.4 Results

6.4.1 Variation in rhizome growth

Rhizome dry weight differed significantly among 142 bermudagrass ecotypes ($P < 0.001$, Fig. 6.2a). Rhizome dry weight varied largely between ecotypes and even within ecotypes from arid regions. The ecotypes with the greatest rhizome dry weight originated from arid environments with aridity indices less than 0.65. The number of ecotypes with rhizome at harvest was 87.5% from arid environments and 95.7% from non-arid environments. However, there were no significant differences between arid and non-arid regions in rhizome dry weights ($P > 0.05$, Fig. 6.2b) and belowground to aboveground dry weight ratios ($P > 0.05$, Fig. 6.2).

6.4.2 Relationships between rhizome growth and local bioclimatic variables

Correlation analyses showed different responses of rhizome dry weight, leaf length and leaf width between ecotypes from arid and non-arid regions to their local climatic conditions. Rhizome dry weight was correlated with aridity index ($r = 0.42$, $P < 0.001$, arid, and $r = 0.17$, $P < 0.05$; arid & non-arid), annual rainfall ($r = 0.41$, $P < 0.001$; arid, and $r = 0.22$, $P = 0.01$; arid & non-arid) and annual reference evapotranspiration ($r = -0.25$, $P < 0.05$; arid, and $r = -0.21$, $P < 0.05$; arid & non-arid) (Fig. 6.3a-c). These relationships were not found in bermudagrass ecotypes from non-arid environments (Supplementary Table S6.2). Nevertheless, the similar relationships between all groups of bermudagrass ecotypes were that belowground to aboveground dry weight ratio showed a positive response to an increase in winter to summer rainfall ratio ($r = 0.30$, $P < 0.01$; arid, $r = 0.68$, $P < 0.001$; non-arid, and $r = 0.45$, $P < 0.001$; arid & non-arid) (Fig. 6.3d).

The relationships between leaf length and leaf width of ecotypes in arid and non-arid environments and their local bioclimatic variables were different (Fig. 6.4). The similarity between ecotypes from both regions were that leaf length was correlated with aridity index ($r = -0.40$, $P < 0.001$; arid, and $r = -0.46$, $P < 0.01$; non-arid) and annual rainfall ($r = -0.40$, $P < 0.001$; arid, and $r = -0.45$, $P < 0.01$; non-arid) (Fig. 6.4a-d). In ecotypes from arid regions only, leaf length was correlated with annual evapotranspiration ($r = 0.27$, $P < 0.01$) (Fig. 6.4e), and leaf width was correlated with annual reference evapotranspiration ($r = -0.22$, $P < 0.05$), aridity index ($r = -0.30$, $P < 0.05$) and annual rainfall ($r = 0.29$, $P < 0.01$) (Fig. 6.4e-g).

6.4.3 Plant traits associated with rhizome growth

PCA analysis revealed no significant differences between PC scores of grasses from arid and non-arid regions ($P > 0.05$, Fig. 6.2a). Some bermudagrass ecotypes from both environments had high PC scores values in PC 1 (32%) that captured positive loadings on rhizome dry weight, belowground to aboveground dry weight ratio, root to shoot dry weight ratio, and belowground dry weight (Fig. 6.2b). Similarly, PC scores of some ecotypes had large values of aboveground dry weight, stolon length, and internode length in positive loadings of PC2 (28%).

In both regions, rhizome dry weight significantly increased with a higher ratio of belowground to aboveground dry weight ($r = 0.61$, $P < 0.001$), leaf width ($r = 0.55$, $P < 0.001$), but significantly decreased with increasing aboveground dry weight ($r = -0.54$, $P < 0.001$) (Fig. 6.5c-e). Moreover, ratio of belowground to aboveground dry weight significantly decreased with greater internode length ($r = -0.44$, $P < 0.001$) (Fig. 6.5f). Aboveground dry weight significantly increased with increased internode length ($r = 0.27$, $P < 0.01$) and increased stolon length ($r = 0.53$, $P < 0.001$) (Fig. 6.5g & h).

6.5 Discussion

6.5.1 Variation between genotypes in rhizome growth

Rhizome weight at the end of the 14-week experiment was similar between ecotypes from both environments and largely varied across the temperate regions in this study. High ecotypic variability in rhizome growth among all ecotypes in this study, even within bermudagrass ecotypes from arid and non-arid environments, can be associated with preferential aboveground growth to belowground growth during the establishment, as indicated in Fig. 6.5e. Bermudagrasses with more rapid aboveground growth tend to delay rhizome growth during the first growing season, as observed in a previous study using 920 bermudagrass ecotypes collected in Australia (Hacker *et al.* 2013), and another study using commercial bermudagrass cultivars (Pornaro *et al.* 2019). The growth of rhizomes commences when those grasses become more mature in the second year of the establishment (Lulli *et al.* 2012; Munshaw *et al.* 2001; Pornaro *et al.* 2019). In contrast, bermudagrasses with the rapid rhizome growth during the establishment were among ecotypes that had preferential belowground growth to aboveground growth, indicated in Fig. 6.5c. In previous studies that included the same reference ecotypes as this study, bermudagrass ecotypes such as 573 originating from arid environments with sandy profiles allocated a greater proportion of assimilates to rhizomes than shoots (Zhou *et al.* 2014; 2015). In this study, bermudagrasses with the largest dry weight during the establishment were found amongst ecotypes in arid environments. The rapid rhizome growth during the establishments can be the characteristics of bermudagrass ecotypes that may have evolved to survive in extreme climatic conditions of Australia (Jewell *et al.* 2012a; Paula and Pausas 2006; Zhou *et al.* 2015).

6.5.2 Response of rhizome growth to arid climatic conditions

There appeared to be a slight reduction in rhizome dry weight with increased annual evapotranspiration. As there was a lot of variability in the data, rhizome growth may be influenced by factors, which were unaccounted for in the present experiment. Rhizome growth in arid regions significantly decreased as the evapotranspiration around their habitats increased. However, the positive responses of rhizome growth to increasing aridity index and rainfall indicated that bermudagrass ecotypes from arid and non-arid regions may have had a long-term adaptation to their local ecosystems. This study also found that neither aridity index nor annual rainfall was correlated with root dry weight ($P > 0.05$, Supplementary Table S6.3). Therefore, the positive responses of rhizome growth to more humid climate conditions in arid environments can be a mechanism of adaptive evolution. Rhizome growth during the wet season is the most reliable survival strategy of bermudagrass genotypes in arid regions with a large rainfall variation and high evapotranspiration (Jewell *et al.* 2012b; Pandeya and Ahirwalb 2020; Paula and Pausas 2006). The rainfall in the regions from which the ecotypes were collected ranges between 50 and 150 mm during the winter, dropping to below 10 mm for the rest of the year (Bureau of Meteorology 2020), which could explain why the winter to summer rainfall ratio was positively correlated with belowground to aboveground dry weight ratio. Regardless of growth environments, a large proportion of belowground biomass in bermudagrasses is allocated to the growth of rhizomes (Hutchings and John 2004; Pornaro *et al.* 2019; Price *et al.* 2002). Therefore, this study found that the increase in belowground to aboveground dry weight ratio promoted rhizome growth.

Through long-term adaptive evolution, bermudagrass ecotypes in the arid region may have enlarged their leaves to optimise net photosynthesis for rhizome growth during the rainy season, indicated by the positive relationship between leaf width and evapotranspiration and the positive relationship between leaf width and rhizome growth. Compared with bermudagrass ecotypes from non-arid environments, the greater leaf width of bermudagrass ecotypes from arid environments can be an advantageous trait associated with increased rhizome growth. Enlargement of leaves can increase net photosynthesis that promotes the growth of reproductive organs, such as rhizomes in bermudagrasses in this study or seeds of other crop species originating from arid regions (Berger *et al.* 2017; Lowry *et al.* 2014; Thompson 2005; Volis *et al.* 2002). Negative relations between rhizome growth and internode length and stolon length can be explained by the observation that the growth of aboveground stolons to increase total leaf area for assimilate production invests a large amount of fixed carbon (Fustec *et al.* 2005; Lambers and Poorter 2004). Stolon expansion requires a large amount of fixed carbon to construct internodes, nodes and tillers to establish leaf clusters (Fustec *et al.* 2005; Matthew *et al.* 2001; Sbrissia *et al.* 2003). Accordingly, an increase in internode length and shoot growth leads to reduced biomass allocation to belowground growth in bermudagrass in this research and other stoloniferous grass species in previous research (da Silva *et al.* 2015; Lulli *et al.* 2012; Munshaw *et al.* 2001; Poorter *et al.* 2012; Pornaro *et al.* 2019). In this study, the enlargement of leaves may be a long-term adaptive evolution strategy of bermudagrass ecotypes from arid regions to optimise rhizome growth.

6.5 Conclusion

This study revealed that rhizome growth was not significantly different between bermudagrass ecotypes from arid and non-arid regions, despite a large variation in rhizome growth among bermudagrass ecotypes and within ecotypes in each aridity index group. Only rhizome growth of bermudagrass ecotypes from arid regions showed positive responses to more humid climate conditions, in which precipitation predominantly falls during the winter. The enlargement of leaf dimensions was likely a mechanism to optimise rhizome growth during the rainy season in arid regions. The greatest rhizome dry weight during establishment was observed amongst the ecotypes from arid environments. Those ecotypes can be valuable genetic resources for plant breeding, forage options and recreational use.

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6.7 Figures

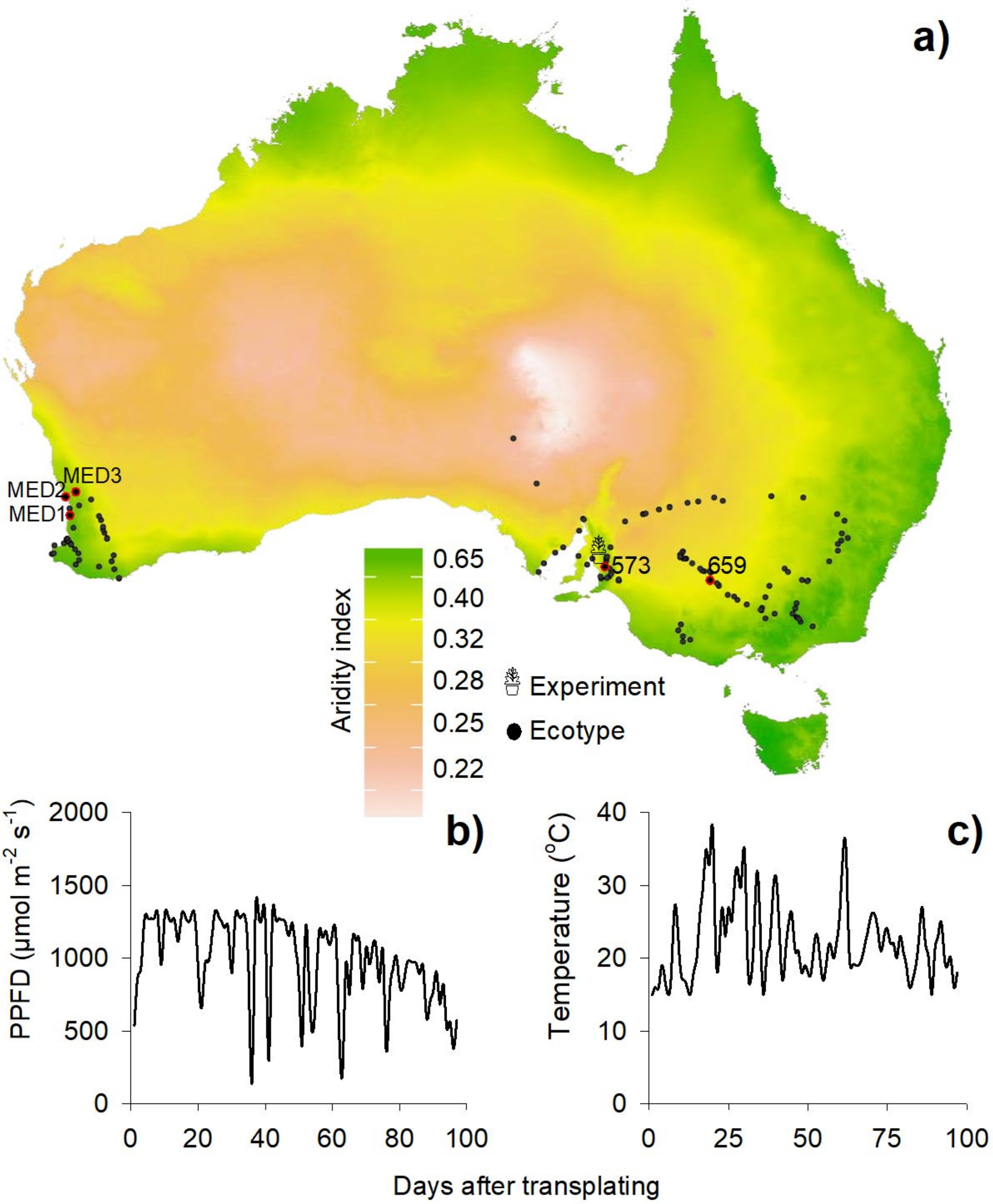

Fig. 6.1 Geographical distribution of bermudagrass ecotypes (details in Supplementary Table S6.1) (a). Experimental conditions: sunlight measured as photosynthetic photon flux density (PPFD) (b) and daily temperature (c). The aridity index map was adapted from Casadebaig *et al.* (2016). Climatic data were obtained from the Roseworthy SA station (Bureau of Meteorology, 2020).

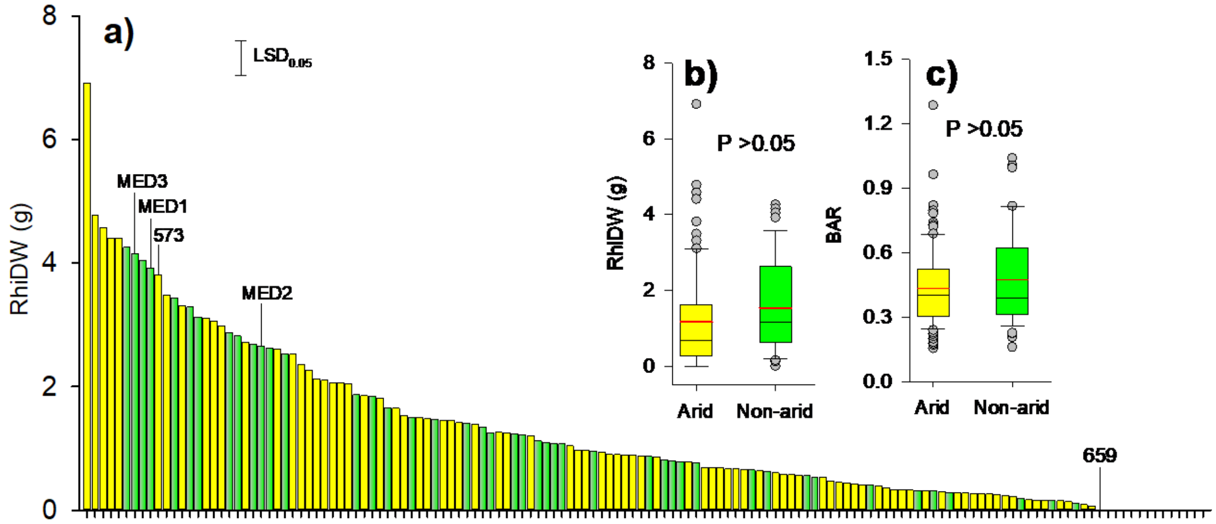

Fig. 6.2 Variation in rhizome dry weight (RhiDW), arid regions (n = 96) and non-arid regions (n = 46) (a). Each bar represents a mean of 3 pots. Comparison of rhizome dry weight (b) and belowground to aboveground dry weight (c) between bermudagrass ecotypes from arid and non-arid regions.

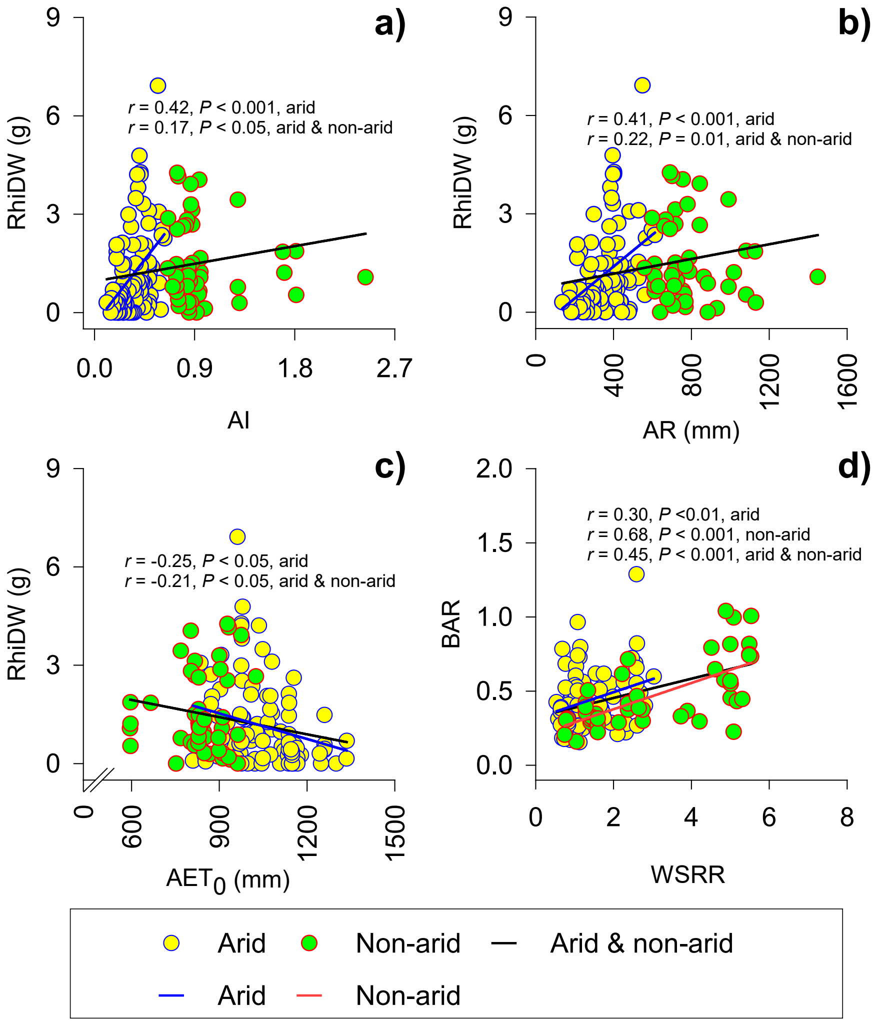

Fig. 6.3 Belowground growth in relation to local climatic condition: relationships between rhizome dry weight (DW) and aridity index (AI) (a), annual rainfall (AR) (b) and annual reference evapotranspiration (AET_o) (c), and aboveground dry weight ratio (BAR) and winter to summer rainfall ratio (WSRR) (d). Each point represents a mean of 3 pots.

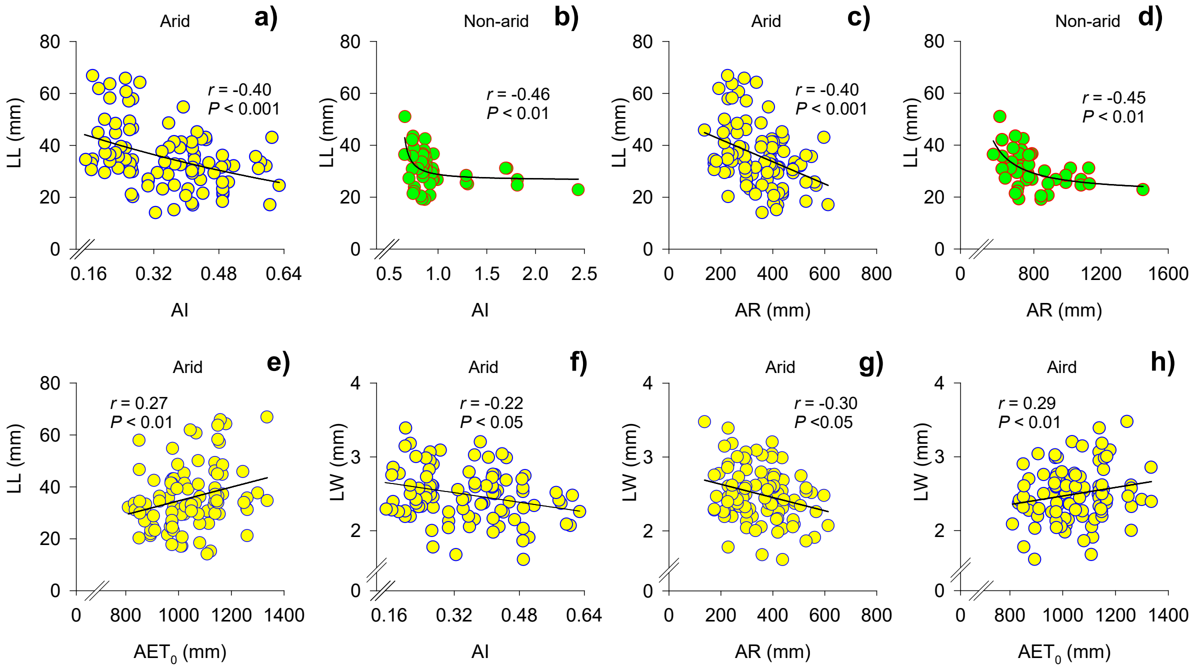

Fig. 6.4 Differences between leaf dimensions of ecotypes in arid and non-arid regions in response to environmental gradients: relationships between leaf length (LL) and aridity index (AI) (a & b), annual rainfall (AR) (c & d) and annual reference evapotranspiration (AET$_0$) (e). Relationships between leaf width (LW) and aridity index (AI) (f), annual rainfall (AR) (g) and annual reference evapotranspiration (AET$_0$). Leaf dimensions measured in the first leaf at the first node.

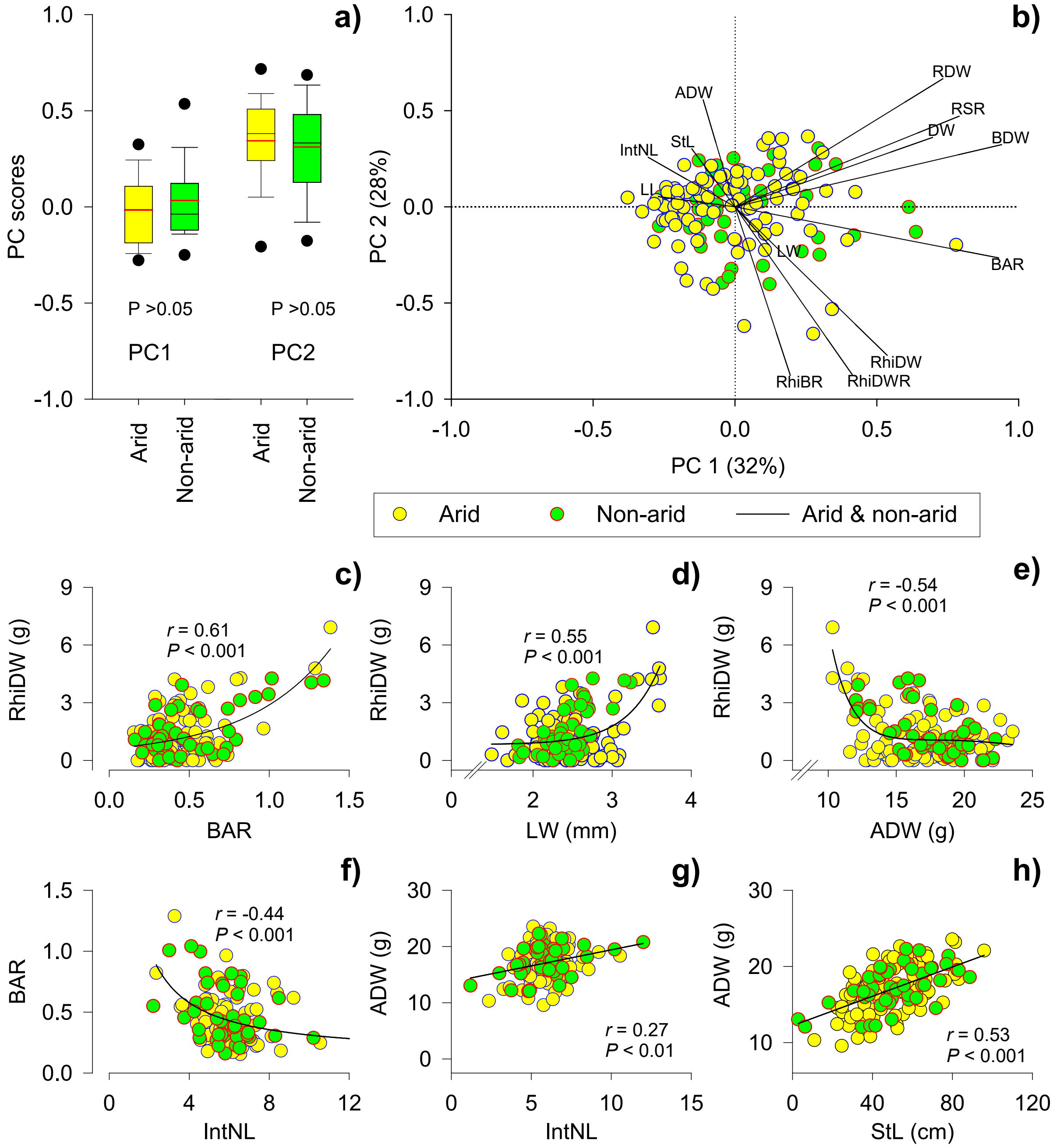

Fig. 6.5 Differentiation between bermudagrass ecotypes from arid and non-arid regions and traits related to rhizome growth of grasses from both regions: PCA biplot differentiating grasses from regions (aridity indices less than 0.65) and grasses from non-arid regions (a) and comparison between PC scores of grasses from arid and non-arid regions (b). Variables include stolon length (StL), internode length (IntNL), leaf width (LW), leaf length (LL), belowground to aboveground dry weight (ADW), belowground dry weight (BDW), rhizome dry weight (RhiDW), root dry weight (RDW), total dry weight (DW), belowground to aboveground dry

weight ratio (BAR), root to shoot ratio (RSR), rhizome to belowground ratio (RhiBR), and rhizome to total dry weight ratio (RhiDWR). Relationships between rhizome dry weight (RhiDW) and belowground to aboveground dry weight ratio (BAR) (c), leaf width (d) and aboveground dry weight (e); belowground to aboveground dry weight ratio (BAR) and internode length (IntNL) (f); aboveground dry weight (ADW) and internode length (IntNL) (g) and stolon length (StL) (g). Leaf dimensions were measured from the leaf at the first node.

6.8 Supplementary data

Table S6.1 Geographical distribution of collected bermudagrass ecotypes: Global positioning system (GPS), annual rainfall (AR), annual mean temperature (AT), annual evapotranspiration ($\mathrm{AET_o}$) and aridity index (AI). MED1, MED2, MED3, 573, and 659 (red) were used in previous studies to drought resistance (Zhou *et al.* 2014) and vertical root growth rates (Chapter 4).

Ecotypes	GPS	AR (mm)	AT (°C)	ETo	AI
Y131	-36.88909,147.06465	1449.8	8.08	594.92	2.437
Y132	-36.72862,146.95397	1081.7	8.08	596.42	1.814
Y125	-36.69963,146.91005	1081.7	8.08	596.69	1.813
Y127	-36.70299,146.92227	1018.6	8.08	596.66	1.707
Y134	-36.77175,147.02970	1126.9	9.47	665.7	1.693
Y081	-34.44621,116.02549	1131.1	20.48	867.43	1.304
Y052	-33.97705,114.98680	990.9	21.39	768.49	1.289
Y079	-33.95955,115.06931	990.9	21.39	768.67	1.289
WG	-27.502668, 153.016821	979	26.63	913.73	1.071
Y135	-36.31662,146.83759	931.3	22.24	938.16	0.993
Y034	-35.01316,138.78646	789.4	15.57	829.17	0.952
Y048	-31.89999,116.16290	864	22.6	908.96	0.951
Y057	-33.61508,115.11181	756.1	23.12	802.6	0.942
Y058	-33.61508,115.11181	756.1	23.12	802.6	0.942
Y072	-33.48053,115.72873	763.9	23.15	815.39	0.937
Y128	-36.55459,146.72669	884.9	20.96	963.33	0.919
Y129	-36.54138,146.74757	884.9	20.96	963.53	0.918
Y071	-33.34489,115.65713	717.7	23.15	816.8	0.879
Y069	-33.33960,115.62424	717.7	23.15	816.85	0.879
Y065	-33.31998,115.63237	717.7	23.15	817.06	0.878
Y076	-33.78425,115.98577	736.3	22.44	849.06	0.867
Y194	-33.30325,149.10704	780.5	18.46	900.23	0.867
MED1	-33.311790, 117.339770	843.1	23	975.01	0.865
Y077	-33.95667,116.13544	726.6	22.44	847.1	0.858
Y130	-37.09894,147.59000	640.2	18.78	752.64	0.851
Y082	-34.23861,116.14791	713.5	22.44	843.89	0.845
Y084	-35.02931,117.87712	769.7	19.53	911.32	0.845
Y088	-35.02652,117.87815	769.7	19.53	911.35	0.845
Y089	-35.00836,117.86057	769.7	19.53	911.55	0.844
Y083	-34.50999,117.01140	699.5	20.92	830.11	0.843
Y139	-36.55312,145.98008	711	18.67	851.44	0.835
Y064	-33.52150,115.51009	668.6	23.12	803.59	0.832
MED2	-32.853120, 115.924160	843.1	21.92	1025.1	0.822
Y032	-34.95442,138.87994	661	15.57	829.87	0.797
Y107	-32.03784,115.74416	706	24.06	931.64	0.758
Y195	-33.42136,148.81643	676.8	18.46	898.75	0.753
MED3	-31.996687, 115.751509	701.7	24.06	932.1	0.753
Y074	-33.58241,115.82427	690.1	22.68	927.72	0.744
Y073	-33.56300,115.81302	690.1	22.68	927.96	0.744
Y090	-34.62975,117.66329	612.1	20.92	828.78	0.739
Y043	-37.74213,142.03157	611.3	19.18	829.35	0.737
Y044	-37.65134,142.34242	611.3	19.18	830.56	0.736
Y045	-37.52704,142.03748	611.3	19.18	832.2	0.735
Y196	-33.55828,148.66716	629.9	18.46	897.02	0.702
Y033	-34.82329,138.96431	597.8	19.06	904.02	0.661
Y141	-36.75027,145.57191	559.2	18.67	848.87	0.659
Y193	-33.09291,148.85903	567.2	18.46	902.88	0.628

Table S6.2 (continued)

Ecotypes	GPS	AR (mm)	AT (°C)	ETo	AI
Y198	-34.30062,148.30341	596.9	22.33	977.9	0.61
Y137	-36.0885535,146.9075003	613	21.58	1012.77	0.605
Y047	-36.99284,141.93640	482.8	21.01	810.06	0.596
Y092	-34.29753,117.55265	485	20.92	832.47	0.583
Y046	-37.24713,141.83920	482.8	19.18	835.9	0.578
Y197	-33.83724,148.67824	549.2	23.83	962.51	0.571
Y024	-35.03476,138.51691	506.9	21.57	981.63	0.516
Y191	-32.25754,148.63736	561.1	24.7	1113.73	0.504
Y160	-34.27209,138.77012	508.7	23.7	1029.63	0.494
Y213	-36.40346,145.39532	437.8	22.43	893.34	0.49
Y214	-36.38489,145.35463	437.8	22.43	893.59	0.49
Y212	-36.29801,145.43547	437.8	22.43	894.77	0.489
Y202	-35.15697,147.37418	528.1	22.23	1080.26	0.489
Y201	-35.15595,147.37403	528.1	22.23	1080.27	0.489
Y199	-35.13341,147.37915	528.1	22.23	1080.61	0.489
Y093	-34.04420,117.64422	424.3	22.52	871.63	0.487
Y204	-35.08886,147.12818	478.5	23.89	1021.75	0.468
Y111	-34.36992,136.07351	398.5	21.33	875.55	0.455
Y162	-34.08569,138.78462	435.6	21.44	972.06	0.448
Y035	-34.94663,138.50714	440.1	21.57	982.8	0.448
Y211	-35.84211,145.55967	422.4	23.01	958.21	0.441
Y041	-34.60037,138.89163	397	19.06	906.83	0.438
Y110	-34.74310,135.84878	379.1	21.33	871.32	0.435
Y192	-32.54845,148.95408	477.1	24.7	1109.48	0.43
Y161	-34.16209,138.74842	435.6	21.18	1021.12	0.427
Y039	-35.12338,139.26528	389.6	23.35	927.74	0.42
Y036	-35.12301,139.28671	389.6	23.35	927.75	0.42
Y208	-34.75309,146.54794	419.2	24.18	1008.55	0.416
Y206	-34.74434,146.54977	419.2	24.18	1008.67	0.416
Y102	-33.05659,117.24126	405.9	23	978.27	0.415
Y207	-34.60001,146.40604	419.2	24.18	1010.77	0.415
Y099	-33.31103,117.33865	403	23	975.02	0.413
Y097	-33.3098588,117.3390095	403	23	975.04	0.413
Y101	-33.3091964,117.3390299	403	23	975.05	0.413
Y103	-32.93469,117.17523	396	23	979.82	0.404
Y051	-31.64054,116.66747	404	25.66	1013.79	0.399
Y163	-33.68330,138.93693	383.6	21.44	977.38	0.392
Y104	-32.8197729,117.1793671	396	23.46	1022.42	0.387
Y105	-32.53354,117.08276	396	23.46	1026.08	0.386
Y209	-35.35754,145.73409	397.9	22.96	1035.74	0.384
573	-35.082453, 139.285123	389.6	23.04	1048.43	0.372
Y217	-35.95710,144.37053	359.1	23.68	969.06	0.371
Y218	-35.8077389,144.2242030	359.1	23.68	971.15	0.37
Y215	-36.14099,144.76511	414.5	22.32	1121.49	0.37
Y113	-33.91222,136.56909	355.4	22.64	993.95	0.358
Y114	-33.68173,136.91765	355.4	22.64	996.9	0.357
Y028	-34.05176,137.56422	321.2	23.77	905.01	0.355
Y106	-32.3665776,117.0067502	369.2	24.33	1064.02	0.347
Y123	-35.33235,140.51540	311	23.39	910.27	0.342
Y190	-31.55655,147.18358	442.2	25.79	1299.71	0.34
Y108	-34.4244974,137.9201883	379.5	22.62	1154.67	0.329
659	-35.741564, 143.945841	359.1	22.94	1108.07	0.324
Y219	-35.3416819,143.5620423	298.6	23.97	975.02	0.306
Y220	-35.3326478,143.5636560	298.6	23.97	975.15	0.306
Y221	-35.2401615,143.4831955	298.6	23.97	976.41	0.306
Y188	-31.50002,145.82688	337.1	26.08	1179.37	0.286
Y235	-34.1111006,141.9089379	228.1	17.14	850.31	0.268

Table S6.1 (continued)

Ecotypes	GPS	AR (mm)	AT (°C)	ETo	AI
Y239	-34.0916291,141.9199303	228.1	17.14	850.53	0.268
Y238	-34.0101495,141.9257121	228.1	17.14	851.46	0.268
Y237	-33.9104461,141.9957781	228.1	17.14	852.59	0.268
Y236	-33.8521054,142.0265266	228.1	17.14	853.25	0.267
Y222	-34.7644681,143.2342083	333.5	24.37	1259.1	0.265
Y223	-34.7242014,143.1738606	333.5	24.37	1259.74	0.265
Y227	-34.3896599,142.4264919	296.6	23.94	1134.69	0.261
Y229	-34.1865071,142.1695618	296.6	23.94	1137.71	0.261
Y230	-34.1792565,142.1454561	296.6	23.94	1137.82	0.261
Y231	-34.1708576,142.1305859	296.6	23.94	1137.94	0.261
Y226	-34.5991655,142.7722786	298.5	23.77	1154.93	0.258
Y116	-32.99185,137.57967	268.4	23.78	1066.35	0.252
Y232	-34.1693230,142.0740165	285	23.94	1137.97	0.25
Y233	-34.1664841,142.0551015	285	23.94	1138.01	0.25
Y025	-34.18512,138.15241	290.1	22.62	1158.41	0.25
Y228	-34.1709977,142.1821296	272.3	23.94	1137.94	0.239
Y224	-34.7117086,142.9398523	293.1	24.37	1259.94	0.233
Y225	-34.6916679,142.8640682	293.1	24.37	1260.25	0.233
Y186	-31.69530,143.74134	263.5	27.34	1165.09	0.226
Y185	-31.55867,143.37769	263.5	27.34	1167.07	0.226
Y184	-31.55794,143.38052	263.5	27.34	1167.08	0.226
Y175	-31.98569,141.45366	243.4	24.68	1148.7	0.212
Y176	-31.98401,141.45442	243.4	24.68	1148.73	0.212
Y178	-31.97345,141.46983	243.4	24.68	1148.88	0.212
Y174	-31.96139,141.45925	243.4	24.68	1149.05	0.212
Y234	-34.1113912,141.9856528	228.1	23.94	1138.83	0.2
y015	-32.49937,137.77939	214.1	26.34	1074.28	0.199
y011	-32.47958,137.75131	214.1	26.34	1074.55	0.199
Y014	-32.47958,137.75131	214.1	26.34	1074.55	0.199
Y165	-32.58155,139.56360	192.8	24.66	1044.63	0.185
Y170	-32.07815,140.99437	209.9	24.68	1147.37	0.183
Y179	-31.78008,142.22808	226.5	25.99	1335.04	0.17
Y181	-31.72295,142.68564	226.5	25.99	1335.95	0.17
Y167	-32.28076,140.33006	174.7	24.66	1048.57	0.167
Y168	-32.27415,140.34087	174.7	24.66	1048.66	0.167
Y169	-32.23242,140.42880	174.7	24.68	1145.15	0.153
Y010	-30.97039,135.75058	183.2	25.88	1247.61	0.147
Y007	-29.01812,134.75372	137.3	27.78	1243.32	0.11

Table S6.2 Pearson's correlation coefficient (*r*) of studied bermudagrasses that included 11 reference cultivars and 137 genotypes. Plant variables include stolon length (StL), first internode length (INL1), second internode length (INL2), first leaf width (LW1), first leaf length (LL1), second leaf width (LW2), second leaf length (LL2), aboveground dry weight (SDW), belowground dry weight (RDW), rhizome dry weight (RhiDW), total dry weight (DW), belowground to aboveground dry weight (BAR), root to shoot ratio (RSR), rhizome dry weight to belowground dry weight ratio (RhiBR) and rhizome dry weight tot total dry weight ratio (RhiDWR). Climatic variables include annual rainfall (AR), annual mean temperature (AT), Winter rainfall (WR), winter to summer rainfall ratio (WSRR), annual evapotranspiration (AET), aridity index (AI). Symbols indicate *, significant at $P < 0.05$,**, significant at $P < 0.01$, ***, significant at $P < 0.001$, and ns, not significant.

Plant variables	Bioclimatic variables					
	AR	Atemp	WR	WSR	AET	AI
Arid						
StL	0.17ns	-0.16ns	0.14ns	0.05ns	-0.16ns	0.21*
IntNL	-0.10ns	0.04ns	-0.14ns	-0.14ns	0.06ns	-0.09ns
LW	-0.29**	0.19ns	-0.17ns	0.05ns	0.22*	-0.30**
LL	-0.39***	0.15ns	-0.34***	-0.15ns	0.27**	-0.40***
ADW	0.12ns	0.02ns	0.09ns	-0.01ns	-0.01ns	0.1ns
BDW	0.09ns	0.05ns	0.19ns	0.27**	-0.17ns	0.13ns
RhiDW	0.41***	-0.16ns	0.43***	0.31**	-0.25*	0.42***
DW	0.24*	0.01ns	0.29**	0.24*	-0.18ns	0.25*
BAR	0.06ns	0.04ns	0.18ns	0.30**	-0.17ns	0.11ns
RSR	-0.13ns	0.12ns	-0.02ns	0.15ns	-0.05ns	-0.10ns
RhiBR	0.41***	-0.23*	0.43***	0.30**	-0.24*	0.42***
RhiDWR	0.39***	-0.18ns	0.41***	0.31**	-0.24*	0.41***
Non-arid						
StL	0.11ns	-0.32*	-0.20ns	-0.44**	-0.28ns	0.21ns
IntNL	-0.15ns	-0.19ns	-0.22ns	-0.25ns	-0.09ns	-0.07ns
LW	-0.29ns	0.18ns	-0.11ns	0.24ns	0.02ns	-0.2ns
LL	-0.45**	0.05ns	-0.40**	-0.11ns	-0.01ns	-0.31*
ADW	-0.02ns	-0.41**	-0.46**	-0.69***	-0.2ns	0.08ns
BDW	-0.20ns	0.44**	0.20ns	0.50***	0.19ns	-0.24ns
RhiDW	-0.11ns	0.17ns	0.17ns	0.44**	0.11ns	-0.1ns
DW	-0.22ns	0.14ns	-0.10ns	0.07ns	0.05ns	-0.18ns
BAR	-0.18ns	0.52***	0.32*	0.68***	0.21ns	-0.24ns
RSR	-0.16ns	0.49***	0.26ns	0.55***	0.17ns	-0.22ns
RhiBR	0.06ns	-0.12ns	0.11ns	0.17ns	-0.07ns	0.10ns
RhiDWR	-0.07ns	0.13ns	0.17ns	0.40**	0.10ns	-0.07ns

Table S6.2 (continued)

Plant variables	Bioclimatic variables					
	AR	Atemp	WR	WSR	AET	AI
Arid & non-arid						
StL	0.15ns	-0.25**	0.05ns	-0.14ns	-0.20*	0.19*
IntNL	-0.04ns	-0.11ns	-0.07ns	-0.14ns	-0.02ns	-0.02ns
LW	-0.13ns	0.09ns	-0.05ns	0.12ns	0.08ns	-0.11ns
LL	-0.33***	0.17*	-0.3***	-0.18*	0.27**	-0.29***
ADW	0.09ns	-0.18*	-0.01ns	-0.22**	-0.10ns	0.11ns
BDW	0.07ns	0.15ns	0.20*	0.37***	-0.12ns	0.04ns
RhiDW	0.22**	-0.07ns	0.27**	0.35***	-0.21*	0.17*
DW	0.17*	-0.04ns	0.20*	0.20*	-0.20*	0.14ns
BAR	0.06ns	0.20*	0.21*	0.45***	-0.1ns	0.02ns
RSR	-0.04ns	0.24**	0.11ns	0.32***	-0.02ns	-0.06ns
RhiBR	0.26**	-0.22**	0.27**	0.25**	-0.24**	0.25**
RhiDWR	0.22*	-0.09ns	0.26**	0.33***	-0.20*	0.18*

Table S6.3 Pearson's correlation coefficient (*r*) of studied bermudagrasses that included 11 reference cultivars and 137 genotypes. Plant variables include stolon length (StL), first internode length (INL1), second internode length (INL2), first leaf width (LW1), first leaf length (LL1), second leaf width (LW2), second leaf length (LL2), aboveground dry weight (SDW), belowground dry weight (RDW), rhizome dry weight (RhiDW), total dry weight (DW), belowground to aboveground dry weight (BAR), root to shoot ratio (RSR), rhizome dry weight to belowground dry weight ratio (RhiBR) and rhizome dry weight tot total dry weight ratio (RhiDWR). Symbols indicate *, significant at $P < 0.05$,**, significant at $P < 0.01$, ***, significant at $P < 0.001$, and ns, not significant.

Variables	StL	IntNL1	IntNL2	LW1	LL1	LW2	LL2
IntNL1	0.62***	-					
IntNL2	0.59***	0.82***	-				
LW1	-0.23**	0.03ns	-0.04ns	-			
LL1	0.04ns	0.55***	0.4***	0.54***	-		
LW2	-0.06ns	0.22**	0.12ns	0.79***	0.61***	-	
LL2	-0.01ns	0.48***	0.35***	0.47***	0.9***	0.57***	-
ADW	0.53***	0.25**	0.27**	-0.08ns	-0.01ns	-0.03ns	-0.1ns
BDW	-0.01ns	-0.23**	-0.15ns	0.1ns	-0.18*	0.01ns	-0.14ns
RhiDW	-0.12ns	-0.27**	-0.21*	0.23**	-0.15ns	0.16ns	-0.1ns
DW	0.25**	-0.07ns	0.02ns	0.08ns	-0.17*	0.04ns	-0.18*
BAR	-0.21*	-0.35***	-0.27**	0.15ns	-0.18*	0.04ns	-0.1ns
RSR	-0.13ns	-0.23**	-0.17*	0.05ns	-0.13ns	-0.04ns	-0.07ns
RhiBR	-0.01ns	-0.14ns	-0.12ns	0.13ns	-0.13ns	0.11ns	-0.1ns
RhiDWR	-0.14ns	-0.25**	-0.22*	0.2*	-0.14ns	0.14ns	-0.09ns

	ADW	BDW	RhiDW	DW	BAR	RSR	RhiBR
BDW	0.07ns						
RhiDW	-0.29***	0.3***	-				
DW	0.61***	0.79***	0.3***	-			
BAR	-0.32***	0.91***	0.56***	0.52***	-		
RSR	-0.13ns	0.89***	-0.02ns	0.5***	0.88***	-	
RhiBR	-0.26**	-0.06ns	0.86***	0.03ns	0.08ns	-0.36***	-
RhiDWR	-0.39***	0.14ns	0.97***	0.12ns	0.33***	-0.15ns	0.93***

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSION

7.1 Introduction

This thesis investigated mechanisms of adaptation to abiotic constraints relevant to ecosystems with rapid surface-drying soils and hardpan soils. Two complementary lines of inquiry, namely the traits that enable a rapid establishment of root systems for overcoming rapid surface drying after establishment and compacted layers access subsoil moisture, and traits that enable the established grasses to survive for a longer period under drought, were examined in a series of 5 experiments in Chapters 3 to 6. From the combined findings of these experiments, traits and contributing mechanisms were identified among a cohort of forage grass species (Table 7.1) and among bermudagrass ecotypes collected in Australia.

Table 7.1 Root vertical rates and root penetration studied in this thesis, and adaptation to drought. Colours indicate high (green cells), moderate (yellow cells), and poor (red cells). Colours labelled as 'drought' and 'yield' were taken from Philp *et al.* (2019). Vertical root growth and root penetration were marked based on Fig. 3.2 (Chapter 3) and Fig. 5.2 (Chapter 5). Blank cells in *Paspalum scrobiculatum* and *Urochloa ruziniensis* were not studied in Chapter 3.

Species[4]	This thesis		Previous review	
	Vertical root growth	Root penetration	Drought	Yield
Megathyrsus maximus	🟩	🟩	🟩	🟩
Paspalum scrobiculatum		🟥	🟥	🟩
Setaria sphacelata	🟨	🟨	🟨	🟩
Urochloa brizantha	🟨	🟨	🟩	🟩
Urochloa decumbens	🟨	🟨	🟩	🟩
Urochloa humidicola	🟥	🟥	🟩	🟩
Urochloa hybrid Mulato II	🟨	🟨	🟩	🟩
Urochloa mosambicensis	🟩	🟨	🟨	🟥
Urochloa ruziniensis		🟩	🟥	🟩

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[4] Chapter 5 used *Megathyrsus maximus* as the basionym of *Panicum maximum* and *Urochloa* grasses as *the* basionym of *Brachiaria* grasses.

7.2 Mechanisms that enable plants to overcome the abiotic stresses due to rapid surface drying after establishment

Increased vertical root growth was associated with greater post-establishment drought resistance (Chapter 4). Under field conditions in a previous study on bermudagrass ecotypes (Zhou *et al.* 2014), drought resistance promoted by deep roots accessing water in the greater depth of soil profiles appeared to be associated with active roots. The rapid establishment of deep roots promotes the growth of roots that extract water at a greater depth from soil profiles (Bodner *et al.* 2015; Boeri *et al.* 2020; Kulmatiski and Beard 2013; Thorup-Kristensen *et al.* 2020). The greater vertical root growth rate of *M. maximus* (Chapter 3) appears to be associated with the ability to extract water at depth from the soil profile and is likely to confer drought resistance in sandy soils with rapid surface drying after the establishment (Table 7.1).

In both Chapter 3 and 4, increased vertical root growth rate was associated with increased shoot growth, which is a desired characteristic for forage production. In forage grass species (Chapter 3), the rapid growth of deep roots and shoots was associated with greater leaf area and greater photosynthetic rate, as part of a drought avoidance strategy (Fig. 2.4, Chapter 2); in addition, an exploitative growth strategy was observed in *M. maximus* and *U. mosambicensis* (Fig. 3.5, Chapter 3). The exploitative growth strategy is also associated with greater nitrogen uptake (Acuña *et al.* 2010; da Pontes *et al.* 2015; Reich 2014; Wright *et al.* 2004). Compared with the forage grass species in Chapter 3, *M. maximus* has been reported to have greater nitrogen uptake (Philp *et al.* 2021; Philp *et al.* 2019) that may be associated with the rapid establishment of deep roots in sandy soil profiles.

The positive relationships between vertical root growth rate and shoot growth (Chapter 3 & 4) demonstrated that increased vertical root growth rate can be associated with greater post-harvest regrowth rates of perennial grass species, as observed in previous studies on *M.*

maximus (Carnevalli *et al.* 2021; da Silva *et al.* 2021). Similar to *M. maximus*, *U. mosambicensis* had a rapid vertical root growth rate that is likely to be the reason that this species has enhanced water extraction at 60 to 100 cm depth (Mazzacavallo and Kulmatiski 2015) and nitrogen uptake leaching into soil depths (Knox *et al.* 2010). Both *M. maximus* and *U. mosambicensis* are well-adapted and are preferred forage grass species (Treydte *et al.* 2013) that are used to restore degraded pasturelands in tropical savanna ecosystems (Ravhuhali *et al.* 2019). In contrast, the negative relationships between vertical root growth rate, proportion of root lengths between 0 and 10 cm depth, and shoot growth, as observed in perennial forage grass species (Chapter 3 and bermudagrass ecotypes (Chapter 4), suggest that a shallow rooting habit is disadvantageous in perennial grass species. Shallow root distribution is a negative drought-responsive characteristic of perennial grasses (Su *et al.* 2008). Although the increased vertical root growth rate in relation to water extraction at soil depths is a drought avoidance mechanism (Fig. 4.6, Chapter 4), the results in Fig. 4.5 in Chapter 4 demonstrated that there was no set of specific traits in drought resistant perennial grasses.

The post-establishment survival of perennial grasses in relation to rapid surface-drying and hardpan soils in sandy soils (Table 7.1) likely involves interplays of multiple traits. In Chapter 3, larger average root diameter and a greater proportion of root length with higher diameters were related to the increased exponential rate of root depth development and wide root angle. The increased exponential rate of root depth development associated with greater average root diameter in *U. humidicola* and *U.* hybrid Mulato II may indicate a conservative growth strategy in perennial forage grass species (da Pontes *et al.* 2015; Duchini *et al.* 2018; Reich 2014). Past studies on *Urochloa* hybrid Mulato II (Cardoso *et al.* 2015) and *U. humidicola* (Guenni *et al.* 2004) found that these two species possess the ability to conservative water use. Therefore, their drought resistance is classed as high (Table 7.1) (Philp *et al.* 2019). Both species might be well adapted to prolonged drought after establishing well on sandy soil.

7.3 Mechanisms that enable plants to avoid abiotic stresses due to hardpan soils

A positive relationship between vertical root growth rate and root penetration (Chapters 3 & 5) indicated that grass species with rapid root growth potentially avoid the abiotic stresses in sandy soils due to hardpan soils or compaction up to 2.12 MPa resistance. In both Chapters 3 and 5, rapid shoot growth was associated with increased vertical root growth rate and greater root penetration. The rapid growth of root length with diameters greater than 0.5 mm at the seedling stage was associated with increased shoot growth, root penetration at the higher wax resistance of 2.12 MPa, vertical root growth rate and total length of fibrous roots during the establishment. Therefore, root length with diameters greater than 0.5 mm measured at seedling may be a characteristic to select perennial forage grass species that are potentially well adapted to sandy soils with rapid surface drying and hardpan soils.

7.4 Mechanisms that enable established forage grasses to survive prolonged drought after establishment

Reference bermudagrass ecotypes with established drought resistance in previous field experiments (Zhou *et al.* 2014) were used to analyse root and shoot growth during establishment (Chapter 4). The results revealed that there was a relative consistency of variation in vertical root growth rate between winter and summer of a Mediterranean type of climate in South Australia. The characteristic of rapid vertical root growth rate was associated with the drought resistance of established bermudagrasses under surface drying conditions in the fields.

In previous studies using the reference bermudagrass ecotypes, an extensive rhizome system during establishment was the mechanism for drought resistance after establishment and promoted post-harvest regrowth rate (Zhou *et al.* 2014; 2015). Therefore, in Chapter 6 the mechanisms for rhizome growth in bermudagrass ecotypes were investigated. Findings showed that bermudagrass ecotypes from arid regions had a positive response to more humid climate conditions in winter, while the rest of the year is dry. Bermudagrasses may have evolved to grow extensive rhizomes as a survival mechanism in extreme climatic conditions of Australian arid regions (Jewell *et al.* 2012a; Paula and Pausas 2006; Zhou *et al.* 2015), where rainfall is low and occurs only in winter (Bureau of Meteorology 2020). Therefore, the ecotypes with the largest rhizomes originated from arid regions with aridity indices less than 0.65. These ecotypes, which originate from arid regions, can be of potential use to meet production niches in sandy soils with highly variable watered conditions, a wide range of temperatures, and especially in drought-prone pasturelands. Larger rhizomes with a greater capacity to recover after severe drought play an essential role in developing the resistance of arid or marginal pastures under extreme drought events (Pandey and Ahirwal 2020; Zwicke *et al.* 2015).

7.5 Further research

Wet season rainfall in the lower Mekong regions (Fig. 2.2, Chapter 2) often causes waterlogged conditions that may reduce the productivity or survival of some forage grass species after establishment. A perennial grass species is unlikely to have the combined traits of rapid vertical root growth, efficient root penetration, drought resistance and waterlogging tolerance. Therefore, it would be beneficial to investigate intercropping using multiple forage grass species in Table 7.1 under field conditions in tropical regions where a variable cycle of drought and flooding occurs, based on local hydrological and climatic data.

In forage grass species (Chapter 5), root penetration was associated with shoot growth and vertical root growth rate. The seasonal conditions likely affected mechanisms for shoot growth and vertical root growth rate of bermudagrasses (Chapter 4). Specifically, the vertical root growth rate was promoted by tiller appearance rate in summer but negatively correlated with inactive roots developed during winter. These mechanisms regarding seasons may lead to the differences in mechanisms for root penetrations of bermudagrasses. Therefore, further studies are required to investigate the mechanisms for root penetration of bermudagrasses under different climatic conditions.

7.6 Conclusion

The thesis identified traits associated with vertical root growth rate and root penetration as mechanisms of perennial grasses for accessing water at the greater depths of soil profiles in ecosystems with rapid surface-drying soils and hardpan soils. In forage grass species, the mechanisms for increased vertical root rate were a narrow root angle, higher photosynthetic rate, higher ratio of root length to leaf area and higher percentage of fine branch roots. In bermudagrasses, vertical root growth rate was promoted by tiller appearance rate in summer but negatively influenced by inactive roots developed during winter. Most importantly, vertical root growth rate of bermudagrasses during establishment was a trait of drought resistance after establishment. Root penetration of forage grass species was promoted by large root diameter, rapid growth of roots with diameters greater than 0.5 mm, measured at seedling stages, and vertical root growth rate. Based on findings in Chapters 3 to 5, the combined traits associated with vertical root growth rate and root penetration may assist in selecting perennial forage species regions with rapid surface drying and soil compaction. Bermudagrasses with the highest rhizome dry weight during establishment were found amongst the ecotypes originating from arid environments and can be used to select potential forage species better adapted to pasturelands in arid regions.

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