

Report on Identification of best genotypes of *Panicum maximum* (*Megathyrsus maximus*) genebank collection for the trait forage production (Biomass/Quality)

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Report on Identification of best genotypes of *Panicum maximum* (*Megathyrsus maximus*) genebank collection for the trait forage production (Biomass/Quality)

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Rationale

Megathyrsus maximus (syn. *Panicum maximum*), is a grass native from subtropical Africa but used as a forage to feed livestock (mostly cattle) all over the tropics. There are a number of commercially available cultivars of *M. maximus* (e.g., cvv. Mombaca, Tanzania, Centauro, Vencedor, Makueni, Tobiata) that are highly productive (over 13 ton dry matter ha^{-1} per year) and show good forage quality and palatability for cattle. As a result, these cultivars are recommended as an alternative to *Brachiaria* grasses in the American tropics (syn. *Urochloa*) and Napier grass (*Cenchrus purpureus*) in East Africa. In particular, cultivars of *M. maximus* are recommended for: 1) intensive cattle production systems —e.g. cut and carry systems in East Africa or confined systems in Brazil—; and 2) for well-drained soils of medium to high soil fertility regions and annual rainfall ranges from 800 to 1800 mm.

Albeit the qualities of existing *M. maximus* cultivars are promising for their use in different regions, several drawbacks are also present. Among them, low tolerance to spittlebugs, waterlogging (under pHs below 6) and trampling resistance are a concern. Therefore, there is need to identify genotypes of *M. maximus* that have the benefits of existing cultivars (or more), but also have novel traits.

Currently, the Alliance of Bioversity International and the Center for Tropical Agriculture maintains over 400 accessions of *M. maximus*. A literature and database review resulted in a selection of 126 germplasm accessions based on diversity of morpho-physiological traits (Cardoso, 2019). Five out of those 126 accesions are commercially available cultivars *(*cvv. Tanzania, Mombaza, Tobiata, Vencedor, Makueni) These germplasm accessions constitute a core collection for selection of promising genotypes for their release as cultivars, but also for breeding purposes.

The following report describes advances in:

1) Establishing a field trial to perform analyses of the core germplasm accession collection of *M. maximus.*
2) Genotypic differences in biomass production and forage quality (i.e. water soluble carbohydrates and crude protein)
3) Future outlook

1) Field trial to perform analyses of the core germplasm accession collection of *M. maximus.*

There is a need to evaluate the core germplasm accession collection of *M. maximus* for selection of promising genotypes for their release as cultivars, but also for breeding purposes. As such, an experiment was established initially to obtain vegetative material for distribution to Colombia's national agricultural research organization (NARS) and later for evaluation purposes.

Site description

The experiment is located in the department of Valle del Cauca in the municipality of Palmira in the experimental fields of regional office of the Alliance Bioversity-CIAT, located at 3o 50' 71''N, -76o 34' 29'' W. The climate of the area is classified as tropical dry forest according to the Holdridge life zone classification system. The altitude above sea level is 965 m, average temperature is 24o C for the year 2020 and precipitation is 811.4 mm/year.

The soils where the experiment is located are classified as Typic Pellustert, which are developed from fine to moderately fine sediments. The clay content is between 40-60%, clayey texture (>40% clay and <40% silt). The pH ranges between 7.5 and 7.8 and the phosphorus content (Bray II) is greater than 40 parts per million. Before planting, the soil was conditioned by mechanization; two passes of harrow and one pass of polisher were made. Subsequently, an herbicide was applied for post-mechanization weed control and then 130 plots of 3 meters long by 2.5 meters wide were laid out. A distance between plots of 1 meter and between furrows of 2 meters.

Establishment of propagation plots for later distribution of plant material across Colombia

In February 2019, 130 accessions of *Megathyrusus maximus* (49 seedlings or botanical seeds per plot) were established in order to obtain plant material for distribution to the Research Centers (C.I) of the Colombian Agricultural Research Corporation (AGROSAVIA). The basic seed for this trial was obtained from an experiment established in the municipality of Patía in southwestern Colombia where there was a collaborative effort between the Universidad Del Cauca and CIAT. The 130 accessions represent the core germplasm collection of *M. maximus* plus other four accessions

Establishment of plants for different evaluations

Once the commitment to distribute the plant material to the C.I. of AGROSAVIA was fulfilled, in January 2020 two extra blocks were add to the original trial design. This was to have three replications in a complete randomized block design, Plants were established as described before. To date, the following activities have been carried out:

- Botanical seed collection was carried out from plants that presented inflorescence.
- From the plants that did not produce botanical seed, plant material was extracted to be multiplied and sown in greenhouses to later be transplanted in the experimental fields.
- Maintenance and monitoring of the materials in the field and greenhouse.
- Re-sowing of material with low germination %.
- Establishment in the field of 130 accessions of *M. maximus*.
- Maintenance and monitoring of materials in the field: Each plot was irrigated at an interval of two days, manual weeding was done in each of the plots.

Problems encountered

Among the problems encountered, there was a low germination percentage in the materials sown with botanical seed. Therefore, seed had to be requested again from CIAT's Genetic Resources Bank. The seed obtained was sown in a greenhouse in Jiffys to minimize the loss of the materials, and once the seedlings reached a good development, they were sown in the field for the second time in March of the same year. Due to the contingency experienced by (COVID 19), the management team of Alliance Bioversity-CIAT implemented preventive isolation measures during the March-August period; therefore, work at CIAT-Palmira was restricted, reducing the number of field personnel in charge of maintenance and monitoring of the experiments. In addition, there was an atypical period of drought in the area. As a result, the materials were affected by water stress and weed invasion, which caused delays in establishment and/or the loss of a large number of plots and materials. Seed was again requested from the CIAT Genetic Resources Bank to complete the planting of the accessions that have not been able to establish; however, the bank is not distributing seed due to the low availability of field personnel to carry out collections because of the pandemic. In view of this, once the gradual return of the technical personnel to the Campus activities was allowed, the plots were cleaned and it was decided to continue obtaining vegetative and botanical seed from the materials that are in the initial multiplication fields of the experiment.

To date, work is being done to establish the third block of 130 accessions of *M. maximus* in order to begin agronomic evaluations. Figure 1 illustrates the advances in the establishment of field trials

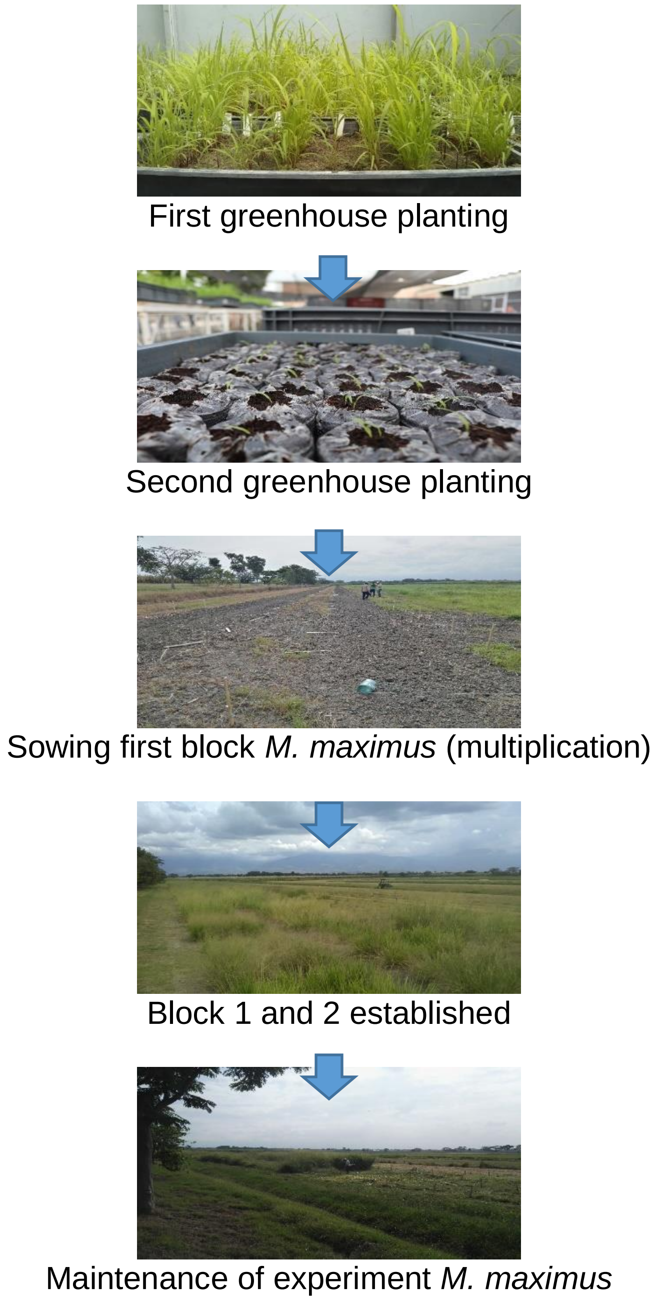

Figure 1. Timeline of set up of trial for the field evaluation of 130 accessions of *M. maximus*

2) Genotypic differences in biomass production, water-soluble carbohydrates and crude protein among *M. maximus* germplasm accessions

High biomass linked to high forage quality are characteristics sought among the core collection of *M. maximus*. Both forage quantity and quality are highly dynamic in time, change over ontogenetic drift and are sensitive to external stresses and management (e.g. cutting intervals). Forage quality is an integration of traits including fibers, protein, carbohydrates to name a few. Among quality traits, water-soluble carbohydrates—WSC—and crude protein—CP— are inversely correlated (the greater The CP, the lower the WSC). Excess of nitrogen leads to more nitrogen-excreted from the animal. As a result, greater WSC is a way to improve nitrogen efficiency of the animal, which might also be translated into greater milk production. Variation of WSC might be result of genetic variation or environmental influence. Therefore, the purpose of this study was to test variation of shoot biomass, WSC and CP among 119 accessions of *M. maximus* (plus eleven checks) after 21 days of growth under well-watered and high soil fertility under greenhouse conditions.This information should serve as a first approximation to identify *M. maximus* accessions with high biomass and high WSC. Such information might be relevant to identify genetic sources for the development of high sugar grasses.

Methodology

The methodology used in the present experiment is similar to what previously by Cardoso et al., 2015. The trial was carried out in a greenhouse at the campus of the Alliance of Bioversity-CIAT, 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 29/22 ° C (day / night) , a relative humidity of 39/70% (day / night) and a maximum photosynthetic active radiation (PAR) of 1200 μmol m2 s− 1 (average daily value of PAR of 750 μmol m2 s− 1.The soil used in this study was a Mollisol collected at CIAT facilities at 0–0.20 m from the soil surface, and the soil was sieved to pass a 2 mm mesh. The plant material used in this study consisted of vegetative propagules of 118 accessions of *M. maximus* plus some checks (*M. maximus* cvv. Tanzania, Mombaza, Tobiata, Vencedor, Makueni; *Urochloa* spp cvv. Tully, Piata, Toledo, Mulato II, Cayman, and once accession of *Cenchrus purpureus* CIAt 26850). The accessions were planted 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 good irrigation conditions. A total of 8 replicates per accession were planted. The soil mixture presented an apparent density (ρ soil) of 1.4g / cm3, organic matter of 6% and a pH of 7.5. Soil was kept under fieldd capacity. The plants were allowed to establish for 30 days and a standardization cut at 7 cm above soil surface was performed to all plants. After that plants were allowed to grow for 21 days and harvested at 10 cm above soil surface. Leaf and stem were separated and oven-dried for 5 days at 50-60 °C and their mass determined. After that, dry leaves and stems were mixed together for determination of WSC and Crude protein according to Borrero et al., (2017). The data were then analyzed using a one-way ANOVA. A post hoc analysis using the LSD test (α = 0.05) was used to identify differences between genotypes by treatments.

Key results

- There is significant variation for all the traits measured ($p < 0.05$, Table 1)
- The preliminary results showed that genotypes with high WSC are more vigorous, yet less leafy. This suggest that vigorous genotypes invest more photo-assimilates in the development of stem tissue, which in turn serves as storage tissue of WSC. Greater storage of WSC might be beneficial to these genotypes for rapid regrowth after intensive grazing or cutting.
- More leafy genotypes (as measured as a leaf to stem ratio) show more CP.

Table 1. Genotypic differences in biomass production, water-soluble carbohydrates and crude protein among *M. maximus* germplasm accessions. Data are means of 8 replicates. WSC (wate-soluble carbohydrates); CP (crude protein); DM (dry mass).

CIAT accession number	WSC (g kg DM)	CP (%)	Shoot (g DM plant^{-1})	Leaf to Stem ratio
673	77.4	11.7	1.8	1.7
685	70.6	10.8	1.7	1.0
688	77.8	8.9	2.1	1.5
691	77.4	12.0	1.9	1.7
692	80.9	9.9	1.8	1.7
6095	79.6	12.1	1.9	1.6
6171	73.6	12.7	1.5	2.2
6175	66.9	9.7	1.7	1.1
6461	85.5	10.1	2.2	1.8
6497	78.8	10.7	2.2	1.7
6500	82.5	9.0	1.8	1.7
6501	79.1	12.6	1.8	2.1
6525	78.7	11.0	2.1	1.5
6526	55.7	16.3	1.6	1.5
6536	77.7	10.1	1.5	2.6
6571	86.1	8.9	2.4	1.6
6658	77.2	10.9	1.6	1.8
6783	87.9	7.5	2.3	1.6
6784	77.3	10.6	1.8	2.5
6787	74.0	9.6	1.7	0.9
6796	75.6	12.7	1.4	2.8
6805	81.9	9.7	1.8	1.7

Continue below

CIAT accession number	WSC (g kg DM)	CP (%)	Shoot (g DM plant^{-1})	Leaf to Stem ratio
6826	76.8	11.6	1.9	1.8
6831	84.7	4.3	2.3	1.2
6836	80.1	10.1	2.1	1.5
6837	69.0	12.2	1.2	1.8
6839	82.5	5.3	2.3	0.9
6840	75.2	6.1	2.0	1.1
6842	77.9	10.6	1.9	1.7
6843	73.0	12.0	1.9	1.4
6855	80.3	9.6	2.2	1.6
6857	77.2	10.4	1.7	1.6
6864	76.1	12.0	1.7	1.9
6866	75.4	11.6	2.1	1.3
6868	87.6	5.0	2.1	1.8
6872	76.7	12.6	1.6	2.0
6890	86.9	3.1	1.3	
6893	68.7	8.5	2.0	1.7
6897	77.6	12.8	2.0	1.9
6898	78.1	12.8	1.8	1.7
6900	78.8	12.4	1.9	1.6
6901	85.9	10.7	2.0	2.0
6903	65.9	9.1	1.6	1.1
6906	63.2	15.0	1.0	1.9
6912	74.5	11.8	1.6	1.7
6915	73.5	9.9	1.4	1.3
6918	77.9	12.0	1.8	1.9
6923	81.5	11.4	2.1	1.6
6927	86.6	2.8	1.9	1.4
6928	55.8	16.4	0.4	
6929	82.0	11.0	2.0	2.0
6944	81.8	8.6	1.9	1.5
6945	87.2	6.4	2.0	1.6
6948	73.2	11.6	1.5	2.1
6949	63.3	10.3	1.7	1.4
6951	65.3	14.9	1.4	2.0
6954	75.9	12.9	1.7	2.4
6955	80.5	9.8	1.9	1.5
6960	85.5	9.2	2.6	1.6
6963	71.7	11.9	1.8	1.6
6967	77.0	8.0	1.7	1.4

Continue below

CIAT accession number	WSC (g kg DM)	CP (%)	Shoot (g DM plant^{-1})	Leaf to Stem ratio
6968	54.6	14.6	1.3	1.4
6969	73.0	12.0	1.5	3.0
6975	57.8	14.3	1.0	2.6
6981	79.2	8.3	1.9	1.4
6982	86.1	3.6	2.2	1.2
6983	71.3	11.2	1.6	1.7
6984	76.0	9.7	1.7	1.7
6986	69.2	9.4	1.5	1.7
6990	73.3	12.1	1.8	1.4
6996	54.3	13.3	0.7	1.0
16003	79.9	11.2	2.0	1.3
16004	88.0	6.4	2.3	1.6
16005	92.4	5.2	2.1	1.9
16011	79.9	10.4	1.9	1.6
16017	80.6	8.4	2.0	1.4
16018	54.8	13.8	1.4	1.4
16019	79.5	12.2	1.6	2.5
16021	78.2	13.4	1.4	3.3
16023	77.4	11.0	1.8	2.0
16025	81.7	11.4	2.1	2.7
16027	76.8	13.4	1.8	2.0
16028	85.0	10.2	2.0	1.8
16035	79.8	10.3	2.0	1.9
16036	68.0	15.1	1.9	1.3
16038	81.0	8.8	1.9	1.5
16039	63.4	10.0	1.9	1.2
16041	65.9	13.8	1.5	1.5
16044	78.5	11.4	2.4	1.2
16046	83.9	11.9	2.1	2.3
16048	53.9	12.9	1.1	1.5
16049	73.0	9.3	1.6	1.4
16054	64.2	14.1	1.3	2.5
16055	73.8	5.6	1.6	1.1
16057	73.6	13.3	1.8	2.0
16058	72.4	13.3	1.6	2.0
16059	79.2	11.2	1.7	1.9
16060	80.6	4.3	1.8	1.7
16061	91.3	7.1	2.2	1.9
16062	77.2	13.3	2.2	1.6
16064	65.6	14.3	1.3	2.3

Continue below

CIAT accession number	WSC (g kg DM)	CP (%)	Shoot (g DM plant^{-1})	Leaf to Stem ratio
16065	72.7	14.0	1.3	2.6
16068	71.7	13.9	1.4	2.2
16069	82.7	10.8	2.0	1.6
16071	63.7	14.3	1.6	2.1
26360	72.6	10.8	1.4	2.0
26723	71.8	13.8	1.6	1.9
26906	82.6	7.9	2.0	1.5
26911	67.1	14.8	1.3	1.9
26917	72.9	14.6	1.5	2.9
26923	71.6	14.4	1.7	1.4
26924	73.0	11.9	1.7	1.3
26925	72.2	14.8	1.7	1.7
26937	63.3	12.8	1.5	1.7
26939	60.9	13.5	0.4	1.3
26944	66.7	10.2	1.3	1.1
26947	82.7	8.7	1.8	2.3
36000	73.7	14.6	1.6	3.2
Average	75.3	10.9	1.7	1.8
LSD 0.05	2.4	1.3	1.3	1.2
				Checks
M. maximus cvv	WSC (g kg DM)	CP (%)	Shoot (g DM plant^{-1})	Leaf to Shoot
Makueni	70.5	12.5	1.7	1.5
Sabanera	78.9	8.0	2.1	1.1
Tobiata	88.2	4.8	2.7	1.2
Vencedor	77.3	10.3	2.0	1.2
Tanzania	84.3	10.5	1.9	1.9
Mombaca	82.5	5.4	2.7	1.2
*Urochlo*a spp. cvv	WSC (g kg DM)	CP (%)	Shoot (g DM plant^{-1})	Leaf to Shoot
cv. Tully	73.5	6.8	1.9	0.7
cv. Toledo	77.6	4.4	2.0	1.0
Cv. Piata	76.8	11.4	1.9	1.5
Mulato 2	80.1	9.3	1.8	1.8
Cayman	87.7	6.6	2.6	1.0
Cenchrus purpureus	WSC (g kg DM)	CP (%)	Shoot (g DM plant^{-1})	Leaf to Shoot
CIAT 26850	69.7	13.6	1.4	2.2

- Genotypes showing similar values of biomass production, high WSC and low CP to those show by cv. Mombaza (the most outstanding one in those aspects) are accessions 6831, 6839, 6868, 6890, 6963, 6982, 16004, 16005, 16055, 16060, 16061. These genotypes were previously identified as accessions of mostly erect growth, long leaves, "water-spending" mode of water use, and slightly lower biomass production than cvv. Mombaza or Tanzania (Cardoso, 2019).
- Further research is needed to test this promising accessions under field conditions

3) Future outlook

Knowledge of forage quantity and quality is vital for the release of new cultivars from existing germplasm accessions or development of new ones by breeding. One of the largest problems in the determination of forage quantity and quality is that both are highly dynamic in time, change over ontogenetic drift and are sensitive to external stresses and management (e.g. cutting intervals). As such, there is a need to implement or develop reliable methods of high throughput to determine such traits. Among tools currently in place, proximal sensing by or remote sensing of plant material are promising approaches for estimating field-scale forage quantity and quality compared with traditional methods. Implementation of field based spectroscopy, as well as satellite and drone based imaging spectroscopy are underway for such purpose.

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