

IDENTIFICATION OF THE MOST SUITABLE BRACHIARIA GENOTYPES FOR IMPROVED LIVESTOCK PRODUCTION IN WATER-LIMITED CONDITIONS IN SOUTH AFRICA

A Report To

Water Research Commission of South Africa

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EXECUTIVE SUMMARY

Background

Climate change can either directly or indirectly impact livestock production. One of the key indirect impacts of climate change on livestock production is the effects of elevated temperatures and reduced water availability on forage production and quality. This has motivated the world to look for forages that are adapted to extreme bioclimatic conditions and at the same time have the ability to potentially reduce greenhouse gas emissions from livestock production systems. *Brachiaria* grass, a genus of the subfamily Panicoideae, and has been proposed as a suitable candidate for production under adverse bioclimatic conditions. Although cultivars of commercial *Brachiaria* species and hybrids have been performing well in the Americas, especially Brazil, only small areas have been sown in Africa, despite many of the species naturally occurring on the continent. Recently improved cultivars of *B. brizantha*, *B. humidicola*, *B. decumbens*, *B. nigropedata*, and hybrid *Brachiaria* cultivars (*B. brizantha* x *decumbens* x *ruziziensis*) have been released in South Africa as pasture grasses. *Brachiaria humidicola* and *B. nigropedata* are considered as grazing pastures, while *B. brizantha*, *B. decumbens* and the Hybrid cultivars are suitable for grazing, hay and silage production. However, similarly to other African countries, the adoption of these forages has been poor.

This study was therefore aimed to identify the best performing, water-limitation tolerant *Brachiaria* genetic resources for improved livestock production in South Africa. To achieve this, the following specific objectives will be pursued:

1. Collection of *Brachiaria* plant genetic resources from various seed distributors and genebanks, multiplying the seeds from genebanks, and subsequently including these *Brachiaria* genetic resources into the South African – National Forage Genebank collection.
2. Morphologically and physiologically, screening selected *Brachiaria* plant genetic resources for their adaptation to water-limited growing conditions, selecting only the most suitable genetic resources for further evaluation, and selecting the best performing genetic resources for further characterisation. The water-limitation screening will look at:
 - a. the germination ecology of different *Brachiaria* genotypes under different temperatures and water-limited conditions
 - b. seedling establishment abilities under different soil moisture conditions
 - c. water-limitation tolerance and the potential to recover under variable moisture conditions
3. Characterizing the *Brachiaria* genetic resources for agronomic traits (production, persistence and quality) and selecting the best performing genetic resources for further evaluation.
4. Evaluating the potential of using *Brachiaria* grass as silage for livestock feeding.

Key Findings

Chapter 2:

- A total of 50 *Brachiaria* accessions were obtained from seed distributors and genebanks. Commercial cultivars of *Brachiaria* grass were obtained from South African seed distributors such as Barenbrug, DLF, Agricol and Brasuda, while breeding lines of non-commercial accessions were obtained from the seed companies as well as genebanks, including the SA-NFG, ILRI (International Livestock Research Institute) and CIAT (International Centre for Tropical Agriculture).

Chapter 3:

- This study aimed to determine the impacts of different temperature and moisture conditions on the seed germination dynamics of *B. humidicola*, *B. brizantha*, *B. decumbens*, *B. nigropedata* and a hybrid *Brachiaria* species, (*B. brizantha* x *B. decumbens* x *B. ruziziensis*). Results showed that although the majority of the species evaluated were able to tolerate a wide germination temperature range, as soon as moisture became more limiting, none of the *Brachiaria* species could tolerate even moderate moisture stress conditions at their germination stages.

Chapter 4:

- Results from the current research trials indicate that *Brachiaria* display different degrees of drought tolerance at different stages of development. Prior work on seed germination showed that seed germination stage in *Brachiaria* is not tolerant to water-limitation and even the slightest decrease in moisture availability could significantly decrease the number of seeds germinating. This work gave further evidence that as seedlings, *Brachiaria* may also not be suitably tolerant to moisture stress, but if the plants survive to maturity, they become more tolerant/resilient. The different genotypes evaluated displayed an array of morphological mechanisms to cope with water-limitation. Certain genotypes were able to allocate more resources to root growth, which, under water-limited conditions, will allow for access to more water and nutrients as soil moisture decreases. These genotypes should be prioritised for further evaluation under field conditions.

Chapter 5:

- This study aimed to evaluate *Brachiaria* genetic resources which are not currently available or included in breeding programs in South Africa to determine their suitability for inclusion in breeding programs for developing drought tolerant *Brachiaria* forages. Results from the trial shows that the *Brachiaria* genetic resources are suitable for inclusion in breeding trials with the majority of the forages surviving the cold frost periods in year 1 and year 2. Furthermore, the biomass yields of these forages significantly increased from year 1 to year 2 even without fertilization or irrigation. Although the yields increased, the lack of fertilisation did result in

reduced quality, especially crude protein content and several deficiency symptoms were becoming visible in year 2. Even though these deficiency symptoms were visible, generally the mineral nutrient content of the forages produced were sufficient for livestock production, while the majority of the mineral nutrients were even in excess of what is required by livestock.

Chapter 6:

- In this study, 24 *Brachiaria* grass genotypes were evaluated under dryland and irrigated conditions. It was shown that there were significant differences in the yield potential of the different genotypes and that these differences was also found between genotypes within a species. The majority of *Brachiaria* genotypes evaluated produced feed during Autumn, Spring and Summer, with no feed harvested in winter. Although biomass production was generally higher under irrigated conditions, all of the genotypes evaluated were able to produce significant amounts of biomass even under dryland conditions. Furthermore, growing the grasses under dryland conditions did not significantly impact the quality of the forages produced.

Chapter 7:

- This study showed that mixing *Brachiaria* grass with *Melia azedarach* leaf meal improved fermentation quality by reducing silage pH closer to a recommended level of 4.2. Since silage with a pH level of 4.2 and below is regarded as well fermented and preserved silage, a 20% inclusion of *Melia azedarach* leaf meal to *Brachiaria* is the recommended level for it resulted in the pH of 4.27. However, further studies are required to determine the silage microbial composition and nutritional quality to verify the overall quality of the silage produced.

Research Products

Except for the knowledge and skills that were collected and developed by the project team members, the most important research products were:

1. Training materials and demonstrations on *Brachiaria* grass production, used during community workshops.
2. These research products contributed to:
 - a. Knowledge transfer among farmers in targeted communal areas.
 - b. Farmer capacity building through hands-on engagement and skills development.

Conclusion and Recommendations

Brachiaria forages represents a sustainable, water-efficient feed for livestock in regions where irrigation of pastures may not be an option. To realize its full potential, scaling up production in South Africa should be considered as this will definitely improve livestock production in areas where the natural veld

has been severely degraded due to overutilisation. While these high yielding forages offers climate-smart solutions for resource-constrained farmers, critical challenges, including the equipment needs for harvesting hay and producing silage must be systematically addressed in order to reach the full potential of the forages. Similarly, based on the nutritional quality of the forages appropriate supplementation regimes should also be considered when implementing these pastures. Strategic implementation through targeted farmer training and supportive policies will also be essential to facilitate widespread adoption. With these measures in place, the use of *Brachiaria* forages can evolve from an innovative alternative to a mainstream component of resilient livestock production systems, effectively balancing productivity with sustainability in water-scarce environments.

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CHAPTER 1:

Introduction

In South Africa, livestock farming is the only option on more than 70 % of the available agricultural land. This is primarily because of marginal edaphic and bioclimatic conditions over most part of the country. Emerging farmers in South Africa, especially those who only farm extensively, often experience low livestock productivity due to their over dependence on poor quality and inadequate feed supply from natural pastures. This is especially true during the dry season when reduced rainfall quantities, and reduced availability of mineral nutrients, result in very poor feed sources for the livestock (Müller et al. 2019).

Apart from reduced livestock production on these extensive systems, the low-quality diets that these livestock often have to eat, results in cattle reared extensively often producing more greenhouse gas (GHG) per kilogram of feed intake (Scholtz et al. 2013). With South Africa being part of the Kyoto protocol, and the government setting reduction targets in line with the Kyoto Protocol (UNFCCC, 2007) i.e., a reduction of 30 – 40 % in CO₂-equivalent emissions from 2003 levels by 2050, these extensive farmers could have a significant impact on the targets set by the government (Scholtz et al. 2013). This is because with increased rainfall variability, and the lack of irrigation water, the quality of available forages for livestock is rapidly decreasing, potentially resulting in increased GHG emissions, mostly methane (CH₄), from livestock on these extensive systems. If GHG reduction targets set by government is to be met, more emphasis needs to be put on improving the quality of the diets used by extensively reared livestock, especially during the dry season. This can be done by planting high quality forage plants during the wet season and harvesting the materials to supplement poor quality rangeland diets with high quality feeds during the dry season. Therefore, we propose the evaluation of various *Brachiaria* accessions, known for their high biomass production (more than most other grass species), and their beneficial effects on both beef and milk production, for their production ability under water-limited conditions in South Africa.

We aim to select the best-adapted genotypes for further development to be implemented into improved fodder flow programs for improved livestock production in the emerging farmer sector in South Africa we also aim to quantify the impacts of implementing these improved, high-quality forages as supplementary feed sources to rangeland diets on the GHG emissions from extensively reared livestock.

1.1. Literature review

With the global human population estimated to reach nine billion by the year 2050, it is estimated that approximately 70 – 80 % more food will be required in 2050 (Bruinsma 2009, Herrero et al. 2009, 2015, Godfray et al. 2010, Nardone et al. 2010, Alexandratos and Bruinsma 2012, Wright et al. 2012, Rao et al. 2015). With this increase in the demand for food products, it is expected that the global demand for livestock products (meat, milk and eggs) will double by 2050, with the largest increases in demand occurring in the developing countries (Delgado et al. 2001; Herrero et al. 2009; Rao et al. 2015). Just

like in other developing countries (Thornton and Herrero 2014), livestock farming in South Africa is an important contributor to food security, poverty alleviation and socio-economic upliftment (Meissner et al. 2013a). However, with the South African human population rapidly increasing, the demand for livestock products is becoming more than what can sustainably be produced under current livestock production systems, primarily as a result of deteriorating rangelands conditions and increased rainfall variability.

In South Africa, emerging farmers (including communal farmers) own a third of the livestock in the country (Palmer, 1999; Coetzee et al., 2005; DAFF, 2018), but their contribution to the total agricultural production value in South Africa is very low (Coetzee et al., 2005; Meissner et al., 2013a,b; Goni et al., 2018). These farmers often experience low livestock productivity partially due to their over dependence on poor quality and inadequate feed supply from natural pastures and reduced access to, and knowledge about improved forage varieties/cultivars (Samuels et al. 2016, Negawo et al. 2017, Goni et al. 2018, Müller et al. 2019). The contribution of these farmers to the South African agricultural sector is also believed to further deteriorate with the projected future bioclimatic changes, which predict hotter and drier conditions with more erratic and unpredictable rainfall events (Mukheibir 2008, DEA 2013, Meissner et al. 2013b, Roffe et al. 2020, Engelbrecht and Monteiro 2021) resulting in existing natural pastures becoming less productive, and current commercially available forage options rapidly becoming obsolete (Muller et al. 2017; Trytsman et al. 2019).

Although climate change will have significant negative impacts on livestock production, livestock are also a large contributor to the climate change problem, contributing approximately 18 % of the global anthropogenic greenhouse gas (GHG) emissions (Steinfeld et al. 2006, IPCC 2007, Tubiello et al. 2007, Thornton et al. 2009, Thornton and Herrero 2010). The largest source of greenhouse gases in ruminants, originate from the methane produced in the gastrointestinal tract, accounting for 17% of global methane emissions (Garnsworthy et al. 2019). In order to compare emissions of GHG, the global warming potential (GWP) of the individual gases is used, with carbon dioxide (CO₂) used as the reference gas (Boadi et al. 2004). The GWP of methane is 23 times more than that of CO₂, resulting in it being a significant role player in greenhouse gas production. Methane can thus affect the climate directly, through its interaction with long-wave infrared energy and indirectly through atmospheric oxidation reactions that produce CO₂. Enteric methane production is a by-product of ruminant anaerobic fermentation in the rumen and large intestine by microbes in a process called methanogenesis or biomethanation. The animals then release the methane into the atmosphere, by exhaling the gas mainly through the mouth and nostrils. Enteric methane produced by cattle can thus be seen as a loss of feed energy from the diet and reflects the inefficient utilization of the feed (Grobler et al. 2014). The need for accurate estimates of enteric methane production in ruminants under different management systems has been emphasized (Chagunda et al. 2009; Chagunda et al. 2013). Therefore, understanding the differences in methane production from cattle grazing different forages is important for the development of successful mitigation strategies. Furthermore, mitigation strategies aimed at reducing emission in the livestock sector are needed to limit environmental burden and ensure sufficient food production for a growing world population. Increasing animal productivity per unit of methane emitted can be a viable strategy for reducing GHG emissions from livestock production (Peters et al. 2012).

To address the problem of feed shortages experienced by emerging farmers, especially during the dry season, and periods of drought, and to simultaneously reduce the contribution that livestock production systems has on greenhouse gas emissions, there is a need to develop improved fodder flow programs. These programs should reduce feed gaps and minimise greenhouse gas emissions from livestock production systems, under current, and the predicted future bioclimatic conditions. One of the ways to do this is to evaluate climate-smart forage species that has been shown to mitigate greenhouse gas emissions in livestock production systems (Thornton and Herrero 2010) and have the potential to produce high biomass yields under the erratic South African bioclimatic conditions. These species should be suitable for use with low capital and irrigation requirements for establishment and management and can easily be taken up by the emerging farmers. *Brachiaria* grass has recently emerged as a suitable candidate for these evaluations under tropical and sub-tropical bioclimatic conditions. *Brachiaria* grass is native to Africa and has been used widely as a tropical forage in Kenya (Ondiko et al. 2016), Rwanda (Mutimura and Everson 2012), and Brazil (Maia et al., 2014). Grasses in the genus *Brachiaria* have advantage over those in other genera due to their deep and an abundant root system which make them drought tolerant and adapted to low fertility soils (Subbarao et al., 2009; Gichangi et al. 2016; Mureithi and Djikeng, 2016). These grasses have the potential to produce a higher biomass yield than most tropical grasses (Jank et al. 2014; Maia et al. 2014; Ondiko et al. 2016) and has been reported to contain a crude protein (CP) content ranging between 13 to 15 % for cultivars Marandu, MG4, Xareas and Piata of *Brachiaria brizantha* (Maia et al. 2014). The agronomic performance and chemical composition of *Brachiaria* grasses are however, depended on the genetic potential of each accession and in the adaptation of those accessions to the bioclimatic and edaphic conditions of the environment under which it is grown (Mutimura and Everson 2012; Maia et al. 2014).

Even though species in the genus *Brachiaria* have a huge potential to improve emerging farmer productivity in South Africa, currently very little is known about the productivity, adaptation, and nutritional quality of different *Brachiaria* species grown under the agro-ecological conditions of South Africa. Furthermore, *Brachiaria* grasses have been reported to improve milk yield in dairy cows and average daily gain of goats in Kenya (Muinga et al., 2016; Ngila et al., 2016), but information on the potential of *Brachiaria* grasses to improve livestock production output per unit of methane emitted is limited. It is therefore important to quantify the productivity of livestock fed on *Brachiaria* based diets per unit of methane emitted.

Apart from this, 16 *Brachiaria* species are naturally found in southern Africa, with the South African National Forage Genebank (SA-NFG) housing 9 *Brachiaria* species that has not yet been evaluated for their productivity, adaptability and nutrient concentration to water-limited agro-ecological conditions in South Africa (Trytsman et al. 2020). Furthermore, as it stands, a number of *Brachiaria* cultivars (MG4, MG5, Llanero, Marandu and Sabia) are commercially available in South Africa, but no cultivar has been developed specifically for the South African livestock industry, especially the emerging livestock industry which is characterised primarily by resource poor farmers farming under dryland and poor rangeland conditions. This, in turn, results in the need to compare existing breeding lines of *Brachiaria* from various seed companies and different accessions from genebanks from across the world to commercially available cultivars, which would allow for the selection of lines to be included into breeding

and improvement programs for the identification and development of adapted cultivars for the South African market.

1.2. Aims and Objectives

The overarching aim of this project is to select the best performing, water-limitation tolerant *Brachiaria* genetic resources for improved livestock production in South Africa. To achieve this, the following specific objectives will be pursued:

1. Collection of *Brachiaria* plant genetic resources from various seed distributors and genebanks, multiplying the seeds from genebanks, and subsequently including these *Brachiaria* genetic resources into the South African –National Forage Genebank collection.
2. Morphologically and physiologically, screening selected *Brachiaria* plant genetic resources for their adaptation to water-limited conditions, selecting only the most suitable genetic resources for further evaluation, and selecting the best performing genetic resources for further characterisation. The water-limitation screening will look at:
 - a. the germination ecology of different *Brachiaria* genotypes under different temperatures and water-limited conditions
 - b. seedling establishment abilities under different soil moisture conditions
 - c. water-limitation tolerance and the potential to recover under variable moisture conditions
3. Characterizing the *Brachiaria* genetic resources for agronomic traits (production, persistence and quality) and selecting the best performing genetic resources for further evaluation.

CHAPTER 2:

Collection of *Brachiaria* genetic resources and determining their initial germination potential with subsequent seed multiplication and entering new genetic resources into the South African National Forage Genebank

2.1. Aim

This study aimed to collect *Brachiaria* genetic resources from various commercial seed distributors in South Africa and international genebanks for evaluation and inclusion in the South African National Forage Genebank.

2.2. Materials and Methods

Commercial cultivars of *Brachiaria* grass were obtained from South African seed distributors such as Barenbrug, DLF, Agricol and Brasuda, while breeding lines of non-commercial accessions were obtained from the seed companies as well as genebanks, including the SA-NFG, ILRI (International Livestock Research Institute) and CIAT (International Centre for Tropical Agriculture).

2.3. Results and Discussion

Often genebanks cannot supply large quantities of seeds and therefore, where necessary, seed multiplications were done to ensure that viable seeds were entered into the SA-NFG. A total of 50 *Brachiaria* accessions were obtained from seed distributors and genebanks (Table 1). All *Brachiaria* accessions obtained from seed distributors and genebanks were tested for their initial seed germination potential to give an indication as to how the remaining experiments will be conducted. The initial viability of the seeds obtained are outlined in Table 1. It was expected that since most of the genetic resources received were not fully domesticated and had gone through significant breeding to stabilise germination the seeds obtained would have low viability. Therefore, the seeds were tested for their initial germination potential by germinating, in the dark, four replicates of 50 seeds of each genetic resource (only those with more than 100 seeds obtained otherwise 4 replicates of 20 seeds) in 9 cm petri-dishes on filter paper, in a germination chamber set at a constant temperature of 20 °C. The seeds were watered as needed and germination was recorded daily for 15 days. Seeds were regarded as germinated after the emergence of a radicle of approximately 0.5 cm. All germinated seeds were counted and then transplanted into seedling trays which was used for further research or seed multiplication.

Only four accessions obtained had a germination potential greater than 70 %. These were *B. brizantha* cv. MG5 (93 %), *B. brizantha* cv. MG4 (72 %), *B. brizantha* cv. Marandu (80 %) and *B. humidicola* cv. Llanero (89 %). These were all from commercial seed companies and cultivars that are commercially available in South Africa. The remaining species had poor viability with 78 % of the accessions evaluated having a germination potential below 15 %. All accessions obtained with a germination percentage below 70 % were regenerated at the genebank nursery for seed multiplication in order to store the genetic resources at the SA-NFG.

The seeds that were transplanted into seedling trays were allowed to grow for one month in the seedling trays after which they were transplanted into larger pots for seed multiplication. The plants were allowed to grow in a net structure until seeds were physiologically mature from where the seeds were harvested. All harvested seeds were tested again for their viability and all genetic resources were then assigned unique accession numbers and included into the SA-NFG.

2.4. Conclusion

Although a large number of seeds were obtained from the various sources, only certain of the resources were used in subsequent research trials due to various reasons such as limited seed numbers, low seed viability and also duplication in resources from various seed companies. However, with this being said, in all experiments conducted, representative samples of all *Brachiaria* species obtained were used.

Table 1: *Brachiaria* accessions obtained from various seed distributors and genebanks

	Genus species	Accession/Cultivar	Distributor	Germination (%)	Notes
1	<i>Brachiaria</i> sp.	Kupfer	Barenbrug	16 ± 2	Seed regeneration in progress to improve germination percentage for inclusion in NFG
2	<i>Brachiaria nigropedata</i>	Sweet velvet	Barenbrug	3 ± 1	Seed regeneration in progress to improve germination percentage for inclusion in NFG
3	<i>Brachiaria nigropedata</i>	330	Barenbrug	16 ± 3	Seed regeneration in progress to improve germination percentage for inclusion in NFG
4	<i>Brachiaria nigropedata</i>	460	Barenbrug	21 ± 2	Seed regeneration in progress to improve germination percentage for inclusion in NFG
5	<i>Brachiaria nigropedata</i>	473	Barenbrug	7 ± 2	Seed regeneration in progress to improve germination percentage for inclusion in NFG
6	<i>Brachiaria nigropedata</i>	710	Barenbrug	30 ± 3	Seed regeneration in progress to improve germination percentage for inclusion in NFG
7	<i>Brachiaria nigropedata</i>	780	Barenbrug	35 ± 3	Seed regeneration in progress to improve germination percentage for inclusion in NFG
8	<i>Brachiaria nigropedata</i>	Tosca	Barenbrug	4 ± 1	Seed regeneration in progress to improve germination percentage for inclusion in NFG
9	<i>Brachiaria brizantha</i>	Piata	ILRI	10 ± 3	Seed regeneration in progress to improve germination percentage for inclusion in NFG
10	<i>B. ruziziensis</i> x <i>B. decumbens</i> x <i>B. brizantha</i>	Mulato II	ILRI	37 ± 3	Seed regeneration in progress to improve germination percentage for inclusion in NFG
11	<i>Brachiaria brizantha</i>	Xaraes	ILRI	2 ± 1	Seed regeneration in progress to improve germination percentage for inclusion in NFG
12	<i>Brachiaria decumbens</i>	Basilisk	ILRI	45 ± 3	Seed regeneration in progress to improve germination percentage for inclusion in NFG
13	<i>Brachiaria brizantha</i>	MG5	Brasuda	93 ± 3	Seed viability high enough for inclusion in NFG
14	<i>Brachiaria brizantha</i>	MG4	Brasuda	72 ± 2	Seed viability high enough for inclusion in NFG
15	<i>Brachiaria brizantha</i>	Marandu	Brasuda	80 ± 2	Seed viability high enough for inclusion in NFG
16	<i>Brachiaria humidicola</i>	Humidicola	Brasuda	5 ± 1	Seed regeneration in progress to improve germination percentage for inclusion in NFG
17	<i>Brachiaria humidicola</i>	Llanero	Brasuda	89 ± 2	Seed viability high enough for inclusion in NFG
18	<i>B. ruziziensis</i> x <i>B. decumbens</i> x <i>B. brizantha</i>	Sabia	Barenbrug	12 ± 2	Seed regeneration in progress to improve germination percentage for inclusion in NFG
19	<i>Brachiaria humidicola</i>	Humco	DLF	5 ± 2	Seed regeneration in progress to improve germination percentage for inclusion in NFG
20	<i>Brachiaria humidicola</i>	Hyma	Barenbrug	7 ± 2	Seed regeneration in progress to improve germination percentage for inclusion in NFG
21	<i>Brachiaria humidicola</i>	Brimar	DLF	3 ± 1	Seed regeneration in progress to improve germination percentage for inclusion in NFG
22	<i>Brachiaria humidicola</i>	Cagana	Barenbrug	9 ± 3	Seed regeneration in progress to improve germination percentage for inclusion in NFG
23	<i>Brachiaria humidicola</i>	Debase	Barenbrug	3 ± 1	Seed regeneration in progress to improve germination percentage for inclusion in NFG
24	<i>Brachiaria nigropedata</i>	Nigropedata	ALLUT	2 ± 1	Seed regeneration in progress to improve germination percentage for inclusion in NFG
25	<i>Brachiaria brizantha</i>	SA-NFG 464	NFG	< 10	Seed regeneration in progress to improve germination percentage
26	<i>Brachiaria decumbens</i>	SA-NFG 465	NFG	< 10	Seed regeneration in progress to improve germination percentage
27	<i>Brachiaria dictyoneura</i>	SA-NFG 466	NFG	< 10	Seed regeneration in progress to improve germination percentage
28	<i>Brachiaria nigropedata</i>	SA-NFG 477	NFG	< 10	Seed regeneration in progress to improve germination percentage
29	<i>Brachiaria nigropedata</i>	SA-NFG 478	NFG	< 10	Seed regeneration in progress to improve germination percentage
30	<i>Brachiaria nigropedata</i>	SA-NFG 476	NFG	< 10	Seed regeneration in progress to improve germination percentage
31	<i>Brachiaria nigropedata</i>	SA-NFG 468	NFG	< 10	Seed regeneration in progress to improve germination percentage
32	<i>Brachiaria nigropedata</i>	SA-NFG 493	NFG	< 10	Seed regeneration in progress to improve germination percentage
33	<i>Brachiaria nigropedata</i>	SA-NFG 487	NFG	< 10	Seed regeneration in progress to improve germination percentage
34	<i>Brachiaria nigropedata</i>	SA-NFG 490	NFG	< 10	Seed regeneration in progress to improve germination percentage
35	<i>Brachiaria nigropedata</i>	SA-NFG 489	NFG	< 10	Seed regeneration in progress to improve germination percentage
36	<i>Brachiaria nigropedata</i>	SA-NFG 7517	NFG	< 10	Seed regeneration in progress to improve germination percentage
37	<i>Brachiaria nigropedata</i>	SA-NFG 7476	NFG	< 10	Seed regeneration in progress to improve germination percentage
38	<i>Brachiaria nigropedata</i>	SA-NFG 7477	NFG	< 10	Seed regeneration in progress to improve germination percentage
39	<i>Brachiaria nigropedata</i>	SA-NFG 7478	NFG	< 10	Seed regeneration in progress to improve germination percentage
40	<i>Brachiaria ruziziensis</i>	SA-NFG 494	NFG	< 10	Seed regeneration in progress to improve germination percentage
41	<i>Brachiaria serrata</i>	SA-NFG 495	NFG	< 10	Seed regeneration in progress to improve germination percentage
42	<i>Brachiaria serrata</i>	SA-NFG 499	NFG	< 10	Seed regeneration in progress to improve germination percentage
43	<i>Brachiaria serrata</i>	SA-NFG 496	NFG	< 10	Seed regeneration in progress to improve germination percentage
44	<i>Brachiaria serrata</i>	SA-NFG 497	NFG	< 10	Seed regeneration in progress to improve germination percentage
45	<i>Brachiaria brizantha</i>	CIAT16169	CIAT	< 10	Seed regeneration in progress to improve germination percentage
46	<i>Brachiaria brizantha</i>	CIAT6384	CIAT	< 10	Seed regeneration in progress to improve germination percentage
47	<i>Brachiaria brizantha</i>	CIAT16483	CIAT	< 10	Seed regeneration in progress to improve germination percentage
48	<i>Brachiaria brizantha</i>	CIAT16339	CIAT	< 10	Seed regeneration in progress to improve germination percentage
49	<i>Brachiaria brizantha</i>	CIAT16320	CIAT	< 10	Seed regeneration in progress to improve germination percentage
50	<i>Brachiaria brizantha</i>	CIAT16350	CIAT	< 10	Seed regeneration in progress to improve germination percentage

CHAPTER 3:

Seed germination responses to temperature and osmotic stress in *Brachiaria humidicola*, *Brachiaria brizantha*, *Brachiaria decumbens*, *Brachiaria nigropedata* and a hybrid *Brachiaria* species (*B. brizantha* x *B. decumbens* x *B. ruziziensis*)

3.1. Materials and Methods

From the regenerated seeds as well as commercial varieties, seeds of *Brachiaria humidicola* (cv. Llanero), *Brachiaria brizantha* (cv. Marandu), *B. nigropedata* (cv. Nigropedata), *B. decumbens* (cv. Basilisk) and the hybrid *Brachiaria* species, *B. brizantha* x *B. decumbens* x *B. ruziziensis* (cv. Mulato II) were used for evaluating the drought tolerance levels of the different *Brachiaria* species at the germination stage. Prior to final inclusion in the trial, these seeds were again tested for their germination potential by germinating, in the dark, four replicates of 100 seeds of each cultivar in 9 cm petri-dishes on filter paper, in a germination chamber set at a constant temperature of 20°C. The seeds were watered as needed and germination was recorded daily for 15 days. Seeds were regarded as germinated after the emergence of a radicle greater than 0.5 cm. Even after regeneration, the seeds still had a germination potential below 95 %, which resulted in all subsequent germination obtained in the subsequent seed germination experiments being calculated as a percentage of the initial germination. This was done following equation 1.

Equation 1: $\% = (X_n/X_i) \times 100$: where % is the final germination percentage expressed as a percentage of the initial germination potential of the species, X_n is the germination percentage obtained under the different experimental treatments and X_i is the initial germination percentage.

After the initial germination potential determination, four replicates of 100 seeds of each temperature and osmotic treatment combination within a species were placed in 9 cm petri-dishes on a layer of filter paper. Germination chambers were calibrated to constant temperatures of 5°C to 45°C at increments of 5°C, under continuous dark conditions. Within each temperature treatment, five osmotic treatments (0 MPa, -0.1 MPa, -0.3 MPa, -0.5 MPa, and -0.7 MPa) were imposed on the seeds. The osmotic treatments were prepared using polyethylene glycol 6000 (PEG6000) in accordance with the methods and equations established by Michael and Kaufmann (1973), seen in equation 2.

Equation 2: $OP = (-1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C + (2.67 \times 10^{-4}) \times C \times T + (8.39 \times 10^{-7}) \times C^2T$ where C = PEG concentration, T = Temperature

The osmotic solutions were stored in the germination chambers at each of the associated temperature treatments. A total of 5 ml of each osmotic solution was added to the petri-dishes and distilled water was used as the 0 MPa or control treatment. After watering, the petri-dishes were sealed using parafilm to prevent excessive water loss. The filter paper and osmotic solutions were replaced every four days in order to keep osmotic conditions within the petri-dishes relatively constant. Seed germination was recorded daily for 20 days, and germinated seeds were removed from the petri-dishes as required to minimize excessive uptake of available water resources by germinated seeds. At the end of the

germination period, the day of first seed germination for each species within each osmotic and temperature treatment was noted.

3.2. Statistical analyses

All data were statistically analyzed using IBM SPSS for Windows Version 22.0 (IBM Corporation 2013, Armonk, NY, USA). One-way analysis of variance (ANOVA) with a Tukey HSD post hoc test was used to determine whether significant differences ($p \geq 0.05$) were obtained between temperature and osmotic treatments within each species. Similarly, one-way analysis of variance (ANOVA) with a Tukey HSD post hoc test was used to determine differences between different species at different temperatures within each osmotic treatment and between different species at different osmotic treatments within each temperature treatment

3.3. Results

The initial germination potential of the species used were 89%, 80%, 85%, 81% and 72% for *B. humidicola*, *B. brizantha*, *B. decumbens*, *B. nigropedata* and the hybrid *Brachiaria*, respectively. Therefore, all further results discussed are expressed as a percentage of this initial seed germination potential. Results from the experiments indicated that seed germination in all *Brachiaria* species evaluated were negatively impacted by osmotic stress conditions as well as low and high temperatures. For all species evaluated, germination only occurred between 15 and 40°C, with no seed germination at 5°C, 10°C and 45°C.

3.3.1. *Brachiaria humidicola*

Under optimum moisture conditions, *B. humidicola* had an optimum germination temperature range of between 15 °C and 35 °C. However, as water-limitation became more severe, the optimum germination temperature range changed to between 15 °C to 25 °C at -0.1 MPa, 20 °C to 25 °C at -0.3 MPa and 20 °C at -0.5 and -0.7 MPa (Table 2). Generally, no differences ($p \geq 0.05$) were observed in seed germination under optimum moisture (0 MPa) conditions and low moisture stress (-0.1 MPa) conditions between 15 °C and 25 °C but decreased significantly ($p < 0.05$) at 30 °C (Table 2). Within this 15 °C to 25 °C temperature range, at 15 °C and 20 °C *B. humidicola* seed germination further decreased significantly ($p < 0.05$) from low osmotic stress conditions (-0.1 MPa) to moderate osmotic stress conditions (-0.3 MPa) but did not differ significantly at 25 °C (Table 2). From moderate osmotic stress conditions, seed germination significantly decreased ($p < 0.05$) to severe osmotic stress conditions (-0.5 MPa and -0.7 MPa), irrespective of the germination temperature (Table 2).

Table 2: Seed germination in *Brachiaria humidicola* at different temperature and osmotic treatments. Statistically significant differences ($p < 0.05$) in seed germination between different osmotic treatments within each temperature are indicated by different lower case letters while statistically significant differences between temperature treatments within each osmotic treatment are indicated by different superscript, upper case letters.

Osmotic Treatment	Temperature (°C)									Significance
	5	10	15	20	25	30	35	40	45	
0 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	86.8 ± 1.7 ^c ^C	90.7 ± 1.6 ^b ^C	81.2 ± 1.2 ^c ^C	82.2 ± 3.7 ^d ^C	82.0 ± 2.1 ^d ^C	41.3 ± 5.5 ^d ^B	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 226.8, p < 0.001$
-0.1 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	83.4 ± 7.3 ^c ^D	85.7 ± 2.4 ^b ^D	80.6 ± 2.1 ^c ^D	67.9 ± 3.7 ^c ^C	62.1 ± 3.4 ^c ^C	23.0 ± 3.1 ^c ^B	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 111.87, p < 0.001$
-0.3 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	53.4 ± 7.6 ^b ^C	79.8 ± 0.8 ^a ^D	73.3 ± 2.2 ^c ^D	62.4 ± 4.9 ^c ^C	14.1 ± 9.8 ^b ^B	4.8 ± 2.4 ^b ^A	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 56.76, p < 0.001$
-0.5 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	29.5 ± 2.5 ^a ^B	75.0 ± 3.4 ^a ^D	43.8 ± 2.1 ^b ^C	25.3 ± 2.8 ^b ^B	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 212.97, p < 0.001$
-0.7 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	12.4 ± 1.5 ^a ^B	67.1 ± 4.7 ^a ^C	15.2 ± 2.5 ^a ^B	5.1 ± 2.1 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 123.2, p < 0.001$
Significance	-	-	$F_{(4,20)} = 43.71, p < 0.001$	$F_{(4,20)} = 9.85, p < 0.001$	$F_{(4,20)} = 195.75, p < 0.001$	$F_{(4,20)} = 51.26, p < 0.001$	$F_{(4,20)} = 64.4, p < 0.001$	$F_{(4,20)} = 36.14, p < 0.001$	-	

3.3.2. *Brachiaria brizantha*

Under optimum moisture conditions, *B. brizantha* had an optimum germination temperature range of between 15 °C and 20 °C (Table 3). As water limitation became more severe (-0.1 MPa and -0.3 MPa), the optimum germination temperature changed to 20 °C and 25 °C (Table 3) even though 80 % germination could only be achieved at 20 °C under optimum osmotic conditions. Unlike *B. humidicola*, *B. brizantha* could not tolerate even low (-0.1 MPa) moisture stress conditions, even at the optimum germination temperature of 20 °C. It was found that seed germination in *B. brizantha* significantly decreases ($p \geq 0.05$) from 85 % to 66 % when water limitation decreases from 0 MPa to -0.1 MPa (Table 3).

Table 3: Seed germination in *Brachiaria brizantha* at different temperature and osmotic treatments. Statistically significant differences ($p < 0.05$) in seed germination between different osmotic treatments within each temperature are indicated by different lower case letters while statistically significant differences between temperature treatments within each osmotic treatment are indicated by different superscript, upper case letters.

Osmotic Treatment	Temperature (°C)									Significance
	5	10	15	20	25	30	35	40	45	
0 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	74.4 ± 4.9 ^d ^D	85.3 ± 0.8 ^c ^D	63.4 ± 4.8 ^c ^C	65.3 ± 4.4 ^c ^C	60.3 ± 2.9 ^d ^C	1.9 ± 0.6 ^b ^B	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 226.80, p < 0.001$
-0.1 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	49.4 ± 3.6 ^d ^D	66.9 ± 3.6 ^b ^F	60.3 ± 5.8 ^c ^{EF}	50.9 ± 4.9 ^d ^E	39.4 ± 5.1 ^c ^C	1.9 ± 1.5 ^b ^B	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 111.87, p < 0.001$
-0.3 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	27.8 ± 2.9 ^b ^{BC}	59.7 ± 3.1 ^{ab} ^D	46.3 ± 4.6 ^b ^{CD}	16.3 ± 4.2 ^c ^B	7.2 ± 4.0 ^b ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 56.76, p < 0.001$
-0.5 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	10.6 ± 2.9 ^a ^B	48.4 ± 3.1 ^{ab} ^D	17.5 ± 2.6 ^a ^C	5.3 ± 1.1 ^b ^B	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 212.97, p < 0.001$
-0.7 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	2.5 ± 1.1 ^a ^A	26.3 ± 3.6 ^a ^B	1.9 ± 0.6 ^a ^A	2.8 ± 1.1 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 123.00, p < 0.001$
Significance	-	-	$F_{(4,20)} = 43.71, p < 0.001$	$F_{(4,20)} = 9.85, p < 0.001$	$F_{(4,20)} = 195.75, p < 0.001$	$F_{(4,20)} = 51.26, p < 0.001$	$F_{(4,20)} = 64.40, p < 0.001$	$F_{(4,20)} = 36.1, p < 0.001$	-	

3.3.3. *Brachiaria decumbens*

Under optimum moisture conditions, *B. decumbens* had an optimum germination temperature range of between 15 °C and 25 °C (Table 4). As water limitation became more severe, the optimum germination temperature range changed to 15 °C and 20 °C at -0.1 MPa to -0.7 MPa (Table 4). Generally, no differences ($p \geq 0.05$) were observed in seed germination under optimum moisture (0 MPa), and low moisture stress (-0.1 MPa) conditions at 15 °C and 20 °C but decreased significantly ($p \geq 0.05$) at 25 °C (Table 4). Within the 15 °C – 25 °C temperature range seed germination decreased significantly ($p < 0.05$) from low osmotic stress conditions (-0.1 MPa) to moderate (-0.3 MPa) and severe (-0.5 MPa and -0.7 MPa) osmotic stress conditions, irrespective of the germination temperature (Table 4).

Table 4: Seed germination in *Brachiaria decumbens* at different temperature and osmotic treatments. Statistically significant differences ($p < 0.05$) in seed germination between different osmotic treatments within each temperature are indicated by different lower case letters while statistically significant differences between temperature treatments within each osmotic treatment are indicated by different superscript, upper case letters.

Osmotic Treatment	Temperature (°C)									Significance
	5	10	15	20	25	30	35	40	45	
0 MPa	0.0 ± 0.0 ^{aA}	0.0 ± 0.0 ^{aA}	90.9 ± 0.3 ^{dE}	91.5 ± 3.5 ^{dE}	90.2 ± 0.7 ^{eE}	65.3 ± 0.6 ^{dD}	37.4 ± 1.6 ^{cC}	10.6 ± 0.8 ^{cB}	0.0 ± 0.0 ^{aA}	$F_{(8,36)} = 961.99, p < 0.001$
-0.1 MPa	0.0 ± 0.0 ^{aA}	0.0 ± 0.0 ^{aA}	85.0 ± 0.6 ^{dE}	84.4 ± 1.9 ^{dE}	77.1 ± 1.0 ^{dD}	49.1 ± 1.0 ^{cC}	13.5 ± 1.7 ^{bB}	2.9 ± 0.6 ^{bA}	0.0 ± 0.0 ^{aA}	$F_{(8,36)} = 1449.27, p < 0.001$
-0.3 MPa	0.0 ± 0.0 ^{aA}	0.0 ± 0.0 ^{aA}	71.2 ± 0.3 ^{eE}	71.8 ± 0.5 ^{eE}	52.6 ± 1.7 ^{dD}	19.4 ± 1.6 ^{cC}	3.8 ± 0.3 ^{bB}	0.0 ± 0.0 ^{aA}	0.0 ± 0.0 ^{aA}	$F_{(8,36)} = 1587.99, p < 0.001$
-0.5 MPa	0.0 ± 0.0 ^{aA}	0.0 ± 0.0 ^{aA}	46.2 ± 1.3 ^{bD}	41.2 ± 1.3 ^{bD}	14.7 ± 1.0 ^{bC}	9.7 ± 0.6 ^{bB}	0.0 ± 0.0 ^{aA}	0.0 ± 0.0 ^{aA}	0.0 ± 0.0 ^{aA}	$F_{(8,36)} = 662.37, p < 0.001$
-0.7 MPa	0.0 ± 0.0 ^{aA}	0.0 ± 0.0 ^{aA}	6.5 ± 1.1 ^{cC}	7.6 ± 0.6 ^{cC}	3.8 ± 0.6 ^{aB}	4.1 ± 0.3 ^{aB}	0.0 ± 0.0 ^{aA}	0.0 ± 0.0 ^{aA}	0.0 ± 0.0 ^{aA}	$F_{(8,36)} = 42.78, p < 0.001$
Significance	-	-	$F_{(4,20)} = 1705, p < 0.001$	$F_{(4,20)} = 333.6, p < 0.001$	$F_{(4,20)} = 1232, p < 0.001$	$F_{(4,20)} = 832.5, p < 0.001$	$F_{(4,20)} = 22.19, p < 0.001$	$F_{(4,20)} = 101.33, p < 0.001$	-	

3.3.4. *Brachiaria nigropedata*

B. nigropedata had an optimum germination temperature range of between 15 °C and 20 °C at optimum osmotic stress conditions (0 MPa) to moderate osmotic stress conditions (-0.3 MPa) (Table 5). However, seed germination reached 90 % and 81 % even at 25 °C under optimum (0 MPa) and low (-0.1 MPa) osmotic stress conditions, respectively. Generally, seed germination decreased significantly ($p < 0.05$) from optimum osmotic conditions (0 MPa) to low osmotic stress conditions (-0.1 MPa) and decreased even further as water-limitation became more severe, irrespective of the germination temperature (Table 5).

Table 5: Seed germination in *Brachiaria nigropedata* at different temperature and osmotic treatments. Statistically significant differences ($p < 0.05$) in seed germination between different osmotic treatments within each temperature are indicated by different lower case letters while statistically significant differences between temperature treatments within each osmotic treatment are indicated by different superscript, upper case letters.

Osmotic Treatment	Temperature (°C)									Significance
	5	10	15	20	25	30	35	40	45	
0 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	96.9 ± 1.3 ^e ^E	95.1 ± 0.5 ^e ^E	90.7 ± 0.61 ^e ^D	75.0 ± 1.1 ^e ^C	37.7 ± 0.8 ^e ^B	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 4557.6, p < 0.001$
-0.1 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	87.0 ± 0.6 ^f ^F	84.3 ± 1.1 ^d ^E	81.5 ± 0.5 ^d ^D	54.9 ± 0.6 ^d ^C	30.2 ± 0.4 ^d ^B	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 6265.7, p < 0.001$
-0.3 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	71.9 ± 0.8 ^e ^E	71.0 ± 0.8 ^e ^E	60.5 ± 0.5 ^e ^D	21.0 ± 1.3 ^e ^C	14.8 ± 0.5 ^e ^B	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 2608.7, p < 0.001$
-0.5 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	43.2 ± 1.3 ^b ^D	43.8 ± 2.3 ^b ^D	24.7 ± 1.1 ^b ^C	4.3 ± 0.4 ^b ^B	10.2 ± 0.8 ^b ^B	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 344.2, p < 0.001$
-0.7 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	9.9 ± 0.9 ^a ^B	9.3 ± 0.4 ^a ^B	6.5 ± 0.3 ^a ^B	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	$F_{(8,36)} = 174.1, p < 0.001$
Significance	-	-	$F_{(4,20)} = 1210.9, p < 0.001$	$F_{(4,20)} = 813.2, p < 0.001$	$F_{(4,20)} = 2914.5, p < 0.001$	$F_{(4,20)} = 1575, p < 0.001$	$F_{(4,20)} = 717.1, p < 0.001$	-	-	

3.3.5. Hybrid *Brachiaria* species

The hybrid *Brachiaria* species (*B. brizantha* x *B. decumbens* x *B. ruziziensis*) had an optimum germination temperature of 20 °C, irrespective of the osmotic conditions under which the seeds were germinated (Table 6). Although seed germination exceeded 70 % even under low osmotic stress conditions (-0.1 MPa) at 15 °C, seed germination decreased significantly ($p < 0.05$) from optimum osmotic conditions (0 MPa) to low osmotic stress conditions (-0.1 MPa) and significantly ($p < 0.05$) decreased further as the osmotic stress became more severe (Table 6).

Table 6: Seed germination in the *Brachiaria* hybrid (*B. brizantha* x *B. decumbens* x *B. ruziziensis*) at different temperature and osmotic treatments. Statistically significant differences ($p < 0.05$) in seed germination between different osmotic treatments within each temperature are indicated by different lower case letters while statistically significant differences between temperature treatments within each osmotic treatment are indicated by different superscript, upper case letters.

Osmotic Treatment	Temperature (°C)									Significance
	5	10	15	20	25	30	35	40	45	
0 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	75.3 ± 1.8 ^c ^D	91.0 ± 1.8 ^d ^E	77.1 ± 1.7 ^e ^D	32.6 ± 1.7 ^d ^C	21.5 ± 4.3 ^b ^B	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	F_(8,36) = 429.55, p < 0.001
-0.1 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	71.5 ± 3.8 ^c ^{CD}	84.0 ± 7.0 ^c ^D	62.5 ± 1.0 ^d ^C	17.4 ± 0.7 ^c ^B	16.0 ± 2.3 ^b ^B	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	F_(8,36) = 153.68, p < 0.001
-0.3 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	30.9 ± 6.1 ^b ^B	69.4 ± 4.0 ^b ^C	35.8 ± 2.3 ^c ^B	6.9 ± 1.0 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	F_(8,36) = 92.50, p < 0.001
-0.5 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	3.1 ± 0.7 ^a ^A	24.0 ± 3.7 ^c ^C	11.5 ± 0.3 ^b ^B	2.8 ± 0.6 ^{ab} ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	F_(8,36) = 41.34, p < 0.001
-0.7 MPa	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	3.5 ± 0.4 ^a ^A	16.3 ± 2.6 ^a ^B	1.7 ± 0.3 ^a ^A	1.7 ± 0.3 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	0.0 ± 0.0 ^a ^A	F_(8,36) = 34.69, p < 0.001
Significance	-	-	F_(4,20) = 112.95, p < 0.001	F_(4,20) = 67.24, p < 0.001	F_(4,20) = 563.93, p < 0.001	F_(4,20) = 181.28, p < 0.001	F_(4,20) = 23.29, p < 0.001	-	-	

3.3.6. Time to germination

When considering the impact of temperature and osmotic conditions on the time to germination (Table 7), it was clear that both temperature and osmotic conditions had a significant ($p < 0.05$) impact on the time to seed germination. Generally, seed germination was initiated faster at warmer temperatures, but as moisture became more limiting, time to germination increased i.e., became longer. *Brachiaria humidicola*, *B. brizantha* and the *Brachiaria* hybrid were all very slow to initiate germination at 15 °C even at optimum osmotic conditions. In all instances however, irrespective of the germination temperatures, as moisture limitation became more severe, the time to germination increased.

Table 7: Time to first seed germination in five *Brachiaria* species under different temperature and osmotic treatments

	Osmotic Treatment	Temperature (°C)								
		5	10	15	20	25	30	35	40	45
<i>B. humidicola</i>	0 MPa	- ± -	- ± -	10.0 ± 0.0	4.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	- ± -
	-0.1 MPa	- ± -	- ± -	10.3 ± 0.3	4.3 ± 0.3	3.0 ± 0.0	3.0 ± 0.0	2.3 ± 0.3	2.0 ± 0.0	- ± -
	-0.3 MPa	- ± -	- ± -	12.0 ± 0.7	4.3 ± 0.3	3.0 ± 0.0	3.0 ± 0.0	5.0 ± 0.0	5.3 ± 0.7	- ± -
	-0.5 MPa	- ± -	- ± -	12.5 ± 0.3	4.3 ± 0.3	3.0 ± 0.0	3.0 ± 0.0	- ± -	- ± -	- ± -
	-0.7 MPa	- ± -	- ± -	13.0 ± 0.0	4.5 ± 0.3	4.0 ± 0.7	5.5 ± 1.2	- ± -	- ± -	- ± -
<i>B. brizantha</i>	0 MPa	- ± -	- ± -	10.8 ± 0.3	5.0 ± 0.4	3.0 ± 0.0	3.0 ± 0.0	2.0 ± 0.0	9.0 ± 0.0	- ± -
	-0.1 MPa	- ± -	- ± -	11.8 ± 0.5	4.3 ± 0.3	3.0 ± 0.0	3.0 ± 0.0	2.0 ± 0.0	9.5 ± 0.5	- ± -
	-0.3 MPa	- ± -	- ± -	11.8 ± 0.5	4.5 ± 0.5	3.3 ± 0.3	3.8 ± 0.5	5.0 ± 2.5	- ± -	- ± -
	-0.5 MPa	- ± -	- ± -	14.0 ± 1.7	5.0 ± 1.0	3.0 ± 0.0	4.0 ± 0.4	- ± -	- ± -	- ± -
	-0.7 MPa	- ± -	- ± -	18.3 ± 1.7	6.3 ± 1.3	6.3 ± 0.0	4.0 ± 0.6	- ± -	- ± -	- ± -
<i>B. decumbens</i>	0 MPa	- ± -	- ± -	4.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	5.0 ± 0.0	- ± -	- ± -
	-0.1 MPa	- ± -	- ± -	5.0 ± 0.0	4.3 ± 0.3	4.0 ± 0.0	3.0 ± 0.0	9.0 ± 0.0	- ± -	- ± -
	-0.3 MPa	- ± -	- ± -	6.0 ± 0.0	4.5 ± 0.5	6.0 ± 0.0	4.0 ± 0.0	11.0 ± 0.0	- ± -	- ± -
	-0.5 MPa	- ± -	- ± -	8.0 ± 0.0	5.0 ± 1.0	12.0 ± 0.0	11.0 ± 0.0	- ± -	- ± -	- ± -
	-0.7 MPa	- ± -	- ± -	15.0 ± 0.0	6.8 ± 1.6	13.3 ± 0.3	12.0 ± 0.0	- ± -	- ± -	- ± -
<i>B. nigropedata</i>	0 MPa	- ± -	- ± -	4.0 ± 0.0	4.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	- ± -	- ± -
	-0.1 MPa	- ± -	- ± -	5.0 ± 0.0	4.0 ± 0.0	3.0 ± 0.0	4.0 ± 0.0	5.0 ± 0.0	- ± -	- ± -
	-0.3 MPa	- ± -	- ± -	9.0 ± 0.0	6.0 ± 0.0	5.0 ± 0.0	6.0 ± 0.0	10.0 ± 0.0	- ± -	- ± -
	-0.5 MPa	- ± -	- ± -	11.0 ± 0.0	9.0 ± 0.0	7.0 ± 0.0	1.0 ± 0.0	11.0 ± 0.0	- ± -	- ± -
	-0.7 MPa	- ± -	- ± -	15.0 ± 0.0	12.0 ± 0.0	9.0 ± 0.0	- ± -	- ± -	- ± -	- ± -
<i>B. hybrid</i>	0 MPa	- ± -	- ± -	12.0 ± 0.6	6.3 ± 0.3	4.5 ± 0.5	3.0 ± 0.0	2.0 ± 0.0	- ± -	- ± -
	-0.1 MPa	- ± -	- ± -	11.5 ± 0.5	6.0 ± 0.4	4.5 ± 0.9	3.0 ± 0.0	3.0 ± 0.0	- ± -	- ± -
	-0.3 MPa	- ± -	- ± -	13.5 ± 0.5	6.5 ± 0.6	8.0 ± 0.6	3.3 ± 0.3	- ± -	- ± -	- ± -
	-0.5 MPa	- ± -	- ± -	14.0 ± 0.0	9.0 ± 1.2	11.3 ± 0.3	5.8 ± 1.1	- ± -	- ± -	- ± -
	-0.7 MPa	- ± -	- ± -	15.0 ± 0.0	8.5 ± 1.3	13.3 ± 0.3	4.3 ± 0.3	- ± -	- ± -	- ± -

3.4. Discussion

This study evaluated the germination responses of five *Brachiaria* species at different temperature and osmotic conditions. We hypothesized that the species would respond differently to temperature and osmotic stress conditions, but that all of the species will be able to germinate under severe moisture and temperature stress conditions. Results from the experiment indicated that all *Brachiaria* species evaluated have a wide temperature range under which they are able to germinate (15 – 40 °C) even though the optimum germination temperature ranges differed between the species which depended on water-availability. Germination was however found to generally be lower at higher temperatures, and this was especially true as osmotic stress became more severe. These responses are likely associated with enzyme activity and oxygen availability of seeds, which are known to decrease when germinated at unfavourable temperatures and limiting moisture conditions (Bewley and Black 1994). Furthermore, higher incubation temperatures and increased moisture stress during germination likely induced secondary dormancy of seeds, leading to prolonged seed germination over non-optimal temperatures and increased osmotic stress levels (Xiao et al. 2020).

Both temperature and osmotic stress conditions are known to impact seed germination (Brown 1995; Bewley and Black 2013). High temperatures are known to damage seeds and stop seed germination from either commencing or completing (Qi and Redmann 1993; Bonvissuto and Busso 2007; Bewley and Black 2013), and even at the most favourable osmotic conditions, temperature can limit the capacity and rate of seed germination in most plants (McDonough and Harniss 1974). This was evident in the current study, where germination was lowest at higher temperatures (35 - 40 °C), with no germination occurring at 45 °C for all species evaluated, even at optimum moisture availability. The fact that germination did not occur at 5 and 10 °C could be attributed to the natural conditions under which these species occur, or for the conditions that the specific cultivars were bred for. Several studies have shown that the environmental and topographical conditions from where seeds are collected can significantly affect the germination rate and success of the seeds at various combinations of osmotic stresses and temperatures (Ascough et al. 2007; Luna et al. 2012; Hu et al. 2015). All *Brachiaria* species evaluated have a tropical to sub-tropical distribution where the rainfall and establishment season is in summer and generally correspond to moderately warm conditions. This corresponds to the results indicating that optimum seed germination occurred at 20 °C (with some species having optimum ranges of 15 – 25 °C), where more than 80 % of seeds germinated.

Results from this study also show that the *Brachiaria* species evaluated do well under low osmotic stress conditions. Generally, seed germination decreased with an increase in osmotic stress, and this was found to be the case for all species evaluated. This was similar to the results of a study conducted by Bonvissuto and Busso (2007) on several other perennial grass species. Among the many *Brachiaria* grasses used as pastures, the species evaluated in this trial are known to be drought tolerant as mature plants (Humphreys 1987; Thomas & Grof 1986). In this study, however, it was clear that all of the species evaluated could only tolerate moderate drought (osmotic) stress with seed germination only exceeding 80 % at osmotic potentials up to -0.1 MPa for most of the species. *Brachiaria brizantha*

however, was found not to tolerate drought well, as germination for this species only exceeded 80 % under optimum osmotic conditions (0 MPa).

3.5. Conclusion

This study aimed to determine the impacts of different temperature and moisture conditions on the seed germination dynamics of *B. humidicola*, *B. brizantha*, *B. decumbens*, *B. nigropedata* and a hybrid *Brachiaria* species, (*B. brizantha* x *B. decumbens* x *B. ruziziensis*). It was hypothesized that although optimum germination temperatures and osmotic conditions will differ between the species, all of the species evaluated will be able to germinate under severe moisture and temperature stress conditions. Contrary to this, however, our results showed that although the majority of the species evaluated were able to tolerate a wide germination temperature range, as soon as moisture became more limiting, none of the *Brachiaria* species could tolerate even moderate drought stress conditions at their germination stages. From these findings, it is important to undertake further evaluations on early seedling responses to temperature and osmotic stress and quantify survival and growth responses to these stresses to develop more detailed planting time guidelines for farmers.

CHAPTER 4:

Water-limitation tolerance in *Brachiaria* genotypes. Towards the identification of tolerant and susceptible genotypes

4.1. Materials and Methods

Brachiaria seeds were obtained from local seed distributors. The seeds were germinated in petri-dishes and germinated seeds with a radicle of 1 cm was transplanted into pots containing a commercial potting soil mix (hygro-mix Hygrotech). After planting the pots were watered as needed and the first water-limitation trial was imposed on the plants 1 month after establishment.

4.1.1. Experiment 1: Water-limitation tolerance at seedling stage

After 1 month, the pots were arranged in a complete random design on greenhouse benches and watering was withheld for the water-limited pots. Each water-limitation period imposed on each genotype was replicated 4 times and water-limitation was imposed for 4, 8, 12, 16, 20 and 24 days with 4 well-watered control pots for each of the water-limitation periods. Imposing of the water-limited periods were staggered so that all plants could be harvested at the same time (Figure 1). By the end of the experimental period, the plants were uprooted and morphological measurements were done. These measurements included: the number of stems, number of leaves, culm thickness, root and shoot length, root and shoot wet mass, root and shoot dry mass, crown thickness, leaf length and leaf width. All data that was collected were statistically analyzed using IBM SPSS for Windows Version 22.0 (IBM Corporation 2013, Armonk, NY, USA). One-way analysis of variance (ANOVA) with a LSD post hoc test was used to determine whether significant differences ($p \geq 0.05$) were observed between the different water-limitation treatments within each genotype. Pearsons correlations were performed for each of the measured variables and soil moisture, measured at the end of the trial using a hand-held soil moisture meter.

4.1.2. Experiment 2: Water-limitation tolerance in mature *Brachiaria* plants

After 4 months of growing under well-watered conditions, pots containing mature *Brachiaria* plants were arranged in a random design on greenhouse benches, and watering was withheld for the water-limited plants. Each water-limitation period imposed on each genotype was replicated 4 times and water-limitation was imposed for 7, 15 and 30 days with 4 well-watered control pots for each of the water-limitation periods. The imposing of the water-limited periods were staggered so that all plants could be harvested at the same time (Figure 2).

A1	BASILISK	T1	Well-watered	R1	Replicate 1
A2	PIATA	T2	4 Days water-limitation	R2	Replicate 2
A3	BAR710	T3	8 Days water-limitation	R3	Replicate 3
A4	MULATO II	T4	12 Days water-limitation	R4	Replicate 4
A5	BAR330	T5	16 Days water-limitation		
A6	BAR7222	T6	20 Days water-limitation		
A7	MARANDU	T7	24 Days water-limitation		
A8	BAR780				
A9	SABIA				
A10	BAR460				
A11	BAR473				
A12	Xaraes				

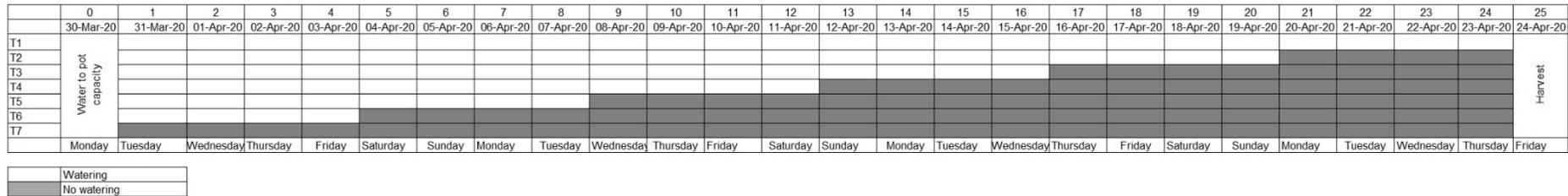


Figure 1: Experimental design of drought evaluation of different *Brachiaria* genotypes subjected to increasing days of water-limitation at seedling stage (1 month after establishment).

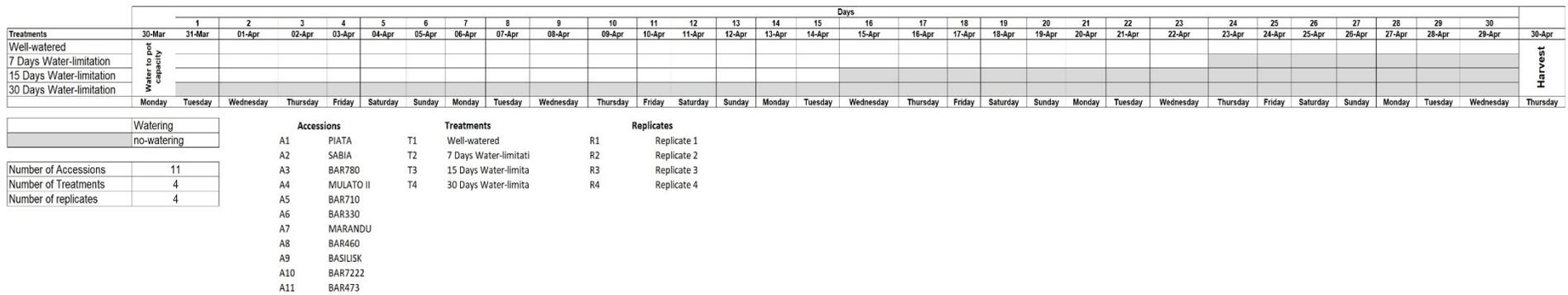


Figure 2: Experimental design of drought evaluation of different *Brachiaria* genotypes subjected to increasing days of water-limitation at 4 months.

4.2. Results and Discussion

4.2.1. Water-limitation tolerance at seedling stage

Results for each genotype collected at seedling stage are indicated in tables 8 – 19. Generally, genotypes such as Basilisk (Table 8), Line 710 (Table 9), Mulato II (Table 10), Marandu (Table 12), Xaraes (Table 14), Piata (only roots) (Table 15), Line 473 (Table 16), Line 460 (Table 17) and Line 330 (Table 18) did not show any significant decreases in root and/or shoot length measurements while genotypes such as Sabia (Table 11), and 780 (Table 13) has significant decreases in either root or shoot length growth, or both. Generally, for the genotypes that did not display any changes in root or shoot growth, there was also preferential allocation of resources between roots and shoots, suggesting the drought tolerance in these *Brachiaria* grasses could be more of a physiological mechanism rather than due to phenotypic plasticity from preferential allocation of resources to the organs tasked with taking up the limited resource.

When considering the mass of the plants subjected to the different durations of water-limitation, it was clear that all of the genotypes evaluated as a significant reduction in mass in either their roots or shoots, or both (Tables 8 – 18). Interestingly, even though the mass fractions decreased with increasing moisture limitation, genotypes such as Basilisk (Table 8), Line 710 (Table 9), Marandu (Table 12), Piata (Table 15), and Line 460 (Table 17) displayed no changes in resource allocation in terms of mass. Genotypes such as Line 473 (Table 16) and Line 710 (Table 9) showed an increase in either fresh or dry mass resource allocation, suggesting that these genotypes invested more into shoot growth rather than root growth, which could limit growth if water-limitation could become more severe. On the other hand, genotypes such as Sabia (Table 11) and Line 710 (Table 9) showed a decrease in root:shoot ratio (either wet or dry), suggesting that these genotypes invest more in root growth during droughts. This finding corresponds to the optimum partitioning theory (Bloom et al. 1985; Mao et al. 2012; Gargallo-Garriga et al. 2014; Eziz et al. 2017) in that these genotypes under water-limited conditions allocated more resources towards the roots, which are tasked with capturing the limited resource.

Table 8: The impacts of different durations of water-limitation on early growth of *Brachiaria decumbens* cv. Basilisk. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	Number of stems	Number of leaves	Culm Thickness	Root Length (cm)	Shoot Length (cm)	Root:Shoot ratio (length)	Shoot Fresh Mass (g)	Root Fresh Mass (g)	Root:Shoot ratio (Fresh Mass)	Shoot Dry Mass (g)	Root Dry Mass (g)	Root:Shoot ratio (Dry Mass)	Crown Thickness	Leaf Length (mm)	Leaf Width (mm)
WW	3.0 ± 0.0a	15.0 ± 0.8b	0.6 ± 0.02	20.6 ± 4.6a	28.8 ± 1.8a	0.7 ± 0.20a	2.6 ± 0.2b	1.8 ± 0.1b	0.7 ± 0.1a	0.9 ± 0.01d	0.6 ± 0.01b	0.7 ± 0.01a	1.2 ± 0.08b	167.2 ± 2.7b	12.1 ± 0.2d
4DWL	3.4 ± 0.2a	15.0 ± 1.0b	0.5 ± 0.20	23.5 ± 3.3a	28.5 ± 0.3a	0.8 ± 0.10a	2.4 ± 0.3b	1.7 ± 0.3b	0.7 ± 0.2a	0.8 ± 0.05c	0.7 ± 0.05b	0.8 ± 0.07a	1.2 ± 0.09b	166.5 ± 8.5b	12.2 ± 0.2d
8DWL	3.2 ± 0.2a	14.6 ± 1.3b	0.5 ± 0.20	23.4 ± 2.0a	28.0 ± 0.3a	0.8 ± 0.10a	2.4 ± 0.1b	1.7 ± 0.1b	0.7 ± 0.1a	0.8 ± 0.01c	0.6 ± 0.05b	0.8 ± 0.07a	1.1 ± 0.05a	165.3 ± 7.6b	10.2 ± 0.5c
12DWL	3.2 ± 0.2a	14.6 ± 0.2b	0.5 ± 0.04	23.2 ± 1.0a	28.0 ± 0.5a	0.8 ± 0.03a	2.3 ± 0.1b	1.7 ± 0.2b	0.7 ± 0.1a	0.8 ± 0.03c	0.7 ± 0.09b	0.9 ± 0.11a	1.1 ± 0.06a	161.1 ± 6.1b	10.2 ± 0.4c
16DWL	3.0 ± 0.3a	13.6 ± 0.6a	0.5 ± 0.02	21.2 ± 1.5a	27.0 ± 2.8a	0.9 ± 0.20a	1.0 ± 0.1a	1.1 ± 0.1a	1.2 ± 0.1a	0.8 ± 0.04c	0.6 ± 0.05b	0.9 ± 0.09a	1.0 ± 0.07a	161.9 ± 3.1b	9.3 ± 0.5bc
20DWL	2.6 ± 0.4a	11.4 ± 0.4a	0.4 ± 0.04	21.2 ± 0.4a	24.7 ± 2.6a	0.9 ± 0.10a	1.0 ± 0.2a	1.0 ± 0.2a	1.3 ± 0.5a	0.5 ± 0.05b	0.5 ± 0.06a	0.9 ± 0.13a	0.9 ± 0.05a	155.3 ± 5.2ab	8.3 ± 0.5b
24DWL	2.6 ± 0.2a	11.6 ± 0.9a	0.3 ± 0.02	19.2 ± 2.8a	22.0 ± 2.6a	0.9 ± 0.20a	0.5 ± 0.1a	0.5 ± 0.1a	1.0 ± 0.2a	0.4 ± 0.04a	0.3 ± 0.03a	0.9 ± 0.10a	0.9 ± 0.09a	139.7 ± 7.0a	6.9 ± 0.3a
F	1.42	3.651	8.667	0.403	1.726	0.262	23.884	7.889	1.343	26.917	5.423	0.622	2.521	2.52	23.155
p	0.242	0.008	< 0.001	0.871	0.152	0.95	< 0.001	< 0.001	0.271	< 0.001	0.001	0.711	0.041	0.03	< 0.001

Table 9: The impacts of different durations of water-limitation on early growth of *Brachiaria brizantha* line 710. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	Number of stems	Number of leaves	Culm Thickness	Root Length (cm)	Shoot Length (cm)	Root:Shoot ratio (length)	Shoot Fresh Mass (g)	Root Fresh Mass (g)	Root:Shoot ratio (Fresh Mass)	Shoot Dry Mass (g)	Root Dry Mass (g)	Root:Shoot ratio (Dry Mass)	Crown Thickness	Leaf Length (mm)	Leaf Width (mm)
WW	2.6 ± 0.2a	13.2 ± 0.7a	1.5 ± 0.02d	19.0 ± 0.5a	11.1 ± 0.6a	1.7 ± 0.10a	1.2 ± 0.33c	1.0 ± 0.20c	0.9 ± 0.2a	0.6 ± 0.05c	0.6 ± 0.05c	1.2 ± 0.17a	0.6 ± 0.03a	83.9 ± 2.8a	15.6 ± 0.5c
4DWL	3.0 ± 0.3a	13.2 ± 0.4a	1.4 ± 0.09cd	19.0 ± 0.6a	11.5 ± 0.8a	1.7 ± 0.15a	1.0 ± 0.08bc	1.0 ± 0.10c	0.9 ± 0.1a	0.6 ± 0.02c	0.7 ± 0.05c	1.1 ± 0.05a	0.6 ± 0.03a	83.2 ± 1.6a	15.3 ± 0.3c
8DWL	2.4 ± 0.5a	13.8 ± 0.8a	1.3 ± 0.02bc	17.4 ± 1.1a	12.1 ± 0.8a	1.5 ± 0.12a	0.9 ± 0.04bc	0.8 ± 0.10c	0.9 ± 0.0a	0.5 ± 0.02b	0.5 ± 0.04b	1.0 ± 0.05a	0.6 ± 0.04a	79.7 ± 3.2a	15.8 ± 0.6c
12DWL	2.0 ± 0.3a	13.2 ± 0.4a	1.2 ± 0.13bc	16.1 ± 0.6a	10.5 ± 0.2a	1.5 ± 0.05a	0.9 ± 0.23bc	0.8 ± 0.10c	1.0 ± 0.2a	0.5 ± 0.03b	0.5 ± 0.04b	1.1 ± 0.09a	0.6 ± 0.02a	78.2 ± 5.0a	13.6 ± 0.8b
16DWL	2.2 ± 0.2a	13.2 ± 0.6a	1.1 ± 0.10ab	16.8 ± 1.2a	10.6 ± 0.5a	1.6 ± 0.06a	0.6 ± 0.05ab	0.5 ± 0.10b	0.9 ± 0.1a	0.5 ± 0.03b	0.4 ± 0.05a	0.9 ± 0.16a	0.5 ± 0.05a	79.3 ± 3.9a	11.6 ± 1.3b
20DWL	2.4 ± 0.2a	13.8 ± 1.0a	1.1 ± 0.04ab	16.8 ± 1.0a	10.7 ± 0.2a	1.6 ± 0.13a	0.4 ± 0.03a	0.4 ± 0.04ab	1.0 ± 0.1a	0.4 ± 0.04a	0.4 ± 0.03a	1.0 ± 0.08a	0.5 ± 0.04a	78.8 ± 1.9a	9.1 ± 0.4a
24DWL	2.0 ± 0.4a	11.8 ± 1.8a	0.9 ± 0.04a	16.5 ± 1.6a	10.3 ± 1.4a	1.7 ± 0.21a	0.3 ± 0.04a	0.3 ± 0.04a	0.8 ± 0.1a	0.3 ± 0.04a	0.3 ± 0.04a	0.8 ± 0.07a	0.5 ± 0.02a	78.1 ± 5.5a	7.8 ± 0.7a
F	1.073	0.522	5.515	1.336	0.72	0.654	4.561	9.07	0.441	7.923	7.945	1.205	0.681	0.422	20.544
p	0.402	0.787	0.001	0.275	0.637	0.687	0.002	< 0.001	0.845	< 0.001	< 0.001	0.333	0.666	0.861	< 0.001

Table 10: The impacts of different durations of water-limitation on early growth of the *Brachiaria hybrid* cultivar Mulato II. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	Number of stems	Number of leaves	Culm Thickness	Root Length (cm)	Shoot Length (cm)	Root:Shoot ratio (length)	Shoot Fresh Mass (g)	Root Fresh Mass (g)	Root:Shoot ratio (Fresh Mass)	Shoot Dry Mass (g)	Root Dry Mass (g)	Root:Shoot ratio (Dry Mass)	Crown Thickness	Leaf Length (mm)	Leaf Width (mm)
WW	2.6 ± 0.2a	13.0 ± 0.9a	0.4 ± 0.02c	18.9 ± 0.6a	15.9 ± 0.3a	1.2 ± 0.04a	1.8 ± 0.2c	3.0 ± 0.3c	0.6 ± 0.08a	0.6 ± 0.03c	0.9 ± 0.09c	1.4 ± 0.1a	1.2 ± 0.04a	12.0 ± 0.3c	1.4 ± 0.03c
4DWL	2.6 ± 0.2a	12.2 ± 1.0a	0.4 ± 0.02c	18.2 ± 0.9a	15.1 ± 0.2a	1.2 ± 0.08a	1.6 ± 0.2b	3.0 ± 0.4c	0.6 ± 0.07a	0.5 ± 0.04b	0.8 ± 0.08bc	1.5 ± 0.2a	1.1 ± 0.10a	11.5 ± 0.2b	1.4 ± 0.01c
8DWL	2.4 ± 0.2a	12.8 ± 1.6a	0.3 ± 0.02b	17.5 ± 0.2a	15.9 ± 0.5a	1.1 ± 0.03a	1.6 ± 0.1b	2.8 ± 0.3c	0.6 ± 0.04a	0.5 ± 0.02b	0.8 ± 0.04bc	1.6 ± 0.1a	1.1 ± 0.08a	11.1 ± 0.3b	1.5 ± 0.02c
12DWL	3.0 ± 0.0b	12.6 ± 0.2a	0.3 ± 0.02b	17.6 ± 0.2a	15.9 ± 0.2a	1.1 ± 0.01a	1.6 ± 0.1b	2.8 ± 0.1c	0.6 ± 0.01a	0.5 ± 0.01b	0.8 ± 0.03bc	1.6 ± 0.1a	1.1 ± 0.04a	11.5 ± 0.2b	1.4 ± 0.02c
16DWL	3.4 ± 0.2b	12.8 ± 0.5a	0.3 ± 0.03b	17.5 ± 0.2a	15.3 ± 0.3a	1.4 ± 0.03a	0.7 ± 0.2a	2.2 ± 0.3b	0.3 ± 0.11a	0.4 ± 0.02a	0.7 ± 0.01b	1.6 ± 0.1a	1.0 ± 0.03a	11.5 ± 0.2b	0.9 ± 0.07b
20DWL	3.2 ± 0.2b	12.6 ± 0.7a	0.2 ± 0.02a	17.4 ± 0.4a	15.7 ± 0.8a	1.1 ± 0.07a	0.6 ± 0.1a	1.8 ± 0.3b	0.4 ± 0.11a	0.4 ± 0.04a	0.7 ± 0.02b	1.9 ± 0.2a	1.0 ± 0.02a	11.3 ± 0.3b	0.7 ± 0.10a
24DWL	3.0 ± 0.0b	12.4 ± 0.5a	0.2 ± 0.02a	17.9 ± 0.5a	15.2 ± 1.3a	1.2 ± 0.08a	0.4 ± 0.0a	0.7 ± 0.1a	0.6 ± 0.08a	0.3 ± 0.02a	0.5 ± 0.03a	1.6 ± 0.1a	1.0 ± 0.05a	9.5 ± 1.0a	0.6 ± 0.03a
F	3.286	0.094	10.245	1.278	0.301	0.698	20.616	8.39	2.666	13.274	4.774	1.315	2.221	2.948	63.881
p	0.014	0.996	< 0.001	0.299	0.931	0.654	< 0.001	< 0.001	0.36	< 0.001	0.002	0.285	0.072	0.013	< 0.001

Table 11: The impacts of different durations of water-limitation on early growth of the *Brachiaria hybrid* cultivar SABIA. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	Number of stems	Number of leaves	Culm Thickness	Root Length (cm)	Shoot Length (cm)	Root:Shoot ratio (length)	Shoot Fresh Mass (g)	Root Fresh Mass (g)	Root:Shoot ratio (Fresh Mass)	Shoot Dry Mass (g)	Root Dry Mass (g)	Root:Shoot ratio (Dry Mass)	Crown Thickness	Leaf Length (mm)	Leaf Width (mm)
WW	2.6 ± 0.2a	12.0 ± 0.5a	0.6 ± 0.04a	21.2 ± 0.7b	19.8 ± 0.4b	1.1 ± 0.02a	4.5 ± 2.8c	2.8 ± 0.1d	1.3 ± 0.3a	0.6 ± 0.01c	0.9 ± 0.02c	1.5 ± 0.05b	1.5 ± 0.02b	138 ± 2.0d	15.4 ± 2.7c
4DWL	2.8 ± 0.2a	12.2 ± 0.2a	0.6 ± 0.02a	21.0 ± 0.5b	20.2 ± 0.5b	1.0 ± 0.03a	2.0 ± 0.2b	2.4 ± 0.2cd	1.2 ± 0.1a	0.6 ± 0.03c	1.0 ± 0.05c	1.6 ± 0.04b	1.5 ± 0.04b	136 ± 3.7d	15.3 ± 0.4c
8DWL	3.0 ± 0.0a	12.8 ± 0.5a	0.6 ± 0.04a	21.0 ± 0.4b	20.0 ± 1.2b	0.1 ± 0.06a	1.8 ± 0.1b	2.2 ± 0.2c	1.3 ± 0.1a	0.6 ± 0.02c	0.9 ± 0.04c	1.4 ± 0.08b	1.4 ± 0.06b	129 ± 2.9c	14.5 ± 0.4c
12DWL	3.2 ± 0.2a	12.4 ± 0.4a	0.6 ± 0.02a	21.4 ± 1.0b	19.0 ± 1.2b	0.2 ± 0.14a	1.6 ± 0.3b	2.0 ± 0.1c	1.5 ± 0.4a	0.6 ± 0.02c	0.8 ± 0.04b	1.4 ± 0.03b	1.3 ± 0.13b	118 ± 2.6b	13.0 ± 0.5b
16DWL	3.2 ± 0.2a	12.8 ± 0.8a	0.5 ± 0.02a	20.2 ± 0.5b	17.2 ± 0.2b	1.2 ± 0.03a	0.7 ± 0.1a	1.4 ± 0.1b	2.0 ± 0.2a	0.5 ± 0.02b	0.7 ± 0.03b	1.4 ± 0.03b	1.1 ± 0.04a	118 ± 3.5b	11.0 ± 0.0b
20DWL	3.0 ± 0.5a	11.2 ± 1.9a	0.5 ± 0.02a	17.8 ± 0.5a	15.8 ± 1.1a	1.2 ± 0.08a	0.4 ± 0.0a	0.5 ± 0.1a	1.2 ± 0.2a	0.4 ± 0.02a	0.5 ± 0.03a	1.2 ± 0.05a	1.0 ± 0.07a	115 ± 1.1b	9.4 ± 8.0a
24DWL	2.4 ± 0.2a	9.4 ± 0.8a	0.5 ± 0.03a	17.6 ± 0.2a	15.1 ± 1.1a	1.2 ± 0.09a	0.3 ± 0.0a	0.4 ± 0.0a	1.1 ± 0.1a	0.3 ± 0.02a	0.3 ± 0.03a	1.1 ± 0.05a	1.0 ± 0.04a	102 ± 2.6a	8.7 ± 0.5a
F	1.185	1.751	2.069	7.365	6.184	0.656	1.864	52.481	1.579	44.26	43.953	9.004	9.345	21.008	24.629
p	0.343	0.146	0.089	< 0.001	< 0.001	0.685	0.023	< 0.001	0.185	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 12: The impacts of different durations of water-limitation on early growth of *Brachiaria brizantha* cultivar Marandu. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	Number of stems	Number of leaves	Culm Thickness	Root Length (cm)	Shoot Length (cm)	Root:Shoot ratio (length)	Shoot Fresh Mass (g)	Root Fresh Mass (g)	Root:Shoot ratio (Fresh Mass)	Shoot Dry Mass (g)	Root Dry Mass (g)	Root:Shoot ratio (Dry Mass)	Crown Thickness	Leaf Length (mm)	Leaf Width (mm)
WW	2.8 ± 0.2a	14.6 ± 0.2c	0.6 ± 0.02a	21.2 ± 0.4a	22.5 ± 0.3c	0.9 ± 0.03a	1.6 ± 0.11c	1.9 ± 0.02d	1.2 ± 0.1a	0.8 ± 0.03c	0.7 ± 0.06b	1.0 ± 0.1a	1.4 ± 0.1d	138.2 ± 5.1a	12.8 ± 0.5d
4DWL	2.4 ± 0.4a	13.0 ± 0.8c	0.5 ± 0.02a	22.1 ± 0.5a	21.5 ± 1.3c	1.0 ± 0.06a	1.5 ± 0.12c	1.7 ± 0.07cd	1.1 ± 0.1a	0.8 ± 0.03c	0.9 ± 0.09b	1.1 ± 0.1a	1.3 ± 0.1c	135.3 ± 11.9a	12.6 ± 0.4d
8DWL	2.8 ± 0.2a	13.6 ± 0.7c	0.5 ± 0.02a	22.5 ± 1.3a	21.8 ± 1.3c	1.0 ± 0.11a	1.5 ± 0.12c	1.5 ± 0.14c	1.0 ± 0.1a	0.7 ± 0.03bc	0.8 ± 0.08b	1.2 ± 0.1a	1.3 ± 0.1c	133.0 ± 4.6a	11.7 ± 1.0cd
12DWL	2.8 ± 0.5a	13.4 ± 0.2c	0.5 ± 0.02a	21.6 ± 1.0a	17.4 ± 1.4b	1.3 ± 0.15a	1.1 ± 0.22b	1.6 ± 0.26c	1.5 ± 0.3a	0.6 ± 0.02b	0.7 ± 0.05b	1.2 ± 0.1a	1.2 ± 0.1c	131.1 ± 9.3a	10.4 ± 0.7c
16DWL	3.0 ± 0.3a	13.4 ± 0.8c	0.6 ± 0.02a	21.8 ± 1.1a	16.2 ± 0.8b	1.4 ± 0.08a	1.0 ± 0.21b	1.2 ± 0.26b	1.3 ± 0.4a	0.6 ± 0.04b	0.6 ± 0.05a	1.1 ± 0.1a	1.1 ± 0.1b	117.2 ± 9.3a	8.4 ± 0.5b
20DWL	2.4 ± 0.2a	11.8 ± 0.6b	0.5 ± 0.02a	21.8 ± 1.4a	15.0 ± 1.0a	1.5 ± 0.10a	0.7 ± 0.03ab	1.1 ± 0.18b	1.7 ± 0.3a	0.5 ± 0.04a	0.6 ± 0.06a	1.2 ± 0.1a	1.1 ± 0.1b	114.3 ± 1.3a	8.7 ± 0.5b
24DWL	2.2 ± 0.4a	9.6 ± 1.5a	0.5 ± 0.02a	18.9 ± 0.4a	15.8 ± 1.8a	0.3 ± 0.23a	0.4 ± 0.03a	0.5 ± 0.06a	1.1 ± 0.1a	0.4 ± 0.03a	0.4 ± 0.04a	1.0 ± 0.1a	0.9 ± 0.1a	114.3 ± 3.8a	7.3 ± 0.5a
F	0.769	4.171	0.493	1.527	6.708	2.317	11.087	8.519	1.231	19.666	6.026	0.89	3.232	1.961	13.037
p	0.6	0.004	0.808	0.206	< 0.001	0.061	< 0.001	< 0.001	0.32	< 0.001	< 0.001	0.516	0.015	0.085	< 0.001

Table 13: The impacts of different durations of water-limitation on early growth of *Brachiaria brizantha* line 780. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	Number of stems	Number of leaves	Culm Thickness	Root Length (cm)	Shoot Length (cm)	Root:Shoot ratio (length)	Shoot Fresh Mass (g)	Root Fresh Mass (g)	Root:Shoot ratio (Fresh Mass)	Shoot Dry Mass (g)	Root Dry Mass (g)	Root:Shoot ratio (Dry Mass)	Crown Thickness	Leaf Length (mm)	Leaf Width (mm)
WW	2.6 ± 0.4a	13.6 ± 0.7c	1.4 ± 0.10c	13.9 ± 1.4a	13.9 ± 2.3a	1.01 ± 0.24a	1.8 ± 0.04c	1.4 ± 0.06c	0.8 ± 0.05a	0.6 ± 0.02b	0.9 ± 0.09c	1.4 ± 0.15b	0.5 ± 0.05c	10.7 ± 0.6a	1.5 ± 0.10c
4DWL	2.8 ± 0.2a	13.4 ± 0.9c	1.3 ± 0.07c	19.7 ± 0.6b	19.5 ± 0.5c	1.01 ± 0.08a	1.8 ± 0.19c	1.4 ± 0.03c	0.8 ± 0.10a	0.6 ± 0.05b	0.8 ± 0.09bc	1.3 ± 0.06b	0.5 ± 0.04c	11.4 ± 0.5a	1.6 ± 0.03c
8DWL	2.6 ± 0.2a	13.0 ± 0.9c	1.2 ± 0.08bc	20.9 ± 2.6c	19.2 ± 1.0c	1.11 ± 0.17a	1.7 ± 0.08c	1.4 ± 0.05c	0.8 ± 0.01a	0.6 ± 0.03b	0.8 ± 0.04bc	1.3 ± 0.09b	0.4 ± 0.02b	10.1 ± 1.3a	1.5 ± 0.06c
12DWL	3.2 ± 0.5a	13.0 ± 0.8c	1.1 ± 0.05b	20.2 ± 0.3c	19.0 ± 1.0c	1.07 ± 0.06a	0.8 ± 0.12b	1.3 ± 0.11bc	1.7 ± 0.30c	0.6 ± 0.02b	0.6 ± 0.07b	1.0 ± 0.10a	0.4 ± 0.04b	11.6 ± 0.9a	0.9 ± 0.15b
16DWL	2.8 ± 0.2a	12.4 ± 0.9b	1.1 ± 0.06b	20.7 ± 0.5c	18.1 ± 1.3b	1.18 ± 0.12a	0.8 ± 0.10b	1.2 ± 0.16b	1.5 ± 0.13b	0.6 ± 0.06b	0.6 ± 0.08b	1.0 ± 0.13a	0.4 ± 0.04b	11.0 ± 0.4a	0.7 ± 0.06b
20DWL	2.8 ± 0.2a	12.4 ± 0.5b	1.0 ± 0.03ab	20.0 ± 0.4c	17.3 ± 1.2b	1.19 ± 0.12a	0.5 ± 0.04a	0.8 ± 0.15a	1.4 ± 0.23b	0.4 ± 0.02a	0.5 ± 0.08a	1.2 ± 0.20a	0.3 ± 0.04a	10.7 ± 0.4a	0.7 ± 0.08b
24DWL	2.0 ± 0.0a	9.4 ± 0.5a	0.8 ± 0.04a	18.2 ± 2.0b	14.7 ± 1.0a	1.27 ± 0.18a	0.4 ± 0.03a	0.5 ± 0.07a	1.3 ± 0.16b	0.3 ± 0.03a	0.4 ± 0.04a	1.1 ± 0.11a	0.3 ± 0.04a	10.4 ± 0.5a	0.4 ± 0.11a
F	1.586	3.371	8.837	2.624	3.042	0.387	38.842	11.877	5.121	11.987	7.456	1.995	4.06	0.518	24.784
p	0.188	0.013	< 0.001	0.038	0.02	0.875	< 0.001	< 0.001	0.001	< 0.001	< 0.001	0.01	0.005	0.793	< 0.001

Table 14: The impacts of different durations of water-limitation on early growth of *Brachiaria brizantha* cultivar Xaraes. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	Number of stems	Number of leaves	Culm Thickness	Root Length (cm)	Shoot Length (cm)	Root:Shoot ratio (length)	Shoot Fresh Mass (g)	Root Fresh Mass (g)	Root:Shoot ratio (Fresh Mass)	Shoot Dry Mass (g)	Root Dry Mass (g)	Root:Shoot ratio (Dry Mass)	Crown Thickness	Leaf Length (mm)	Leaf Width (mm)
WW	2.3 ± 0.5a	12.3 ± 0.8a	0.6 ± 0.04a	21.0 ± 0.4a	28.9 ± 2.2a	0.7 ± 0.05a	2.2 ± 0.05c	2.4 ± 0.2d	1.1 ± 0.1	0.7 ± 0.06b	0.6 ± 0.04a	1.0 ± 0.06a	1.4 ± 0.09c	219 ± 3.7a	11.4 ± 0.4b
4DWL	3.0 ± 0.0a	12.3 ± 1.1a	0.6 ± 0.03a	21.8 ± 0.8a	30.0 ± 0.5a	0.7 ± 0.01a	2.2 ± 0.18c	2.3 ± 0.4d	1.0 ± 0.1a	0.7 ± 0.05b	0.6 ± 0.02a	0.9 ± 0.11a	1.3 ± 0.06c	215 ± 1.6a	12.1 ± 0.4b
8DWL	2.5 ± 0.3a	12.0 ± 1.2a	0.6 ± 0.05a	22.3 ± 0.8a	31.5 ± 1.3	0.7 ± 0.02a	1.9 ± 0.20bc	1.6 ± 0.2c	0.8 ± 0.1a	0.7 ± 0.03b	0.6 ± 0.02a	0.9 ± 0.04a	0.3 ± 0.05a	212 ± 10.7a	11.9 ± 0.2b
12DWL	2.5 ± 0.5a	12.0 ± 0.6a	0.6 ± 0.05a	24.8 ± 3.3a	30.5 ± 2.3a	0.8 ± 0.10a	1.4 ± 0.31b	1.3 ± 0.2c	1.0 ± 0.3a	0.6 ± 0.04ab	0.5 ± 0.04a	0.9 ± 0.06a	0.1 ± 0.13a	209 ± 15.6a	11.4 ± 0.3b
16DWL	3.0 ± 0.0a	12.3 ± 0.3a	0.6 ± 0.03a	24.5 ± 1.7a	27.8 ± 2.7a	0.9 ± 0.18a	0.7 ± 0.11a	0.9 ± 0.3b	1.3 ± 0.5a	0.5 ± 0.03a	0.5 ± 0.04a	0.9 ± 0.09a	1.0 ± 0.03b	187 ± 13.6a	7.1 ± 0.7a
20DWL	2.5 ± 0.3a	10.5 ± 0.3a	0.6 ± 0.03a	25.3 ± 2.5a	25.8 ± 3.8a	1.1 ± 0.22a	0.8 ± 0.10a	0.6 ± 0.0a	0.8 ± 0.1a	0.5 ± 0.03a	0.5 ± 0.06a	1.1 ± 0.07a	1.0 ± 0.03b	181 ± 14.9a	8.5 ± 0.8a
F	0.871	0.746	0.48	0.21	0.766	1.343	13.975	7.448	0.54	3.336	0.845	1.102	6.65	1.932	17.034
p	0.52	0.6	0.787	0.50	0.586	0.291	< 0.001	< 0.001	0.744	0.026	0.535	0.394	<0.001	0.109	< 0.001

Table 15: The impacts of different durations of water-limitation on early growth of *Brachiaria brizantha* cultivar Piata. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	Number of stems	Number of leaves	Culm Thickness	Root Length (cm)	Shoot Length (cm)	Root:Shoot ratio (length)	Shoot Fresh Mass (g)	Root Fresh Mass (g)	Root:Shoot ratio (Fresh Mass)	Shoot Dry Mass (g)	Root Dry Mass (g)	Root:Shoot ratio (Dry Mass)	Crown Thickness	Leaf Length (mm)	Leaf Width (mm)
WW	2.4 ± 0.2a	10.4 ± 1.1b	0.6 ± 0.04a	18.8 ± 0.5a	26.2 ± 2.0c	0.7 ± 0.04a	1.6 ± 0.2d	1.3 ± 0.1c	0.8 ± 0.1a	0.8 ± 0.08b	0.7 ± 0.06c	1.0 ± 0.1a	1.2 ± 0.07a	235 ± 11.2d	11.2 ± 0.7c
4DWL	2.6 ± 0.2a	12.8 ± 0.7b	0.6 ± 0.04a	19.4 ± 0.2a	27.8 ± 2.1c	0.7 ± 0.07a	1.5 ± 0.1d	1.3 ± 0.2c	0.8 ± 0.2a	0.9 ± 0.06b	0.7 ± 0.06c	0.8 ± 0.1a	1.2 ± 0.04a	232 ± 5.5d	11.5 ± 0.6c
8DWL	2.2 ± 0.4a	11.4 ± 1.9b	0.6 ± 0.05a	19.2 ± 0.5a	28.8 ± 3.0c	0.7 ± 0.10a	1.4 ± 0.2d	1.3 ± 0.1c	1.0 ± 0.3a	0.7 ± 0.08b	0.6 ± 0.03b	0.9 ± 0.1a	0.1 ± 0.05a	213 ± 5.3c	11.2 ± 0.4c
12DWL	2.0 ± 0.4a	10.8 ± 1.4b	0.5 ± 0.02a	19.6 ± 1.0a	29.0 ± 3.0c	0.7 ± 0.10a	1.0 ± 0.3c	1.1 ± 0.2b	1.7 ± 0.8a	0.6 ± 0.13b	0.6 ± 0.04b	1.3 ± 0.5a	0.1 ± 0.04a	213 ± 15.7c	9.0 ± 0.5b
16DWL	1.6 ± 0.4a	8.0 ± 1.2a	0.5 ± 0.05a	19.6 ± 0.9a	27.2 ± 1.8c	0.7 ± 0.04a	0.7 ± 0.1b	0.9 ± 0.1b	1.3 ± 0.1a	0.6 ± 0.06b	0.5 ± 0.03ab	0.9 ± 0.4a	1.0 ± 0.09a	199 ± 17.1b	8.1 ± 0.5b
20DWL	1.6 ± 0.2a	8.2 ± 0.9a	0.5 ± 0.02a	18.0 ± 0.5a	21.8 ± 3.4b	1.0 ± 0.20b	0.5 ± 0.1ab	0.6 ± 0.1a	1.6 ± 0.5a	0.4 ± 0.08a	0.4 ± 0.03a	1.4 ± 0.4a	1.0 ± 0.04a	177 ± 11.2b	6.6 ± 0.7a
24DWL	1.4 ± 0.2a	8.0 ± 0.9a	0.5 ± 0.04a	18.0 ± 0.3a	14.8 ± 2.8a	1.4 ± 0.30c	0.4 ± 0.1a	0.5 ± 0.1a	1.7 ± 0.5a	0.3 ± 0.07a	0.4 ± 0.04a	1.6 ± 0.5a	1.0 ± 0.05a	144 ± 9.4a	6.4 ± 0.6a
F	1.946	2.486	1.908	1.242	3.817	3.234	9.165	6.922	0.777	7.095	9.384	0.988	2.147	7.806	10.236
p	0.108	0.047	0.114	0.316	0.007	0.015	< 0.001	< 0.001	0.595	< 0.001	< 0.001	0.452	0.079	< 0.001	< 0.001

Table 16: The impacts of different durations of water-limitation on early growth of *Brachiaria brizantha* line 473. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	Number of stems	Number of leaves	Culm Thickness	Root Length (cm)	Shoot Length (cm)	Root:Shoot ratio (length)	Shoot Fresh Mass (g)	Root Fresh Mass (g)	Root:Shoot ratio (Fresh Mass)	Shoot Dry Mass (g)	Root Dry Mass (g)	Root:Shoot ratio (Dry Mass)	Crown Thickness	Leaf Length (mm)	Leaf Width (mm)
WW	3.0 ± 0.0a	12.2 ± 0.5a	0.6 ± 0.03a	22.0 ± 2.4a	16.3 ± 0.5a	1.4 ± 0.2a	1.9 ± 0.2c	2.5 ± 0.3c	1.3 ± 0.2a	0.6 ± 0.03b	0.6 ± 0.01a	1.0 ± 0.04a	1.5 ± 0.05c	129 ± 2.6c	14.8 ± 0.2b
4DWL	2.5 ± 0.3a	12.5 ± 0.9a	0.6 ± 0.03a	20.5 ± 0.6a	16.0 ± 0.9a	1.3 ± 0.1a	1.9 ± 0.2c	2.3 ± 0.4c	1.2 ± 0.2a	0.6 ± 0.02b	0.7 ± 0.08a	1.1 ± 0.11a	1.3 ± 0.08b	120 ± 3.3c	14.8 ± 0.4b
8DWL	3.0 ± 0.0a	12.8 ± 0.4a	0.6 ± 0.02a	20.0 ± 0.3a	15.6 ± 2.0a	1.4 ± 0.3a	1.9 ± 0.1c	2.0 ± 0.2bc	1.0 ± 0.1a	0.6 ± 0.05b	0.7 ± 0.02a	1.2 ± 0.11ab	1.3 ± 0.06b	118 ± 3.5b	14.5 ± 0.3b
12DWL	2.8 ± 0.2a	12.2 ± 0.7a	0.5 ± 0.02a	19.6 ± 0.2a	15.0 ± 1.6a	1.4 ± 0.2a	1.8 ± 0.1c	1.8 ± 0.2b	1.0 ± 0.1a	0.6 ± 0.05b	0.7 ± 0.02a	1.2 ± 0.11ab	1.2 ± 0.09b	118 ± 1.6b	14.2 ± 0.2b
16DWL	2.4 ± 0.4a	10.0 ± 1.1a	0.6 ± 0.02a	19.2 ± 0.5a	15.7 ± 1.4a	1.3 ± 0.1a	1.7 ± 0.1c	1.8 ± 0.1b	1.1 ± 0.1a	0.5 ± 0.05a	0.7 ± 0.05a	1.3 ± 0.04b	1.2 ± 0.05	115 ± 7.5b	0
20DWL	2.8 ± 0.2a	11.2 ± 1.0a	0.6 ± 0.04a	18.8 ± 0.5a	14.1 ± 0.7a	1.3 ± 0.1a	1.3 ± 0.2b	1.7 ± 0.3b	1.4 ± 0.1a	0.5 ± 0.04a	0.7 ± 0.06a	1.5 ± 0.09c	1.2 ± 0.12b	109 ± 5.4a	10.0 ± 0.7a
24DWL	2.8 ± 0.2a	11.6 ± 0.7a	0.5 ± 0.04a	17.9 ± 0.6a	14.1 ± 1.1a	1.3 ± 0.1a	0.5 ± 0.1a	1.0 ± 0.2a	2.0 ± 0.3b	0.4 ± 0.05a	0.6 ± 0.09a	1.5 ± 0.16c	1.0 ± 0.02a	104 ± 0.0a	9.5 ± 0.0a
F	0.959	1.82	0.217	2.099	0.423	0.13	11.118	3.318	4.394	3.483	0.356	2.805	4.674	3.125	27.621
p	0.472	0.134	0.968	0.088	0.857	0.991	< 0.001	0.015	0.003	0.012	0.9	0.031	0.002	0.01	< 0.001

Table 17: The impacts of different durations of water-limitation on early growth of *Brachiaria brizantha* line 460. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	Number of stems	Number of leaves	Culm Thickness	Root Length (cm)	Shoot Length (cm)	Root:Shoot ratio (length)	Shoot Fresh Mass (g)	Root Fresh Mass (g)	Root:Shoot ratio (Fresh Mass)	Shoot Dry Mass (g)	Root Dry Mass (g)	Root:Shoot ratio (Dry Mass)	Crown Thickness	Leaf Length (mm)	Leaf Width (mm)
WW	3.2 ± 0.2b	13.8 ± 0.6b	0.6 ± 0.04a	20.0 ± 0.4a	17.2 ± 0.5a	1.2 ± 0.05a	2.1 ± 0.2c	2.5 ± 0.04c	1.2 ± 1.1a	0.7 ± 0.02b	0.8 ± 0.03b	1.1 ± 0.04a	1.5 ± 0.03b	11.9 ± 0.5a	1.5 ± 0.04b
4DWL	2.6 ± 0.2b	11.2 ± 0.7b	0.6 ± 0.01a	20.2 ± 1.1a	17.3 ± 0.8a	1.2 ± 0.11a	2.0 ± 0.1c	2.4 ± 0.06c	1.2 ± 0.1a	0.6 ± 0.05ab	0.8 ± 0.03b	1.3 ± 0.08a	1.4 ± 0.10b	11.8 ± 0.4a	1.5 ± 0.03b
8DWL	2.2 ± 0.2b	10.4 ± 0.9ab	0.6 ± 0.03a	22.8 ± 0.9a	17.8 ± 0.4a	1.3 ± 0.03a	1.8 ± 0.2c	2.1 ± 0.43c	1.1 ± 0.1a	0.6 ± 0.03ab	0.8 ± 0.06b	1.2 ± 0.07a	1.2 ± 0.02a	11.4 ± 0.5a	1.5 ± 0.05b
12DWL	2.8 ± 0.4b	9.0 ± 1.4a	0.6 ± 0.02a	22.2 ± 0.4a	17.0 ± 0.3a	1.3 ± 0.03a	1.2 ± 0.1b	1.6 ± 0.07b	1.3 ± 0.1a	0.6 ± 0.05ab	0.7 ± 0.03ab	1.2 ± 0.05a	1.2 ± 0.10a	11.4 ± 0.3a	1.4 ± 0.05b
16DWL	2.6 ± 0.2b	9.6 ± 0.4a	0.6 ± 0.04a	22.2 ± 1.2a	17.0 ± 1.5a	1.4 ± 0.16a	1.2 ± 0.2b	1.6 ± 0.14b	1.5 ± 0.3a	0.5 ± 0.05a	0.6 ± 0.05a	1.1 ± 0.13a	1.2 ± 0.05a	11.6 ± 0.5a	0.6 ± 0.11a
20DWL	2.8 ± 0.2b	9.6 ± 0.2a	0.6 ± 0.02a	22.6 ± 0.5a	17.8 ± 0.9a	1.3 ± 0.07a	0.7 ± 0.2a	1.1 ± 0.27a	1.6 ± 0.3a	0.5 ± 0.03a	0.6 ± 0.05a	1.3 ± 0.05a	1.1 ± 0.09a	11.8 ± 0.6a	0.4 ± 0.03a
24DWL	1.8 ± 0.2a	8.8 ± 0.4a	0.6 ± 0.05a	22.0 ± 4.6a	15.9 ± 2.0a	1.3 ± 0.14a	0.5 ± 0.1a	0.6 ± 0.13a	1.2 ± 0.2a	0.4 ± 0.01a	0.4 ± 0.07a	1.0 ± 0.21a	1.1 ± 0.05a	11.4 ± 0.5a	0.3 ± 0.04a
F	3.429	5.243	0.185	0.354	0.353	0.562	13.577	10.508	0.747	8.57	8.455	0.85	4.178	0.204	78.135
p	0.012	0.001	0.979	0.901	0.902	0.757	< 0.001	< 0.001	0.617	< 0.001	< 0.001	0.543	0.004	0.974	< 0.001

Table 18: The impacts of different durations of water-limitation on early growth of *Brachiaria brizantha* line 330. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	Number of stems	Number of leaves	Culm Thickness	Root Length (cm)	Shoot Length (cm)	Root:Shoot ratio (length)	Shoot Fresh Mass (g)	Root Fresh Mass (g)	Root:Shoot ratio (Fresh Mass)	Shoot Dry Mass (g)	Root Dry Mass (g)	Root:Shoot ratio (Dry Mass)	Crown Thickness	Leaf Length (mm)	Leaf Width (mm)
WW	2.8 ± 0.2a	13.2 ± 0.9b	0.6 ± 0.04a	21.4 ± 2.0a	19.2 ± 0.6a	1.1 ± 0.1a	1.9 ± 0.2c	3.0 ± 0.3d	1.6 ± 0.2a	0.6 ± 0.03b	0.7 ± 0.05a	1.2 ± 0.07a	1.48 ± 0.02c	119.7 ± 3.0a	15.1 ± 0.5b
4DWL	2.8 ± 0.2a	13.4 ± 0.7b	0.6 ± 0.02a	23.6 ± 1.0a	18.0 ± 0.6a	1.3 ± 0.1a	1.8 ± 0.2c	2.8 ± 0.6d	1.5 ± 0.2a	0.5 ± 0.06b	0.7 ± 0.04a	1.3 ± 0.09a	1.36 ± 0.05bc	119.6 ± 6.7a	14.4 ± 0.3b
8DWL	2.4 ± 0.2a	13.2 ± 0.5b	0.6 ± 0.02a	27.2 ± 3.5a	18.2 ± 1.5a	1.5 ± 0.2a	1.7 ± 0.1bc	2.2 ± 0.2c	1.3 ± 0.1	0.5 ± 0.02b	0.6 ± 0.03a	1.4 ± 0.05a	1.34 ± 0.08b	119.9 ± 5.5a	14.8 ± 0.4b
12DWL	3.0 ± 0.0a	13.0 ± 0.4b	0.6 ± 0.05a	22.2 ± 1.2a	17.8 ± 0.7a	1.2 ± 0.1a	1.7 ± 0.2bc	2.2 ± 0.1c	1.3 ± 0.1a	0.5 ± 0.07b	0.6 ± 0.03a	1.3 ± 0.06a	1.32 ± 0.12b	117.8 ± 1.4a	14.6 ± 0.4b
16DWL	3.0 ± 0.0a	12.8 ± 0.4b	0.6 ± 0.02a	22.4 ± 2.1a	16.2 ± 1.3a	1.4 ± 0.2a	1.5 ± 0.1b	2.2 ± 0.3c	1.4 ± 0.2a	0.5 ± 0.12b	0.6 ± 0.02a	1.4 ± 0.11a	1.26 ± 0.09b	118.9 ± 3.6a	12.9 ± 0.7ab
20DWL	2.6 ± 0.2a	10.6 ± 0.1a	0.6 ± 0.02a	20.6 ± 1.5a	16.2 ± 0.4a	1.3 ± 0.1a	0.7 ± 0.2a	1.6 ± 0.3b	2.5 ± 0.6a	0.4 ± 0.02a	0.6 ± 0.05a	1.6 ± 0.14a	1.08 ± 0.04a	115.2 ± 1.9a	9.7 ± 0.4a
24DWL	2.6 ± 0.2a	10.6 ± 0.9a	0.6 ± 0.04a	20.0 ± 0.4a	16.2 ± 0.4a	1.1 ± 0.4a	0.6 ± 0.2a	0.9 ± 0.2a	1.6 ± 0.1a	0.4 ± 0.03a	0.6 ± 0.09a	1.5 ± 0.16a	1.10 ± 0.04a	106.8 ± 1.9a	8.7 ± 0.7a
F	1.333	2.926	0.456	1.582	1.862	1.207	13.753	5.172	2.145	4.414	0.78	1.297	4.03	1.475	28.201
p	0.276	0.024	0.834	0.189	0.123	0.332	< 0.001	0.001	0.079	0.003	0.593	0.299	0.005	0.201	< 0.001

Table 19: Pearsons correlations coefficients (r) between soil water content and plant growth characteristics in 11 *Brachiaria* genotypes subjected to different periods of water-limitation at seedling stage

	Genotypes										
	Basilisk	710	Mulato	Marandu	780	Xaraes	Piata	473	Sabia	460	330
	Soil moisture content										
Number of Stems	.567	.617	-.671	.414	.276	-.348	.719	.284	-.110	.626	.144
Number of Leaves	.810*	.350	.397	.828*	.804*	.601	.696	.710	.514	.894*	.805*
Culm Thickness	.858*	.947*	.924*	.241	.987*	.613	.955*	-.043	.988**	.466	.588
Root Length	.300	.792*	.740	.473	-.515	-.935*	.419	.998*	.771*	-.715	.389
Shoot Length	.798*	.570	.376	.909**	-.011	.452	.645	.856*	.840*	.385	.949*
RootL:Shoot Length Ratio	-.983*	.243	-.036	.058	-.757*	-.792	-.705	.431	-.782*	-.801*	-.251
Shoot Fresh Mass	.890*	.971*	.893*	.939*	.926*	.907*	.951*	.772*	.977*	.944*	.877*
Root Fresh Mass	.836*	.962*	.853*	.931*	.836*	.946*	.899*	.932*	.931*	.943*	.928*
Root:Shoot Fresh Mass Ratio	-.787*	-.009	.407	-.312	-.728	.123	-.844*	-.439	.026	-.459	-.330
Shoot Dry Mass	.820*	.889*	.954*	.945*	.824*	.635	.844*	.911*	.818*	.973*	.941*
Root Dry Mass	.651	.924*	.901*	.743	.979*	.829*	.971*	.272	.865*	.902*	.919*
Root:Shoot Dry Mass Ratio	-.957*	.856*	-.731	-.131	.647	-.336	-.676	-.911*	.835*	.157	-.882*
Crown Thickness	.944*	.897*	.947*	.962*	.974*	.962*	.965*	.981*	.881*	.953*	.953*
Leaf Length	.758*	.848*	.728	.938*	.127	.867*	.914*	.970*	.914*	.402	.742
Leaf Width	.894*	.908*	.829*	.957*	.925*	.673	.896*	.760*	.906*	.886*	.861*

*p < 0.05

4.2.2. Water-limitation tolerance in mature *Brachiaria* genotypes

Results for each mature *Brachiaria* genotype is indicated in tables 20 – 29. In general, water-limitation seems to have a lesser negative impact on mature *Brachiaria* plants compared to results obtained at seedling stage. For genotypes Basilisk, 710, Sabia, Marandu, 780, Piata, 473 and 460, no significant differences were observed in both root and shoot length with increasing water-limitation. However, the genotypes Mulato II and 330 showed a significant decrease in root length with increasing degrees of water-limitation. In contrast however, nearly all of the genotypes had a reduction in root and/or shoot mass (fresh or dry). For Mulato II, it was found that although root length significantly decreased with increasing water-limitation, root mass did not decrease. In most of the genotypes evaluated, no differences were found in resource allocation between roots and shoots. However, genotypes such as Basilisk, Mulato II, 780, Piata and 330 were found to allocate significantly more resources towards root growth. The increased root:shoot ratio observed under moisture stress conditions can be explained by greater inhibition of shoot growth rather than root growth under moisture stressed conditions. This is in accordance with the optimal partitioning theory, which states that plants will allocate more resources to the structures by which the limiting resources are captured to optimize the plants' performance, and therefore improve the fitness and the success of those plants (Beebe et al. 2013).

Table 20: The impacts of different durations of water-limitation on the growth of mature *Brachiaria decumbens* cv. Basilisk plants. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	WW	7 DWL	15 DWL	30 DWL	F	p
Number of stems	30.0 ± 3.2b	26.5 ± 1.7b	27.5 ± 1.8b	20.8 ± 0.5a	3.66	0.044
Number of leaves	147.3 ± 22.5c	128.0 ± 4.6b	127.3 ± 2.0b	94.0 ± 2.9a	3.627	0.045
Culm Thickness	0.5 ± 0.03b	0.5 ± 0.05b	0.3 ± 0.00a	0.2 ± 0.02a	12.789	< 0.001
Crown Thickness	4.4 ± 0.4b	3.9 ± 0.1b	3.4 ± 0.2a	3.0 ± 0.1a	7.046	0.005
Root Length (cm)	40.9 ± 2.2b	38.3 ± 2.0b	30.5 ± 0.7a	31.1 ± 1.5a	9.232	0.002
Shoot Length (cm)	74.9 ± 1.5a	73.0 ± 2.4a	70.8 ± 3.0a	67.0 ± 0.5a	2.648	0.097
Root:Shoot Length Ratio	0.5 ± 0.02a	0.5 ± 0.02a	0.4 ± 0.02a	0.5 ± 0.02a	5.774	0.111
Shoot Fresh Mass (g)	66.8 ± 10.3d	58.6 ± 1.0c	26.9 ± 1.8b	11.8 ± 1.5a	23.809	< 0.001
Root Fresh Mass (g)	14.2 ± 0.8b	14.3 ± 1.4b	13.4 ± 1.5b	7.0 ± 0.5a	9.605	0.002
Root:Shoot Fresh Mass Ratio	0.2 ± 0.03a	0.2 ± 0.03a	0.5 ± 0.07b	0.6 ± 0.05b	16.513	< 0.001
Shoot Dry Mass (g)	32.4 ± 4.5c	31.0 ± 1.0c	21.1 ± 2.2b	9.4 ± 0.9a	17.107	< 0.001
Root Dry Mass (g)	7.4 ± 0.6b	7.3 ± 0.3b	7.4 ± 0.2b	4.9 ± 0.2a	12.274	0.001
Root:Shoot Dry Mass Ratio	0.2 ± 0.02a	0.2 ± 0.01a	0.4 ± 0.04b	0.5 ± 0.05b	17.108	< 0.001
Culm node number	5.0 ± 0.0b	5.0 ± 0.0b	4.8 ± 0.3ab	4.3 ± 0.3a	4.00	0.035
Internode Length	8.8 ± 0.5b	8.9 ± 0.3b	8.1 ± 0.3b	6.9 ± 0.3a	6.34	< 0.001
Leaf length (cm)	20.5 ± 1.0a	20.0 ± 0.9a	19.7 ± 0.9a	19.1 ± 1.1a	0.419	0.74
Leaf width	1.5 ± 0.03b	1.1 ± 0.04a	1.0 ± 0.03a	1.0 ± 0.05a	39.299	< 0.001

Table 21: The impacts of different durations of water-limitation on the growth of mature *Brachiaria brizantha* line 710 plants. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	WW	7 DWL	15 DWL	30 DWL	F	p
Number of stems	17.0 ± 2.4c	14.0 ± 1.1b	13.8 ± 1.4ab	11.5 ± 0.6a	3.557	0.048
Number of leaves	89.5 ± 17.4c	73.8 ± 9.7b	69.8 ± 10.6b	63.0 ± 2.9a	4.257	0.029
Culm Thickness	0.6 ± 0.03a	0.7 ± 0.03a	0.4 ± 0.03a	0.3 ± 0.03a	2.079	0.156
Crown Thickness	4.5 ± 0.2b	3.8 ± 0.1a	3.5 ± 0.2a	3.4 ± 0.2a	5.851	0.011
Root Length (cm)	26.8 ± 1.1a	27.2 ± 2.2a	25.8 ± 1.1a	22.3 ± 1.0a	0.379	0.77
Shoot Length (cm)	43.0 ± 2.6a	42.5 ± 4.0a	46.7 ± 2.2a	49.1 ± 1.4a	0.66	0.592
Root:Shoot Length Ratio	0.6 ± 0.10a	0.5 ± 0.04a	0.6 ± 0.08a	0.5 ± 0.03a	0.195	0.898
Shoot Fresh Mass (g)	77.7 ± 13.7d	44.6 ± 7.7c	24.1 ± 34.0b	13.7 ± 0.4a	65.475	< 0.001
Root Fresh Mass (g)	20.8 ± 5.2c	15.1 ± 4.3b	16.8 ± 2.0b	11.5 ± 0.8a	7.056	0.005
Root:Shoot Fresh Mass Ratio	0.7 ± 0.1a	1.2 ± 0.1a	1.7 ± 0.2a	2.1 ± 0.7a	3.144	0.065
Shoot Dry Mass (g)	29.6 ± 4.3c	17.8 ± 2.7b	17.5 ± 2.9b	11.1 ± 0.3a	20.698	< 0.001
Root Dry Mass (g)	11.4 ± 1.6a	11.0 ± 2.2a	9.1 ± 0.2a	9.0 ± 0.6a	3.422	0.053
Root:Shoot Dry Mass Ratio	0.6 ± 0.10a	1.0 ± 0.04a	0.9 ± 0.08a	1.7 ± 0.05a	2.759	0.088
Culm node number	4.3 ± 0.5a	4.8 ± 0.3a	3.0 ± 0.0a	2.8 ± 0.3a	0.228	0.803
Internode Length	4.8 ± 0.2b	4.4 ± 0.2b	3.6 ± 0.2a	3.0 ± 0.1a	17.18	< 0.001
Leaf length (cm)	27.8 ± 0.9a	26.9 ± 0.8a	27.0 ± 0.8a	27.3 ± 0.9a	0.384	0.765
Leaf width	2.1 ± 0.03b	1.9 ± 0.02ab	1.5 ± 0.03a	1.6 ± 0.03a	15.818	< 0.001

Table 22: The impacts of different durations of water-limitation on the growth of mature *Brachiaria hybrid* cv. Mulato II plants. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	WW	7 DWL	15 DWL	30 DWL	F	p
Number of stems	13.3 ± 1.3a	13.0 ± 0.7a	12.0 ± 2.0a	11.8 ± 1.9a	0.218	0.882
Number of leaves	62.5 ± 5.7a	61.6 ± 1.8a	60.3 ± 2.5a	60.5 ± 5.6a	0.059	0.98
Culm Thickness	0.7 ± 0.10a	0.6 ± 0.03a	0.6 ± 0.05a	0.6 ± 0.03a	1.189	0.355
Crown Thickness	3.6 ± 0.2a	3.3 ± 0.1a	3.1 ± 0.1a	2.9 ± 0.1a	2.966	0.075
Root Length (cm)	32.5 ± 1.4b	32.6 ± 2.7b	25.8 ± 2.3ab	20.5 ± 0.6a	14.18	< 0.001
Shoot Length (cm)	49.6 ± 1.5a	47.9 ± 3.8a	45.7 ± 1.2a	45.0 ± 0.5a	0.98	0.435
Root:Shoot Length Ratio	0.7 ± 0.04b	0.7 ± 0.05b	0.6 ± 0.06ab	0.5 ± 0.02s	5.018	0.018
Shoot Fresh Mass (g)	52.5 ± 6.5d	45.2 ± 4.1c	22.6 ± 2.8b	10.4 ± 1.2s	22.536	< 0.001
Root Fresh Mass (g)	27.9 ± 6.9a	27.7 ± 1.3a	18.3 ± 2.8a	13.9 ± 1.2a	3.319	0.057
Root:Shoot Fresh Mass Ratio	0.5 ± 0.06a	0.6 ± 0.05a	0.8 ± 0.06a	1.4 ± 0.10b	23.994	< 0.001
Shoot Dry Mass (g)	21.6 ± 2.1c	19.0 ± 1.5bc	14.9 ± 1.7b	8.1 ± 1.0a	12.753	< 0.001
Root Dry Mass (g)	14.8 ± 2.7a	14.2 ± 1.2a	12.5 ± 1.7a	10.1 ± 1.1a	1.322	0.313
Root:Shoot Dry Mass Ratio	0.7 ± 0.05a	0.8 ± 0.10a	0.8 ± 0.03a	1.3 ± 0.20b	5.463	0.013
Culm node number	4.3 ± 0.3a	3.5 ± 0.3a	3.5 ± 0.3a	3.5 ± 0.3a	1.8	0.201
Internode Length	4.8 ± 0.2b	3.5 ± 0.1a	3.5 ± 0.1a	3.3 ± 0.1a	22.505	< 0.001
Leaf length (cm)	24.0 ± 0.9a	24.3 ± 0.8a	24.9 ± 0.8a	24.8 ± 1.0a	0.231	0.874
Leaf width	1.9 ± 0.04b	1.9 ± 0.03b	1.3 ± 0.03a	1.4 ± 0.04a	73.228	< 0.001

Table 23: The impacts of different durations of water-limitation on the growth of mature *Brachiaria hybrid* cv. Sabia plants. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	WW	7 DWL	15 DWL	30 DWL	F	p
Number of stems	13.5 ± 1.5a	13.0 ± 0.6	13.0 ± 1.5a	12.5 ± 1.9a	0.055	0.982
Number of leaves	73.0 ± 2.0a	65.0 ± 6.7a	60.3 ± 7.0a	56.0 ± 7.1a	0.876	0.489
Culm Thickness	0.5 ± 0.00a	0.5 ± 0.03a	0.5 ± 0.08a	0.5 ± 0.06a	0.187	0.903
Crown Thickness	3.2 ± 0.2a	3.0 ± 0.3a	2.9 ± 0.4a	2.3 ± 0.2a	1.315	0.329
Root Length (cm)	33.8 ± 12.5a	40.8 ± 11.0a	41.7 ± 6.1a	43.6 ± 2.4a	0.252	0.858
Shoot Length (cm)	42.5 ± 2.5a	41.1 ± 1.5a	40.6 ± 0.9a	39.8 ± 2.1a	0.349	0.791
Root:Shoot Length Ratio	0.8 ± 0.2a	1.0 ± 0.2a	1.0 ± 0.1a	1.1 ± 0.1a	0.541	0.666
Shoot Fresh Mass (g)	67.2 ± 3.7d	51.6 ± 4.2c	22.8 ± 3.4b	10.3 ± 2.5a	51.113	< 0.001
Root Fresh Mass (g)	29.9 ± 4.5b	29.4 ± 2.2b	21.0 ± 4.7b	7.6 ± 1.7a	8.707	0.005
Root:Shoot Fresh Mass Ratio	0.4 ± 0.04a	0.6 ± 0.04a	0.9 ± 0.17a	0.8 ± 0.11a	2.428	0.132
Shoot Dry Mass (g)	20.8 ± 2.1c	19.7 ± 1.7bc	15.5 ± 2.6b	7.9 ± 1.9a	6.831	0.011
Root Dry Mass (g)	10.4 ± 0.8b	9.9 ± 0.9b	8.5 ± 1.4b	4.6 ± 0.7a	5.801	0.017
Root:Shoot Dry Mass Ratio	0.5 ± 0.01a	0.5 ± 0.05a	0.6 ± 0.05a	0.7 ± 0.16a	0.645	0.606
Culm node number	3.5 ± 0.5b	3.3 ± 0.3b	2.8 ± 0.3b	1.8 ± 0.5a	3.923	0.048
Internode Length	3.1 ± 0.4a	3.0 ± 0.1a	2.9 ± 0.5a	2.5 ± 0.4a	0.283	0.637
Leaf length (cm)	25.1 ± 1.0a	24.7 ± 0.7a	24.7 ± 0.8a	24.1 ± 1.0a	0.215	0.886
Leaf width	1.9 ± 0.03b	1.8 ± 0.03b	1.4 ± 0.04a	1.4 ± 0.05a	39.553	< 0.001

Table 24: The impacts of different durations of water-limitation on the growth of mature *Brachiaria brizantha* cv. Marandu plants. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	WW	7 DWL	15 DWL	30 DWL	F	p
Number of stems	13.0 ± 0.6a	11.8 ± 0.6a	11.5 ± 0.5a	9.8 ± 1.3a	2.82	0.084
Number of leaves	68.5 ± 1.8b	64.0 ± 2.7b	62.5 ± 5.4b	48.5 ± 6.3a	3.748	0.041
Culm Thickness	0.8 ± 0.04b	0.8 ± 0.06b	0.6 ± 0.03a	0.6 ± 0.05a	6.947	0.006
Crown Thickness	3.9 ± 0.4a	3.5 ± 0.2a	3.6 ± 0.1a	3.3 ± 0.1a	1.238	0.339
Root Length (cm)	36.8 ± 4.1a	32.9 ± 4.5a	28.5 ± 2.3a	28.3 ± 3.3a	1.231	0.341
Shoot Length (cm)	54.8 ± 4.2a	54.4 ± 0.6a	49.2 ± 4.2a	49.9 ± 4.1a	6.66	0.592
Root:Shoot Length Ratio	0.7 ± 0.06a	0.6 ± 0.09a	0.6 ± 0.05a	0.6 ± 0.10a	0.282	0.851
Shoot Fresh Mass (g)	40.3 ± 2.8c	37.5 ± 3.5c	26.0 ± 4.5b	11.0 ± 1.3a	17.026	< 0.001
Root Fresh Mass (g)	21.1 ± 2.3b	20.5 ± 0.1b	16.2 ± 2.7ab	7.2 ± 1.1a	11.842	0.001
Root:Shoot Fresh Mass Ratio	0.5 ± 0.03a	0.6 ± 0.05a	0.6 ± 0.02a	0.7 ± 0.1a	0.918	0.482
Shoot Dry Mass (g)	23.0 ± 1.0b	20.4 ± 2.3b	20 ± 3.1b	9.3 ± 1.1a	8.619	0.003
Root Dry Mass (g)	13.1 ± 0.4b	12.9 ± 1.2b	12.2 ± 1.5b	6.1 ± 1.0a	9.246	0.002
Root:Shoot Dry Mass Ratio	0.6 ± 0.01a	0.6 ± 0.03a	0.6 ± 0.03a	0.7 ± 0.16a	0.402	0.755
Culm node number	2.3 ± 0.3a	2.3 ± 0.3a	2.3 ± 0.3a	2.0 ± 0.0a	0.333	0.802
Internode Length	3.6 ± 0.2b	3.1 ± 0.2a	3.0 ± 0.2a	2.9 ± 0.2a	2.898	0.041
Leaf length (cm)	30.3 ± 0.9b	29.7 ± 0.9b	27.0 ± 1.3ab	26.3 ± 1.3a	3.197	0.025
Leaf width	1.7 ± 0.03b	1.7 ± 0.03b	1.1 ± 0.04a	1.1 ± 0.04a	29.058	< 0.001

Table 25: The impacts of different durations of water-limitation on the growth of mature *Brachiaria brizantha* line 780 plants. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	WW	7 DWL	15 DWL	30 DWL	F	p
Number of stems	17.0 ± 2.4a	14.0 ± 1.1a	13.8 ± 1.4a	11.5 ± 0.6a	2.114	0.152
Number of leaves	89.5 ± 17.4a	73.8 ± 9.7a	69.8 ± 10.6a	63.0 ± 2.9a	0.979	0.435
Culm Thickness	0.6 ± 0.03b	0.7 ± 0.03b	0.4 ± 0.03a	0.3 ± 0.03a	38.385	< 0.001
Crown Thickness	4.5 ± 0.2b	3.8 ± 0.1a	3.5 ± 0.2a	3.4 ± 0.2a	6.29	0.008
Root Length (cm)	26.8 ± 1.1a	27.2 ± 2.2a	25.8 ± 1.1a	22.3 ± 1.0a	2.275	0.132
Shoot Length (cm)	43.0 ± 2.6a	42.5 ± 4.0a	46.7 ± 2.2a	49.1 ± 1.4a	1.29	0.323
Root:Shoot Length Ratio	0.6 ± 0.06a	0.7 ± 0.08a	0.6 ± 0.05a	0.5 ± 0.02a	2.507	0.108
Shoot Fresh Mass (g)	77.7 ± 13.7d	44.6 ± 7.7c	24.1 ± 34.0b	13.7 ± 0.4a	12.161	0.001
Root Fresh Mass (g)	20.8 ± 5.2a	15.1 ± 4.3a	16.8 ± 2.0a	11.5 ± 0.8a	1.19	0.355
Root:Shoot Fresh Mass Ratio	0.3 ± 0.02a	0.3 ± 0.06a	0.7 ± 0.09b	0.8 ± 0.06b	21.066	< 0.001
Shoot Dry Mass (g)	29.6 ± 4.3c	17.8 ± 2.7b	17.5 ± 2.9b	11.1 ± 0.3a	6.836	0.006
Root Dry Mass (g)	11.4 ± 1.6a	11.0 ± 2.2a	9.1 ± 0.2a	9.0 ± 0.6a	0.8	0.518
Root:Shoot Dry Mass Ratio	0.4 ± 0.02a	0.6 ± 0.1ab	0.6 ± 0.1ab	0.8 ± 0.03b	5.742	0.011
Culm node number	4.3 ± 0.5b	4.8 ± 0.3b	3.0 ± 0.0a	2.8 ± 0.3a	10.529	0.001
Internode Length	4.8 ± 0.2c	4.4 ± 0.2c	3.6 ± 0.2b	3.0 ± 0.1a	13.682	< 0.001
Leaf length (cm)	27.8 ± 0.9a	26.9 ± 0.8a	27.0 ± 0.8a	27.3 ± 0.9a	0.249	0.862
Leaf width	2.1 ± 0.03b	1.9 ± 0.02ab	1.5 ± 0.03a	1.6 ± 0.03a	26.871	< 0.001

Table 26: The impacts of different durations of water-limitation on the growth of mature *Brachiaria brizantha* cv. Piata plants. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	WW	7 DWL	15 DWL	30 DWL	F	p
Number of stems	10.0 ± 1.1a	10.5 ± 1.3a	9.8 ± 0.6a	9.3 ± 0.9a	0.268	0.847
Number of leaves	61.3 ± 4.6c	53.3 ± 3.7b	52.0 ± 3.9b	42.0 ± 1.8a	4.943	0.018
Culm Thickness	0.6 ± 0.04a	0.6 ± 0.03a	0.5 ± 0.04a	0.5 ± 0.03a	1.522	0.259
Crown Thickness	3.9 ± 0.3a	3.5 ± 0.3a	3.6 ± 0.2a	3.0 ± 0.2a	2.292	0.13
Root Length (cm)	33.8 ± 1.6a	33.6 ± 1.9a	28.2 ± 1.2a	27.6 ± 2.3a	3.458	0.051
Shoot Length (cm)	88.6 ± 3.0a	89.0 ± 6.0a	79.5 ± 4.0a	73.8 ± 6.7a	2.081	0.156
Root:Shoot Length Ratio	0.4 ± 0.03a	0.4 ± 0.01a	0.4 ± 0.01a	0.4 ± 0.05a	0.194	0.898
Shoot Fresh Mass (g)	102.7 ± 4.1d	64.2 ± 2.9c	31.6 ± 3.6b	13.2 ± 1.1a	54.637	< 0.001
Root Fresh Mass (g)	32.5 ± 2.6c	25.2 ± 1.9b	23.4 ± 3.1b	8.0 ± 0.2a	20.473	< 0.001
Root:Shoot Fresh Mass Ratio	0.3 ± 0.02a	0.4 ± 0.04a	0.7 ± 0.07b	0.6 ± 0.06b	14.67	< 0.001
Shoot Dry Mass (g)	32.6 ± 1.2c	29.9 ± 0.9c	20.8 ± 2.0b	10.6 ± 1.0a	54.099	< 0.001
Root Dry Mass (g)	11.5 ± 0.6b	11.3 ± 0.9b	11.6 ± 1.3b	5.1 ± 0.2a	13.975	< 0.001
Root:Shoot Dry Mass Ratio	0.4 ± 0.01a	0.4 ± 0.03a	0.6 ± 0.05b	0.5 ± 0.07b	4.72	0.021
Culm node number	5.3 ± 0.5b	4.5 ± 0.3b	3.8 ± 0.3b	2.8 ± 0.5a	7.552	0.004
Internode Length	8.3 ± 0.4b	8.3 ± 0.4b	7.1 ± 0.4ab	6.0 ± 0.4a	6.461	< 0.001
Leaf length (cm)	39.4 ± 1.2a	36.3 ± 1.0a	36.1 ± 1.0a	36.0 ± 1.2a	2.497	0.062
Leaf width	1.7 ± 0.04b	1.4 ± 0.02a	1.3 ± 0.22a	1.2 ± 0.02a	2.826	0.041

Table 27: The impacts of different durations of water-limitation on the growth of mature *Brachiaria brizantha* line 473 plants. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	WW	7 DWL	15 DWL	30 DWL	F	p
Number of stems	17.7 ± 2.4a	21.0 ± 1.1a	16.7 ± 1.4a		0.39	0.693
Number of leaves	95.0 ± 17.4a	106.7 ± 9.7a	81.3 ± 10.6a		0.451	0.657
Culm Thickness	0.6 ± 0.03a	0.6 ± 0.03a	0.5 ± 0.03a		2.286	0.183
Crown Thickness	4.7 ± 0.2a	4.3 ± 0.1a	3.3 ± 0.2a		2.889	0.132
Root Length (cm)	29.0 ± 1.1a	33.5 ± 2.2a	28.0 ± 1.1a		2.687	0.147
Shoot Length (cm)	49.6 ± 2.6a	47.3 ± 4.0a	44.7 ± 2.2a		0.664	0.549
Root:Shoot Length Ratio	0.6 ± 0.02a	0.7 ± 0.04a	0.6 ± 0.02a		4.926	0.054
Shoot Fresh Mass (g)	106.3 ± 13.7c	77.3 ± 7.7b	33.5 ± 3.4a		5.279	0.048
Root Fresh Mass (g)	59.3 ± 5.2a	58.1 ± 4.3a	41.4 ± 2.0a		0.757	0.509
Root:Shoot Fresh Mass Ratio	0.5 ± 0.0a	0.7 ± 0.1a	1.3 ± 0.1a		0.823	0.483
Shoot Dry Mass (g)	30.6 ± 4.3b	29.6 ± 2.7b	22.2 ± 2.9a		16.216	0.004
Root Dry Mass (g)	16.1 ± 1.6a	18.9 ± 2.2a	12.7 ± 0.2a		3.68	0.091
Root:Shoot Dry Mass Ratio	3.7 ± 0.5a	4.0 ± 0.3a	3.7 ± 0.0a		0.393	0.691
Culm node number	3.9 ± 0.2a	3.9 ± 0.2a	3.9 ± 0.2a		0.005	0.995
Internode Length	0.6 ± 0.23a	0.5 ± 0.04a	0.6 ± 0.08a		0.125	0.885
Leaf length (cm)	26.1 ± 0.9a	25.5 ± 0.8a	25.0 ± 0.8a		0.472	0.625
Leaf width	1.9 ± 0.03b	1.9 ± 0.02b	1.5 ± 0.03a		47.158	<0.001

Table 28: The impacts of different durations of water-limitation on the growth of mature *Brachiaria brizantha* line 460 plants. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	WW	7 DWL	15 DWL	30 DWL	F	p
Number of stems	16.0 ± 2.0c	12.3 ± 0.7b	12.0 ± 0.6b	8.7 ± 0.9a	6.473	0.016
Number of leaves	78.3 ± 12.4a	56.0 ± 6.7a	55.7 ± 3.7a	51.0 ± 13.3a	1.55	0.275
Culm Thickness	0.6 ± 0.03b	0.5 ± 0.03b	0.3 ± 0.03a	0.3 ± 0.03a	26.25	< 0.001
Crown Thickness	3.7 ± 0.2a	3.5 ± 0.5a	3.5 ± 0.3a	3.2 ± 0.4a	0.317	0.813
Root Length (cm)	30.1 ± 3.0a	30.0 ± 2.3a	29.4 ± 0.7a	28.0 ± 2.8a	0.162	0.919
Shoot Length (cm)	36.6 ± 1.9a	36.2 ± 0.4a	35.5 ± 1.1a	35.2 ± 3.2a	0.1	0.958
Root:Shoot Length Ratio	0.8 ± 0.1a	0.8 ± 0.1a	0.8 ± 0.1a	0.8 ± 0.2a	0.002	1
Shoot Fresh Mass (g)	46.3 ± 7.0d	31.2 ± 2.8c	14.4 ± 1.7b	7.8 ± 3.6a	16.54	0.001
Root Fresh Mass (g)	21.0 ± 2.9c	16.1 ± 1.2b	12.1 ± 1.0b	5.0 ± 0.8a	15.809	0.001
Root:Shoot Fresh Mass Ratio	0.5 ± 0.01a	0.5 ± 0.04a	0.9 ± 0.05a	0.9 ± 0.31a	1.96	0.199
Shoot Dry Mass (g)	18.5 ± 2.7c	12.1 ± 1.7b	10.7 ± 1.2b	6.6 ± 3.0a	4.639	0.037
Root Dry Mass (g)	9.6 ± 1.2b	9.2 ± 0.2b	8.5 ± 0.3b	4.0 ± 0.9a	11.395	0.003
Root:Shoot Dry Mass Ratio	0.5 ± 0.02a	0.8 ± 0.1a	0.8 ± 0.1a	0.8 ± 0.3a	0.604	0.631
Culm node number	2 ± 0a	2 ± 0a	2 ± 0	2 ± 0a		
Internode Length	2.8 ± 0.2a	2.5 ± 0.2a	2.2 ± 0.2a	2.1 ± 0.3a	2.429	0.078
Leaf length (cm)	27.0 ± 0.8a	25.0 ± 0.7a	25.0 ± 0.7a	24.7 ± 0.8a	2.035	0.113
Leaf width	1.9 ± 0.02b	1.7 ± 0.06b	1.3 ± 0.03a	1.4 ± 0.03a	48.746	< 0.001

Table 29: The impacts of different durations of water-limitation on the growth of mature *Brachiaria brizantha* line 330 plants. Statistically significant differences ($p < 0.05$) within each measured variable between the different water-limitation treatments are indicated by different letters.

	WW	7 DWL	15 DWL	30 DWL	F	p
Number of stems	22.3 ± 1.3a	23.8 ± 1.7a	20.0 ± 2.3a	18.5 ± 1.8a	1.647	0.231
Number of leaves	109.0 ± 5.8c	116.8 ± 14c	91.5 ± 5.1b	81.3 ± 5.0a	3.715	0.042
Culm Thickness	0.7 ± 0.03b	0.6 ± 0.03b	0.5 ± 0.03ab	0.3 ± 0.03a	24.733	< 0.001
Crown Thickness	4.5 ± 0.4a	4.4 ± 0.2a	4.3 ± 0.1a	3.7 ± 0.2a	2.159	0.146
Root Length (cm)	30.8 ± 1.4c	28.3 ± 0.6bc	27.0 ± 1.3b	22.8 ± 0.9a	9.542	0.002
Shoot Length (cm)	38.9 ± 1.8a	43.4 ± 1.3a	39.6 ± 1.1a	38.3 ± 1.2a	2.831	0.083
Root:Shoot Length Ratio	0.8 ± 0.02b	0.7 ± 0.03b	0.7 ± 0.05b	0.6 ± 0.03a	6.749	0.006
Shoot Fresh Mass (g)	72.4 ± 5.7d	65.7 ± 5.0c	27.0 ± 0.9b	10.2 ± 1.3a	59.675	< 0.001
Root Fresh Mass (g)	54.7 ± 1.6c	54.2 ± 3.8c	28.7 ± 1.9b	15.4 ± 1.2a	68.236	< 0.001
Root:Shoot Fresh Mass Ratio	0.8 ± 0.09a	0.8 ± 0.02a	1.1 ± 0.11ab	1.6 ± 0.17b	10.743	0.001
Shoot Dry Mass (g)	26.7 ± 1.2c	26.1 ± 1.5c	19.3 ± 0.7b	7.4 ± 0.8a	64.813	< 0.001
Root Dry Mass (g)	20.3 ± 2.2c	18.9 ± 2.4b	17.0 ± 0.4b	11.3 ± 0.7a	5.87	0.01
Root:Shoot Dry Mass Ratio	0.8 ± 0.11a	0.7 ± 0.07a	0.9 ± 0.05a	1.6 ± 0.16b	13.355	< 0.001
Culm node number	3.3 ± 0.3bc	3.8 ± 0.3c	3.0 ± 0.0b	2.3 ± 0.3a	8.333	0.003
Internode Length	3.3 ± 0.1b	3.2 ± 0.1b	3.2 ± 0.1b	2.5 ± 0.1a	7.208	< 0.001
Leaf length (cm)	21.7 ± 0.7a	21.7 ± 0.7a	20.8 ± 0.7a	20.0 ± 0.5a	1.602	0.191
Leaf width	2.1 ± 0.05b	1.8 ± 0.02ab	1.6 ± 0.02a	1.6 ± 0.04a	38.652	< 0.001

4.3. Conclusion

Results from the current research trials indicate that *Brachiaria* display different degrees of water-limitation tolerance at different stages of development. Prior work on seed germination showed that seed germination stage in *Brachiaria* is not tolerant to water-limitation and even the slightest decrease in moisture availability could significantly decrease the number of seeds germinating. The work done at the seedling stage shows that all of the *Brachiaria* lines/cultivars displayed biomass and leaf size reductions under water-limited conditions, but the magnitude and timing of when these decreases were visible differed. Cultivars and lines like Mulato II, Xaraes, Line 473 and Line 710 were relatively more resilient to water-limitation, maintaining leaf numbers and root traits for longer. In contrast, cultivars and lines like Sabia, Piata, Basilisk and line 460 were more sensitive to water-limited conditions, showing early and strong biomass and leaf area reductions. As mature plants, several cultivars and lines had some degree of phenotypic plasticity as the main mechanism to cope with water-limitation. Cultivars such as Basilisk, Piata and Lines 780 and 330 show significant increases in root:shoot ratios despite biomass losses. Basilisk also maintains relatively higher root length into the water-limited period, while Line 780 and Line 330 show strong structural thinning and clear allocation adjustments. Piata on the other hand, couples major biomass loss with significant Root:Shoot ratio increases. These traits for these cultivars and lines fit a tolerant or avoidance learning ideotype under prolonged water-limitation stress. On the other hand, cultivars and lines such as Marandu, Sabia, 710 and 460 showed higher sensitivity with consistent biomass losses, leaf width narrowing and no significant root:shoot shifts. Therefore, this work showed that although there are differences in water-limitation tolerance amongst *Brachiaria* genotypes, there are some lines and cultivars such as Line 710, 780, 460, Basilisk and Sabia which maintain their seedling tolerance to water-limitation into maturity. Others such as Mulato II, Piata and Marandu adjust their strategies as they mature, either becoming more conservative (Mulato II) or more sensitive (Piata and Marandu).. This is because at different stages of development, plants are often exposed to different degrees of a particular stress, impacting differently on their survival. This is due to the ecological niche requirements of plants varying as they develop, resulting in changes in the plant traits that determine the success with which a plant can survive in a specific environment (Grubb 1977).

In conclusion, the different genotypes evaluated displayed an array of morphological mechanisms to cope with water-limitation. Certain genotypes were able to allocate more resources to root growth, which, under water-limited conditions, will allow for access to more water and nutrients as soil moisture decreases. These genotypes should be prioritised for further drought tolerance evaluation under field conditions. However, further scrutinization of the results are needed to get a better indication of drought tolerance.

CHAPTER 5:

Agronomic Characterisation and nutritional quality of exotic *Brachiaria* breeding lines

5.1. Materials and Methods

This study was conducted at Roodeplaats Experimental Farm of the Agricultural Research Council (ARC), which is located in the northern region of Gauteng province, South Africa. It is located between the longitude of 25°36'08.2"S, and the latitude of 28°21'01.3"E, and at an altitude of 1168 m above sea level. Monthly average of surface air temperature for the study area was hot (16 °C to 32 °C) during the summer months, and cold (1 °C to 23 °C) in winter, with some occasional frost. The study area experienced unimodal rainfall with uneven distribution during the spring and summer months therefore, leading to the occurrence low rainfall and high rainfall intensity taking place successively during the raining season as shown in Table 1. During the study period, the average annual rainfall of the area was 809 mm, and a range of 757 mm to 860 mm (AGROMET, 2022).

Table 30: Average monthly minimum and maximum temperatures (°C) and monthly total rainfall over the two years of *Brachiaria* evaluation at Roodeplaats Experimental Farm (AGROMET, 2022)

Temperature (°C)												
Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
2020	17/30	16/31	14/29	12/26	6/22	1/20	1/21	11/25	10/29	14/30	15/31	16/34
2021	17/36	15/36	11/37	7/37	4/28	2/23	0/21	5/25	9/30	11/28	14/31	15/28

Rainfall (mm)												
Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
2020	118.87	37.84	68.08	128.51	0	2.03	0	0.51	5.33	72.90	213.08	110.75
2021	160.28	57.15	41.4	75.69	0.51	0.76	0	0	46.73	60.7	123.95	293.62

5.1.1 Field plot preparation

Soil was randomly sampled from field plots using a steel soil augur at 15 and 30 cm soil depth, and a composite sample was collected at each of the two depths for chemical composition analyses following the composite sampling method outlined by Carter and Gregorich (2008). The collected soil samples were air-dried and sieved through a 2 mm diameter mesh following the protocol as described by Kleinman et al. (2002). For the determination of soil phosphorus (P) content, the Bray-1 extraction protocol of Bray and Kurtz (1945) was followed as modified by Kleinman et al. (2001). To determine the cation exchange capacity of the soil, 30 ml of 0.1 mol dm⁻³ barium chloride was added to 2.5 g of air-dried soil sample contained in a hermetically sealed polyethylene centrifuge tube to prepare an extract in accordance to the protocol by Jarenko et al. (2014). Magnesium (Mg²⁺) concentration was determined from extracts using inductively coupled plasma optical emission spectrometry (ICP-OES), and the difference between the added Mg²⁺ ions and the Mg²⁺ ions, which remained in the solution, was

designated as the CEC value. The values of aluminium (Al^{3+}), iron (Fe^{2+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), manganese (Mn^{2+}), potassium (K^+) and sodium (Na^+), which are collectively referred to as exchangeable cation contents were determined by ICP-OES using the barium chloride method followed by Jarenko et al. (2014). To determine the soil pH value, distilled water was mixed with air-dried soil samples in a volume-to-volume ratio of 1:2.5, and blended to a uniform mixture. A probe connected to a calibrated pH meter was inserted in the mixture, and the value on the pH meter was recorded. Soil particle size was determined by following the Bouyoucos Hydrometer method (Sikora and Moore, 2004). Field plots were prepared for seedling establishment by using a Husqvarna rear-tine cultivator (TR430 Cultivator, Husqvarna SA (Pty) Ltd, Pietermaritzburg, South Africa). Results from the soil analyses are presented in Table 31.

Table 31: Soil characteristics for the study site

Soil parameters	Sampling depth (cm)	
	15 cm	30 cm
P (mg/kg)	10.9	2.5
K (mg/kg)	338	170
Ca (mg/kg)	2660	2870
Mg (mg/kg)	644	628
Na (mg/kg)	31	87.4
pH	7.06	7.14
Total Cations (cmol $+/kg$)	19.57	20.31

5.1.2. Experimental design and evaluation of *Brachiaria* genotypes

A total of 11 grass cultivars composed of two locally adapted grass species and nine *Brachiaria* grass cultivars were used in this study. The two locally adapted grass species, *Chloris gayana* cv. Sakambora and *Panicum maximum* cv. PUK8 were obtained from Agricol (Pty) Ltd, Silverton, South Africa. Two of the nine *Brachiaria* grass are commercial cultivars, *B. brizantha* cv. Marandu and *B. hybrid* cv. Sabia (*B. ruziziensis* × *B. decumbens* × *B. brizantha*) and were sourced from Barenbrug South Africa Seeds (Pty) Ltd, Pretoria, South Africa. Two other commercial *Brachiaria* accessions, *B. brizantha* cv. Piata and *B. decumbens* cv. Basilisk were obtained from the International Livestock Research Institute genebank (ILRI Kenya) based in Nairobi, Kenya. Five breeding lines, which are *B. brizantha* cv. CIAT 16169, CIAT 6384, CIAT 16483, CIAT 16339, and CIAT 16320, were obtained from ILRI based in Cali, Colombia. The two locally adapted grass species were designated as controls, and the nine *Brachiaria* grass cultivars were designated as treatments.

To prepare for seed germination, a seed germination medium, hygromix (Hygrotech Seed Pty Ltd, Pretoria, South Africa) was mixed with water in a weight/weight ratio of 1:2. The hygromix mixture was then filled into a multi pot cavity plastic seedling tray (Plastilon Packaging Pty Ltd, Centurion, South

Africa) with an individual cavity volume of 60 mL. Seeds from each of the cultivars were drilled into hygromix-containing seedling tray at a seeding rate of one seed per seedling tray cavity. The seedling trays were immediately placed in a tunnel greenhouse under natural light with average ambient temperature of $25 \pm 2^\circ\text{C}$ on a seedling tray platform with a matted surface at a height of 60 cm from the floor. All seedling trays were watered at every second day to achieve field capacity and was continued for 21 days. At day 21 of germination, the germinated seedlings of all grass cultivars were transplanted to the prepared 8 m^2 ($4 \text{ m} \times 2 \text{ m}$) individual field plots and were arranged in a randomized complete block design (RCBD) with three replicates per cultivar. The seedlings were transplanted into field plots at 0.5 m inter-row spacing and 0.3 m intra-row spacing and a spacing of 1.0 m between field plots. Weeds were removed manually within and between plots using a hand-hoe. The established grasses were evaluated for two calendar years under rainfed conditions without fertilizer application for the whole of the evaluation period. Agronomic performance (plant counts, plot cover, plant height, plant spread and plant tillers) of the *Brachiaria* grass varieties and control grass species were recorded at harvesting as described by Ondiko *et al.* (2016). The biomass yield of all cultivars was recorded at 50% flowering to enable comparison of the cultivars at the same phenological stage by cutting the grasses at 5 cm stubble height. Accession adaptability was determined using both plant survival after exposure to low winter temperatures and incidences of disease/pests. A plant survival percentage of at least 75 % was declared an indication of adaptability, which is similar to the 75 % germination percentage required to declare viability in grass seed.

5.1.3. Chemical analysis

Grass samples from each grass accession were collected (1 kg) over a period of 2 years. Samples of each accession were oven-dried at 60°C for 72 hours. After drying, the grass samples were ground in a hammer mill through a 1mm screen (DIETZ hammer mill, Type WRB 90 LB /4P, Model No. 2858490, Dettingen-Teck, Germany) for chemical analyses. The ground samples of each grass accession were subjected to chemical analyses to determine both the ADF and NDF following the procedure developed by Van Soest *et al.* (1991). The NDF was determined by hydrolyses of starch using heat-stable α -amylase (Sigma-Aldrich Co. LTD. Gillingham, UK, No. A-1278) and Sodium Lauryl Sulphate which formed soluble complexes with proteins. Triethylene glycol removed non-fibre soluble material, and Aminopolycarboxylic acid, ethylenediaminetetraacetic acid (EDTA) prevented insoluble calcium-pectin matrices from forming, and thus dissolving pectin according to EN ISO 16472 (2006). The ADF was determined by treating samples with an acid detergent solution, which is composed of Cetyltrimethylammonium bromide and concentrated sulphuric acid according to EN ISO 13906 (2008). The fiber results were used to calculate the digestible dry matter (DDM), metabolisable energy (ME), total digestible nutrients (TDN), digestible forage energy (DFE), net energy for lactation (NEL), net energy for maintenance (NEM) and net energy for gain/growth (NEG) using equations 1 to 7. Nitrogen levels of grass accessions were measured using the Kjeldahl method according to the Association of Official Analytical Chemists (AOAC) (1990, ID 968.06), and the crude protein (CP) content was derived by multiplying the nitrogen concentration by 6.25, as per standard calculation. The method used to

analyse the fat content involved utilizing ether extraction within the Solvent Extractor System - Tecator Soxtec System HT6, which encompassed a three-step extraction process involving boiling, rinsing, and recovery according to AOAC (2000, ID 920.39). The grass samples were combusted at 550°C in a muffle furnace and subsequently cooled in a desiccator to determine the ash content according to the AOAC (2000, ID 968.08) The ground grass samples underwent acid digestion to determine the phosphorus (P) concentration using a flow injection auto analyser following the protocol outlined by Allen et al. (1974). The assessment of macro-minerals sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca) was conducted using atomic absorption spectrophotometry following AOAC methods (2000, ID 929.03, 920.09, and 956.01, respectively). Similarly, the analysis of micro-minerals including copper (Cu), manganese (Mn), and zinc (Zn) followed AOAC methods (2000, ID 921.02, 953.03, and 941.03, respectively).

Equation 1: DDM (%) = 88.9 - (ADF × 0.779) (Rasby et al. 2008)

Equation 2: ME (Mcal kg⁻¹ DM) = (1.01 × DFE) - 0.45 (Meissner et al. 2000)

Equation 3: TDN (%) = 87.84 - (0.7 × ADF) (Schroeder 2009)

Equation 4: DFE (Mcal kg⁻¹ DM) = 0.04409 × TDN (Meissner et al. 2000)

Equation 5: NEL (Mcal kg⁻¹ DM) = 1.044 - (0.0119 × %ADF) (Rasby et al. 2008)

Equation 6: NEM (Mcal kg⁻¹ DM) = [(1.37 × ME) - (0.3042 × ME) + (0.051 × ME)] - 0.508 (Rasby et al. 2008)

Equation 7: NEG (Mcal kg⁻¹ DM) = [(1.42 × ME) - (0.3836 × ME) + (0.0593 × ME)] - 0.7484 (Rasby et al. 2008)

5.1.4. Statistical analysis

Data on the adaptability and agronomic performance of *Brachiaria* grass accessions and control grass species were subjected to analysis of variance (ANOVA) using general linear model procedure (Proc GLM) of Statistical Analysis Systems (SAS, 2017). Where there was a significant difference (P≤0.05) between the treatments means, Tukey's honestly significant difference was used for mean separation. After separation of the samples for nutritional characterization into low and high rainfall periods, the results were analyzed using a one way analyses of variance (ANOVA) when more than two seasons data were available and independent sample t-test where only two seasons of data were available. These data were analyzed using SPSS v. 21.

5.2. Results and Discussion

The agronomic characterization of the forage genetic resources is given in tables 32 (year 1) and 33 (year 2). In general, plant survival was good with 100 % of the plants surviving in the first year. In the second year however, although most plants survived, there was some mortality, with CIAT accession 16483 having nearly 40 % mortality. The mortality was attributed to the frost conditions experienced in the second year. However, because most of the production areas where these forages are proposed to be planted experiences frost, this result suggests that this accession is not well adapted to the prevailing agro-ecological conditions and should be excluded from inclusion in breeding programs. Although plant

survival was good, there were minor occurrence of plant disease such as grey leaf spot and necrosis, although these did not affect the DM yield of the grasses.

Total biomass production in year 1 ranged between 3.9 and 13.8 tons/Ha (Table 32) while in year two it ranged between 6.6 and 26.7 tons/Ha. In both years the highest biomass yields were recorded for commercial varieties, with *B. decumbens* cv. Basilisk and *B. brizantha* cv. Marandu having the highest yields in year 1 and year 2, respectively. From the results, it was clear that the production in the second year of growth was significantly greater than in year 1, even though for some of the accessions evaluated, the plants reached the stage for harvesting more times than in the second year. In the first year, the harvesting intervals ranged from 1 to 6 times, while in the second year harvesting ranged from 1 to 4 times. The increase in biomass yields can be attributed mainly the increase in the number of tillers from the first to the second growing season. Where a significant increase in the number of tillers did not lead to an increase in DM yield, a reduction in stem thickness of the grasses was observed. The highest DM yielding *Brachiaria* grass accession all have thick, long, and hard stems, and this may have implications on their nutrient composition, voluntary feed intake and performance by ruminants.

Although biomass yields increased from year 1 to year 2, the quality of the forages produced decreased (Table 34 and Table 35). The nutritional quality of the forages harvested over the two years were grouped into samples collected in the high rainfall period and those collected in the low rainfall period. Here samples in each year for each season were grouped to obtain an average quality over the high rainfall period vs. low rainfall period. Generally crude protein decreased from year 1 (Table 34) to year 2 (Table 35), with the lowest CP content being 3 % in year 2 for PIATA. The majority of the samples analyzed contained crude protein in excess of 6 – 8 % which is the minimum requirements for maintenance by livestock. However, several of the accessions evaluated contained a CP of approximately 12 – 13 % and thus is able to maintain highly productive livestock herds. The decreases in protein content in the forages can be attributed to the reduction in N in the soils due to the lack of fertilization. In the second year, more visual nutrient deficiencies were visible.

When considering the digestible dry matter content (Table 35), the DDM content in the samples ranged between 45.7 % and 66.5 %, with the majority of the samples having a DDM below 60%. Generally, forages with a DDM below 60 % is indicative of forages with low digestibility and energy content. This corresponds to the observations from above which states that the highest DM yielding *Brachiaria* grass accessions all have thick, long, and hard stems, and this may have implications on their nutrient composition, voluntary feed intake and performance by ruminants. From the results the metabolizable energy (ME) content of the forages ranged from 2.2 Mcal/kg DM (9.2 MJ/kg DM) and 3 Mcal/kg DM (12.6 MJ/kg DM). Therefore, although not very high, the ME content of the forages is sufficient for lambs up to 20 kg (3.9 – 10.5 MJ/kg DM) as well as dry ewes of 40 – 60 kg (7.6 – 10.2 MJ/kg DM). However, the energy content of these forages are not sufficient to sustain pregnant ewes which requires an energy content of 14.5 – 17.7 MJ/kg DM, or lactating ewes which requires an energy content of 15.5 – 19.4 MJ/kg DM.

With regard to the mineral nutrient content of the forages (Table 35), in the second growing season, there was an increased occurrence of symptoms of nutrient deficiency in the grass leaves such purple-colored leaves which is a sign of phosphorus deficiency as well as yellowing of the leaves, which is a sign of chlorosis and could possibly be attributed to nitrogen deficiencies (Figure 3). Although these deficiencies were visible, the P content in the forages ranged from 0.1 % to 0.3 %. Therefore, the majority of the samples contained P in concentrations within the recommended range of 1.6 g/kg to 3.7 g/kg. Generally, the mineral nutrient content of the forages was in excess of the recommended concentrations for livestock. Copper concentrations were mostly below the recommended ranges of 0.005 to 0.1 g/kg. Calcium concentrations ranged between 0.1 % and 1.6 % with the majority of the forages having Ca concentrations within the recommended range of 1.8 and 10 g/kg. Iron concentrations ranged between 0.07 g/kg and 0.73 g/kg. The majority of the samples thus contained Fe concentrations in excess of the upper range of what is required by livestock (0.03 – 0.1 g/kg). Potassium concentrations ranged from 13 – 30 g/kg. These results indicate that the majority of the forages contained K concentrations close to or in excess of the upper requirement limits for livestock. The majority of the forages evaluated contained Na in concentrations which fall within the recommended ranges of 0.4 g/kg and 1.8 g/kg. However, *P. maximum* and *C. gayana* contained Na in excess of the maximum permissible levels. Several of the forages evaluated contained Zn concentrations below the recommended range of 0.02 – 0.05 g/kg.

Table 32: Agronomic performance, adaptability and phenological attributes of *Brachiaria* grass accessions for year 1

Accessions	Variables									
	DM (g/kg)	DM yield (tons/ha/harvest)	Total DM yield (tons/ha/year)	Plot cover (%)	Plant survival (%)	Disease/pests (1 – 10)	Regrowth rate (No. of harvests/year)	Plant height (cm)	Plant spread (cm)	Plant tillering
CIAT 16169	227 ± 1 ^a	0.8 ± 0.1 ^{ab}	3.9 ± 0.5 ^{ab}	55.7 ± 6.0 ^{ab}	100.0	1.0 ± 0.0 ^a	6.0 ^c	38.0 ± 1.7 ^a	70 ± 4	52 ± 4 ^a
CIAT 6384	258 ± 2 ^{ab}	1.2 ± 0.3 ^{ab}	5.9 ± 1.4 ^{bab}	51.8 ± 6.4 ^a	100.0	1.0 ± 0.0 ^a	6.0 ^c	32.0 ± 5.0 ^a	73 ± 6	101 ± 13 ^{ab}
Basilisk	250 ± 1 ^{ab}	3.4 ± 0.2 ^{bc}	13.8 ± 0.6 ^d	77.3 ± 7.6 ^{ab}	100.0	2.33 ± 0.12 ^c	5.0 ^d	28.3 ± 4.7 ^a	95 ± 12	136 ± 13 ^b
CIAT 16483	245 ± 1 ^{ab}	2.3 ± 0.6 ^{ab}	7.0 ± 1.8 ^{abc}	59.0 ± 5.0 ^{ab}	100.0	1.0 ± 0.0 ^a	5.0 ^d	53.3 ± 6.1 ^{ab}	96 ± 22	69 ± 15 ^{ab}
P. maximum	242 ± 2 ^{ab}	3.4 ± 1.1 ^{bc}	10.1 ± 3.3 ^{cd}	62.0 ± 8.2 ^{ab}	100.0	1.0 ± 0.0 ^a	5.0 ^d	37.0 ± 4.3 ^a	83 ± 5	46 ± 8 ^a
CIAT 16339	239 ± 1 ^{ab}	3.3 ± 0.6 ^{bc}	9.9 ± 1.9 ^{cd}	64.3 ± 5.1 ^{ab}	100.0	1.0 ± 0.0 ^a	4.0 ^c	56.6 ± 5.8 ^{ab}	88 ± 16	57 ± 15 ^a
C. gayana	242 ± 2 ^{ab}	2.3 ± 0.6 ^{abc}	6.9 ± 1.8 ^{abc}	70.0 ± 6.6 ^{ab}	100.0	1.0 ± 0.0 ^a	5.0 ^d	35.0 ± 5.6 ^a	69 ± 3	78 ± 20 ^{ab}
Piata	311 ± 2 ^c	4.3 ± 1.0 ^{cd}	8.6 ± 2.1 ^{abc}	82.0 ± 11.5 ^{bc}	100.0	1.0 ± 0.0 ^a	2.0 ^b	55.0 ± 17.4 ^{ab}	116 ± 18	60 ± 6 ^a
Marandu	307 ± 1 ^c	3.2 ± 0.6 ^{bc}	6.4 ± 1.3 ^{abc}	81.0 ± 3.6 ^{bc}	100.0	1.0 ± 0.0 ^a	2.0 ^b	28.3 ± 4.0 ^a	95 ± 16	58 ± 14 ^a
CIAT 16320	301 ± 0 ^c	7.5 ± 2.1 ^d	7.5 ± 2.1 ^{abc}	81.6 ± 7.6 ^{bc}	100.0	1.67 ± 0.12 ^b	1.0 ^a	96.7 ± 30.4 ^c	92 ± 30	50 ± 17 ^a
Sabia	267 ± 4 ^{ab}	6.6 ± 1.4 ^d	6.6 ± 1.4 ^{abc}	88.3 ± 10.4 ^c	100.0	1.73 ± 0.31 ^b	1.0 ^a	38.8 ± 3.8 ^a	88 ± 21	110 ± 14 ^{ab}
<i>p</i> – value (Trt.)	<0.001	<0.001	<0.001	<0.001	0.026	<0.001	<0.001	<0.001	0.099	0.004
<i>p</i> – value (Blk.)	0.253	0.614	0.365	0.830	0.489	0.172	-	0.986	0.941	0.598

Table 33: Agronomic performance, adaptability and phenological attributes of *Brachiaria* grass accessions for year 2

Accessions	Variables									
	DM (g/kg)	DM yield (tons/ha/harvest)	Total DM yield (tons/ha/year)	Plot cover (%)	Plant survival (%)	Disease/pests (1 – 10)	Regrowth rate (No. of harvests/year)	Plant height (cm)	Plant spread (cm)	Plant tillering
CIAT 16169	307 ± 1 ^{ab}	2.2 ± 0.3 ^{ab}	8.8 ± 1.1 ^{ab}	47.3 ± 2.1 ^{ab}	94 ± 10 ^{ab}	1.0 ± 0.0 ^a	4.0 ^d	31.0 ± 1.4 ^a	78 ± 3	114 ± 14 ^{ab}
CIAT 6384	292 ± 1 ^{ab}	2.9 ± 0.4 ^{ab}	11.4 ± 1.5 ^{ab}	69.5 ± 16.9 ^{bc}	100 ± 0 ^b	1.0 ± 0.0 ^a	4.0 ^d	29.8 ± 4.0 ^a	69 ± 3	170 ± 29 ^b
Basilisk	285 ± 1 ^{ab}	3.6 ± 0.5 ^{bc}	10.9 ± 1.6 ^{ab}	62.8 ± 6.9 ^{bc}	100 ± 0 ^b	1.0 ± 0.0 ^a	3.0 ^c	34.1 ± 4.0 ^{ab}	80 ± 7	278 ± 14 ^c
CIAT 16483	288 ± 1 ^{ab}	1.7 ± 0.6 ^{ab}	6.6 ± 2.2 ^a	30.8 ± 1.7 ^a	61 ± 40 ^a	1.0 ± 0.0 ^a	4.0 ^d	43.6 ± 1.6 ^{bc}	99 ± 11	180 ± 58 ^{bc}
P. maximum	202 ± 4 ^a	3.2 ± 1.2 ^{bc}	6.6 ± 1.9 ^a	55.0 ± 7.6 ^{ab}	100 ± 0 ^b	1.0 ± 0.0 ^a	3.0 ^c	45.8 ± 12.7 ^{ab}	96 ± 20	99 ± 24 ^{ab}
CIAT 16339	267 ± 1 ^{ab}	3.3 ± 1.1 ^{bc}	9.6 ± 3.3 ^{ab}	61.7 ± 7.3 ^{ab}	100 ± 0 ^b	1.4 ± 0.3 ^b	3.0 ^c	51.0 ± 9.0 ^{bc}	96 ± 11	147 ± 41 ^{ab}
C. gayana	282 ± 2 ^{ab}	2.6 ± 0.6 ^{bc}	10.2 ± 2.3 ^{ab}	52.5 ± 6.6 ^{ab}	100 ± 0 ^b	1.0 ± 0.0 ^a	4.0 ^d	47.5 ± 4.4 ^{ab}	82 ± 1	163 ± 27 ^{ab}
Piata	356 ± 2 ^c	10.5 ± 3.2 ^{cd}	21.1 ± 6.5 ^{cd}	68.3 ± 16.7 ^{bc}	100 ± 0 ^b	1.0 ± 0.0 ^a	2.0 ^b	62.8 ± 10.7 ^{bc}	105 ± 20	74 ± 22 ^a
Marandu	327 ± 3 ^{bc}	13.4 ± 3.4 ^{cd}	26.7 ± 6.8 ^d	74.2 ± 13.8 ^{bc}	100 ± 0 ^b	1.1 ± 0.1 ^a	2.0 ^b	66.9 ± 27.6 ^{bc}	113 ± 23	124 ± 56 ^{ab}
CIAT 16320	367 ± 3 ^b	10.1 ± 2.6 ^{cd}	20.2 ± 5.3 ^{cd}	81.6 ± 16.3 ^{bc}	100 ± 0 ^b	1.0 ± 0.0 ^a	2.0 ^b	90.1 ± 14.6 ^c	104 ± 31	72 ± 22 ^a
Sabia	317 ± 1 ^{bc}	22.0 ± 5.4 ^d	22.0 ± 5.4 ^{cd}	86.7 ± 12.6 ^c	100 ± 0 ^b	1.0 ± 0.0 ^a	1.0 ^a	79.3 ± 48.7 ^c	88 ± 21	124 ± 35 ^{ab}
<i>p</i> – value (Trt.)	0.002	<0.001	<0.001	0.003	<0.001	0.004	<0.001	0.013	0.151	<0.0001
<i>p</i> – value (Blk.)	0.731	0.709	0.491	0.781	0.436	0.585	-	0.786	0.993	0.516

Table 34: Nutritional quality of different forage genetic resources. Comparisons were made within each accession between years and seasons and not between different accessions. Statistically significant differences are indicated by different lower case letters.

	ADF (%)	NDF (%)	Ash (%)	Fat (%)	Protein (%)	Moisture (%)	DDM (%)	TDN (%)	DFE (MCal/kg DM)	ME (MCal/kg DM)	NEL (MCal/kg DM)	NEM (MCal/kg DM)	NEG (MCal/kg DM)	
Basilisk	Year 1_High Rainfall	33.7 ± 0.6b	61.5 ± 0.6b	11.8 ± 0.5a	1.8 ± 0.1a	13.4 ± 0.5b	7.3 ± 0.09b	62.7 ± 0.5b	74.3 ± 0.5b	3.3 ± 0.02b	2.9 ± 0.02b	0.6 ± 0.01b	2.7 ± 0.02b	2.4 ± 0.02b
	Year 1_Low Rainfall	47.9 ± 1.3c	60.3 ± 1.2ab	11.1 ± 1.0a	2.7 ± 0.1c	12.1 ± 0.7b	8.7 ± 0.2c	51.6 ± 1.0a	64.3 ± 0.9a	2.8 ± 0.04a	2.4 ± 0.04a	0.5 ± 0.02a	2.2 ± 0.04a	1.9 ± 0.04a
	Year 2_High Rainfall	35.5 ± 0.3b	68.7 ± 0.5c	10.3 ± 0.3a	1.9 ± 0.1ab	6.3 ± 1.3a	8.1 ± 0.03c	61.2 ± 0.3b	73.0 ± 0.2b	3.2 ± 0.01b	2.8 ± 0.01b	0.6 ± 0.004b	2.6 ± 0.01b	2.3 ± 0.01b
	Year 2_Low Rainfall	28.8 ± 0.8a	57.0 ± 1.8a	11.5 ± 0.8a	2.3 ± 0.2bc	6.4 ± 0.5a	4.7 ± 0.1a	66.5 ± 0.6c	77.7 ± 0.6c	3.4 ± 0.02c	3.01 ± 0.02c	0.7 ± 0.009c	2.9 ± 0.03c	2.5 ± 0.03c
	F _(3,12)	95.838	18.9	0.829	8.666	19.951	284.801	95.838						
	p	< 0.001	0.001	0.514	0.007	< 0.001								
C. gayana	Year 1_High Rainfall	40.4 ± 1.0a	75.9 ± 0.7bc	9.5 ± 0.4a	1.7 ± 0.2a	10.5 ± 0.6a	8.2 ± 0.2b	57.4 ± 0.8a	69.5 ± 0.7a	3.1 ± 0.03a	2.6 ± 0.03a	0.6 ± 0.01a	2.4 ± 0.04a	2.2 ± 0.03a
	Year 1_Low Rainfall	55.5 ± 9.8a	73.7 ± 1.3b	12.7 ± 1.0b	2.9 ± 0.5a	12.2 ± 2.4a	9.1 ± 0.3b	45.7 ± 7.7a	59.0 ± 6.9a	2.6 ± 0.30a	2.2 ± 0.30a	0.4 ± 0.10a	1.9 ± 0.30a	1.6 ± 0.30a
	Year 2_High Rainfall	37.4 ± 3.7a	77.9 ± 0.1c	9.4 ± 0.3a	2.0 ± 0.1a	7.7 ± 0.2a	8.4 ± 0.07b	59.8 ± 2.9a	71.6 ± 2.6a	3.2 ± 0.10a	2.7 ± 0.10a	0.6 ± 0.04a	2.6 ± 0.10a	2.3 ± 0.10a
	Year 2_Low Rainfall	37.8 ± 0.7a	70.9 ± 0.3a	10.8 ± 0.6ab	2.0 ± 0.1a	9.5 ± 0.6a	4.6 ± 0.07a	59.4 ± 0.5a	71.3 ± 0.5a	3.1 ± 0.02a	2.7 ± 0.02a	0.6 ± 0.01a	2.5 ± 0.02a	2.2 ± 0.02a
	F _(3,12)	2.613	17.261	5.517	3.574	2.247	98.433	2.613	2.613	2.613	2.613	2.613	2.613	2.613
	p	0.123	0.001	0.024	0.066	0.16	< 0.001	0.123	0.123	0.123	0.123	0.123	0.123	0.123
CIAT16169	Year 1_High Rainfall	32.7 ± 1.0a	62.2 ± 2.1a	10.1 ± 0.3b	2.3 ± 0.2bc	13.6 ± 0.9b	7.6 ± 0.2b	63.4 ± 0.8c	74.9 ± 0.7c	3.3 ± 0.03c	2.9 ± 0.03c	0.7 ± 0.01c	2.7 ± 0.03c	2.4 ± 0.03c
	Year 1_Low Rainfall	38.0 ± 0.6b	66.3 ± 0.5b	10.1 ± 0.2b	2.9 ± 0.1c	13.4 ± 1.2b	8.6 ± 0.1b	59.3 ± 0.5b	71.2 ± 0.4b	3.1 ± 0.02b	2.7 ± 0.02b	0.6 ± 0.01b	2.5 ± 0.02b	2.2 ± 0.02b
	Year 2_High Rainfall	42.4 ± 1.3c	74.4 ± 0.8c	8.9 ± 0.3a	1.9 ± 0.03ab	6.9 ± 0.2a	8.0 ± 0.3b	55.9 ± 1.0a	68.2 ± 0.9a	3.0 ± 0.04a	2.6 ± 0.04a	0.5 ± 0.02a	2.4 ± 0.05a	2.1 ± 0.04a
	Year 2_Low Rainfall	42.0 ± 0.9c	71.1 ± 0.4c	8.5 ± 0.1a	1.4 ± 0.3a	7.1 ± 1.8a	4.3 ± 0.05a	56.2 ± 0.7a	68.4 ± 0.6a	3.0 ± 0.03a	2.6 ± 0.03a	0.5 ± 0.01a	2.4 ± 0.03a	2.1 ± 0.03a
	F _(3,12)	21.364	21.12	14.153	8.542	10.028	107.442	21.364						
	p	< 0.001	< 0.001	0.001	0.007	0.004	< 0.001							
CIAT16320	Year 1_Low Rainfall	42.3 ± 1.7b	71.8 ± 0.8b	7.4 ± 0.3a	1.8 ± 0.2a	7.4 ± 0.2b	7.9 ± 0.20b	55.9 ± 1.3a	68.2 ± 1.2a	3.0 ± 0.05a	2.6 ± 0.05a	0.5 ± 0.02a	2.4 ± 0.06a	2.1 ± 0.06a
	Year 2_High Rainfall	48.5 ± 2.3b	80.0 ± 1.3c	6.5 ± 0.1a	1.5 ± 0.2a	2.9 ± 0.2a	7.7 ± 0.01b	51.1 ± 1.8a	63.9 ± 1.6a	2.8 ± 0.07a	2.4 ± 0.07a	0.5 ± 0.03a	2.2 ± 0.08a	1.9 ± 0.08a
	Year 2_Low Rainfall	32.7 ± 1.5a	60.8 ± 2.1a	11.2 ± 0.4b	3.5 ± 0.3b	6.9 ± 0.5b	4.4 ± 0.05a	63.4 ± 1.2b	74.9 ± 1.1b	3.3 ± 0.05b	2.9 ± 0.05b	0.7 ± 0.02b	2.7 ± 0.05b	2.4 ± 0.05b
	F _(2,9)	18.138	42.196	72.512	30.32	66.295	399.306	18.138						
p	0.003	< 0.001	< 0.001	0.001	< 0.001	< 0.001	0.003							

Table 34: Continue

	ADF (%)	NDF (%)	Ash (%)	Fat (%)	Protein (%)	Moisture (%)	DDM (%)	TDN (%)	DFE (MCal/kg DM)	ME (MCal/kg DM)	NEL (MCal/kg DM)	NEM (MCal/kg DM)	NEG (MCal/kg DM)	
CIAT16339	Year 1_High Rainfall	33.0 ± 1.4a	61.6 ± 1.6a	10.5 ± 0.3a	1.8 ± 0.1a	11.0 ± 0.6bc	7.4 ± 0.2b	63.2 ± 1.1a	74.8 ± 1.0a	3.3 ± 0.04a	2.9 ± 0.04a	0.7 ± 0.02a	2.7 ± 0.05a	2.4 ± 0.05a
	Year 1_Low Rainfall	39.4 ± 5.3a	62.5 ± 1.0a	10.5 ± 0.2a	2.4 ± 0.5a	13.7 ± 0.5c	8.8 ± 0.1b	58.2 ± 4.2a	70.3 ± 3.7a	3.1 ± 0.20a	2.7 ± 0.20a	0.6 ± 0.06a	2.5 ± 0.20a	2.2 ± 0.2a
	Year 2_High Rainfall	42.3 ± 0.8a	74.8 ± 1.2b	10.4 ± 0.1a	2.1 ± 0.1a	9.7 ± 0.8ab	8.0 ± 0.1b	56.0 ± 0.6a	68.3 ± 0.5a	3.0 ± 0.02a	2.6 ± 0.02a	0.5 ± 0.01a	2.4 ± 0.03a	2.1 ± 0.03a
	Year 2_Low Rainfall	31.1 ± 2.7a	59.5 ± 4.0a	10.1 ± 0.2a	2.6 ± 0.1a	8.9 ± 0.6a	4.3 ± 0.1a	64.6 ± 2.1a	76.0 ± 1.9a	3.4 ± 0.08a	2.9 ± 0.08a	0.7 ± 0.03a	2.8 ± 0.09a	2.5 ± 0.09a
	F _(3,12)	2.894	9.05	0.763	1.821	11.037	154.586	2.894	2.894	2.894	2.894	2.894	2.894	2.894
	p	0.102	0.006	0.546	0.221	0.003	< 0.001	0.102	0.102	0.102	0.102	0.102	0.102	0.102
CIAT16483	Year 1_High Rainfall	35.8 ± 1.5a	67.8 ± 1.0a	10.4 ± 0.3a	1.8 ± 0.1a	13.3 ± 1.2c	7.5 ± 0.2b	61.0 ± 1.2a	72.8 ± 1.1a	3.2 ± 0.05a	2.8 ± 0.05a	0.6 ± 0.02a	2.6 ± 0.05a	2.3 ± 0.1a
	Year 1_Low Rainfall	43.6 ± 6.0a	67.7 ± 1.1a	12.0 ± 0.6a	2.4 ± 0.3b	13.7 ± 0.8c	8.6 ± 0.1c	54.9 ± 4.7a	67.3 ± 4.2a	3.0 ± 0.20a	2.5 ± 0.20a	0.5 ± 0.07a	2.3 ± 0.20a	2.0 ± 0.2a
	Year 2_High Rainfall	38.7 ± 1.0a	71.1 ± 0.3a	10.5 ± 0.2a	2.3 ± 0.02ab	8.9 ± 0.2b	8.0 ± 0.1c	58.8 ± 0.8a	70.8 ± 0.7a	3.1 ± 0.03a	2.7 ± 0.03a	0.6 ± 0.01a	2.5 ± 0.03a	2.2 ± 0.03a
	Year 2_Low Rainfall	37.4 ± 1.1a	69.4 ± 3.6a	9.8 ± 0.8a	2.8 ± 0.04b	5.7 ± 0.4a	4.4 ± 0.1a	59.7 ± 0.8a	71.6 ± 0.8a	3.2 ± 0.03a	2.7 ± 0.03a	0.6 ± 0.01a	2.6 ± 0.04a	2.3 ± 0.04a
	F _(3,12)	1.124	0.656	3.362	7.641	24.695	228.793	1.124	1.124	1.124	1.124	1.124	1.124	1.124
	p	0.395	0.602	0.076	0.01	<0.001	< 0.001	0.395	0.395	0.395	0.395	0.395	0.395	0.395
CIAT16384	Year 1_Low Rainfall	33.5 ± 0.4a	63.0 ± 0.4a	11.0 ± 0.4a	2.1 ± 0.2a	11.1 ± 0.6a	8.8 ± 0.02b	62.8 ± 0.3a	74.4 ± 0.3a	3.3 ± 0.01a	2.9 ± 0.01a	0.6 ± 0.005a	2.7 ± 0.01a	2.4 ± 0.01a
	Year 1_High Rainfall	38.5 ± 0.5a	69.2 ± 0.3a	9.9 ± 0.5a	1.7 ± 0.1a	9.1 ± 0.6a	7.3 ± 0.06a	58.9 ± 0.4a	70.9 ± 0.4a	3.1 ± 0.02a	2.7 ± 0.02a	0.6 ± 0.006a	2.5 ± 0.02a	2.2 ± 0.02a
	Year 2_High Rainfall	35.0 ± 3.6a	70.0 ± 3.2a	10.7 ± 0.3a	2.1 ± 0.3a	8.1 ± 0.7a	8.4 ± 0.05b	61.6 ± 2.8a	73.3 ± 2.5a	3.2 ± 0.10a	2.8 ± 0.10a	0.6 ± 0.040a	2.6 ± 0.13a	2.3 ± 0.10a
	F _(2,9)	1.429	3.439	2.176	0.93	6.111	273.097	1.429	1.429	1.429	1.429	1.429	1.429	1.429
	p	0.311	0.101	0.195	0.445	0.36	< 0.001	0.311	0.311	0.311	0.311	0.311	0.311	0.311
Marandu	Year 1_Low Rainfall	40.7 ± 4.1a	65.3 ± 1.5a	10.7 ± 0.3b	2.9 ± 0.07b	9.2 ± 0.9b	7.8 ± 0.10b	57.2 ± 3.2a	69.4 ± 2.9a	3.1 ± 0.1a	2.6 ± 0.10a	0.6 ± 0.05a	2.4 ± 0.10a	2.1 ± 0.10a
	Year 2_Low Rainfall	37.3 ± 2.2a	64.0 ± 1.8a	8.8 ± 0.1a	2.6 ± 0.01a	4.1 ± 0.4a	4.6 ± 0.08a	59.8 ± 1.7a	71.7 ± 1.5a	3.2 ± 0.1a	2.7 ± 0.07a	0.6 ± 0.03a	2.6 ± 0.08a	2.3 ± 0.08a
	t _(1,6)	0.716	0.553	5.634	3.914	4.984	25.097	-0.716	-0.716	-0.716	-0.716	-0.716	-0.716	-0.716
	p	0.513	0.61	0.005	0.017	0.008	< 0.001	0.513	0.513	0.513	0.513	0.513	0.513	0.513

Table 34: Continue

		ADF (%)	NDF (%)	Ash (%)	Fat (%)	Protein (%)	Moisture (%)	DDM (%)	TDN (%)	DFE (MCal/kg DM)	ME (MCal/kg DM)	NEL (MCal/kg DM)	NEM (MCal/kg DM)	NEG (MCal/kg DM)
P. maximum	Year 1_Low Rainfall	37.4 ± 0.6a	66.0 ± 0.2a	13.8 ± 0.3c	1.8 ± 0.03a	13.7 ± 1.0b	8.5 ± 0.1a	59.8 ± 0.5a	71.7 ± 0.4a	3.2 ± 0.02a	2.7 ± 0.02a	0.6 ± 0.01a	2.6 ± 0.02a	2.3 ± 0.02a
	Year 1_High Rainfall	36.6 ± 3.0a	67.2 ± 3.2a	11.3 ± 0.4a	1.6 ± 0.1a	10.2 ± 1.0a	7.4 ± 0.1a	60.4 ± 2.3a	72.2 ± 2.1a	3.2 ± 0.09a	2.8 ± 0.09a	0.6 ± 0.04a	2.6 ± 0.10a	2.3 ± 0.10a
	Year 2_High Rainfall	40.4 ± 0.7a	72.9 ± 1.0a	12.6 ± 0.1b	2.2 ± 0.1b	9.2 ± 1.0a	8.2 ± 0.02a	57.4 ± 0.6a	69.6 ± 0.5a	3.1 ± 0.02a	2.6 ± 0.02a	0.6 ± 0.01a	2.4 ± 0.03a	2.2 ± 0.03a
	F _(2,9)	1.236	3.554	16.82	7.203	37.546	1.236	1.236	1.236	1.236	1.236	1.236	1.236	1.236
	p	0.355	0.096	0.003	0.025	< 0.001	0.355	0.355	0.355	0.355	0.355	0.355	0.355	0.355
PIATA	Year 1_Low Rainfall	40.6 ± 5.9a	64.3 ± 1.7a	11.3 ± 0.6b	2.6 ± 0.3b	7.3 ± 1.2b	8.6 ± 0.07c	57.2 ± 4.6a	69.4 ± 4.1a	3.1 ± 0.2a	2.6 ± 0.2a	0.6 ± 0.10a	2.4 ± 0.2a	2.1 ± 0.2a
	Year 2_Low Rainfall	28.9 ± 4.1a	60.8 ± 2.4a	11.5 ± 0.4b	2.8 ± 0.3b	5.0 ± 0.5ab	4.4 ± 0.20a	66.4 ± 3.2a	77.6 ± 2.9a	3.4 ± 0.1a	3.0 ± 0.1a	0.7 ± 0.05a	2.8 ± 0.1a	2.5 ± 0.1a
	Year 2_High Rainfall	44.9 ± 2.4a	78.4 ± 1.1b	7.5 ± 0.3a	1.4 ± 0.1a	3.0 ± 0.1a	7.6 ± 0.02b	53.9 ± 1.8a	66.4 ± 1.a7	2.9 ± 0.1a	2.5 ± 0.1a	0.5 ± 0.03a	2.3 ± 0.1a	2.0 ± 0.1a
	F _(2,9)	3.597	26.711	26.782	10.494	8.515	221.71	3.597	3.597	3.597	3.597	3.597	3.597	3.597
	p	0.094	0.001	0.001	0.011	0.018	< 0.001	0.094	0.094	0.094	0.094	0.094	0.094	0.094
Sabia	Year 1_Low Rainfall	40.7 ± 3.6a	65.4 ± 1.7a	10.0 ± 0.5b	3.0 ± 0.1b	13.6 ± 1.1b	7.9 ± 0.3b	57.2 ± 2.8a	69.3 ± 2.5a	3.1 ± 0.1a	2.6 ± 0.1a	0.6 ± 0.04a	2.4 ± 0.1a	2.1 ± 0.1a
	Year 2_Low Rainfall	36.7 ± 4.2a	64.9 ± 3.2a	7.7 ± 0.3a	2.3 ± 0.2a	3.8 ± 0.2a	4.5 ± 0.1a	60.5 ± 3.3a	72.3 ± 3.0a	3.2 ± 0.1a	2.8 ± 0.1a	0.6 ± 0.05a	2.6 ± 0.1a	2.3 ± 0.1a
	t _(1,6)	0.772	0.128	3.697	2.919	9.009	10.459	-0.772	-0.772	-0.772	-0.772	-0.772	-0.772	-0.772
	p	0.483	0.904	0.021	0.043	0.001	< 0.001	0.483	0.483	0.483	0.483	0.483	0.483	0.483

Table 35: Mineral nutrient content in different forage grass genetic resources. Comparisons were made within each accession between years and seasons and not between different accessions. Statistically significant differences are indicated by different lower case letters.

	Ca (%)	Cu (ppm)	Fe (ppm)	K (%)	K/Ca+Mg (%)	Mg (%)	Mn (ppm)	Na (%)	P (%)	Zn (ppm)	
Basilisk	Year 1_High Rainfall	0.3 ± 0.01a	4.7 ± 0.3b	298.3 ± 41.2a	3.1 ± 0.1c	1.7 ± 0.02b	0.4 ± 0.02b	69.0 ± 1.2a	0.02 ± 0.003a	0.2 ± 0.01a	32.3 ± 4.5a
	Year 1_Low Rainfall	0.3 ± 0.02a	4.3 ± 0.3b	454.7 ± 196.7a	2.8 ± 0.2bc	1.7 ± 0.2b	0.4 ± 0.03b	69.3 ± 22.7a	0.01 ± 0.003a	0.2 ± 0.04a	34.7 ± 5.4a
	Year 2_High Rainfall	0.3 ± 0.02a	2.0 ± 0.0a	193.0 ± 35.3a	2.5 ± 0.01b	2.0 ± 0.1b	0.2 ± 0.01a	41.3 ± 2.6a	0.02 ± 0.003a	0.2 ± 0.01a	24.0 ± 2.0a
	Year 2_Low Rainfall	0.4 ± 0.02b	2.3 ± 0.3a	729.0 ± 221a	1.9 ± 0.0a	1.1 ± 0.1a	0.3 ± 0.01a	63.7 ± 11.9a	0.07 ± 0.007b	0.2 ± 0.01a	29.7 ± 0.7a
	F _(3,12)	10.035	22.222	2.402	19.468	9.526	17.649	1.054	45.333	1.833	1.581
	p	0.004	< 0.001	0.143	< 0.001	0.005	0.001	0.421	< 0.001	0.219	0.268
C. gayana	Year 1_High Rainfall	0.5 ± 0.03b	4.0 ± 0.0b	369.3 ± 21.0a	1.4 ± 0.1bc	1.0 ± 0.03b	0.12 ± 0.01a	45.3 ± 0.7a	0.9 ± 0.05bc	0.2 ± 0.003b	15.7 ± 0.7ab
	Year 1_Low Rainfall	0.5 ± 0.03b	4.0 ± 0.6b	458.3 ± 87.8a	1.6 ± 0.1c	1.2 ± 0.06bc	0.12 ± 0.01a	58.0 ± 10.7a	1.1 ± 0.10c	0.2 ± 0.01b	19.3 ± 0.7bc
	Year 2_High Rainfall	0.3 ± 0.01a	1.0 ± 0.6a	250.7 ± 27.3a	1.2 ± 0.04b	1.4 ± 0.06c	0.08 ± 0.01a	34.0 ± 7.02a	0.6 ± 0.04ab	0.1 ± 0.01a	12.3 ± 1.3a
	Year 2_Low Rainfall	0.4 ± 0.01b	1.3 ± 0.3a	513.3 ± 97.1a	0.9 ± 0.02a	0.8 ± 0.04a	0.10 ± 0.02a	41.7 ± 9.1a	0.7 ± 0.07a	0.2 ± 0.01b	23.3 ± 0.3c
	F _(3,12)	16.249	13.857	2.87	17.122	28.492	2.5	1.617	6.15	9.324	32.32
	p	0.001	0.002	0.104	0.001	< 0.001	0.133	0.261	0.018	0.005	< 0.001
CIAT16169	Year 1_High Rainfall	0.3 ± 0.02a	4.3 ± 0.3c	151.3 ± 23.7a	3.1 ± 0.04d	1.6 ± 0.2a	0.4 ± 0.06b	55.3 ± 0.7c	0.01 ± 0.003a	0.3 ± 0.02	27.3 ± 1.8a
	Year 1_Low Rainfall	0.3 ± 0.02a	3.7 ± 0.3bc	102.0 ± 19.1a	2.7 ± 0.20c	1.5 ± 0.3a	0.4 ± 0.07b	35.7 ± 2.4b	0.01 ± 0.000a	0.2 ± 0.01a	25.3 ± 0.7a
	Year 2_High Rainfall	0.2 ± 0.01a	3.0 ± 0.0b	100.3 ± 8.7a	2.3 ± 0.05b	2.0 ± 0.2a	0.2 ± 0.04a	28.3 ± 2.0a	0.06 ± 0.003b	0.3 ± 0.02b	37.7 ± 0.9b
	Year 2_Low Rainfall	0.3 ± 0.03a	1.0 ± 0.3a	198.3 ± 34.1a	1.8 ± 0.01a	1.7 ± 0.2a	0.2 ± 0.03a	32.0 ± 3.0ab	0.08 ± 0.003b	0.2 ± 0.02a	29.0 ± 1.7a
	F _(3,12)	3.094	15.556	4.015	41.838	1.146	6.484	30.015	129.222	6.159	16.101
	p	0.089	0.001	0.051	< 0.001	0.388	0.016	< 0.001	< 0.001	0.018	< 0.001
CIAT16320	Year 1_Low Rainfall	0.2 ± 0.01a	1.0 ± 0.0a	76.7 ± 21.6a	1.9 ± 0.06b	1.8 ± 0.2b	0.2 ± 0.02b	33.3 ± 8.6a	0.01 ± 0.003a	0.1 ± 0.020a	16.3 ± 0.7a
	Year 2_High Rainfall	0.1 ± 0.01a	2.0 ± 0.6a	70.0 ± 16.2a	1.4 ± 0.10a	2.2 ± 0.08b	0.1 ± 0.006a	21.7 ± 4.5a	0.01 ± 0.000a	0.09 ± 0.02a	13.0 ± 1.2a
	Year 2_Low Rainfall	0.5 ± 0.05b	4.3 ± 0.9b	423.3 ± 93.1b	2.0 ± 0.05b	1.1 ± 0.05a	0.2 ± 0.007b	41.0 ± 7.5a	0.06 ± 0.006b	0.2 ± 0.02a	33.0 ± 0.6b
	F _(2,9)	49.533	7.9	13.037	17.683	19.583	27.152	1.897	52.75	2.967	163.158
	p	< 0.001	0.021	0.007	0.003	0.002	0.001	0.23	< 0.001	0.127	< 0.001

Table 35: Continue

	Ca (%)	Cu (ppm)	Fe (ppm)	K (%)	K/Ca+Mg (%)	Mg (%)	Mn (ppm)	Na (%)	P (%)	Zn (ppm)	
CIAT16339	Year 1_High Rainfall	0.3 ± 0.02a	4.3 ± 0.3a	187.7 ± 61.5a	2.8 ± 0.2b	1.5 ± 0.2a	0.4 ± 0.04ab	44.7 ± 0.9a	0.01 ± 0.0a	0.2 ± 0.02a	26.7 ± 1.3a
	Year 1_Low Rainfall	0.3 ± 0.02a	3.3 ± 0.3a	123.3 ± 41.2a	2.9 ± 0.2b	1.5 ± 0.2a	0.5 ± 0.06b	43.7 ± 2.6a	0.01 ± 0.0a	0.2 ± 0.01a	29.0 ± 1.0a
	Year 2_High Rainfall	0.3 ± 0.02a	4.3 ± 0.3a	230.7 ± 87.5	2.4 ± 0.1a	1.7 ± 0.2a	0.3 ± 0.03a	56.0 ± 32.0a	0.04 ± 0.02b	0.3 ± 0.03a	41.7 ± 3.5b
	Year 2_Low Rainfall	0.4 ± 0.04b	3.0 ± 0.6a	611.0 ± 281.6a	1.9 ± 0.2a	1.1 ± 0.2a	0.3 ± 0.04a	60.7 ± 24.7a	0.08 ± 0.003c	0.2 ± 0.04a	37.7 ± 3.5b
	F _(3,12)	5.137	2.833	2.088	5.422	1.508	4.482	0.172	16.409	2.282	7.354
	p	0.029	0.106	0.18	0.025	0.285	0.04	0.912	0.001	0.156	0.011
CIAT16483	Year 1_High Rainfall	0.23 ± 0.01a	5.0 ± 0.6c	363.3 ± 88.4a	2.7 ± 0.09b	1.8 ± 0.05a	0.3 ± 0.01a	33.0 ± 3.0a	0.01 ± 0.0a	0.2 ± 0.03a	24.7 ± 2.7a
	Year 1_Low Rainfall	1.6 ± 1.40a	5.3 ± 0.3c	379.3 ± 130.4a	2.4 ± 0.2b	1.1 ± 0.5a	1.2 ± 0.8a	69.3 ± 28.1a	1.2 ± 1.2a	0.2 ± 0.05a	60.7 ± 36.7a
	Year 2_High Rainfall	0.2 ± 0.01a	2.0 ± 0.0a	207.3 ± 37.0a	2.4 ± 0.02b	2.1 ± 0.1a	0.2 ± 0.01a	32.0 ± 2.1a	0.01 ± 0.003a	0.2 ± 0.02a	18.0 ± 1.0a
	Year 2_Low Rainfall	0.3 ± 0.04a	3.3 ± 0.3b	251.7 ± 63.9a	1.9 ± 0.03a	1.7 ± 0.2a	0.2 ± 0.02a	20.7 ± 0.7a	0.05 ± 0.003a	0.2 ± 0.003a	30.7 ± 1.2a
	F _(3,12)	0.992	17.267	0.935	9.614	2.479	1.31	2.228	0.968	1.457	1.048
	p	0.444	0.001	0.467	0.005	0.135	0.366	0.162	0.454	0.297	0.423
CIAT6384	Year 1_Low Rainfall	0.3 ± 0.02a	4.3 ± 0.3b	408.0 ± 49.1a	2.9 ± 0.05a	1.8 ± 0.07a	0.3 ± 0.02b	50.3 ± 2.3b	0.01 ± 0.001a	0.3 ± 0.003a	29.7 ± 4.7a
	Year 1_High Rainfall	0.3 ± 0.01a	4.3 ± 0.3b	265.3 ± 78.6a	3.0 ± 0.20a	1.9 ± 0.1a	0.3 ± 0.01b	54.7 ± 3.5b	0.01 ± 0.003a	0.2 ± 0.010a	33.0 ± 5.1a
	Year 2_High Rainfall	0.4 ± 0.07a	2.0 ± 0.0a	192.7 ± 51.9a	2.5 ± 0.10a	1.9 ± 0.2a	0.2 ± 0.01a	41.7 ± 1.5a	0.02 ± 0.001b	0.2 ± 0.020a	24.7 ± 2.4a
	F _(2,9)	0.202	24.5	3.188	3.349	0.192	9.971	6.572	7	1.371	0.973
	p	0.823	0.001	0.114	0.106	0.83	0.012	0.031	0.027	0.323	0.43
Marandu	Year 1_Low Rainfall	0.3 ± 0.02a	3.3 ± 0.3a	606 ± 120.8a	2.2 ± 0.08b	1.4 ± 0.09a	0.3 ± 0.02a	56.3 ± 3.3b	0.01 ± 0.001a	0.2 ± 0.03a	26.0 ± 2.3a
	Year 2_Low Rainfall	0.3 ± 0.04a	3.0 ± 0.0a	258 ± 47.5a	1.7 ± 0.07a	1.2 ± 0.20a	0.2 ± 0.05a	33.0 ± 4.04a	0.06 ± 0.003b	0.1 ± 0.02a	24.7 ± 8.4a
	t _(1,6)	-0.593	1	2.684	4.86	1.12	0.953	4.481	-16	1.379	0.154
	p	0.585	0.374	0.055	0.008	0.325	0.325	0.011	< 0.001	0.24	0.89

Table 35: Continue

	Ca (%)	Cu (ppm)	Fe (ppm)	K (%)	K/Ca+Mg (%)	Mg (%)	Mn (ppm)	Na (%)	P (%)	Zn (ppm)	
P. maximum	Year 1_Low Rainfall	0.6 ± 0.01b	7.3 ± 0.3b	528 ± 13.9a	2.9 ± 0.04a	1.3 ± 0.1a	0.4 ± 0.03b	57.7 ± 0.7c	0.4 ± 0.1a	0.3 ± 0.01a	26.0 ± 0.0a
	Year 1_High Rainfall	0.5 ± 0.05ab	4.3 ± 0.7a	531 ± 169.4a	2.5 ± 0.20a	1.4 ± 0.1a	0.2 ± 0.01a	44.7 ± 5b	0.4 ± 0.2a	0.2 ± 0.04a	27.7 ± 3.7a
	Year 2_High Rainfall	0.4 ± 0.01a	3.3 ± 0.3a	396 ± 61.5a	2.3 ± 0.10a	1.4 ± 0.1a	0.2 ± 0.01a	30.3 ± 0.3a	0.4 ± 0.1a	0.3 ± 0.03a	37.7 ± 0.3b
	F _(2,9)	8.012	19.5	0.54	4.475	1.184	14.904	21.568	0.144	4.942	8.811
p	0.02	0.002	0.609	0.065	0.369	0.005	0.002	0.869	0.054	0.016	
PIATA	Year 1_Low Rainfall	0.4 ± 0.03b	2.0 ± 0.6a	308 ± 98.6b	2.7 ± 0.06c	1.6 ± 0.1b	0.3 ± 0.01b	49.0 ± 2.1a	0.02 ± 0.010a	0.2 ± 0.03a	20.0 ± 0.0a
	Year 2_Low Rainfall	0.5 ± 0.05c	1.3 ± 0.3a	366 ± 40.1b	1.8 ± 0.10b	1.0 ± 0.1a	0.3 ± 0.01b	45.3 ± 6.4a	0.06 ± 0.003b	0.2 ± 0.04a	25.0 ± 4.2a
	Year 2_High Rainfall	0.2 ± 0.01a	1.3 ± 0.3a	86 ± 24.3a	1.3 ± 0.10a	1.7 ± 0.1b	0.1 ± 0.01a	31.0 ± 3.8a	0.02 ± 0.010a	0.1 ± 0.02a	15.7 ± 0.7a
	F _(2,9)	16.971	0.8	5.493	51.008	12.704	92	4.592	22.875	2.948	3.681
p	0.003	0.492	0.044	< 0.001	0.007	< 0.001	0.062	0.002	0.128	0.091	
Sabia	Year 1_Low Rainfall	0.3 ± 0.01a	3.0 ± 1.0a	443 ± 184.1a	2.3 ± 0.3a	1.4 ± 0.04a	0.3 ± 0.05a	57.7 ± 7.8b	0.01 ± 0.00a	0.2 ± 0.01a	30.3 ± 4.3a
	Year 2_Low Rainfall	0.3 ± 0.03a	1.7 ± 0.3a	110 ± 18.4a	1.6 ± 0.1a	1.5 ± 0.08a	0.2 ± 0.01a	24.3 ± 1.5a	0.06 ± 0.003b	0.2 ± 0.02a	31.3 ± 4.3a
	t _(1,6)	2.619	1.265	1.802	2.234	-0.017	2.405	4.226	-16	0.64	-0.163
	p	0.059	0.275	0.146	0.089	0.367	0.074	0.013	< 0.001	0.557	0.878

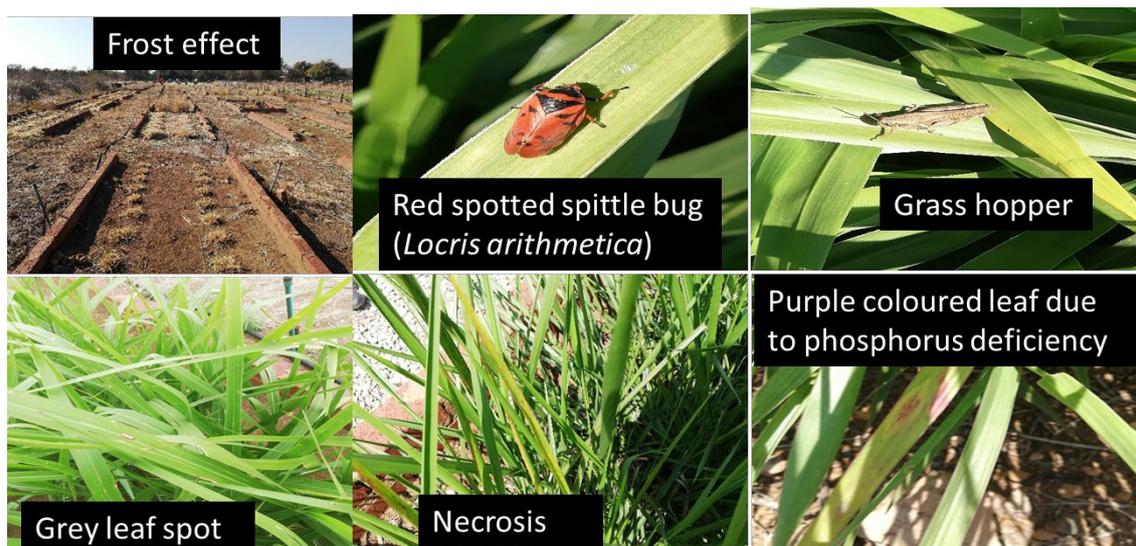


Figure 3: Plant observations: Frost, pests, disease and symptoms of nutrient deficiencies

5.3. Conclusion

This study aimed to evaluate *Brachiaria* genetic resources that are not currently available or included in breeding programs in South Africa to determine their suitability for inclusion in breeding programs for developing water-limitation tolerant *Brachiaria* forages. The forages were grown under dryland conditions without any fertilization from year 1 to year 2 to determine whether these can also be used by resource poor farmers in South Africa. Results from the trial shows that the *Brachiaria* genetic resources are suitable for inclusion in breeding trials with the majority of the forages surviving the cold frost periods in year 1 and year 2. Furthermore, the biomass yields of these forages significantly increased from year 1 to year 2 even without fertilization or irrigation. Although the yields increased, the lack of fertilisation did result in reduced quality, especially crude protein content and several deficiency symptoms were becoming visible in year 2. Even though these deficiency symptoms were visible, generally the mineral nutrient content of the forages produced were sufficient for livestock production, while the majority of the mineral nutrients were even in excess of what is required by livestock.

CHAPTER 6

Characterising the agronomic potential of *Brachiaria* genetic resources grown under rainfed and irrigated conditions.

6.1. Materials and Methods

In order to prioritise the best performing *Brachiaria* genetic resources that will be planted for feeding trials, small plot trials were initially established to quantify agronomic performance over a minimum of three growing seasons. To do this, 24 *Brachiaria* accessions were obtained from various commercial seed companies and seed distributors as well as from ILRI and the SA-NFG. The accessions obtained were within the species *B. brizantha*, *B. decumbens*, *B. nigropedata*, *B. humidicola* and *Brachiaria* hybrids (from ILRI and Barenbrug). Due to the low germination obtained above, seeds that germinated were transplanted into seedling trays and allowed to grow under controlled greenhouse conditions for 1 month after establishment, after which the seedlings were transplanted into 10 m² plots at the ARC-AP Roodeplaat campus. Prior to planting all soil analyses were done and all soil fertiliser corrections were implemented. The small plot trials consisted of two complete random block designs, one under irrigated conditions and another under dryland conditions. Four replicated plots of each *Brachiaria* accession was planted with twelve *Brachiaria* plants within each 10 m² plot. Within the blocks, rainfall was measured after each rainfall event, and for the irrigated plots, at the end of the month, rainfall was supplemented to obtain a minimum of 150 mm of water for the trial area. When more than 150 mm was measured, no additional irrigation was done. For agronomic performance, all grass cultivars were evaluated at 75 % flowering (9 plants flowering in a plot) for dry matter (DM) biomass yield, plant counts, plant height and plant canopy width. For these measurements five plants were selected from the plots. For DM yield, the grass were harvested at 5 cm stubble height, harvested from within each plot using a 0.25 m² quadrant. The harvested material were oven dried at 60 °C for 72 hours to a constant mass. The dry mass was expressed as tons/Ha per cutting and the total biomass production per month as well as per season was calculated as the cumulative production over the growing period. These grasses were scheduled to be evaluated over a period of four growing seasons defined as three full calendar years from the first day of transplantation in the field plots. After each growing season, the dried samples will be milled and analysed for their nutritional quality.

Dryland (Block C)										Open Rows		Irrigated (Block A and B)										Open Rows				
16	S15R3	32	S2R1	48	S2R4	64	S18R1	80	S14R4	96	S5R3		S5R4	96	S23R3	80	S7R1	64	S21R1	48	S12R1	32	S17R1	16		
15	S13R4	31	S24R4	47	S7R1	63	S4R2	79	S18R4	95	S23R4		S21R4	95	S10R1	79	S22R4	63	S14R4	47	S17R3	31	S10R2	15		
14	S15R1	30	S3R2	46	S14R2	62	S3R1	78	S1R4	94	S9R1		S6R1	94	S20R2	78	S8R3	62	S9R4	46	S5R1	30	S13R1	14		
13	S6R1	29	S1R2	45	S2R2	61	S10R2	77	S5R2	93	S6R3		S3R1	93	S9R3	77	S6R2	61	S2R1	45	S4R1	29	S11R2	13		
12	S21R2	28	S19R3	44	S22R2	60	S23R1	76	S12R1	92	S10R1		S24R4	92	S18R4	76	S19R2	60	S13R2	44	S5R2	28	S16R2	12		
11	S12R4	27	S16R1	43	S17R3	59	S7R3	75	S8R2	91	S16R4		S19R4	91	S20R3	75	S23R2	59	S16R1	43	S6R4	27	S11R4	11		
10	S19R2	26	S13R1	42	S20R1	58	S20R4	74	S23R2	90	S4R3		S20R1	90	S19R1	74	S18R3	58	S11R3	42	S23R4	26	S19R3	10		
9	S20R3	25	S17R2	41	S12R3	57	S13R3	73	S10R4	89	S24R3		S2R3	89	S7R3	73	S4R3	57	S3R3	41	S1R3	25	S9R2	9		
8	S3R4	24	S11R3	40	S18R2	56	S8R4	72	S9R4	88	S4R4		S4R2	88	S15R2	72	S7R4	56	S20R4	40	S21R2	24	S10R4	8		
7	S23R3	23	S5R1	39	S22R4	55	S19R4	71	S13R2	87	S15R4		S13R4	87	S14R2	71	S22R1	55	S17R4	39	S14R1	23	S12R3	7		
6	S21R4	22	S4R1	38	S21R1	54	S7R2	70	S24R2	86	S6R4		S16R3	86	S24R1	70	S9R1	54	S7R2	38	S17R2	22	S1R1	6		
5	S15R2	21	S14R1	37	S1R3	53	S21R1	69	S19R1	85	S16R3		S24R3	85	S1R4	69	S14R3	53	S10R3	37	S2R2	21	S22R2	5		
4	S2R3	20	S24R1	36	S17R4	52	S3R3	68	S14R3	84	S10R3		S13R3	84	S24R2	68	S5R3	52	S8R2	36	S15R3	20	S3R2	4		
3	S6R2	19	S1R1	35	S9R3	51	S7R4	67	S22R1	83	S8R3		S15R4	83	S12R2	67	S2R4	51	S22R3	35	S6R3	19	S1R2	3		
2	S17R1	18	S11R2	34	S18R3	50	S21R3	66	S9R2	82	S16R2		S18R1	82	S3R4	66	S21R3	50	S8R1	34	S4R4	18	S16R4	2		
1	S12R2	17	S5R4	33	S22R3	49	S8R1	65	S11R4	81	S20R2		S23R1	81	S8R4	65	S11R1	49	S12R4	33	S18R2	17	S15R1	1		
1		2		3		4		5		6			6		5		4		3		2		1			

S1	330
S2	460
S3	473
S4	710
S5	780
S6	Basilisk
S7	Piata
S8	Mulato II
S9	Marandu
S10	Tosca
S11	Sabia
S12	Sweet Velvet

S13	Kupfer
S14	Xaraes
S15	Lianero
S16	Humidicola
S17	Nigropedata
S18	MG4
S19	MG5
S20	Humco
S21	Hyma
S22	Btima
S23	Cagana
S24	Debase

24 Cultivars (S1 – S24)

4 Replicates per Cultivar (4 plots) (R1 – R4)

12 plants/plot (3 rows of 4 plants). This will be adjusted to the number of seedlings available once I get the final count

Experiment set up as two complete randomized block designs (one irrigated and the other dryland)

R1	Replicate 1	R3	Replicate 3
R2	Replicate 2	R4	Replicate 4

Figure 4: Experimental layout of agronomic characterization trial under dryland and irrigated conditions planted at the ARC Roodeplaat campus

6.2. Results and Discussion

6.2.1. *Brachiaria brizantha* (Figures 5 and 6)

Across almost all cultivars/lines, irrigation significantly improved cumulative yield (Figure 5). Annual yields generally increased from year 1 to year 2 but then decreased again to year 3 and year 4 under dryland and irrigated conditions. The highest cumulative yields over the 4 years were achieved by Lines 330, 460, 473, 780, and cultivars Marandu, Brimar, Piata, and MG5 under irrigation. Line 710 and cv. Xaraes performed relatively better under dryland, suggesting improved adaptation to water-limited conditions. In general, irrigation was found to lift biomass in most *B. brizantha* lines and cultivars, but moisture limitation stable genotypes like Line 710 and cultivar Xaraes could be critical for resilient livestock feed supply. Breeding strategies should exploit this variation to deliver cultivars tailored to both high-input and climate-challenged dryland systems. The cultivars and lines evaluated can be divided into two general categories namely irrigation responsive genotypes i.e., those with higher yields under irrigation and poor yields under dryland (Line 330, 460, 473, 780, cultivars Brimar, Debase, Hyma, Marandu, Piata, MG4, MG5), and water-limitation stable or adaptable genotypes which are those cultivars and lines which either maintains yield stability regardless of the water regime or those that shows only moderate irrigation benefits but has reasonable drought resilience (Line 710 and cultivar Xaraes). When considering seasonal fluctuations in biomass (Figure 6) under dryland conditions *B. brizantha* cultivars and lines differ in how long they retain green matter, with some crashing earlier in

autumn. Under irrigation, yield gains are significant, but the winter biomass dips still persist, indicating that irrigation mostly extends usable forage unto autumn.

The nutritional quality of *B. brizantha* is indicated in Table 36. Generally, the dry matter (%) of the *B. brizantha* lines and cultivars were approximately 93% indicating very low moisture content. This however is typical of tropical grasses and suggest that these grasses are good for hay making but may reduce palatability. The crude protein content ranged from 3.5% to 8.7%, and was higher under irrigation in autumn (8.7%) and lowest under dryland conditions (3.7%). The fiber content increases under dryland conditions in summer with NDF content ranging from 62% to 68% and ADF content ranging from 33% too 40%. The increase in fiber into summer under dryland conditions indicate that the fodders may have reduced digestibility. Based on these results, the forages produced can be used for maintenance by sheep and goats but growth and reproduction will be limited by the low crude protein content, especially in summer when supplementation will be needed. For beef cattle, the crude protein content is generally adequate for maintenance ad low weight gains. However, in summer under dryland conditions, the crude protein content of below 7% is below the critical threshold and therefore supplementation will be needed. For dairy cows, the nutritional quality of the *Brachiaria brizantha* cultivars and lines are inadequate for high milk yields, with crude protein far below the 16 – 18% needed. If *B. brizantha* cultivars are to be used for dairy cows, it should strictly be used as a base forage but must be supplemented with high protein and energy feeds.

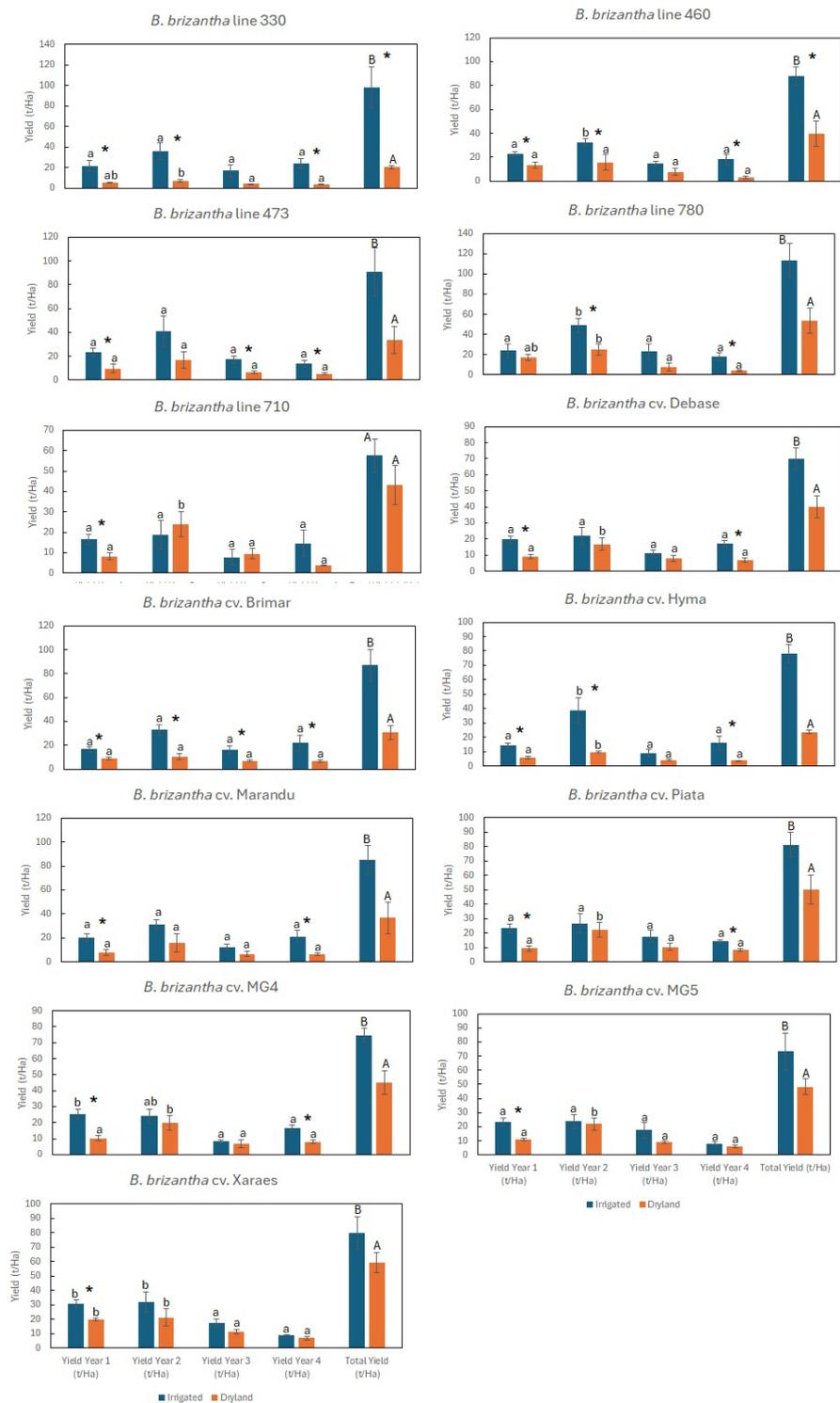


Figure 5: Biomass yield (ton/Ha) of *Brachiaria brizantha* accessions over a four year growing period. Statistically significant differences ($p < 0.05$) in yield between years within a dryland or irrigated treatment is shown by different lower case letters. Differences in yield between dryland and irrigated treatments within each year are indicated by a *. Differences in total yield over the four years between the dryland and irrigated treatments are indicated by different uppercase letters.

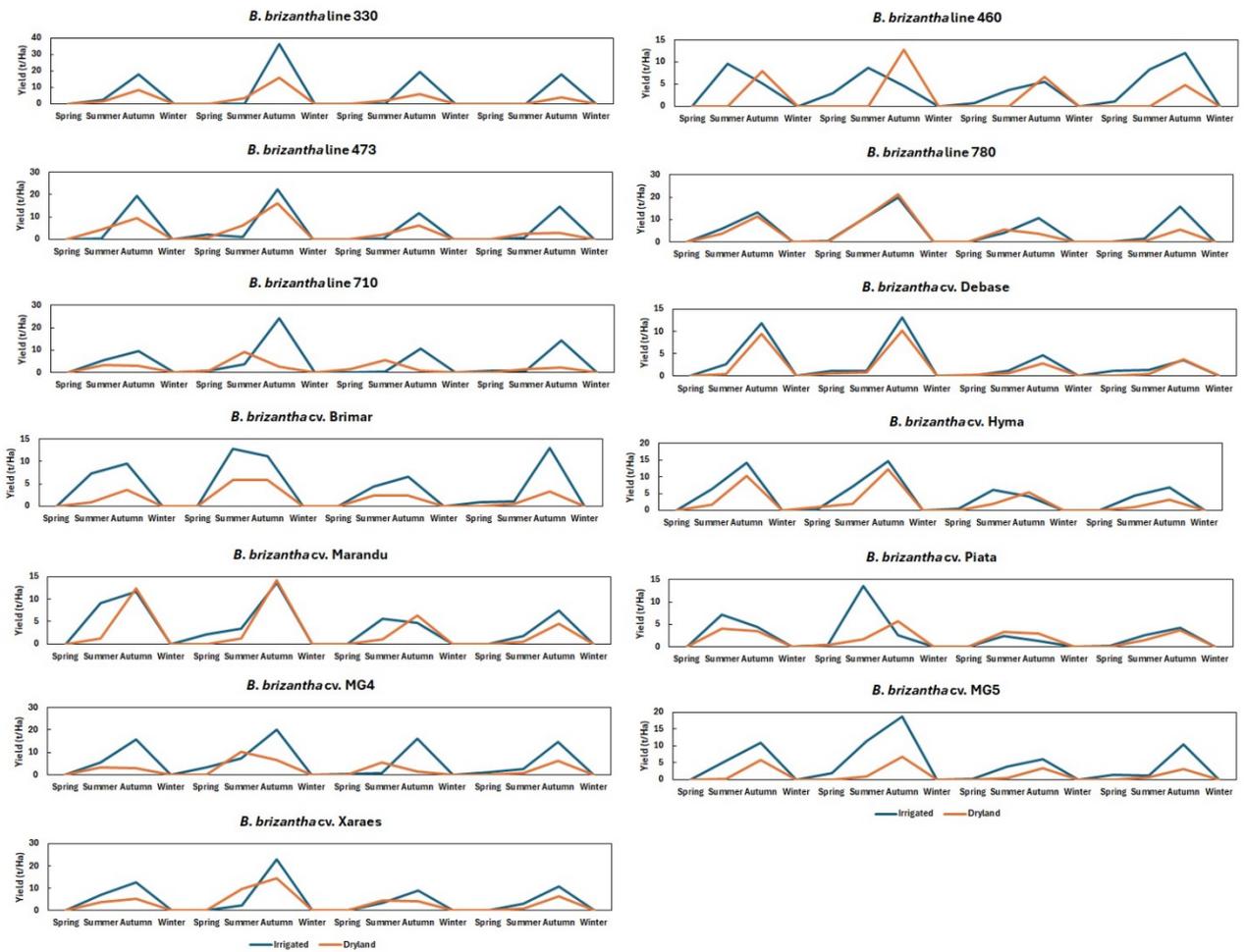


Figure 6: Seasonal biomass yields (ton/Ha) of *Brachiaria brizantha* accessions over a four-year growing period

Table 36: Nutritional quality of *B. brizantha* under dryland and irrigated conditions over the 2022 and 2023 growing season.

		2022						2023											
		Dryland			Irrigated			Dryland			Irrigated								
		Autumn	Spring	Summer															
<i>B. brizantha</i>	DM (%)	93.09±0.189			93.08±0.064			93.01±0.099			93.29±0.17			93.95±0.151			93.77±0.088		
	Ash (%)	11.2±0.694			11.66±0.928			11.67±0.96			8.75±0.179			10.24±0.109			10.18±0.112		
	NDF (%)	63.06±1.597			66.19±2.902			61.72±3.27			61.45±1.66			65.81±0.563			68.31±0.513		
	ADF (%)	33.01±1.512			36.17±2.5			33.62±2.008			33.81±1.632			37.13±0.14			39.79±0.002		
	CP (%)	5.6±0.381			6.44±0.468			8.71±0.236			3.68±0.316			3.48±0.194			3.72±0.067		
	EE	1.14±0.078			1.18±0.066			1.14±0			1.29±0.095			0.99±0.034			1.01±0.055		
	DDM (%)	63.19±1.178			60.72±1.947			62.71±1.564			62.57±1.271			59.97±0.109			57.9±0.001		
	ME (Mcal kg ⁻¹ DM)	2.43±0.047			2.33±0.078			2.41±0.063			2.41±0.051			2.3±0.004			2.22±0		
	TDN (%)	64.74±1.058			62.52±1.75			64.31±1.405			64.18±1.142			61.85±0.098			59.99±0.001		
	DFE (Mcal kg ⁻¹ DM)	2.85±0.047			2.76±0.077			2.84±0.062			2.83±0.05			2.73±0.004			2.64±0		
	DOM (%)	61.65±1.008			59.54±1.666			61.24±1.339			61.12±1.088			58.9±0.094			57.13±0.001		
	NEL (Mcal kg ⁻¹ DM)	0.65±0.018			0.61±0.03			0.64±0.024			0.64±0.019			0.6±0.002			0.57±0		
	NEM (Mcal kg ⁻¹ DM)	2.21±0.053			2.1±0.087			2.19±0.07			2.18±0.057			2.07±0.005			1.97±0		
	NEG (Mcal kg ⁻¹ DM)	1.92±0.052			1.81±0.085			1.9±0.069			1.89±0.056			1.78±0.005			1.69±0		

6.2.2. *Brachiaria nigropedata* (Figures 7 and 8)

A total of 4 *Brachiaria nigropedata* accessions were evaluated (Figure 7 and 8). Differences in annual yield between irrigated and dryland systems were only occasionally observed. No differences in inter-annual yield within irrigated and dryland conditions are seen. *Brachiaria nigropedata* cultivars Kupfer, Nigropedata and Tosca benefit significantly from irrigation over four years. The stable yields observed across years under dryland conditions suggest that *B. nigropedata* is moderately drought tolerant, but irrigation substantially improves long-term productivity. However, cv. Sweetvelvet can be regarded as drought tolerant with no differences in annual and long-term yields between irrigated and dryland conditions. When considering the seasonal yields of *B. nigropedata*, it was clear that these were strong wet season producers, especially under irrigation. The cultivar Kupfer under irrigation provides a longer harvesting period.

The nutritional quality of *B. nigropedata* is indicated in Table 37. Generally, the dry matter (%) of the *B. nigropedata* cultivars were approximately 92 - 93%, indicating very low moisture content. The crude protein content ranged from 8.5% to 15.6% with spring and autumn peaks under irrigation. The fiber content increases under dryland conditions in summer, with NDF content ranging from 58% to 67% and ADF content ranging from 29% to 37%. The increase in fiber into summer under dryland conditions indicates that the fodders may have reduced digestibility. Based on these results, the forages produced are suitable for moderate to high performance in sheep and goats, with spring irrigated values (15%) aligning with requirements for lactating ewes. For beef cattle, the crude protein content is generally adequate for moderate to high weight gains (0.6 – 0.9 kg/day) during the seasons when crude protein exceeds 10%. For dairy cows, with crude protein levels up to 15%, the forages produced can contribute to milk production, although it is still below the optimal levels of 16% to 18%.

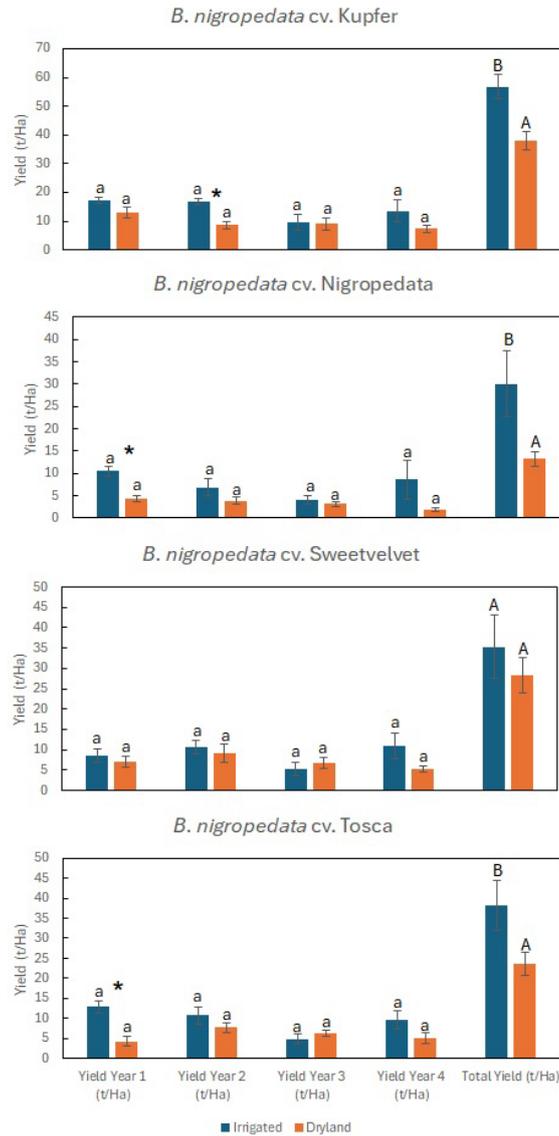


Figure 7: Biomass yield (ton/Ha) of *Brachiaria nigropedata* accessions over a four-year growing period. Statistically significant differences ($p < 0.05$) in yield between years within a dryland or irrigated treatment are shown by different lower case letters. Differences in yield between dryland and irrigated treatments within each year are indicated by a *. Differences in total yield over the four years between the dryland and irrigated treatments are indicated by different uppercase letters.

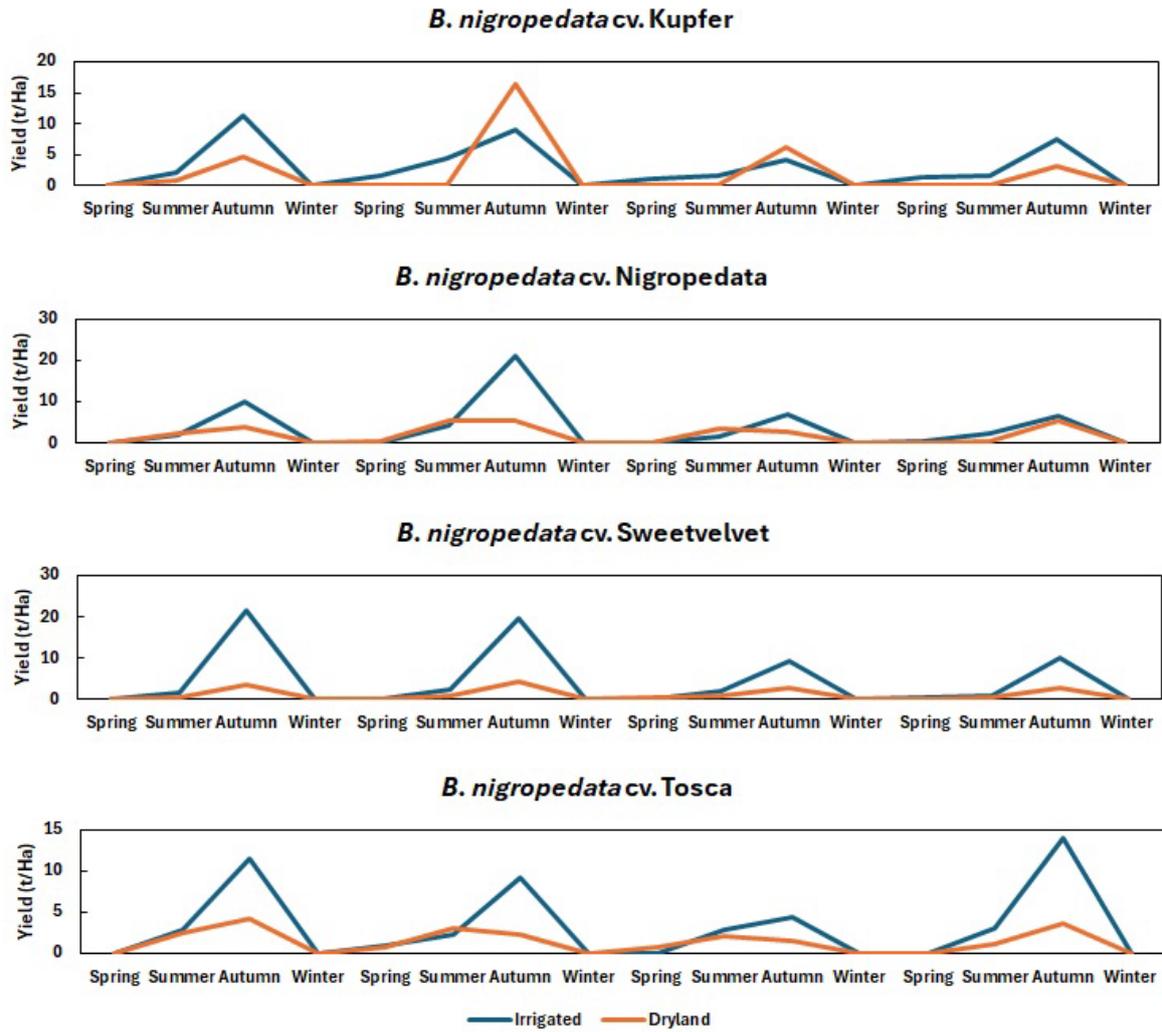


Figure 8: Seasonal biomass yield (ton/Ha) of individual *Brachiaria nigropedata* accessions over a four-year growing period.

Table 37: Nutritional quality of *B. nigropedata* under dryland and irrigated conditions over the 2022 and 2023 growing season.

		2022						2023					
		Dryland			Irrigated			Dryland			Irrigated		
		Autumn	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer
B. nigropedata	DM (%)	92.8±0.061	92.34±0.047	92.49±0.063	92.73±0.098	92.73±0.125	92.73±0.125	92.56±0.128	93.2±0.107	92.98±0.091			
	Ash (%)	13.24±0.976	13.09±0.564	9.86±0.602	15.41±0.791	12.71±0.879	12.71±0.879	11.99±0.466	9.15±0.808	16.03±0.519			
	NDF (%)	60.7±2.354	59.24±2.197	66.86±1.256	63.96±1.367	67.64±0.817	67.64±0.817	61.96±1.588	66.37±2.092	58.02±0.647			
	ADF (%)	30.69±0.987	29.1±0.349	35.87±0.504	34.06±0.102	36.3±0.096	36.3±0.096	32.57±0.647	36.85±1.14	29.74±0.335			
	CP (%)	12.51±0.362	15.62±0.681	9.58±0.708	13.7±0.239	10.52±1.234	10.52±1.234	10.34±0.174	8.51±0.761	13.97±1.265			
	EE	1.38±0.182	1.45±0.072	1.26±0.065	1.18±0.074	1.08±0.074	1.08±0.074	1.53±0.105	1.18±0.076	1.3±0.053			
	DDM (%)	65±0.769	66.23±0.272	60.95±0.393	62.37±0.08	60.63±0.075	60.63±0.075	63.53±0.504	60.19±0.888	65.73±0.261			
	ME (Mcal kg ⁻¹ DM)	2.51±0.031	2.55±0.011	2.34±0.016	2.4±0.003	2.33±0.003	2.33±0.003	2.45±0.02	2.31±0.036	2.53±0.01			
	TDN (%)	66.36±0.691	67.47±0.244	62.73±0.353	64±0.072	62.43±0.067	62.43±0.067	65.04±0.453	62.04±0.798	67.02±0.235			
	DFE (Mcal kg ⁻¹ DM)	2.93±0.03	2.97±0.011	2.77±0.016	2.82±0.003	2.75±0.003	2.75±0.003	2.87±0.02	2.74±0.035	2.95±0.01			
	DOM (%)	63.2±0.658	64.26±0.233	59.74±0.336	60.95±0.068	59.46±0.064	59.46±0.064	61.95±0.431	59.09±0.76	63.83±0.224			
	NEL (Mcal kg ⁻¹ DM)	0.68±0.012	0.7±0.004	0.62±0.006	0.64±0.001	0.61±0.001	0.61±0.001	0.66±0.008	0.61±0.014	0.69±0.004			
	NEM (Mcal kg ⁻¹ DM)	2.29±0.034	2.34±0.012	2.11±0.018	2.17±0.004	2.09±0.003	2.09±0.003	2.22±0.023	2.07±0.04	2.32±0.012			
	NEG (Mcal kg ⁻¹ DM)	1.99±0.034	2.05±0.012	1.82±0.017	1.88±0.003	1.8±0.003	1.8±0.003	1.93±0.022	1.79±0.039	2.03±0.011			

6.2.3. *Brachiaria decumbens* (Figures 9 and 10)

Only one *Brachiaria decumbens* accession was evaluated. Although differences in annual yield (Figure 9) between irrigated and dryland systems were only occasionally observed, *Brachiaria decumbens* cv. Basilisk benefit significantly from irrigation over the four years. Under both irrigated and dryland conditions Basilisk showed no inter annual variation in yield. The stable yields observed across years indicates Basilisk is moderately drought tolerant, but irrigation substantially improves long-term productivity. Under dryland conditions *B. decumbens* cv. Basilisk generally remains stable between the years and maintains a reasonable summer to autumn production (Figure 10). However, there are substantial differences between irrigated and dryland seasonal yield.

The nutritional quality of *B. decumbens* is indicated in Table 38. Generally, the dry matter (%) of the *B. decumbens* was approximately 92 - 93%, indicating very low moisture content. The crude protein content ranged from 5% and 12.6% with spring and autumn peaks under irrigation exceeding 12%. The fiber content increases under dryland conditions in summer with NDF content ranging from 58% to 70% and ADF content ranging from 29% to 38%. The increase in fiber into summer under dryland conditions indicates that the fodders may have reduced digestibility. Based on these results, the forages produced are suitable for moderate to high performance in sheep and goats with spring irrigated values (12%) aligning with requirements for lactating ewes. However, under dryland conditions in summer, crude protein content levels decreased to approximately 5% which is not even sufficient for maintenance and supplementation would be required. For beef cattle, the crude protein content is generally adequate for maintenance and moderate growth under irrigation. However, the low crude protein content under dryland conditions in summer months will restrict performance without protein supplementation. For dairy cows, irrigated spring forages with a crude protein exceeding 12% can support partial milk production but it remains below the optimum threshold of 16 – 18%.

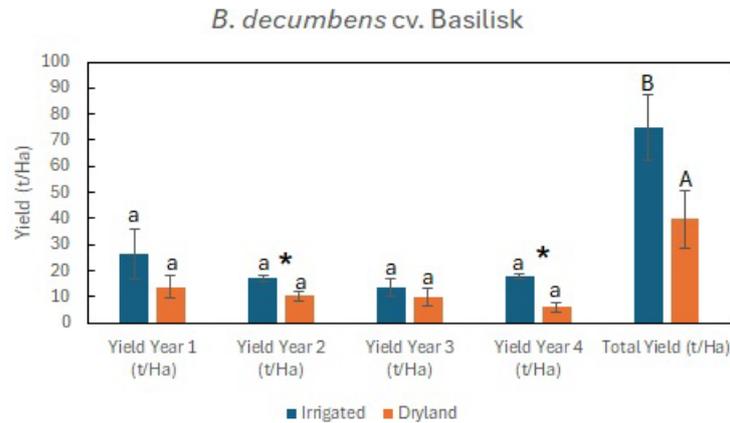


Figure 9: Biomass yield (ton/Ha) of *Brachiaria decumbens* cultivar Basilisk over a four-year growing period. Statistically significant differences ($p < 0.05$) in yield between years within a dryland or irrigated treatment are shown by different lower-case letters. Differences in yield between dryland and irrigated treatments within each year is indicated by a *. Differences in total yield over the four years between the dryland and irrigated treatment are indicated by different uppercase letters.

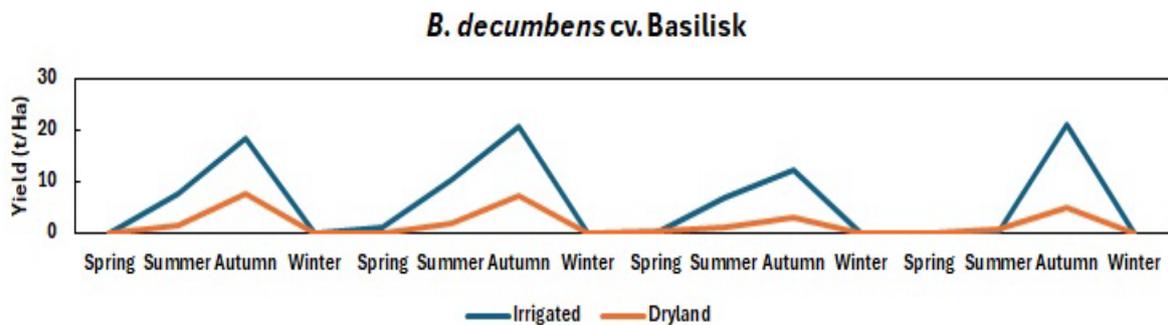


Figure 10: Seasonal biomass yield (ton/Ha) of *Brachiaria decumbens* cultivar Basilisk over a four year growing period

Table 38: Nutritional quality of *B. decumbens* under dryland and irrigated conditions over the 2022 and 2023 growing season

		2022						2023					
		Dryland			Irrigated			Dryland			Irrigated		
		Autumn	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer
<i>B. decumbens</i>	DM (%)	92.84±0.337	92.16±0.202	93.36±0.133	93.22±0.163	93.36±0	92.86±0.056	92.55±0.114	93.18±0.088	93.6±0.108	93.19±0.361		
	Ash (%)	12.14±0.389	13.02±0.071	13.02±0.393	16.41±0.21	13.02±0	11.21±0.199	11.69±0.139	9.47±1.449	13.11±0.019	10.41±0.032		
	NDF (%)	60.59±0.825	56.26±0.895	62.46±0.889	63.58±0.802	62.46±0	70.5±1.311	61.65±1	65.3±2.756	59.59±0.454	67.24±1.998		
	ADF (%)	31.09±0.267	29.25±0.31	33.01±0.197	33.73±1.077	33.01±0	38.46±0.649	31.56±0.178	35.01±1.516	30.91±0.945	38.53±3.128		
	CP (%)	9.18±0.11	8.83±0.161	9.17±0.379	8.42±0.053	9.17±0	5.09±0.188	12.61±0.028	7.99±1.649	10.33±0.011	6.75±0.888		
	EE	1.37±0.027	1.06±0.03	1.02±0.109	1.3±0.195	1.02±0	1.03±0.01	1.29±0.017	1.03±0.013	1.31±0.189	0.91±0.213		
	DDM (%)	64.68±0.208	66.12±0.242	63.19±0.153	62.62±0.839	63.19±0	58.94±0.505	64.31±0.138	61.63±1.181	64.82±0.736	58.88±2.436		
	ME (Mcal kg ⁻¹ DM)	2.49±0.008	2.55±0.01	2.43±0.006	2.41±0.034	2.43±0	2.26±0.02	2.48±0.006	2.37±0.047	2.5±0.029	2.26±0.097		
	TDN (%)	66.08±0.187	67.37±0.217	64.74±0.138	64.23±0.754	64.74±0	60.92±0.454	65.75±0.124	63.33±1.061	66.2±0.661	60.87±2.189		
	DFE (Mcal kg ⁻¹ DM)	2.91±0.008	2.97±0.01	2.85±0.006	2.83±0.033	2.85±0	2.69±0.02	2.9±0.005	2.79±0.047	2.92±0.029	2.68±0.097		
	DOM (%)	62.93±0.178	64.16±0.207	61.65±0.131	61.17±0.718	61.65±0	58.02±0.432	62.62±0.119	60.32±1.01	63.05±0.63	57.97±2.085		
	NEL (Mcal kg ⁻¹ DM)	0.67±0.003	0.7±0.004	0.65±0.002	0.64±0.013	0.65±0	0.59±0.008	0.67±0.002	0.63±0.018	0.68±0.011	0.59±0.037		
	NEM (Mcal kg ⁻¹ DM)	2.28±0.009	2.34±0.011	2.21±0.007	2.18±0.037	2.21±0	2.02±0.023	2.26±0.006	2.14±0.053	2.28±0.033	2.02±0.109		
	NEG (Mcal kg ⁻¹ DM)	1.98±0.009	2.05±0.011	1.92±0.007	1.89±0.037	1.92±0	1.73±0.022	1.97±0.006	1.85±0.052	1.99±0.032	1.73±0.107		

6.2.4. *Brachiaria* hybrids (Figures 11 and 12)

A total of 3 hybrid *Brachiaria* cultivars were evaluated. All three *Brachiaria* hybrid cultivars benefit significantly from irrigation over four years (Figure 10). Under dryland conditions Mulato II and Sabia show no inter annual variation in yield was observed. For rainfed systems *Brachiaria* hybrids are a reliable choice due to their relatively stable production over the years. However, these cultivars expresses higher productivity potential when water is available. Under dryland conditions the *Brachiaria* Hybrids generally outperform all other species, particularly by retaining leaf later. This makes them the most versatile option across water regimes.

The nutritional quality of the *Brachiaria* hybrid cultivars are indicated in Table 39. Generally, the dry matter (%) of the *B. decumbens* was approximately 92 - 93%, indicating very low moisture content. The crude protein content in the hybrids are very low and declined to approximately 5% in autumn/summer, even under irrigation. The fiber content increases under dryland conditions in summer with NDF content ranging from 58% to 66% and ADF content ranging from 30% to 37%. Based on these results, the forages produced cannot sustain high performance in sheep and goats without supplementation. For beef cattle, the crude protein content is adequate for maintenance and low gains. However, supplementation will be needed for finishing or lactating cows. For dairy cows, crude protein levels are too low for milk production and therefore, if the *Brachiaria* hybrids are used for feed for dairy cattle, it should be combined with protein sources.

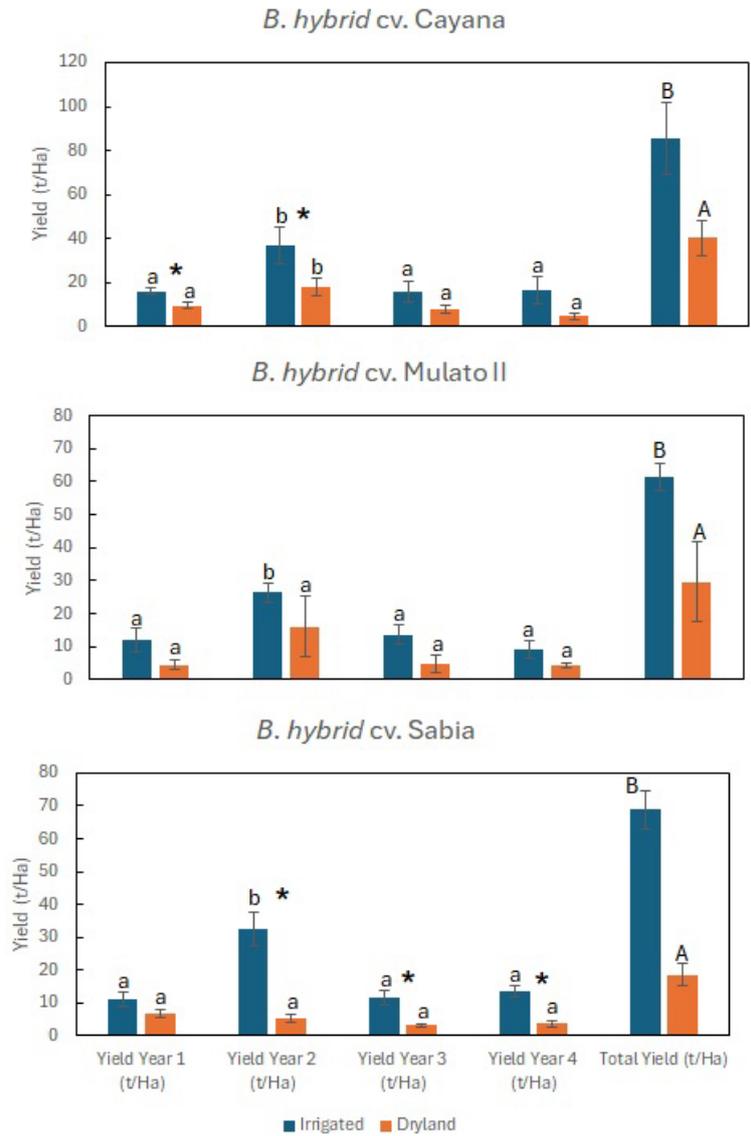


Figure 11: Biomass yield (ton/Ha) of *Brachiaria* hybrid accessions over a four-year growing period. Statistically significant differences ($p < 0.05$) in yield between years within a dryland or irrigated treatment are shown by different lower-case letters. Differences in yield between dryland and irrigated treatments within each year is indicated by a *. Differences in total yield over the four years between the dryland and irrigated treatment are indicated by different uppercase letters.

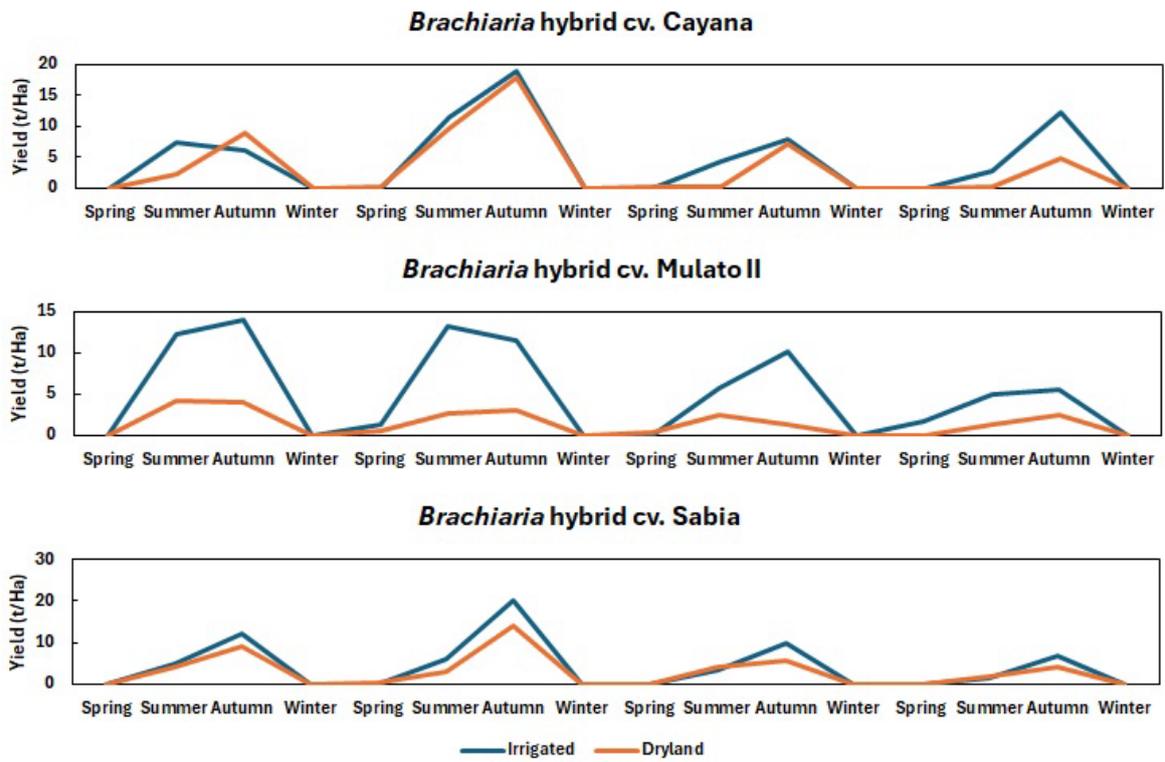


Figure 12: Seasonal biomass yield (ton/Ha) of *Brachiaria* hybrid accessions over a four year growing period.

Table 39: Nutritional quality of *Brachiaria hybrids* under dryland and irrigated conditions over the 2022 and 2023 growing season

		2022						2023					
		Dryland			Irrigated			Dryland			Irrigated		
		Autumn	Spring	Summer									
<i>Brachiaria hybrid</i>	DM (%)	93.07±0.175			92.73±0.282			93.05±0.01			93.56±0.071		
	Ash (%)	14.28±0.354			11.42±0.348			9.94±0.016			11.5±0.397		
	NDF (%)	60.55±1.344			62.65±0.674			58.7±1.631			66.42±0.918		
	ADF (%)	30.84±0.669			33.52±0.526			30.14±1.139			37.11±0.365		
	CP (%)	8.23±0.218			6.63±0.249			5.08±0.36			4.88±0.863		
	EE	1.37±0.141			1.32±0.065			1.83±0.113			1.22±0.056		
	DDM (%)	64.88±0.522			62.79±0.41			65.42±0.887			59.99±0.284		
	ME (Mcal kg ⁻¹ DM)	2.5±0.021			2.42±0.016			2.52±0.035			2.3±0.011		
	TDN (%)	66.25±0.469			64.38±0.368			66.74±0.797			61.87±0.255		
	DFE (Mcal kg ⁻¹ DM)	2.92±0.021			2.84±0.016			2.94±0.035			2.73±0.011		
	DOM (%)	63.1±0.446			61.31±0.351			63.56±0.759			58.92±0.243		
	NEL (Mcal kg ⁻¹ DM)	0.68±0.008			0.65±0.006			0.69±0.014			0.6±0.004		
	NEM (Mcal kg ⁻¹ DM)	2.28±0.023			2.19±0.018			2.31±0.04			2.07±0.013		
	NEG (Mcal kg ⁻¹ DM)	1.99±0.023			1.9±0.018			2.02±0.039			1.78±0.012		

6.2.5. *Brachiaria humidicola* (Figures 13 and 14)

A total of 3 *Brachiaria humidicola* cultivars were evaluated. Only in year 1 and year 4 did annual yields differ between irrigated and dryland conditions. All three *B. humidicola* cultivars benefitted significantly from irrigation over four years, although the magnitude of response varies by cultivar. Under dryland conditions no inter annual variation in yield was observed. *B. humidicola* is a persistent, drought-tolerant grass that maintains stable yields under dryland conditions but expresses higher productivity potential when water is available. Under dryland, *B. humidicola* cultivars differ in how long they retain green material with *B. humidicola* cv. Humco showing significant variation in when the peak biomass yields can be expected between irrigated and dryland conditions. This gives an opportunity for farmers to exploit longer yields when combining irrigated and dryland *B. humidicola* cv. Humco pastures.

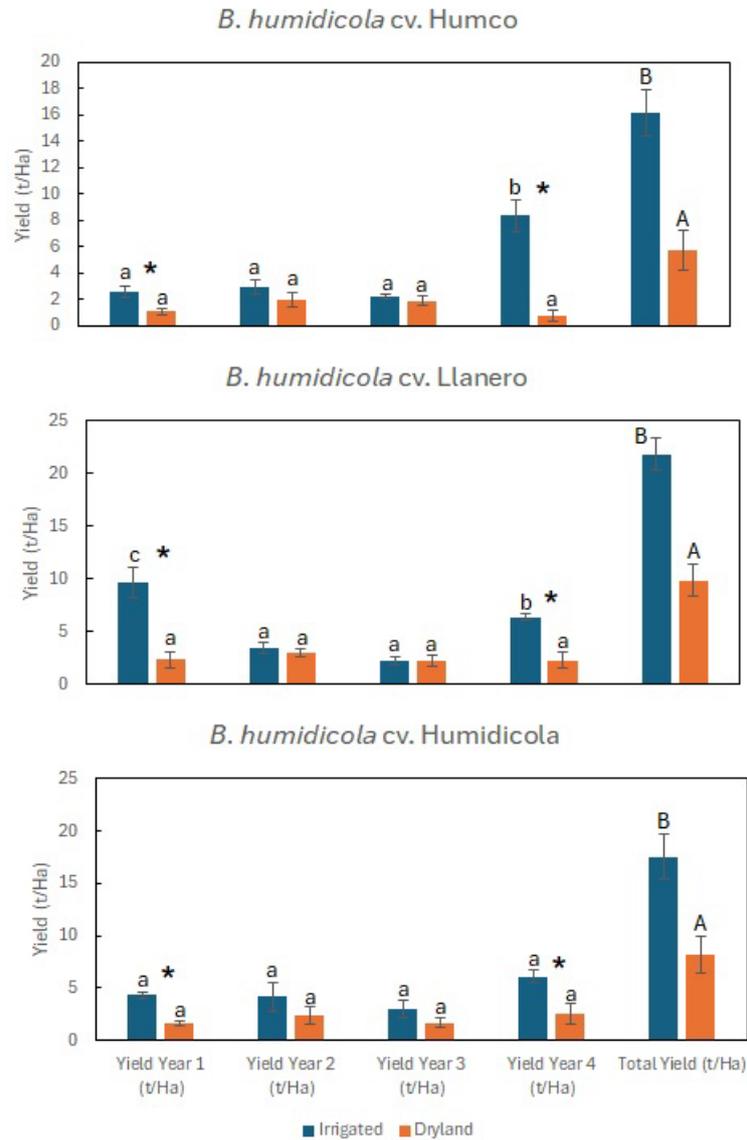


Figure 13: Biomass yield (ton/Ha) of *Brachiaria humidicola* accessions over a four-year growing period. Statistically significant differences ($p < 0.05$) in yield between years within a dryland or irrigated treatment are shown by different lower-case letters. Differences in yield between dryland and irrigated treatments within each year is indicated by a *. Differences in total yield over the four years between the dryland and irrigated treatment are indicated by different uppercase letters.

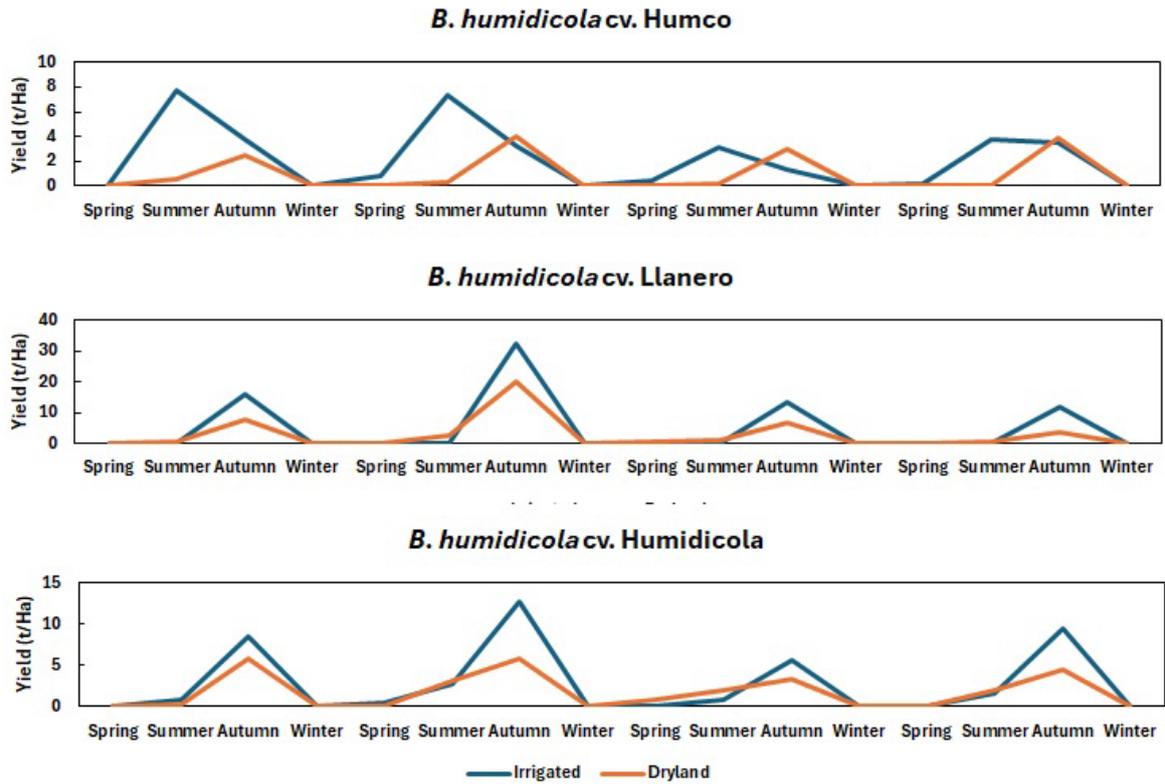


Figure 14: Seasonal biomass yield (ton/Ha) of *Brachiaria humidicola* accessions over a four year growing period.

6.3. Conclusion

A total of 24 *Brachiaria* grass genotypes were evaluated under dryland and irrigated conditions for their agronomic potential and nutritional quality. It was shown that there were significant differences in the yield potential of the different genotypes and that these differences were also found between genotypes within a species. Biomass yields generally increased from the first to the second year and stabilized in the subsequent years. The majority of *Brachiaria* genotypes evaluated produced feed during Autumn, Spring and/or Summer, with no feed harvested in winter. This is because, like all grasses, *Brachiaria* grasses are susceptible to cold temperatures, especially periods where frost is prevalent, and therefore go dormant during this time. Furthermore, although biomass production was generally higher under irrigated conditions, all of the genotypes evaluated were able to produce significant amounts of biomass even under dryland conditions, and growing the grasses under dryland conditions did not significantly impact the quality of the forages produced.

CHAPTER 7:

Fermentative profile and aerobic stability of *Brachiaria* grass mixed with *Melia azedarach* leaf meal

7.1. Background

Feed shortage is the major setback on livestock production in sub-Saharan Africa, particularly under small-scale/ communal farmers. Change in weather pattern as affected by climate change has led to shift in raining pattern and this has a major role it plays on forage availability as feed for livestock. In South Africa for the past three raining season, rain has been delayed from early August to late October, and this has negative impact on grass growth and planting season. This means that forage production from rangeland or planted pasture will be negatively affected. Owing to climate change, research has been conducted where different grass species has been evaluated for adaptability and agronomic performance. Likewise, *Brachiaria* species has been evaluated for agronomic performance in ARC-Roodeplaat, northern region of Gauteng province. The region is characterised by low temperatures during the dry season (winter months) and that negatively affect biomass yield. Therefore, this indicate a need to preserved access forage for dry season period. On the other hand, ensiling the access forage for feeding during the scarcity period is the better option to preserve the material. Ensiling has been a common practiced technology for fresh forage preservation to be used during the dry season period (Zhang et al., 2019; Wang et al., 2020). Less nutrients are loss associated with weather on silage making in relation to hay making (Ntakyo et al., 2020). In addition, the benefit of ensiling forage is the bale technology where storage can be accomplished without buildings (Jayanegara et al., 2018). Ensiled forage material undergoes fermentation process where lactic acid bacteria uses water soluble sugars to produce lactic acid, and that helps in a rapid drop of pH to be below 5 within the first three days after ensiling (Ntakyo et al., 2020). However, it is better to ensiled forage mixture than mono-culture silage, this is based on the fact that different forage have different silage characteristics which affect the quality of silage (Lai et al., 2023). Therefore, strategic mixing two or three forage at ensiling improves silage quality. Hence, in this study brachiaria grass forage was mixed with *Melia azedarach* leaf meal to evaluate it effect on silage quality.

Melia azedarach is one of the invasive and widely distributed tree throughout South Africa. Nevertheless, the plant produces plenty of nutritious leaves during wet season which is shed off during the dry season period. It has been reported that some other trees at maturity shed their leaves and loose nutrients during the dry season period (Mbatha and Bakare 2018). Forages from this plant was reported to contain a crude proteins content which ranges from 124 to 218 g/kg DM, with a calcium and a phosphorus ranges between 23.1 and 25.0 g/kg DM, 2.2 and 2.4 g/kg DM, respectively (Feediedia 2012). Furthermore, communal small-scale subsistence farmers reported to used forage for medicinal purposes in livestock (Mpanza et al. 2009). This is because, *Melia azedarach* contain phytochemicals such as tri-terpenoids, flavonoids and alkaloids with antimicrobial properties (Dinga et al., 2024). Therefore, this means that forage leaves of *Melia azedarach* has an important role to play as animal

feeding for both as therapeutic and protein source. Therefore, this study aimed to evaluate the fermentative characteristics and aerobic stability of *Brachiaria* grass mixed with *Melia azedarach* leaf meal at ensiling.

7.2. Materials and Methods

7.2.1. *Brachiaria* grass production and harvesting for silage making

Brachiaria grass was evaluated for agronomic performance for two growing seasons at Agricultural Research council in Roodeplaat farm, South Africa. For silage making, *Brachiaria* grass was harvested at 50% flowering stage of growth. Grass was harvested using a brush cutter, and it was cut at 5 cm above the ground, to reduce possible soil contamination. After harvesting, forage material was transported to Agricultural Research Council-Animal Production (ARC-AP) Irene farm, which is 32 minutes' drive. Upon arrival at ARC-AP, the forage was milled using a hummer mill to achieve a theoretical length of 2 to 3 cm recommend for proposer compaction. The milled *Brachiaria* grass forage was divided into three equal portions of 8 kg each on a fresh mass basis. Harvesting, milling and ensiling were conducted within the same day.

7.2.2. Treatments and ensiling

Three portion of grass were used mixed with *Melia azedarach* leaf meal at three inclusion levels (i.e. 0, 20% and 30%) of grass fresh mass basis hereafter referred as T1, T2, and T3, respectively. *Melia azedarach* leaves were harvest before flowering at ARC-AP in the Irene farm early in the morning the same day grass was harvested. After harvesting, *Melia azedarach* leaves were milled and wilted for three hours before mixed with grass forage. After mixing the grass with *Melia azedarach* leaf meal to form treatments, molasses meal was added at 5% of the mixture as an additive to boost the sugar content required for proper fermentation. After mixing with the molasses meal, the forage was packed in a plastic bags with a dimension of 600 mm x 300 mm and a thickness of 150 micron, each treatment had four replicates. In order to ensure anaerobiosis, the excess air was sucked out of the bag using an electric vacuum cleaner (HWD35 MAX wet and dry drum). After vacuuming, plastic bags were closed using cable ties (100 mm x 300 mm size). After closure, the bags weights were recorded and an average amount of 1.89 kg \pm 0.067 of forage was packed per bag. Ensiled forages were kept in dark room at room temperature of approximately 25 °C for 40 days.

7.2.3. Characterisation of fresh forage and silage

On ensiling day (day zero), samples of about 40 g of fresh forage mixture (i.e. grass + *Melia azedarach* leaf meal) were taken in duplicates per treatment and put in stomacher bags. Each sample was mixed with 360 mL of distilled water, shook vigorously for 2 minutes and kept in fridge at 5 °C overnight. The following day, the samples were filtered using Whatman No 1 filter paper and the pH was recorded using portable pH meter (Thermo Orion Model 525, Thermo Fisher Scientific, Waltham, MA, USA). In addition, another 100 g samples were taken in duplicates per treatment and used to determine dry matter at ensiling. The samples were weighed to determine the wet mass and oven dried at 60 °C until a constant mass before weighing again to determine the dry weight from which dry matter percentage

was calculated. The ensiled forage mixture was opened on day 3 and 40 post-ensiling. At each opening day, bag weight were recorded per treatment before opening. Samples were taken to determine pH and dry matter as described for fresh forage above.

7.2.4. Aerobic stability test

To assess the aerobic stability of silage after 40 days of ensiling, an extract 500 g samples (two samples per treatment) were packed loosely in a clean plastic bag, following the same procedure as the one used for ensiling, and exposed to oxygen for 8 days. After packing the samples, the plastic bag was covered by a nylon cloth to allow airflow but prevent contamination by dust (Li et al., 2020). The aerobic stability was evaluated by taking 20 g samples (in duplicates per treatment) from the bags, and mixing these with 180 mL of distilled water. These samples were used to determine pH as described under the characterization of fresh forage and silage section above. The pH was recorded on day 0, 2, 4, 6 and 8 of silage exposure to air. Day zero (0) was the day in which the silage was opened (i.e. day 40). Silage was regarded not stable when the pH value of the oxygen exposed silage increased above 5.

7.2.5. Statistical analysis

All data obtained was subjected into one-way analysis of variance (ANOVA) using SAS version 9.0 (SAS Institute, Inc, Cary, NC, USA). Statistical significance was declared at p value ≤ 0.05 . Where a statistically significant difference was observed, post-hoc analysis was conducted and PDIFF statement was used to compare the means.

7.3. Results

7.3.1. Fermentative profile of forage mixture

The fermentative quality of *Brachiaria* grass forage mixed with *Melia azedarach* leaf meal was measured by determining silage pH and dry matter (DM) on day 3 and 40 post-ensiling. Table 36 below showed fermentative quality of *Brachiaria* grass mixed with *Melia azedarach* leaf meal. The result showed that inclusion of *Melia azedarach* leaves in *Brachiaria* grass for forage did not affect fermentation quality. On day zero (ensiling day) the pH was above 5 which is normal as it indicated an unfermented material, however, adding *Melia azedarach* leaf meal numerically reduce pH without a significant effect ($p > 0.05$). What was noticeable is that the pH value of the silage mixture dropped to below 5 with the first three days of ensiling which indicated an effective fermentation process. This showed that during fermentation all silage treatments produced enough lactic acid required to reduce silage pH without treatment effect. It is worth noting that as the ensiling days advanced the pH of treatments where grass was mixed with *Melia azedarach* leaf meal remained numerically lower than silage of *Brachiaria* grass alone.

Table 40: Fermentative quality of *Brachiaria* forage as influenced by *Melia azedarach* leaf meal

Treatment	Opening days		
	D0	D3	D40
T1	5.63	4.82	4.56
T2	5.44	4.25	4.27
T3	5.41	4.45	4.30
SEM	0.052	0.177	0.089
p-value	0.1107	0.2123	0.1763

T1 = *Brachiaria* grass alone; T2 = *Brachiaria* grass + 20% *Melia azedarach* leaf meal, and T3 = *Brachiaria* grass + 30% *Melia azedarach* meal. D0 = ensiling days, D3 = Silage opened 3 days post-ensiling, D40 = Silage opened 40 days post-ensiling. SEM = Standard error of mean.

Table 37 showed dry matter changes of *Brachiaria* mixed with *Melia azedarach* leaf meal as influenced by fermentation process. The DM content of *Brachiaria* mixed with *Melia azedarach* leaf meal was numerically low throughout the ensiling process, however, the difference was not significant ($p > 0.05$). The sample treatment (T2) recorded low dry matter drop due to fermentation as compared to the other two treatments (i.e. T1 and T3). For example, after day 3 of ensiling, T2 dropped by 0.33% of dry matter due to fermentation, whereas T1 and T3 dropped by 5.0% and 6.0%, respectively. While on day 40 post-ensiling, T1 lost 1.9% of dry matter, while T1 and T3 lost 10.2% and 9.3% of dry matter, respectively. On day 40 of post-ensiling *Brachiaria* grass silage recorded numerically lower dry matter with no significant effect ($p > 0.05$). Generally, even though dry matter dropped due to the fermentation process, it did not drop below 28%.

Table 41: Dry matter changes of *Brachiaria* grass/*Melia azedarach* forage mixture as influenced by fermentation

Treatment	Opening days		
	D0	D3	D40
T1	32.86	31.23	29.50
T2	30.72	30.64	30.16
T3	34.90	32.80	31.67
SEM	1.336	2.609	1.551
p-value	0.2337	0.8418	0.6434

T1 = *Brachiaria* grass alone; T2 = *Brachiaria* grass + 20% *Melia azedarach* leaf meal, and T3 = *Brachiaria* grass + 30% *Melia azedarach* meal. D0 = ensiling days, D3 = Silage opened 3 days post-ensiling, D40 = Silage opened 40 days post-ensiling. SEM = Standard error of mean.

7.3.2. Silage aerobic stability

Table 38 showed the pH value of silage exposed to oxygen over a period of 8 days. The pH value of silage at day 2 of oxygen exposure remained the same as at the opening day 40 (D0). There was no treatment effect on pH changes of the silage exposed to oxygen over 6 days of silage exposure, although, T2 and T3 recorded low pH as compared to T1 silage. However, on day 8 of silage exposure

to oxygen a significant change in silage pH was observed. Silages in T2 and T3 showed a significant ($p < 0.05$) increase of pH as compared to T1 silage. Silages produced from *Brachiaria* grass mixed with *Melia azedarach* leaf meal recorded higher pH values than that of *Brachiaria* grass alone at day 8 of oxygen exposure.

Table 42: Changes in pH value of silage exposed to oxygen over a period of time.

Treatment	Days of oxygen exposure				
	D0	D2	D4	D6	D8
T1	4.56	4.56	4.45	4.89	4.95 ^b
T2	4.27	4.27	4.31	4.72	5.43 ^a
T3	4.30	4.30	4.42	4.65	5.58 ^a
SEM	0.089	0.089	0.099	0.053	0.083
p-value	0.1763	0.1763	0.6084	0.1015	0.0256

7.4. Discussion and Conclusion

Fermentative quality of silage is determined by silage pH. The drop of pH to below 5 within the first three days of ensiling indicates that fermentation was effective (Liu et al., 2016; Ntakyo et al., 2020). The drop of pH values in treatments recorded in this study means that *Melia azedarach* leaf meal did not disturb fermentation, and all silage produced adequate lactic acid within the first three days of ensiling. According to a review by Kung et al. (2018), lactic acids produced by lactic bacteria contribute to the reduction in pH for silage. A well preserved silage is recommended to have pH of 4.2 and below (McDonald et al., 1991). However, in this study the pH values recorded from different treatment ranged from 4.27 to 4.56 (Table 36). Even though there was no statistical differences, the addition of *Melia azedarach* to the *Brachiaria* grass assisted in reducing silage pH. This is because T2 and T3 were the only treatments which recorded pH values closer to 4.2. Therefore, in this study, mixing *Melia azedarach* leaf meal with *Brachiaria* grass forage at ensiling influenced fermentation quality of the silage. This is because, fermentation quality of silage is indicated by its pH value (Wang et al., 2020).

The dry matter (DM) content recorded at ensiling ranged between 30 and 35% which was within the range of 30 – 40% the recommended level for good fermentation (Dunière et al., 2013). According to Kharazian et al. (2024) DM content of forage at ensiling is a critical factor that affects silage fermentation quality, and intake of the silage. From this work, the DM decreased with time of ensiling and that was attributed to the fermentation process. However, DM content was still above the value of 28%, which is regarded as the minimum recommended value for DM (Borges et al., 2023).

Aerobic stability of the silage is regarded as the resistance of forage to the spoilage process after oxygen exposure (Kung et al., 2021). Therefore, the longer the resistance of silage to spoilage indicates the safer the silage to use as feed for livestock. This is based on the fact that silage spoilage is associated with an increase of microorganisms that metabolized lactic acids in oxygen exposed silage to produce acetic acid, carbon dioxide (CO₂) and water (Nascimento et al., 2023). In this study, pH was used to measure aerobic stability of silages exposed to oxygen, and when the pH value increased

above 5, the silage was regarded as spoiled silage. In this study it was observed that the silage produced was resistant to spoilage at least of 6 days, with pH values still below 5. This could mean that the silage is safe to feed to livestock up to 6 days after opening. However, T2 and T3 silage were no longer safe to feed livestock after day 8, owing to pH values increases above 5. Chen et al. (2022) and Addah (2022) associated an increase of pH and temperature in oxygen exposed silage with extensive growth of aerobic microorganisms such as yeast, mold and other bacteria such as clostridia. Therefore, the high pH value recorded on day eight of silage exposure to oxygen could be attributed to development of yeast and mold, even though this was not measured in this study. This indicates the toxicity of the silage, hence it is not recommended for livestock feeding.

In conclusion, this study showed that mixing *Brachiaria* grass with *Melia azedarach* leaf meal improved fermentation quality by reducing silage pH closer to a recommended level of 4.2. Since silage with a pH level of 4.2 and below is regarded as well fermented and preserved silage, a 20% inclusion of *Melia azedarach* leaf meal to *Brachiaria* is the recommended level for it resulted in the pH of 4.27. However, further studies are required to determine the silage microbial composition and nutritional quality to verify the overall quality of the silage produced.

CHAPTER 8

General Conclusion and Recommendations

Brachiaria grasses are increasingly valued in tropical and subtropical livestock systems due to their high biomass potential and adaptability. However, water-limitation remains a key production constraint for improved production. This is particularly true for dryland systems, typical of many African smallholder environments. This review synthesizes findings from the current research done on the evaluation of *Brachiaria* germplasm under water-limited growing conditions to identify cultivars and lines that are best suited for water-limited environments, and to highlight priorities for breeding initiatives.

General trends could be observed between the different studies conducted. Across the studies, water-limitation reduced biomass production, leaf size, and crude protein content, with genotypes differing in resilience. Several cultivars displayed adaptive traits such as increased root:shoot ratios, resource allocation to root systems, or extended green leaf retention. These traits underpin drought avoidance or tolerance mechanisms and are critical for forage availability under prolonged moisture stress.

When considering the findings at species level, it was shown that for *Brachiaria brizantha*, Line 710 and cv. Xaraes consistently outperformed other entries, showing stable yields and adaptation to low-moisture systems. This indicates their potential as cultivars for climate-resilient forage supply. *Brachiaria decumbens* cv. Basilisk showed stable yields across the years under dryland conditions and moderate drought tolerance, although irrigation significantly improved long-term productivity. Its relatively consistent seasonal production, particularly into autumn, makes it useful in systems with unpredictable and variable rainfall patterns. *Brachiaria nigropedata* cv. Sweetvelvet demonstrated strong drought tolerance, maintaining yields with no significant differences between irrigated and dryland systems. Other cultivars such as Kupfer, Nigropedata and Tosca benefited substantially from irrigation but were moderately drought tolerant. The *Brachiaria* hybrid cultivars Mulato II, Cayana and Sabia showed the most consistent dryland performance across years, generally outperforming other species under rainfed conditions. Their extended leaf retention into dry seasons enhances forage availability, although their crude protein levels were consistently low (<7% in summer), necessitating supplementation. From this work the best *Brachiaria* options to use under dryland systems are *B. brizantha* Line 710 and cv. Xaraes, *B. decumbens* cv. Basilisk, *B. nigropedata* cv. Sweetvelvet and all three *Brachiaria* hybrid cultivars i.e., Mulato II, Cayana and Sabia.

In order to improve the water-limitation tolerance of these *Brachiaria* species, the following priorities were identified. Firstly, the cultivars and lines that performed better under water-limited conditions were often those with improved root traits thus, breeding for deeper and more extensive root systems for improved water capture should be considered. Secondly, all of the cultivars/lines showed a lack of ability to maintain green leaf material into the winter months and therefore selecting for genotypes with prolonged greenness into dry seasons should be considered. One of the most important findings in the current study was the fact that the *Brachiaria* cultivars and lines valued were generally highly

susceptible to moisture limitation at the germination and seedling stages. Therefore, improving seedling-stage resilience to moisture stress should be a priority. Once the seedlings are able to establish, it is clear in this study that tolerance levels increased at the mature plant stage.

In conclusion, the *Brachiaria* grasses evaluated in this study exhibit considerable genetic variation in response to water-limitation. While irrigation improved productivity, certain species, cultivars and lines perform well in water-limited systems. For immediate use, *B. brizantha* Line 710 and Xaraes, *B. decumbens* Basilisk, *B. nigropedata* Sweetvelvet, and the *Brachiaria* hybrid Mulato II are recommended. For long-term improvement, breeding should target root architecture, seasonal persistence, and nutritional quality to develop forages that sustain productivity and animal performance under increasing climate variability.

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