Genotypic variation in drought resistance among *Urochloa* Hybrids (population Br15)

Juan Andres Cardoso

CGIAR RESEARCH PROGRAM ON Livestock
Alliance Bioversity International CIAT

Genotypic variation in drought resistance among *Urochloa* Hybrids (population Br15)

Juan Andres Cardoso (j.a.cardoso@cgiar.org)
Tropical forages Program, Alliance of Bioversity International and CIAT

Highlights

- **Hybrids with longer and deeper roots extracted more water, in turn reflected in greater shoot dry mass production under water-limited conditions.**

Rationale

Previous research showed a correlation in the ability to uptake water from drying soil and shoot biomass production in *Urochloa* hybrids (Jimenez et al, 2016). The ability to uptake water in drying soil is associated with a larger and deeper root system in *Urochloa* grasses (Cardoso et al., 2015). Bearing that into mind, drought resistance in *Urochloa* grasses has been defined as the ability to produce more biomass under water-limiting conditions. Due to improvements in off-the-shelf imaging sensors and image analyses, routine estimation of visible total root length and deep rooting (root length at depth below 60cm of soil surface) is now a reality (Cardoso and Rao, 2019). However, such estimations are not straightforward. Maximum rooting depth (i.e., length of the longest visible root) holds promise for its use as a proxy for deep rooting. The following work aimed to evaluate the variation in dry mass, water uptake, maximum rooting depth, deep rooting and total root length of 84 hybrids of *Urochloa* hybrids after three weeks of growth under well-watered and water-limiting conditions. We hypothesized that hybrids with larger and deeper roots systems could extract more water in drying soil which in turn is reflected in greater shoot dry mass production. We also hypothesized that maximum rooting depth could be used as a surrogate measurement of deep rooting. Pearson correlation, simple and multiple linear regressions were calculated to test the relationship between individual and combination of root traits upon shoot biomass. Broad sense heritability was calculated for each trait and watering to test their relevance and suitability for screening purposes.

Materials and methods

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 run at an average temperature of 31/22 ° C (day / night), a relative humidity of 41/69% (day / night) and a maximum photosynthetic active radiation (PAR) of 1100 µmol m^2 s^{-1} (average daily value of PAR of 710 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 of 84 interspecific hybrids of *Urochloa* that were grown in pots filled with 4 kg of fertilized soil (milligrams of nutrient added 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 well irrigated conditions. Plant materials were developed and obtained from the interspecific *Urochloa* hybrid program of CIAT (now the Alliance of Bioversity-CIAT). Each propagule was visually selected for its homogeneity (0.04 m in length). The propagules were then replanted in a 2: 1 (weight / weight) 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 mixture was allowed to air dry for a couple of days. The soil mixture presented an apparent density (ρ soil) of 1.5g cm^3, 6% organic matter and a pH of 7.5. After air drying the soil, 8 kg of soil mixture was inserted into transparent 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. After that, field capacity of soil was determined for each soil cylinder. After that, a propagule ~0.01 m below the soil surface was planted in each soil cylinder and watered daily to maintain field capacity under greenhouse conditions for 21 days. Subsequently, a factorial combination of 84 hybrids was established by two conditions of water supply (well-watered and progressive drying of the soil) in a randomized complete block of three repetitions. The experiment was carried out for 21 days.

Water uptake of plants
Water uptake was calculated for each repetition by weighing each cylinder throughout the experiment every 2 days and before harvesting (at 21 days). The well-watered soil treatment was kept at field capacity by adding the same mass of water lost by evapotranspiration in a period of 2 days. The progressive drying of the soil treatment (hereinafter called water-limitation) 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. Water uptake (or transpiration) was calculated according to Cabrera-Bosquet et al. (2009) from the difference between evapotranspiration and evaporation (average value of three cylinders).

Determination of root length and dry mass
Prior to harvesting (21 days after imposition of treatments), two different views of each soil column were photographed using a digital camera (Nikon, Coolpix p6000, Nikon, Japan) mounted on a fixed tripod. Recorded images were then processed in ImageJ software (NIH, USA) to subtract background (i.e., soil) out of roots using K means algorithm. Maximum rooting depth was recorded with a ruler in each plant (length of the longest visible root in the soil column) prior to harvesting. Root length was estimated for the entire root column (0-120 cm) and for the 60-120 cm of soil depth (i.e., deep rooting). After that, shoot (all of the aerial part) and roots were separated and washed under tap water. After that, shoots and roots were oven dried at 60 ° C for 96 hours for the determination of dry mass

Statistical Analyses
Simple correlation (r), regression (R^2) and stepwise multiple linear regression (R2) analysis were performed to test association and prediction value between maximum rooting depth, root length, deep rooting, water uptake, root biomass over shoot dry mass. Broad sense heritability was calculated for each trait under the different watering treatments. Analyses were performed using R software.

Results and discussion

- Water-limitation resulted in smaller plants in most hybrids. *Urochloa* hybrids that did not show significant differences in shoot dry mass production under different treatments were those that were inherently small under well-watered conditions.

- Larger hybrids (i.e., greater shoot dry mass) under well-watered conditions showed greater shoot dry mas under water-limiting conditions (Figure 1A-B).

- Variation in shoot dry mass among hybrids under water-limited could be explained to great extent by the variation of their root systems. In general, hybrids with larger and deeper roots showed greater water uptake (Table 1) and concomitantly, greater shoot biomass production.

- Single root traits that showed greater r and R^2 with shoot biomass under water-limited conditions were: root mass, deep rooting and total root length (Table 1). However, combination of root traits showed better model prediction of shoot biomass under water-limited conditions (Table 2). Taking into account parsimony of model with the greatest prediction, the model that included deep rooting and total root length was the most adequate one (R^2 and adjusted R^2 of 0.8).
- Overall, all measured traits showed broad sense heritabilities above 0.4. This suggests that all are suitable for their use in screening and breeding. Among them, water uptake showed greatest heritability. Whereas shoot dry mass showed the lowest heritability. Determination of water uptake (or transpiration) is a demanding and challenging task. In contrast, shoot dry mass is the easiest among measured traits in the present study. Root traits obtained in the present study sit in the middle regarding ease of measurement and heritability (Table 3).
- Maximum rooting depth was suggested as a proxy for deep rooting. However, maximum rooting depth showed low to moderate values of r to rooting depth (-0.35 for well-watered and -0.26 for water-limited conditions) and very low values of R^2 (0.12 for well-watered and 0.07 for water-limited conditions). This suggested that maximum rooting depth is not a fair surrogate for deep rooting under well-watered or water-liming conditions in *Urochloa* hybrids.
- The importance of root traits were evident in the present study. This can be seen as the increase of values of r, R^2, and heritability for root dry mass, deep rooting and total root length in respect to shoot dry mass under water-limited conditions (as compared to well-watered conditions). In particular, our results highlighted the relevance of longer and deeper rooting available water is stored in deep layers of soil. The identification of hybrids with long and deeper roots could make a difference between availability or lack of forage to feed livestock over long dry seasons.

Future outlook

- Further testing of the evaluated hybrids need to be performed under field conditions combined with measurements of shoot traits relevant to water-limited conditions (control of water loss)

Acknowledgements
This work was conducted as part of the CGIAR Research Program on Livestock and is supported by contributors to the CGIAR Trust Fund. CGIAR is a global research partnership for a food-secure future. Its science is carried out by 15 Research Centers in close collaboration with hundreds of partners across the globe.

Links to references

Cabrera-Bousquet et al. 2009. https://doi.org/10.1093/jxb/erp028

Cardoso et al., 2015. https://doi.org/10.1093/aobpla/plv107

Cardoso and Rao. 2019. https://www.taylorfrancis.com/chapters/edit/10.1201/9781351104609-42/drought-resistance-tropical-forage-grasses-juan-andr%C3%A9s-cardoso-idupulapati-rao

Jimenez et al., 2020 https://uknowledge.uky.edu/cgi/viewcontent.cgi?article=2077&context=igc

Rao et al., 1996. https://doi.org/10.1007/BF00011991

Figure 1. Shoot dry mass production of 84 *Urochloa* hybrids under well-watered and water-limited conditions for 21 days. A) All genotypes, B) genotypes that were above the average under both watering conditions. Plotted data are means of 3 replicates.

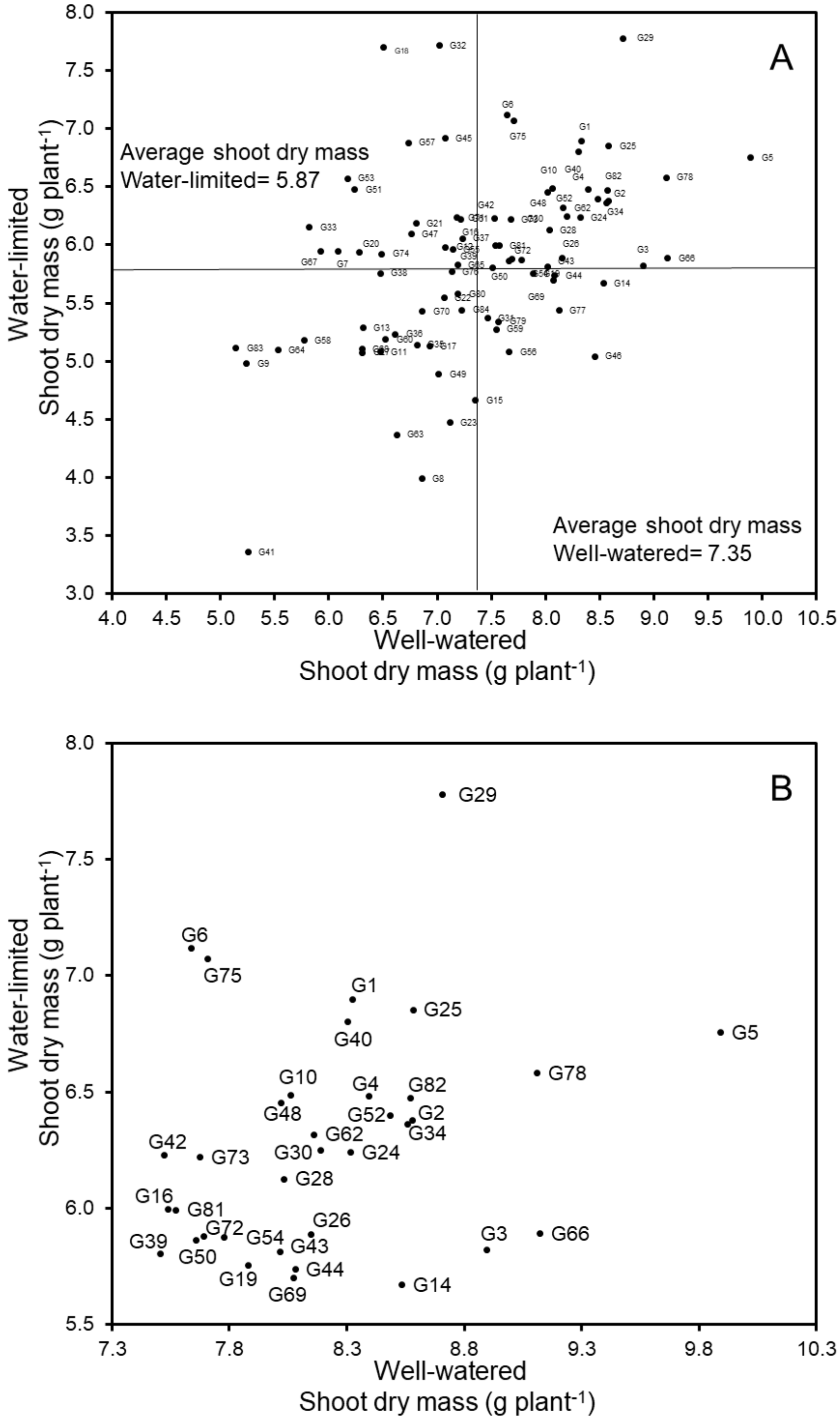

Table 1. Pearson and single regression coefficients of different traits and shoot dry mass under of 84 *Urochloa* hybrids well-watered and water-limited conditions for 21 days

Pearson correlation (r) Regression (R^2)		Root dry mass	Water uptake	Maximum rooting depth	Deep rooting	Total root length
Shoot dry mass	Well-watered	0.17 (r) 0.03 (R^2)	0.9 (r) 0.81 (R^2)	0.19 (r) 0.03 (R^2)	0.25 (r) 0.06 (R^2)	0.35 (r) 0.12 (R^2)
	Water-limited	0.32 (r) 0.10 (R^2)	0.73 (r) 0.54 (R^2)	0.11(r) 0.12 (R^2)	0.45 (r) 0.21 (R^2)	0.34 (r) 0.12 (R^2)

All r values above 0.15 are significant at $\alpha \leq 0.05$.

Table 2. Multiple linear regression coefficients of root traits to predict shoot dry mass of 84 *Urochloa* hybrids under water-limited conditions for 21 days

Multiple linear regression	Model 1 $Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3$	Model 2 $Y=\beta_0+\beta_1X_1+\beta_2X_2$	Model 3 $Y=\beta_0+\beta_1X_1+\beta_3X_3$	Model 4 $Y=\beta_0+\beta_2X_2+\beta_3X_3$
y = Shoot under water-limitation	Root mass (X_1), deep rooting (X_2), Total root length (X_3)	Root mass (X_1), deep rooting (X_2)	Root mass (X_1), total root length (X_3)	Deep rooting (X_2), Total root length (X_3)
R^2	0.847	0.210	0.545	0.832
Adjusted R^2	0.845	0.204	0.541	0.830
p	0	0	0	0

Table 3. Broad sense heritability (H) of different traits of 84 *Urochloa* hybrids well-watered and water-limited conditions for 21 days

H	Shoot mass	Root mass	Water uptake	Maximum rooting depth	Deep rooting	Total root length
Well-watered	0.5	0.5	0.9	0.6	0.7	0.6
Water-limited	0.4	0.6	0.9	0.6	0.8	0.8