INITIATIVE ON
Sustainable Animal Productivity

Alliance Bioversity & CIAT

Evaluation and selection of forage materials water stress

Juan Andrés Cardoso

The Americas Hub
Km 17, Recta Cali-Palmira CP 763537
Apartado Aéreo 6713
Cali, Colombia
Tel. (+57) 602 4450000

alliancebioversityciat.org/
www.cgiar.org

Executive Summary

Identification of forage materials by their adaptation to drought and waterlogging

Having few improved forage options puts the livestock sector at risk of low productivity in the event of extreme abiotic stress events (eg , droughts, floods). Currently, the CIAT genebank (now Alianza Bioversity -International-CIAT) and ILRI (International Livestock Research Institute) has the largest and most diverse collection of tropical forages in the world. This diversity of germplasm allows the selection of new materials with potential for adoption in different agroecological conditions in Colombia.

Information related to the existing diversity in tropical forages in terms of their resistance to drought and tolerance to waterlogging can serve to improve animal production in periods of drought and extreme rainfall. Additionally, knowledge of the development of different pastures and their water demands can better recommend the most appropriate genotypes for various climates and situations, and guide grazing management and irrigation schedules.

1. Evaluation in drought and flooding

1.1. Evaluation under drought conditions of some forage materials

Introduction

It is not true that plants displaying the C_4 photosynthetic pathway are more resistant than plants displaying the C_3 photosynthetic pathway . The development of water deficit in grasses (C_4) and legumes (C_3), as in any plant, occurs when the water supply does not match their water needs. The availability of water is essential to determine the productivity of C_4 grasses and C_3 legumes , and a wide variability has been found in the way they respond to periods of drought. In the tropics, perennial grasses and legumes are widely used for livestock production. The perennial nature of both groups of plants (grasses and legumes) means that they face drought conditions at some point in their productive lives. Thus, avoiding water deficit by controlling water loss is a common response in both tropical grasses and legumes. Plants control water loss through responses in the aerial part. Previous research by the Forage Network conducted at CIAT (now Alianza Bioversity Internacional-CIAT) showed significant differences in transpiration per unit leaf area and leaf senescence in various forage plants. Both responses serve to counteract excessive water loss in conditions of water deficit and are a rapid screening method for the selection of outstanding plants in drought conditions.

Therefore, the objective of this work was to improve the understanding of the responses and coping strategies of eleven genotypes of tropical forages (four legumes and seven grasses) under drought conditions under greenhouse conditions. Therefore, measurements of transpiration (total water extracted by the plants), leaf area and leaf senescence were determined in a period after 21 days of plant growth under well-irrigated or dry conditions. This information can contribute to a good targeting of different genotypes for different agroecological zones with different patterns of drought events.

Materials and methods

The methodology used in the present experiment was previously described in greater detail by Cardoso *et al* ., 2015 . The trial was carried out in a greenhouse at CIAT headquarters, Palmira, Colombia (latitude 3°29' N; longitude 76°21' W; altitude 965 m). During the course of the experiment, atmospheric conditions were recorded at a weather station (WatchDog 2475 Plant Growth Station , Spectrum Technologies Inc., USA) and were run at an average temperature of 30/22°C (day/night), a relative humidity of 42/74% (day/night) and a maximum photosynthetic active radiation (PAR) of 1200 µmol $m^{2} s^{-1}$ (mean daily PAR value of 750 µmol $m^{2} s^{-1}$. The soil used in this study was a Mollisol collected at CIAT facilities at 0–0.20 m from the soil surface. The soil was sieved to pass a 2 mm mesh.

The plant material used in this study consisted of vegetative propagules from 11 accessions of different forage species of grasses and legumes. The accessions studied were *Desmodium vellutinum* 23982, *Stylosanthes guianensis* 11995, *Centrosema molle* 15160, *Centrosema acutifolium* 15816, *B. brizantha* 26124, *M. maximus* 16051, *M. maximus* 6799, *M. maximus* 6172, *M. maximus* 6798, *M. maximus* Sabanera, *M. maximus* 6177 . The accessions came from the Bioversity-CIAT Alliance Genetic Resources Unit, with the exception of cv . Sabanera , from Agrosavía . The accessions are planted in pots filled with 4 kg of fertilized soil (milligrams of added nutrient per kilogram of soil: N 21, P 26, K 52, Ca 56, Mg 15, S 10, Zn 1.0, Cu 1.0, B 0.05 and Mo 0.05) and good irrigation conditions. Each propagule was selected visually for its homogeneity (4 m in length). The propagules were then replanted in a 2:1 (w/w) mix of soil and river sand that was previously fertilized (milligrams of nutrient added per kilogram of soil mix: N 40, P 50, K 100, Ca 101, Mg 28, S20, Zn 2.0, Cu 2.0, B 1.0 and Mo 1.0). This fertilization rate represents the recommended generic fertility level for crop and pasture establishment (Rao et al. 1992). After fertilization, the soil mix was allowed to air dry for a couple of days. The soil mixture presented an apparent density (ρ soil) of 1.4g/cm^{3}, organic matter of 6% and a pH of 7.5. After air-drying the soil, 5 kg of soil mixture were inserted into 4.5-liter pots. The soil mixture was then

saturated with water and allowed to drain for a couple of hours until it reached field capacity. After that, a propagule ~1 cm below the soil surface was planted in each pot with soil and irrigated daily to maintain field capacity under greenhouse conditions for 21 days. Subsequently, the plants were not watered for 28 days. Each genotype had four replicates.

Total plant transpiration

Daily transpiration was calculated for each replicate by weighing each cylinder throughout the experiment every 2 days and before collection (at 21 days). The well-irrigated treatment soil (control) was maintained at field capacity by adding the same mass of water lost through evapotranspiration over a 2-day period. The progressive drying of the soil treatment (hereinafter called drought) was imposed by the cessation of irrigation from the beginning of the experiment. Pots with soil but no plants were used to estimate soil evaporation only under the two levels of water supply. The total transpiration of the plants was calculated from the difference between evapotranspiration and evaporation (average value of four cylinders).

Determination of leaf area and percentage of dead leaves

The plants were collected 21 days after the start of the treatments. Dead and live leaves were separated manually and the leaf area was measured with a leaf area meter (Li-COR 3100, Li-COR Biosciences , USA).

Statistical analysis

All statistical analyzes were performed in R software (v 2.15.2) (R Development Core Team 2012). Data were checked using Levene's test for homogeneity of variance and log-transformed if necessary. The data was then analyzed using a one-way ANOVA. A post hoc analysis using the LSD test ($\alpha = 0.05$) was used to identify differences between genotypes by treatments.

Results and Discussion

The results with respect to transpiration, leaf area and transpiration per unit of leaf area are presented in Table 1. The results indicate that legumes presented lower efficiency in leaf blade production per unit of water transpired compared to grasses (*M maximus* and *U. brizantha*) . This is due to the greater efficiency of plants with C_4 metabolism (grasses) over C_3 plants (legumes). Within the grasses, it was observed that *B. brizantha* CIAT 26124 showed lower transpiration per unit area than the rest of the grasses (*M. maximus*). This may be due to the fact that *U. brizantha*

CIAT 26124 presents lower leaf senescence under stress conditions than the *M. maximus accessions* included in this study (Table 1). It is possible that the low senescence of *U. brizantha leaves* is a genetically controlled attribute, similar to late senescence (' stay green ") observed in many other species. Within the *M. maximus accessions ,* no significant differences were found in terms of leaf area production, transpiration, or transpiration per area unit. However, the CIAT 6172 accession presented greater leaf senescence during the course of the experiment. This may indicate that CIAT 6172 is more sensitive to water scarcity conditions than the other *M. maximus accessions* included in this study. Within the legumes, it was found that *Stylosanthes guianensis* CIAT 11995 *and Desmodium vellutinum* CIAT 23982 are more sensitive to water scarcity than *Centrosema acutifolium* CIAT 15816 and *Centrosema mole* 23982. The best drought adaptation between *Centrosema acutifolium* CIAT 15816 and *Centrosema mole* 23982 may be due to a more efficient use of water and greater soil cover to minimize transpiration, as well as the redirection of the leaves upwards as a mechanism for dissipating excess energy.

Table 1. Transpired water, leaf area, transpiration per unit leaf area and percentage of dead leaves in eleven legume and grass genotypes under drought stress conditions under greenhouse conditions for 21 days. Data are averages of 4 replicates

	Water transpired (g plant $^{-1}$)	Leaf area (cm ^{2} plant $^{-1}$)	transpiration per unit leaf area	% leaves dead
Desmodium vellutinum 23982	169.0	196.6	1.2	100.0
stylosanthes guianensis 11995	185.3	182.4	1.0	100.0
u *brizantha 26124*	273.7	608.4	2.2	15.2
centrosema mole 15160	306.3	418.1	1.4	8.9
M maximus 16051	308.0	608.8	2.0	35.2
centrosema acutifolium 15816	345.3	443.5	1.3	10.2
M maximus 6799	368.5	722.2	2.0	32.4
M maximus 6172	370.3	655.4	1.8	48.5
M maximus 6798	394.8	753.7	1.9	37.2
m maximus Sabanera	397.3	716.3	1.8	32.1
M maximus 6177	399.3	779.2	2.0	29.6
Average	319.8	553.1	1.7	40.8
LSD 0.05	58.4	124.3	0.4	12.3

1.2. Evaluation under flooded conditions of some forage materials

Introduction

Soil waterlogging is an important limitation to the productivity of tropical forages. Soil waterlogging (or soil flooding) occurs when the soil is saturated with water. Due to the slow diffusion of gases in water, the exchange of gases between the soil and the atmosphere is strongly hampered. Soil waterlogging reduces plant growth as $O_{2\ availability}$ in the root zone decreases. The lack of oxygen in roots makes it difficult for the uptake of nutrients by mass flow (for example, nitrogen). This means that, if the plants do not have adaptations to withstand waterlogging, a symptom is the progressive "yellowing" of the leaves (loss of chlorophyll in the leaves). This loss of chlorophyll in leaves can result in the total death of the leaves and perhaps the plant. To cope with waterlogging, plants often develop new roots that are capable of taking up nutrients dissolved in soil or standing water. In previous studies carried out at CIAT, it was identified that the greater production of roots in conditions of waterlogging was an adequate "proxy" to evaluate tolerance for waterlogging in tropical forages (including legumes and grasses), and that plants with greater length of adventitious roots (defined as roots produced during waterlogging) showed lower leaf senescence.

Therefore, the objective of this work was to evaluate the responses and coping strategies of eleven genotypes of tropical forages (four legumes and seven grasses) under flooded conditions. Therefore, determinations of the length of cord roots and leaf senescence were carried out in plants subjected to stress due to waterlogging for 15 days.

Materials and methods

The methodology used in the present experiment was previously described in greater detail by Cardoso *et al* ., 2013 . The trial was carried out in a greenhouse at CIAT headquarters, Palmira, Colombia (latitude 3°29' N; longitude 76°21' W; altitude 965 m). During the course of the experiment, atmospheric conditions were recorded at a weather station (WatchDog 2475 Plant Growth Station , Spectrum Technologies Inc., USA) and were run at an average temperature of 31/22°C (day/night), a relative humidity of 39/68% (day/night) and a maximum photosynthetic active radiation (PAR) of 1000 µmol m *2* s ⁻ 1 (mean daily PAR value of 690 µmol $m^{2} s^{-1}$). Inside the greenhouse, 3 radiators were located to increase the temperature and increase the vapor pressure to constant values between 2.0 and 2.5 kPa and simulate the humid environment of

the rainy season. The soil used in this study was a Mollisol collected at the CIAT facilities at 0–0.20 m from the soil surface. The soil was sieved to pass a 2 mm mesh.

The plant material used in this study consisted of vegetative propagules from 11 accessions of different forage species of grasses and legumes. The accessions studied were *Desmodium vellutinum* 23982, *Stylosanthes guianensis* 11995, *Centrosema molle* 15160, *Centrosema acutifolium* 15816, *B. brizantha* 26124, *M. maximus* 16051, *M. maximus* 6799, *M. maximus* 6172, *M. maximus* 6798, *M. maximus* Sabanera, *M. maximus* 6177 . The accessions came from the Bioversity-CIAT Alliance Genetic Resources Unit, with the exception of cv . Sabanera , from Agrosavía . *The accessions were* planted in pots filled with 4 kg of fertilized soil (milligrams of added nutrient per kilogram of soil: N 21, P 26, K 52, Ca 56, Mg 15, S 10, Zn 1.0, Cu 1.0, B 0.05 and Mo 0.05) and good irrigation conditions. Each propagule was selected visually for its homogeneity (0.04 m in length. The propagules were then replanted in a 1:1 (w/w) mixture of soil and river sand that was previously fertilized (milligrams of added nutrient per kilogram of soil mix: N 40, P 50, K 100, Ca 101, Mg 28, S20, Zn 2.0, Cu 2.0, B 1.0 and Mo 1.0.) This fertilization rate represents the recommended fertility level for the establishment of crops and pastures (Rao et al. 1992).After fertilization, the soil mix was allowed to air dry for a couple of days.The soil mix had a bulk density (ρ soil) of 1.5g/cm ^{3}, 6% organic matter, and a pH of 8.0.After air-drying the soil, 5 kg of soil mix was inserted into 4.5-litre pots.The soil mix was then saturated with water and allowed to drain for a couple of hours to reach field capacity.After that, a ~0.01 m propagule was planted below the surface d the soil in each soil cylinder and irrigated daily to maintain field capacity under greenhouse conditions for 15 days. Subsequently, the pot was introduced into another larger and larger pot to maintain a constant sheet of water 3cm above the ground. Each genotype had four replicates.

Determination of leaf area and percentage of dead leaves

The plants were collected 21 days after the start of the treatments. Dead and live leaves were separated manually and the leaf area was measured with a leaf area meter (Li-COR 3100, Li-COR Biosciences , USA).

Determination of cord root length

The roots separated from the stems. Under a dissecting microscope, roots that appeared to be damaged were graded and discarded. Following this, the roots were placed in a water-filled acrylic tray and carefully arranged so that there was no overlap of lateral roots. These roots

were scanned to record gray images at 600 dpi with a dual scanner (EPSON Expression 1680, Japan). The scanned images were then used for root length determination using WinRhizo (Regent Instruments, Canada).

Statistical analysis

All statistical analyzes were performed in R software (v 2.15.2) (R Development Core Team 2012). Data were checked using Levene's test for homogeneity of variance and log-transformed if necessary. The data was then analyzed using a one-way ANOVA. A post hoc analysis using the LSD test ($\alpha = 0.05$) was used to identify differences between genotypes.

Results and Discussion

Significant differences were found between the different genotypes for the length of cord roots and percentage of dead leaves. Within *M. maximus accessions* , there are differences in the amount of root production induced by waterlogging at surface nodes. In particular, CIAT 6798 and 6177, cv . Sabanera show a greater number of roots. The aforementioned genotypes also show taller stems under flooded conditions than under control conditions and have lower percentages of dead leaves (Table 1). The production of cord roots and greater stem elongation under waterlogged conditions suggests that these genotypes have an escape response (oxygen search) that is beneficial to them. *Desmodium Vellitunim* CIAT 23982 shows hypertrophied lenticels. The genotypes of *Centrosema molle* , *C. angustigolia* and *Stylosanthes guinanensis* show adventitious roots. In general, it is suggested that genotypes capable of producing a greater number of cord roots are more tolerant to waterlogging. It is important to note that the soil used is a soil that does not contain highly concentrated redoximorphic elements (iron and manganese) that can reach toxic concentrations under flooded conditions. It is probable that the outstanding genotypes in this type of soil (Mollisol , pH > 7), do not support waterlogging conditions in acid soils with high iron and manganese contents.

Table 1. Root length and % of dead leaves in eleven genotypes of legumes and grasses under stress conditions due to waterlogging under greenhouse conditions for 15 days. Data are average of four repetitions.

	Cordial roots length (plant cm $^{-1}$)	% leaves dead (floor $^{-1}$)
Desmodium vellutinum 23982	15.2	81.0
S. guianensis 11995	317.3	5.0
B.brizantha 26124 _	0.5	76.2
centrosema mole 15160	1.3	46.9
M maximus 16051	5.7	45.2
centrosema acutifolium 15816	0.8	9.2
M maximus 6799	268.5	22.4
M maximus 6172	70.4	58.5
M maximus 6798	184.2	32.2
M. maximus Sabanera	207.5	28.1
M maximus 6177	179.3	39.6
Average	113.7	40.4
LSD 0.05	49.4	15.3

1.3. Variation of *Cenchrus accessions ciliaris* under progressive drought stress

Introduction

Cenchus ciliaris L. (Poaceae) is a highly cultivated grass in semi-arid zones (rainfall less than 700mm per year) and it grows well in loams. Under these conditions, *Cenchrus ciliaris* is cultivated as a forage plant since its leaves remain green during periods of low water availability and it is palatable to livestock. Previous research at CIAT has suggested that *Cenchrus ciliaris* is more productive than *Urochloa brizantha* cv . Toledo and U. hibrido cv . Mulatto in the sub-humid to humid tropics, with clay loam soils and marked rainy seasons (eg , Santander de Quilichao, Palmira). However, internal research carried out at CIAT itself suggests the opposite (CIAT Rede Forrajes Report, 2018).

Therefore, the objective of this work was to improve the understanding of the responses and coping strategies of 15 accessions of *Cenchrus ciliaris* under drought conditions under greenhouse conditions. Therefore, measurements of dry matter production in stems and roots,

root:stem ratio , height and canopy temperature depression were determined in a period after 21 days of plant growth under well-irrigated or dry conditions.

Materials and methods

The methodology used in the present experiment was previously described in greater detail by Cardoso *et al* ., 2015 . The trial was carried out in a greenhouse at CIAT headquarters, Palmira, Colombia (latitude 3°29' N; longitude 76°21' W; altitude 965 m). During the course of the experiment, atmospheric conditions were recorded at a weather station (WatchDog 2475 Plant Growth Station , Spectrum Technologies Inc., USA) and were run at an average temperature of 30/22°C (day/night), a relative humidity of 40/70% (day/night) and a maximum photosynthetic active radiation (PAR) of 1100 µmol m *2* s ⁻ 1 (mean daily PAR value of 710 µmol $m^{2} s^{-1}$). Inside the greenhouse, 3 radiators were located to increase the temperature and increase the steam pressure to constant values between 3.0 and 3.5 kPa. The soil used in this study was a Mollisol collected at the CIAT facilities at 0–0.20 m from the soil surface. The soil was sieved to pass a 2 mm mesh.

The plant material used in this study consisted of vegetative propagules from 15 accessions of *Chloris gayana* from the ILRI (International Livestock Research Institute, Ethiopia) germplasm bank that were grown in pots filled with 4 kg of fertilized soil (milligrams of added nutrient per kilogram of soil: N 21, P 26, K 52, Ca 56, Mg 15, S 10, Zn 1.0, Cu 1.0, B 0.05 and Mo 0.05) and good irrigation conditions. Each propagule was selected visually for its homogeneity (0.04 m in length. The propagules were then replanted in a 2:1 (w/w) mixture of soil and river sand that was previously fertilized (milligrams of added nutrient per kilogram of soil mix: N 40, P 50, K 100, Ca 101, Mg 28, S20, Zn 2.0, Cu 2.0, B 1.0 and Mo 1.0.) This fertilization rate represents the recommended fertility level for the establishment of crops and pastures (Rao et al. 1992).After fertilization, the soil mix was allowed to air dry for a couple of days.The soil mix had a bulk density (ρ soil) of 1.4g/cm^{3} , 6% organic matter, and a pH of 7.5.After air-drying the soil, 5 kg of soil mix was inserted into clear plastic cylinders (80 cm high x 7.5 cm diameter) inserted into yellow PVC tubes. of the same dimensions.The soil mixture was then saturated with water and allowed to drain for a couple of hours until reaching field capacity. After that, a ~0.01 m propagule was planted below the soil surface in each soil cylinder and irrigated daily to maintain field capacity under greenhouse conditions for 21 days. Subsequently, a factorial combination of 15 *C. ciliaris genotypes was established* (plus two controls: *Urochloa brizantha* cv . Toledo and *Urochloa* hybrid cv . Mulato II) by two conditions of water supply (well irrigated and

progressive drying of the soil) in a randomized complete block of four replications. The experiment was carried out for 21 days.

Total plant transpiration

Daily transpiration was calculated for each replicate by weighing each cylinder throughout the experiment every 2 days and before collection (at 21 days). The well-irrigated treatment soil (control) was maintained at field capacity by adding the same mass of water lost through evapotranspiration over a 2-day period. The progressive drying of the soil treatment (hereinafter called drought) was imposed by the cessation of irrigation from the beginning of the experiment. Soil cylinders without plants were used to estimate soil evaporation only under the two levels of water supply. The total transpiration of the plants was calculated according to Cabrera - Bosquet et al. (2009) from the difference between evapotranspiration and evaporation (average value of four cylinders).

Depression (CTD)

Three leaves were selected per plant to monitor Canopy Temperature Depression (CTD) using an infrared light temperature gun (Spectrum technologies , USA). The CTD was calculated as the immediate difference in temperature in degrees Celsius between the leaves and the environment.

Determination of dry mass and height

The plants were harvested 21 days after starting the treatments. Stems (all of the aerial part) and roots were separated and washed under a delicate stream of water. These were then taken to a forced air oven maintained at 60 °C for 96 hours for the determination of dry mass. The dry mass of roots and stems were used to calculate the ratio between root and stem per plant. Plant height was determined prior to harvest with a tape measure.

Statistical analysis

All statistical analyzes were performed in R software (v 2.15.2) (R Development Core Team 2012). Data were checked using Levene's test for homogeneity of variance and log-transformed if necessary. The data was then analyzed using a one-way ANOVA. A post hoc analysis using the LSD test ($\alpha = 0.05$) was used to identify differences between genotypes by treatments. Controls

were included as reference but were excluded from the ANOVA tests. Hierarchical clustering was then performed using the simple method.

Results and Discussion

In general, the results indicate that *C. ciliaris* is less affected by drought than cvv . Toledo and Mulato II under the growth conditions of the present experiment. Transpiration and CTD data suggest that *C. ciliaris* they have greater stomatal control which reduces transpiration under control and drought conditions than cvv . Toledo and Mulato II. Greater stomatal control in plants is characteristic of grasses adapted to semi-arid and arid zones . The morphophysiological attributes of the different accessions of *C. ciliaris* registered in this experiment did not show significant differences (Table 1 and 2). The dendrogram (Figure 1) shows little variability in the attributes, which indicates that the variation between the accessions is continuous.

Table 1. Production of dry mass in stems, roots and root:stem ratio of 15 *Cenchrus accessions ciliaris* (plus two controls) under irrigation conditions and progressive drought (21 days) under greenhouse conditions. Data are averages of four replicates. LSD is the least significant mean difference test at α = 0.05. NS is non-significant LSD test.

Accession	Stem dry mass (g plant $^{-1}$)		Root dry mass (g plant $^{-1}$)		Root:Stem Ratio	
	Control	Drought	Control	Drought	Control	Drought
15687	5.433034	4.098205	5.433034	4.098205	0.757421	0.66608
777	5.025274	3.931443	5.025274	3.931443	0.650033	0.648
13299	5.340996	4.188672	5.340996	4.188672	0.581003	0.677701
16868	5.525987	4.382516	5.525987	4.382516	0.682743	0.59273
16660	5.68551	4.548925	5.68551	4.548925	0.655494	0.737764
16617	5.206501	4.165855	5.206501	4.165855	0.740069	0.702853
16609	6.924315	6.026191	6.924315	6.026191	0.622421	0.662798
6652	4.723712	4.201192	4.723712	4.201192	0.668035	0.696348
12825	5.058526	4.61117	5.058526	4.61117	0.735293	0.576248
6645	4.097276	3.750378	4.097276	3.750378	0.619916	0.728848
6642	7.58339	6.998513	7.58339	6.998513	0.546415	0.554743
2020	5.828524	5.428823	5.828524	5.428823	0.639421	0.60348
12464	2.78821	2.614411	2.78821	2.614411	1.003124	0.932454

6647	7.782225	7.436251	7.782225	7.436251	0.600339	0.527679
18483	6.24484	6.162738	6.24484	6.162738	0.589884	0.594026
Average	5.549888	4.836352	3.648367	3.139273	0.672774	0.660117
LSD	DK	DK	DK	DK	DK	DK
witnesses						
Toledo	13.90292	6.259815	13.90292	6.259815	0.390005	0.70448
Mulatto	11.99572	6.097491	11.99572	6.097491	0.399542	0.691542

Table 2. Height, total transpiration and CTD (canopy temperature depression) of 15 *Cenchrus accessions ciliaris* (plus two controls) under irrigation conditions and progressive drought (21 days) under greenhouse conditions. Data are averages of four replicates. LSD is the least significant mean difference test at α = 0.05. NS is non-significant LSD test.

Accession	Height (cm plant $^{-1}$)		Transpiration (liters plant water $^{-1}$)		CTD (° *C* plant $^{-1}$)	
	Control	Drought	Control	Drought	Control	Drought
15687	98.75	72.8	1.10	0.91	3.35	3.10
777	79.45	58.15	1.00	0.89	3.28	2.38
13299	90.2	48.2	0.99	0.93	3.20	2.48
16868	98.35	78.8	1.07	0.90	3.90	2.75
16660	92.4	62.75	1.07	1.00	3.33	2.65
16617	92.45	74.9	1.07	0.95	3.30	2.48
16609	98.85	73.45	1.18	1.11	2.70	2.80
6652	67.7	50.1	0.98	0.94	3.38	2.70
12825	101.8	87.8	1.05	0.91	3.33	2.70
6645	79.4	55.6	0.89	0.91	3.28	2.60
6642	113.45	85.6	1.20	1.15	3.00	2.78
2020	78.2	73.55	1.08	1.01	3.08	2.65
12464	71.75	68.6	1.02	0.91	3.20	2.80
6647	113.3	85	1.26	1.18	3.00	2.95
18483	107.1	81.3	1.20	1.18	3.48	2.73
Average	92.21	70.44	1.08	0.99	3.25	2.70
LSD	15.2	22.7	DK	DK	DK	DK
witnesses						
Toledo	152.95	65	1.44	1.10	3.95	1.33
Mulatto	118.05	57.5	1.38	1.16	3.93	1.03

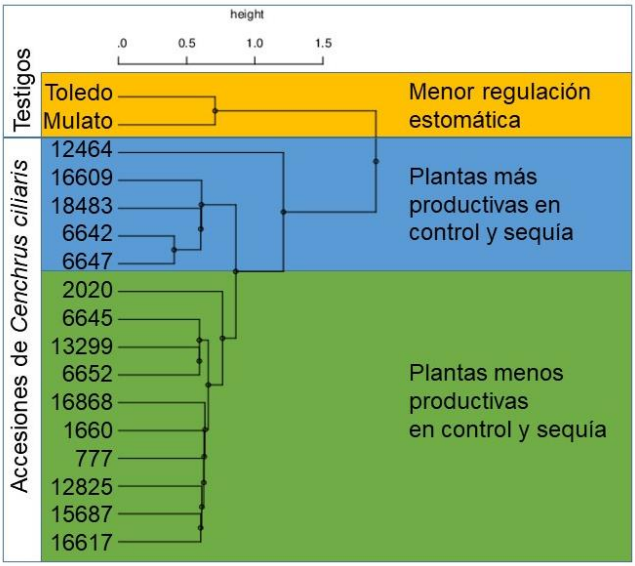


Figure 1. Hierarchical grouping of genotypes according to their morphophysiological attributes

1.4. Variation of *Chloris accessions gayana* under progressive drought stress

Introduction

Chloris gayana is a grass native to Africa, but widely cultivated and naturalized in the tropics and subtropics. *Chloris gayana* exhibits high phenotypic diversity and adaptability, as well as easy agronomic management, which makes this species interesting for its introduction into various production systems in Colombia. For various producers in Colombia, the introduction of new cultivars resistant to drought is a priority. CIAT imported 18 accessions of *Chloris gayana* for evaluation under dry conditions as part of the Forage Network. Preliminary data (see Red de Forrajes report, 2018) showed that *Chloris gayana* has a deep and long root system. Long and deep roots are associated with the maintenance of water status in drought conditions.

Therefore, the objective of this work was to improve the understanding of the responses and coping strategies of 18 accessions of *C. gayana* under drought conditions under greenhouse conditions. Thus, measurements of shoot and root dry matter production, root:shoot ratio , canopy temperature depression, and root attributes (root length density, average root diameter, and maximum root depth) they were determined in a period after 28 days of growth of the plants in conditions of good irrigation or conditions of drought.

Materials and methods

The methodology used in the present experiment was previously described in greater detail by Cardoso *et al* ., 2015 and is similar to that used for the *Cenchrus evaluation. ciliaris* , previously described . The trial was carried out in a greenhouse at CIAT headquarters, Palmira, Colombia (latitude 3°29' N; longitude 76°21' W; altitude 965 m). During the course of the experiment, atmospheric conditions were recorded at a weather station (WatchDog 2475 Plant Growth Station , Spectrum Technologies Inc., USA) and were run at an average temperature of 29/20°C (day/night), a relative humidity of 55/75% (day/night) and a maximum photosynthetic active radiation (PAR) of 1050 µmol $m^{2} s^{-1}$ (mean daily PAR value of 750 µmol $m^{2} s^{-1}$). Inside the greenhouse, 4 humidifiers were located to reduce the vapor pressure to constant values between 2.5 and 3.5 kPa. The soil used in this study was a Mollisol collected at the CIAT facilities at 0–0.20 m from the soil surface. The soil was sieved to pass a 2 mm mesh.

The plant material used in this study consisted of vegetative propagules from 15 accessions of *Chloris gayana* from the ILRI (International Livestock Research Institute, Ethiopia) germplasm bank that were grown in pots filled with 4 kg of fertilized soil (milligrams of added nutrient per kilogram of soil: N 21, P 26, K 52, Ca 56 , Mg 15, S 10, Zn 1.0, Cu 1.0, B 0.05 and Mo 0.05) and good irrigation conditions. Each propagule was selected visually for its homogeneity (0.04 m in length. The propagules were then replanted in a 2:1 (w/w) mixture of soil and river sand that was previously fertilized (milligrams of added nutrient per kilogram of soil mix: N 40, P 50, K 100, Ca 101, Mg 28, S20, Zn 2.0, Cu 2.0, B 1.0 and Mo 1.0.) This fertilization rate represents the recommended fertility level for the establishment of crops and pastures (Rao et al. 1992).After fertilization, the soil mix was allowed to air dry for a couple of days.The soil mix had a bulk density (ρ soil) of 1.4g/cm^{3} , 6% organic matter, and a pH of 7.5.After air-drying the soil, 5 kg of soil mixture was inserted into clear plastic cylinders (120 cm high x 7.5 cm diameter) inserted into yellow PVC tubes. of the same dimensions.The soil mixture was then saturated with water and allowed to drain for a couple of hours until reaching field capacity. After that, a ~0.01 m propagule was planted below the soil surface in each soil cylinder and irrigated daily to maintain field capacity under greenhouse conditions for 15 days. Subsequently, a factorial combination of 18 *Chloris genotypes was established. gayana* by two conditions of water supply (well irrigated and progressive drying of the soil) in a randomized complete block of four replications. The experiment was carried out for 28 days.

Canopy Temperature Depression (CTD)

Three leaves were selected per plant to monitor Canopy Temperature Depression (CTD) using an infrared light temperature gun (Spectrum technologies , USA). The CTD was calculated as the immediate difference in temperature in degrees Celsius between the leaves and the environment.

Determination of dry mass

The plants were harvested 28 days after starting the treatments. Stems (all of the aerial part) were separated and washed under a delicate stream of water. These were then taken to a forced air oven maintained at 60 °C for 96 hours for the determination of dry mass. The dry mass of roots and stems were used to calculate the ratio between root and stem per plant.

Morphological characteristics of the root system.

Prior to harvest, record the visual depth of the roots manually with a counter metric. The roots were washed to remove soil with tap water and then placed in a container with a few drops of wetting agent (polysorbate 20) for 10 min and rinsed again with tap water to remove loose soil. After that, the roots were placed in plastic bags and stored at -20 °C for further analysis. For morphological characterization, roots were carefully placed in a water-filled acrylic tray to minimize root overlap. Dead roots and debris were removed as much as possible from the tray with tongs and an eyedropper. The roots were then scanned to record gray images at 400 dpi with a dual scanner (EPSON Expression 1680, Japan). Recorded images were processed with ImageJ software to remove background noise (ie, <0.3 mm soil particles). The processed images were then used to estimate root length and average root diameter (ARD) using WinRhizo software (Regent Instruments, Canada). Root length density (the length of roots per unit volume of soil, RLD cm cm ^{3}) was calculated. After scanning, roots were carefully harvested to minimize material loss and oven-dried as previously described to determine root dry mass.

Statistical analysis

All statistical analyzes were performed in R software (v 2.15.2) (R Development Core Team 2012). Data were checked using Levene's test for homogeneity of variance and log-transformed if necessary. The data was then analyzed using a one-way ANOVA. A post hoc analysis using the LSD test ($\alpha = 0.05$) was used to identify differences between genotypes by treatments. Hierarchical clustering was then performed using the simple method. Additionally, simple

Pearson correlations were made between the different attributes measured under control and drought conditions.

Results and Discussion

Two contrasting accessions were identified, the most sensitive being accession number 13072; the accession with the best production in both control and drought conditions was 13103. In general, it is observed that the genotypes best adapted to drought conditions under greenhouse conditions were those with longer roots (but not necessarily deeper), thicker roots and a higher root:stem ratio (Tables 1 and 2). Table 3 shows the correlations between attributes under control or drought conditions. Figure 4 shows the hierarchical grouping by genotype based on attribute similarities.

Table 1. Production of dry mass in stems, roots, root:stem ratio and CTD (canopy temperature depression) of 18 *Chloris accessions gayana* under irrigation conditions and progressive drought (28 days) under greenhouse conditions. Data are averages of three replicates. LSD is the least significant mean difference test at α = 0.05. NS is non-significant LSD test.

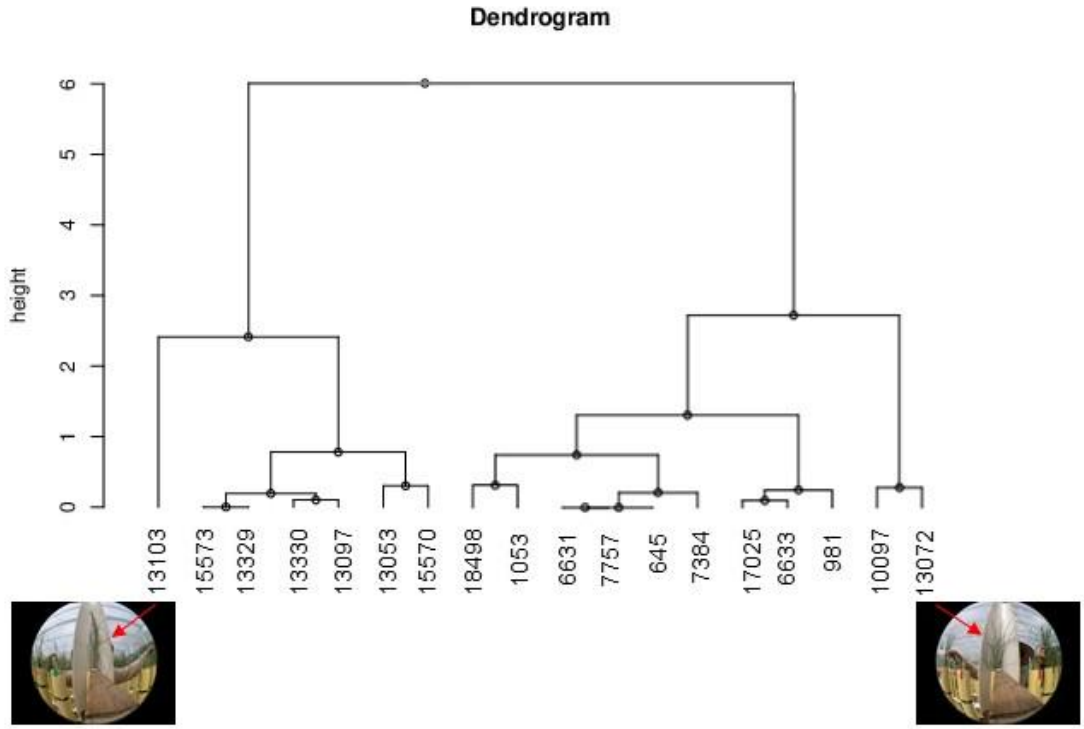
	stem dry mass (g plant $^{-1}$)		root dry mass (g plant $^{-1}$)		Proportion Root:Stem		CTD (° *C* plant $^{-1}$)	
Accession	Control	Drought	Control	Drought	Control	Drought	Control	Drought
13072	10.4	3.2	3,328	1,344	0.42	0.32	4.5	1
1053	11.0	3.8	4.95	2014	0.53	0.45	3.5	0.8
18498	11.1	4.1	5,217	2,419	0.59	0.47	4.2	1.2
7757	11.3	4.3	3,503	2,537	0.59	0.31	5	2.8
6631	10.4	4.3	4,784	2,365	0.55	0.46	3.9	0.9
645	10.1	4.3	3,737	2.15	0.5	0.37	4.2	1.3
13330	11.3	5.0	4,972	2.85	0.57	0.44	4.7	1.3
10097	7.6	3.4	3,572	1,972	0.58	0.47	4.5	1.1
981	10.3	4.8	3,605	2,736	0.57	0.35	3.5	2.2
15573	11.0	5.1	3.41	2,958	0.58	0.31	5	2.7
6633	9.6	4.6	3.84	2,484	0.54	0.4	3.5	1.4
7384	9.2	4.4	2.76	2,508	0.57	0.3	4.3	2.7
13329	10.4	5.1	4,576	2,703	0.53	0.44	4.7	0.9
13053	10.0	5.3	4.2	2,809	0.53	0.42	3.5	1.1

15570	10.4	5.6	4,368	3.36	0.6	0.42	4.1	1.8
13097	9.2	5.0	4,232	2.95	0.59	0.46	3.9	1.3
10225	8.3	4.6	2,988	2,714	0.59	0.36	4.4	23
13103	11.9	6.7	4,165	4.02	0.6	0.35	3.8	2.5
Average	10.2	4.6	4.0	2.6	0.6	0.4	4.2	1.6
LSD	1.03	0.8	DK	1.32	DK	DK	DK	0.8

Table 2. Root length density (RLD), average root diameter (ARD) and maximum root depth of 18 *Chloris accessions gayana* under irrigation conditions and progressive drought (28 days) under greenhouse conditions. Data are averages of three replicates. LSD is the least significant mean difference test at α = 0.05. NS is non-significant LSD test.

	RL D (cm cm ^{3} plant $^{-1}$)		AVR (mm plant $^{-1}$)		deepening roots	
Accession	Control	Drought	Control	Drought	Control	Drought
13072	2.36	0.84	0.33	0.21	63.00	95.00
1053	3.97	1.00	0.44	0.27	93.00	69.00
18498	4.49	1.37	0.47	0.30	110.00	102.00
7757	3.00	2.11	0.39	0.33	99.00	116.00
6631	4.01	1.17	0.45	0.31	76.00	69.00
645	2.98	1.23	0.40	0.30	91.00	80.00
13330	4.15	1.50	0.50	0.37	101.00	113.00
10097	3.48	1.19	0.35	0.25	61.00	120.00
981	3.10	1.86	0.42	0.36	114.00	74.00
15573	2.91	2.13	0.43	0.39	66.00	88.00
6633	3.27	1.41	0.42	0.33	60.00	114.00
7384	2.47	2.03	0.36	0.33	105.00	82.00
13329	3.75	1.23	0.48	0.37	96.00	113.00
13053	3.49	1.34	0.48	0.38	81.00	95.00
15570	3.88	1.89	0.50	0.43	92.00	113.00
13097	3.88	1.56	0.46	0.37	113.00	114.00
10225	2.84	1.93	0.38	0.35	106.00	86.00
13103	3.55	2.30	0.54	0.52	85.00	69.00
Average	3.42	1.56	0.43	0.34	89.56	95.11
LSD	0.79	0.57	0.12	0.10	DK	DK

Figure 1. Hierarchical grouping of *Chloris accessions gayana* based on various morpho-physiological attributes under flooded conditions (for 28 days)



r	Tallo	Raiz	R:S	CTD	RLD	ARD	Profundidad
Tallo	1.0	0.9	-0.1	0.4	0.6	0.9	-0.1
Raiz	0.5	1.0	-0.1	0.5	0.8	0.9	0.0
R:S	0.0	0.1	1.0	-0.8	-0.6	-0.1	0.3
CTD	0.0	-0.3	0.1	1.0	0.9	0.5	-0.2
RLD	0.3	0.9	0.4	-0.2	1.0	0.7	-0.1
ARD	0.6	0.7	0.4	-0.3	0.7	1.0	-0.1
Profundidad	0.1	0.2	0.4	-0.1	0.2	0.2	1.0

Sequía

Control

Table 3. Simple Pearson correlations between quantified attributes. Cells in blue represent correlations under drought conditions. Green cells represent correlations under control conditions. Stem (stem dry mass), Root (root dry mass), R;S (root:stem ratio), RLD (root length density, ARD (mean root diameter), Depth (maximum root depth)

2. Phenotyping of new materials

2.1. Phenotyping of new materials using morphometric and image analysis methods

Introduction

There is a wide variety of promising forage plants, of which not much is known about their morphological characteristics. The characterization of plant morphology requires measuring the vegetative and reproductive structures. This type of characterization has allowed evaluating ecotypes or accessions under conditions of water deficit and their productive potential. This evaluation has made it possible to identify ecotypes or promising accessions for their introduction to the market or improvement programs . However, morphological characterization sometimes becomes a process that requires a lot of time and increases the possibility of generating errors in the measurements of different attributes. Currently, various approaches to plant phenotyping have been developed to assess diversity based on image analysis. Accordingly , the objective of this study was to evaluate a low-cost method using drone images to estimate color pixel values, morphometric parameters, and field coverage area.

Materials and methods

14 material accessions were established under field conditions at CIAT headquarters, Palmira, Colombia (latitude 3°29' N; longitude 76°21' W; altitude 965 m). each accession has 4 or 5 plants that have been imaged weekly with a drone (Phantom IV. DJI, China) equipped with a two-band camera that captures infrared (750-800nm) and red (650- 700) (Sentera , USA), and the drone's own camera (RGB).

Accession	gender	species	observation
9689	*aeschynomene*	*Brazilian*	
8487	*aeschynomene*	*histri x*	VAR.DENSIFLORA
3794	*Desmodium*	*heterocarpon*	VAR.OVALIFOLIUM
10260	*stylosanthes*	*guianensis*	EX IZ- SP,BRA
18866	*alysicarpus*	*rugosus*	
2653	*stylosanthes*	*humilis*	
22087	*phlemingian*	*macrophylla*	
2783	*stylosanthes*	*humilis*	
4079	*macroptilium*	*erythroloma -?*	
10274	*stylosanthes*	*guianensis*	D.E.CIAT 168 _

2935	*stylosanthes*	*capitata*	
11782	*stylosanthes*	*hamata*	
2509	*stylosanthes*	*viscose*	
2110	*stylosanthes*	*viscose*	
106915 accessions op6	*stylosanthes*	*scabra*	

Results and Discussion

Due to the cessation of activities at the CIAT facilities (COVID-19), there were problems (eg , weeds and difficulty in obtaining drone data) in data collection. The weeds were mixed with the plants to be evaluated and it was not possible to accurately distinguish weeds from plants of interest.

3. Characterization of evapo-transpiration rates

3.1. Characterization of evapotranspiration rates in *Urochloa cultivars spp .* and *Megathyrus maximus*

Introduction

Knowledge of water use by forage cultivars (evapotranspiration, ETc) can contribute to the direction of appropriate genotypes for various climates and situations, and guide grazing management and irrigation schedules. An integrating measure to help characterize plant water use is the crop coefficient (k).

Data obtained previously (see final report of Red de Forrajes 2019) empirically showed the relationships between ETc , K and the leaf area index (LAI) in several *Urochloa genotypes.* spp . and *M. maximus* . In general, the relationships between them are described by quadratic equations of the third level as follows:

$K= -0031(ETc^{2}) + 0.40 (ETc)+ 0.11, R^{2} = 0.8, p< 0.001$

$K= -0.026(IAF^{2}) + 0.35 (IAF) + 0.08, R^{2} = 0.8, p< 0.001$

However, the empirical determination of ETc , K or IAF is demanding. Therefore, one of the objectives of this study was to determine crop evapotranspiration rates and crop coefficient in 13 *Urochloa cultivars.* spp . and *Megathyrsus maximus* under field conditions using meteorological information and obtained remotely with a drone. Additionally, special emphasis

was placed on K since the K values represent the integrated effects of changes in leaf area, plant height, crop characteristics, water availability, development rate, and plant life. soil and weather conditions.

Materials and methods

The trial was carried out under field conditions at CIAT headquarters, Palmira, Colombia (latitude 3°29' N; longitude 76°21' W; altitude 965 m). During the course of the experiment, atmospheric conditions were recorded at a weather station. The soil where the plants grew is a Mollisol with an apparent density (ρ soil) of 1.5g/cm ^{3}, organic matter of 8% and a pH of 7.3. The plant material used in this study consisted of 13 cultivars of *Urochloa* spp . and *Megathyrsus maximus* obtained from vegetative material saved by the CIAT tropical forage program. The cultivars were: *U. ruziensis* cv . Kennedy, *U. humidicola* cvv . Tully and Ranger, *U. decumbens* cv . Basilisk , *U. brizantha* cvv . Piata , Toledo , Marandú , *U.* hybrids cvv . Mulatto, Mulatto II, Cayman and Cobra, *M. maximus* cvv . Tanzania and Mombaca . The cultivars were established in an area of 4 m ^{2}. Crop evapotranspiration (ETc) was calculated for these using the Penman-Monteit Fao 56 equation. Plant height was standardized every nine weeks (20 cm above the ground) to record changes in growth. Additionally, crop coefficient (k) was calculated according to evapotranspiration data from a reference surface (ETo) and crop (ETc) according to the formula k = ETc / ETo .

The reference surface evapotranspiration (ETo) is a hypothetical grass reference crop with an assumed crop height of 0.12 m, a fixed surface resistance of 70 sm $^{-1}$ and an albedo of 0.23. The reference area closely resembles a large, well-watered, green grass area of uniform height, actively growing and completely covering the ground. The fixed surface resistance of 70 sm $^{-1}$ implies a moderately dry soil surface as a result of weekly irrigation frequency. For ETc calculations , leaf area index (LAI) data were used remotely calculated with a drone (Phantom IV. DJI, China) equipped with a two-band camera that captures infrared (750-800nm) and Red (650-700) (Sentera , USA), and the drone's own camera (RGB). IAF calculations were based on previous regressions obtained with Random Forests . The study was conducted for one year at weekly intervals (September 2019-September 2020).

Results and Discussion

The data obtained for different genotypes at weekly intervals are shown in the figure. In general, the growth patterns of the crop (eg .., k), followed the precipitation patterns (Figures 1

and 2). It was observed that the two cultivars of *M. maximus* grow faster after standardization. However, the growth rate of *M. maximus* slows down under conditions of minimal rainfall. On the contrary, the cvv . of Urochloa in general maintain constant growth rates during the different periods evaluated. This suggests that cvv . of *M. maximus* they are closer to the anisohydric model of water use (water wasters), than the cvv . of *Urochloa* spp .

The K values shown in the different crops under conditions with different rainfalls showed that the shape of the plant is related to the amount of water transpired. Within the evaluated cultivars, it was evidenced that the height of the canopy and the inclination of the leaves (data not shown) influence the amount of water transpired. The tallest plants with the most vertical position of their leaves (*M. maximus* cvv . Tanzania and Mombaca , and U. brizantha cv . Toledo) showed higher ETc and K values. In contrast, the shorter plants with more horizontal leaves (*U. humidicola* cvv . Llanero and Tully) showed lower ETc and K. Within the Urochloa hybrids , this pattern was repeated: cvv . cayman showed taller plants with more vertically positioned leaves and higher ETc and K values than cv . Mulato II (plants shorter and with leaves located in a plane more horizontally than cv . Cayman).

The data obtained from Etc , K, and IAF indirectly with the Penman - Monteith equation and drones indicate that this methodology can be implemented for large-scale estimation (eg , farms) and support agronomic management of forages and grazing, as well as to guide irrigation application with greater precision and efficiency in water resources

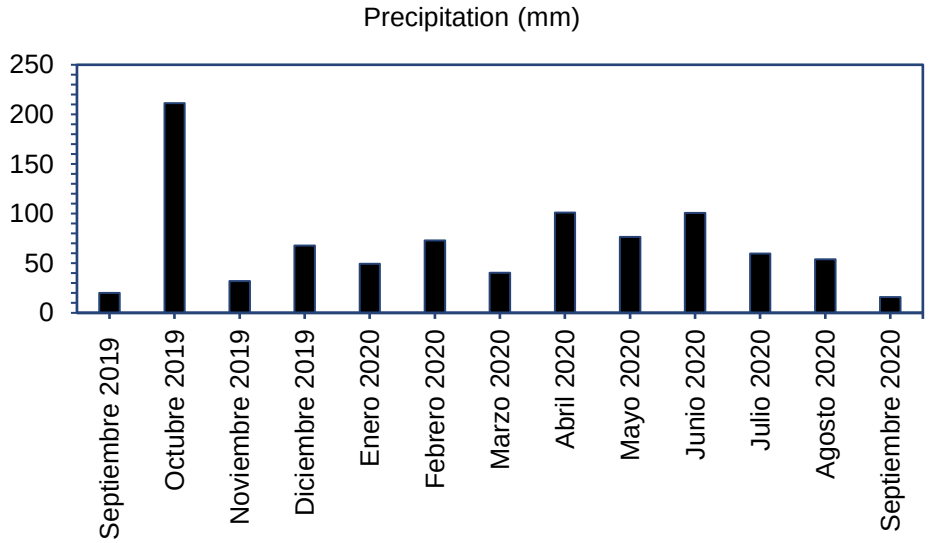
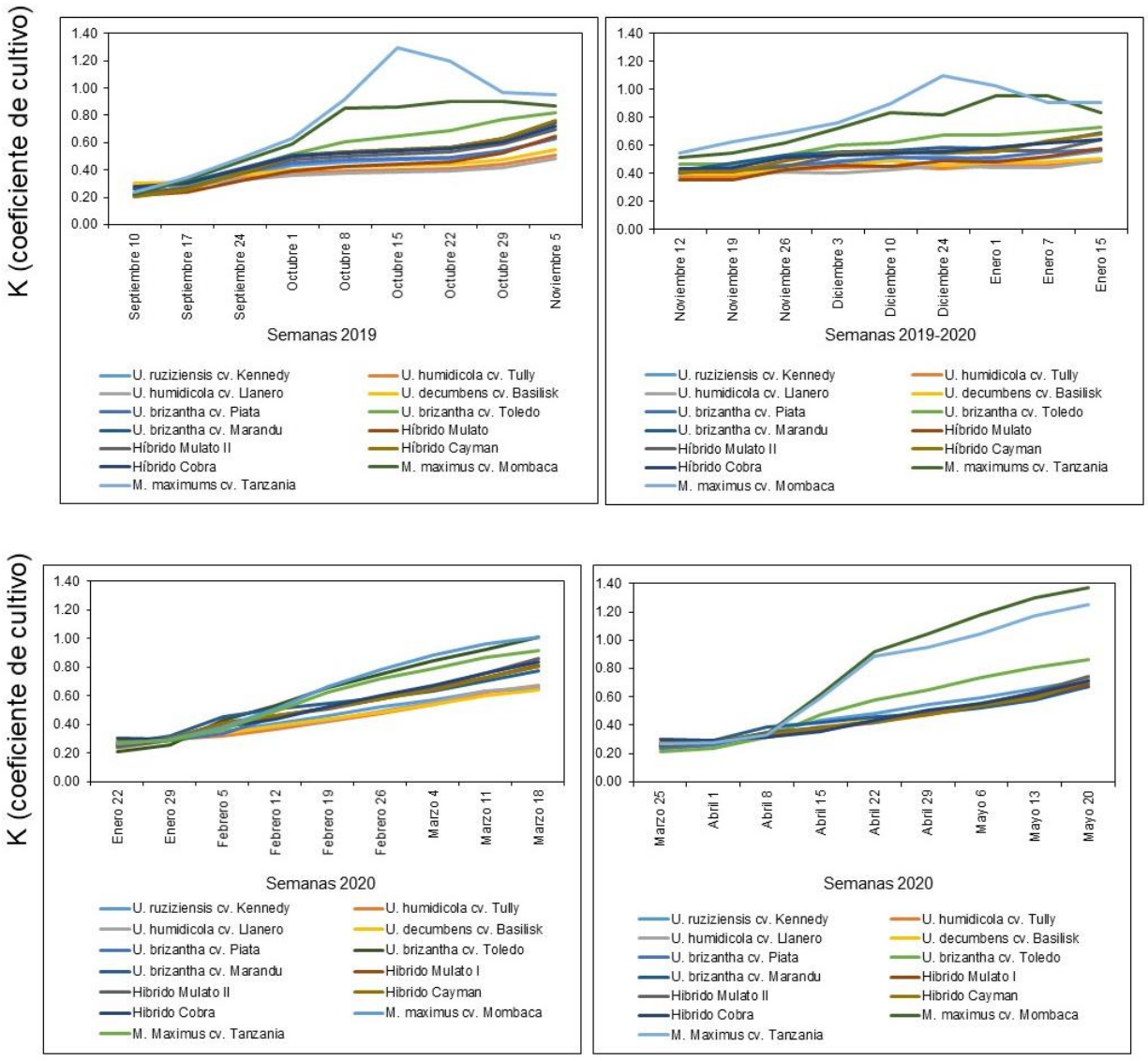


Figure 1. Precipitation averages per month (2019-2020) at the CIAT facilities, Palmira



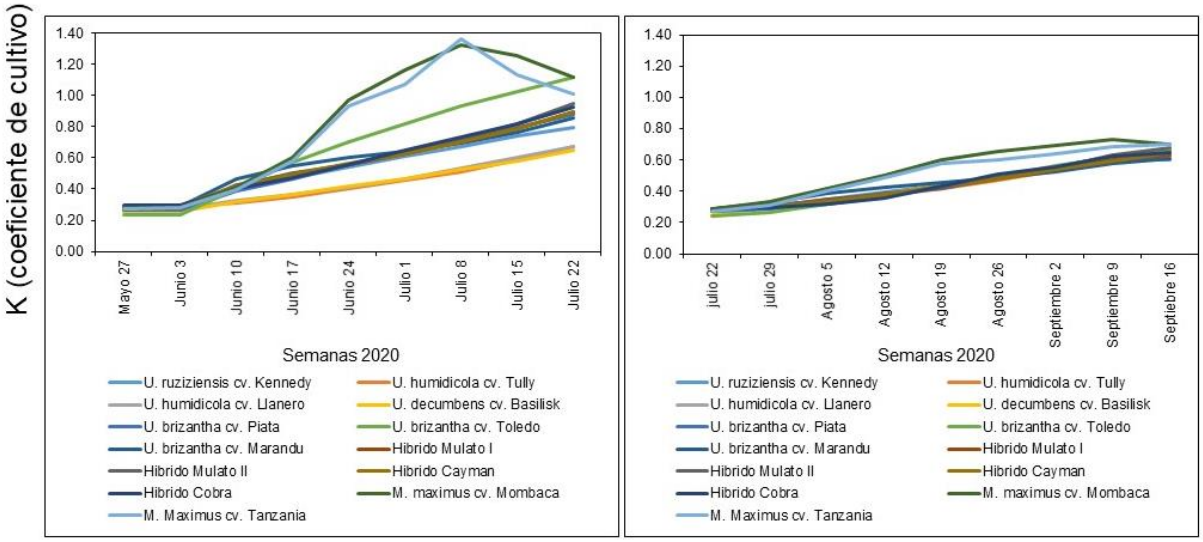


Figure 2. Crop coefficients for 13 cvv . of *Urochloa* spp . and *M. maximus* in periods of 9 weeks during 2019-2020

4. Conclusions

The different genotypes studied differ in terms of resistance to drought and waterlogging, but surely the classifications may vary between different studies, probably due to different climates, soil types, duration of imposed stresses, and evaluation methods. Differences in root growth in constrained soils (for example, high resistance to penetration) may influence resistance to drought.

Likewise , surely the ranges of ETc and K of tropical forages vary widely depending on the environment (evaporative demand and soil water). The K data obtained in this instance of the Forage Network provide valuable reference points for making irrigation recommendations. Further work is needed to understand the genotypic variation of different forages in terms of ET (or K or LAI) and the possible influence of canopy morphology as well as physiological traits (e.g. osmotic adjustment, stomatal regulation, sensitivity to low values). high atmospheric steam demand). both in conditions of field capacity or drought.

5. Recommendations

It is necessary that the information obtained from previous studies carried out under greenhouse and field conditions be integrated. Although phenotypic information under greenhouse conditions may seem irrelevant to some breeders and agronomists, many traits of interest are not easy to accurately record under field conditions. The combination of data from both greenhouse and field conditions should provide relevant information to quantify genotype x environment interactions to establish priorities for breeding key plant traits in stressful environments.

Currently, information obtained remotely by drones makes heavy use of segmentation processes based on pixels or shapes. However, one limitation (as observed in this report) is that it is easy to confuse weeds with plants of interest under this modality of image analysis. It is recommended to use semantic segmentation to face this limitation.