

Physiological analysis and nutritional quality of maize: a comparative study between hybrid and landraces varieties

Thiago Lucas De Oliveira¹, Germano Augusto Oliveira Carneiro², Gisele Machado Fernandes³, Bruno Henrique Rocha⁴, Vinícius Ribeiro Faria⁴ And Willian Rodrigues Macedo^{4,*}

¹ Universidade Federal de Lavras, Campus Universitário, Caixa postal 3037, CEP 37200-000, Lavras/MG, Brazil.

² BASF – Rodovia 365, Uberlândia/MG, Brazil.

³ Universidade de São Paulo, FMRP, Av. Bandeirantes, 3900 - Vila Monte Alegre, Ribeirão Preto/SP, CEP 14049-900, Brazil.

⁴ Universidade Federal de Viçosa, Campus Rio Paranaíba, Rodovia MG 230, Km 8, CEP 38810-000, Brazil.

*Corresponding author: E-mail: willian_rmacedo@yahoo.com.br

KeyWords: *Zea mays L.*, gas exchange, silage quality, Cerrado.

Abstract

Maize (*Zea mays L.*) is important for animal production systems, and the use of unimproved maize varieties has increasingly become a viable option for small farmers. This study aimed to characterize and evaluate the physiological and feed potential of maize populations, as a raw material for silage production, in the mesoregion of the Alto Paranaíba, Minas Gerais (MG). We used nine varieties of unimproved maize from the Germplasm Bank at Embrapa Maize & Sorghum - BAGMilho (Sete Lagoas, MG), as follows: (milho stands for maize; crioulo, for landrace) MG 110 – Milho Amarelão, MS 043 – Crioulo, MG 083 – Milho Branco, RN 013 – Milho Metro, MG 073 – Milho Vermelhinho, MS 016 – Palha Roxa 90, MG 079 – Milho Cunha, RR 040 – Crioulo de Roraima, AC 015 – Milho Boliviano and a commercial hybrid variety of Pioneer, P4285. The hybrid maize Pioneer P4285 showed better physiological and nutritional performance. Among the accessions of the Germplasm Bank, the MG 110 – Milho Amarelão and MG 015 – Milho Boliviano were distinguished by their physiological adaptation to the Cerrado of Minas Gerais, and for showing characteristics suitable for use in the silage process for animal feed.

Introduction

Maize is one of the world's major grain crops. It provides human and animal feed, together with raw material to the industry, depending on the quantity and quality of the yield. Maize plays an important role in animal production systems, because it is often the main energy source provided in the animals' diets. According to Cruz et al (2011), the need to produce bulky food for herds, particularly in the dry season, when the growth of natural pastures becomes increasingly restricted, has increased the use of silage by cattle ranchers dedicated to milk production. Although several annual and perennial forage plants can produce silage, maize is one of the crops most used for this purpose in Brazil, due to its good yield, excellent fermentation quality and maintenance of nutritional value of the ensiled mass. Other advantages are low production costs and good animal acceptability.

The production of silage is a process aimed at conserving the forage with a nutritional value as close as possible to the original, i.e. with a minimum of losses.

Silage is important for being a food reserve in the dry season, to increase milk and/or meat production, to help pasture management, to use forage surpluses and to balance nutritionally the animals' diets.

Tagliari (2001) states that the modern agricultural model has made agricultural production more expensive, because the use of improved species has led small-holders to use expensive technologies, such as hybrid seeds. The genetic diversity in maize allows its cultivation in different environments. According to Hallauer et al. (1998), maize is grown from latitude 58 °N to 40 °S, and from sea level up to 3800 m altitude. In addition, its genetics have been studied more than any other plant species: consequently, the inheritance of numerous characters and their genome are well known. According to Nass and Paterniani (2000), economic importance, genetic structure, chromosome number, reproduction type, ease of manual pollination and the possibility of generating different types of progenies are factors that have contributed to this cereal being propagated by allogamy.

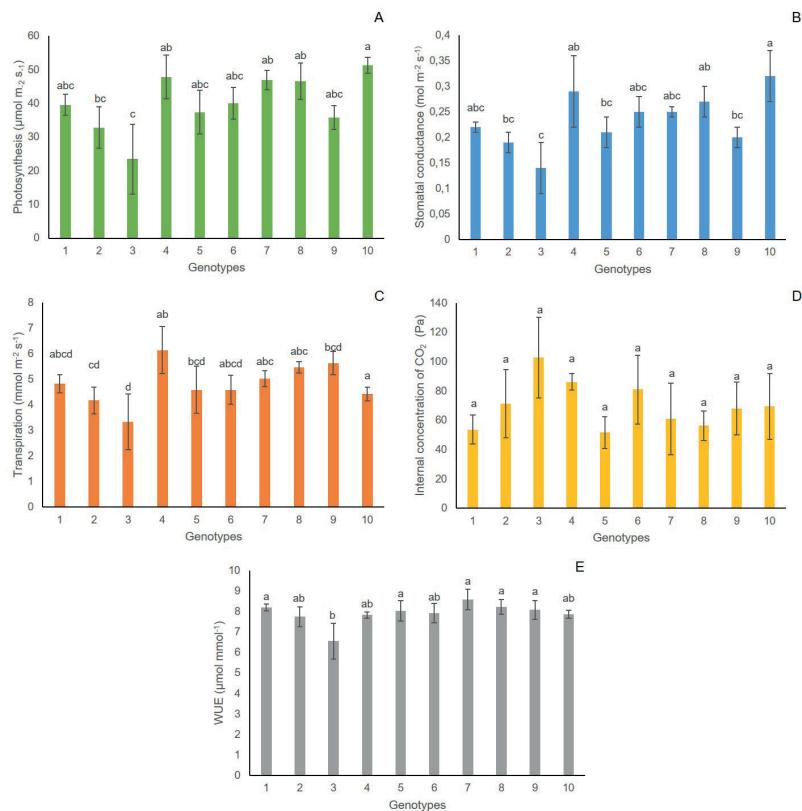


Fig. 1 - V7-V8 phenological stages. Analysis of: A - Photosynthesis (A, $\mu\text{mol m}^{-2} \text{s}^{-1}$), B - stomatal conductance (gs, $\text{mol m}^{-2} \text{s}^{-1}$), C - transpiration (E, $\text{mmol m}^{-2} \text{s}^{-1}$), D - internal concentration of CO_2 (Ci, Pa), and E - water use efficiency (WUE, $\mu\text{mol mmol}^{-1}$) of Zea mays L. genotypes (1 - MG 110 Milho Amarelão; 2 - MS 043 Crioulo; 3 - MG 083 Milho Branco; 4 - RN 013 Milho Metro; 5 - MG 073 Milho Vermelhinho; 6 - MS 016 Palha Roxa 90; 7 - MG 079 Milho Cunha; 8 - RR 040 Crioulo de Roraima; 9 - AC 015 Milho Boliviano and 10 - Pioneer P4285). Averages followed by the same letter in the column do not differ statistically from one another by the Dunnett test with 95% probability, and the bars indicate de \pm SEM.

Maize crops consisting of landrace varieties (also known as local races, unimproved and native varieties) are characterized by their great genetic variability, which makes selection for phyto-enhancement a strategic possibility. Choosing a suitable germplasm is important if a breeding program has to be successful. The use of unimproved maize breeds has increasingly become a viable alternative for small farmers. Miranda et al (2007) found that reusing seeds, harvest after harvest, from selected plants grown under the environmental and nutritional conditions imposed by the socioeconomic level of the farmer, results in the development of maize populations adapted to different situations.

In general, unimproved populations show lower productivity compared to hybrid varieties on the market; however, they are important because of their high genetic variability, which can be studied in the search for genes that are tolerant and/or resistant to biotic and abiotic factors. According to Carpentieri-Pípolo et al. (2010), when cultivated under conditions employing low crop technologies, the performance of native va-

rieties may be equal or even superior to that of commercial varieties.

The aim of this study was to characterize and evaluate the physiological and feed potential of maize populations for silage production in the region of Alto Paranaíba, Minas Gerais

Materials and Methods

Genetic materials

Nine varieties of unimproved maize from the Germplasm Bank of Embrapa Maize and Sorghum (BAGMilho) of Sete Lagoas: MG 110 Milho Amarelão, MS 043 Crioulo, MG 083 Milho Branco, RN 013 Milho Metro, MG 073 Milho Vermelhinho, MS 016 Palha Roxa 90, MG 079 Milho Cunha, RR 040 Crioulo de Roraima and AC 015 Milho Boliviano (supplementary material) were used for this study; additionally a commercial hybrid variety Pioneer - P4285 was used as a control.

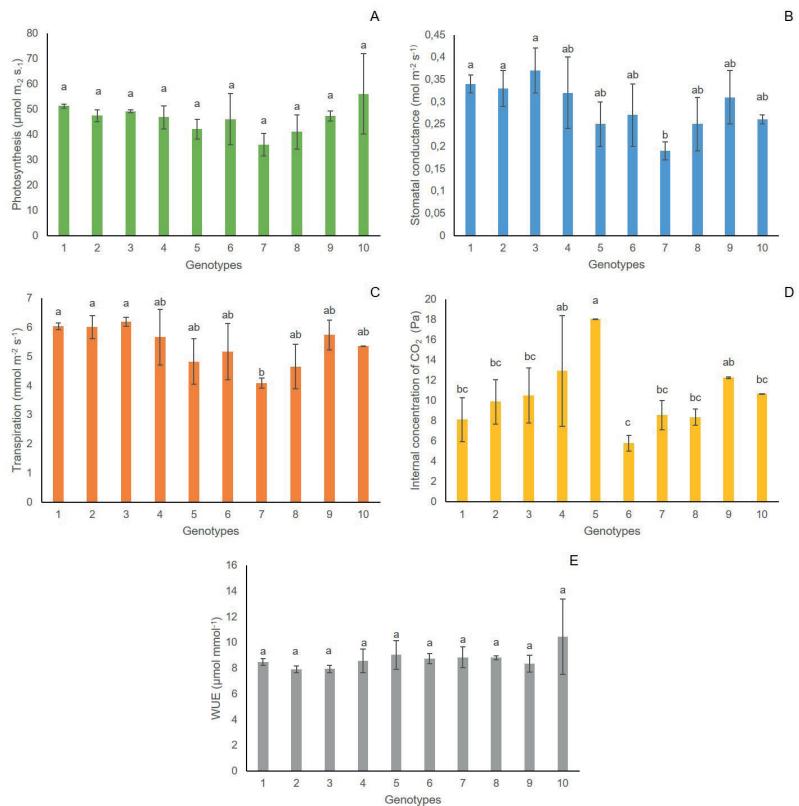


Fig. 2 - R1 - R3 phenological stages Analysis of: A - Photosynthesis (A, $\mu\text{mol m}^{-2} \text{s}^{-1}$), B - stomatal conductance (gs, $\text{mol m}^{-2} \text{s}^{-1}$), C - transpiration (E, $\text{mmol m}^{-2} \text{s}^{-1}$), D - internal concentration of CO_2 (Ci, Pa), and E - water use efficiency (WUE, $\mu\text{mol mmol}^{-1}$) of Zea mays L. genotypes (1 - MG 110 Milho Amarelão; 2 - MS 043 Crioulo; 3 - MG 083 Milho Branco; 4 - RN 013 Milho Metro; 5 - MG 073 Milho Vermelhinho; 6 - MS 016 Palha Roxa 90; 7 - MG 079 Milho Cunha; 8 - RR 040 Crioulo de Roraima; 9 - AC 015 Milho Boliviano and 10 - Pioneer P4285). Averages followed by the same letter in the column do not differ statistically from one another by the Dun-can test with 95% probability, and the bars indicate de \pm SEM.

Agronomical plan and treatments

The experiment was performed under field conditions in the 2015/2016 agricultural year at the Federal University of Viçosa (UFV), Campus Rio Paranaíba. The experimental area was located at coordinates $18^{\circ} 13' \text{S}$ and $46^{\circ} 13' \text{W}$, at an altitude of 1120 m, according Köppen climate classification, the site of experiment is Cwa, humid subtropical climate (Dubreuil et al, 2018), during 2015-2016 crop session, where the seeds were planting on November 5th 2015.

The experiment was laid out in a Randomized Block Design (RBD) in which all varieties were sown in three blocks of $3.0 \times 2.4 \text{ m}$, with three rows per treatment and 0.8 m spacing between rows. We adopted the phenological cycle proposed by Ritchie and Hanaway (1989), and thinning at the phenological stage VE to obtain a density of 4 plants m^{-2} , which equates to a final population of $50,000 \text{ plants ha}^{-1}$.

The base fertilization was 300 kg ha^{-1} of formulated 08-30-10 (N-P2O5-K2O). Top dressing was carried out in two stages: the first at 20 days after planting, at the

phenological stages V4 and V5, providing 125 kg ha^{-1} N and $125 \text{ kg K2O ha}^{-1}$, and the second at 40 days after planting, at the V7 and V8 stages, providing 125 kg N ha^{-1} .

Mechanical weeding was carried out until the rows were closed. Pests were controlled by the application of Klorpan (active ingredient chlorpyrifos), 1.5 L ha^{-1} , under conditions of high insect density. Disease control was by application of the fungicide Priori Xtra (azoxystrobin + cyproconazole), 300 ml ha^{-1} , when the plants were between the phenological stages VT and R2.

Physiological traits

Three gas exchange analyses were performed during the crop development cycle, in the phenological stages: between V7 and V8 (first measurement), between R1 and R3 (second measurement), and between R4 and R5 (third measurement). Three plants of each variety were selected to obtain the following parameters: photosynthetic rate (A), stomatal conductance (gs), inter-

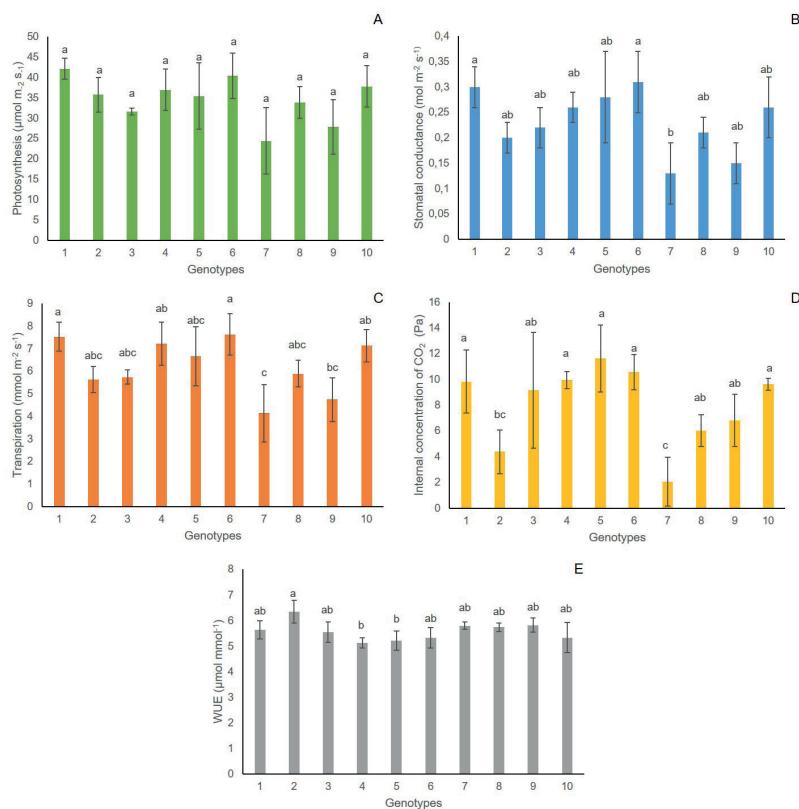


Fig. 3 -R4 - R5 phenological stages. Analysis of: A - Photosynthesis (A, $\mu\text{mol m}^{-2} \text{s}^{-1}$), B - stomatal conductance (gs, $\text{mol m}^{-2} \text{s}^{-1}$), C - transpiration (E, $\text{mmol m}^{-2} \text{s}^{-1}$), D - internal concentration of CO_2 (Ci, Pa), and E - water use efficiency (WUE, $\mu\text{mol mmol}^{-1}$) of Zea mays L. genotypes: (1 - MG 110 Milho Amarelão; 2 - MS 043 Crioulo; 3 - MG 083 Milho Branco; 4 - RN 013 Milho Metro; 5 - MG 073 Milho Vermelhinho; 6 - MS 016 Palha Roxa 90; 7 - MG 079 Milho Cunha; 8 - RR 040 Crioulo de Roraima; 9 - AC 015 Milho Boliviano and 10 - Pioneer P4285). Averages followed by the same letter in the column do not differ statistically from one another by the Duncan test with 95% probability, and the bars indicate $\pm \text{SEM}$.

nal concentration of CO_2 (Ci), leaf transpiration (E) and water use efficiency (WUE). Measurements were taken from leaves of the middle third of each plant, under photosynthetically active radiation of $1,500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. All measurements occurred between 8:30 am and 11:30 am, using an infrared gas analyzer (IRGA) model LI-6400 (LI-COR, Lincoln, Nebraska, USA) coupled to a modular fluorometer (6400-40 LCF, LI-COR, Lincoln, Nebraska, USA).

Chemical analyses

For evaluation of nutritional quality the plant shoots samples, of each genotype, were harvested when the plants were between stage R3 (grains having a pasty texture) and stage R5 (hard farinaceous), at 28 to 35% dry matter content (Pereira et al, 2008), with a cut made at 40 cm from the base of the plant, aiming to reduce the fibrous fraction and increase the proportion of grain in the plant dry matter.

After the samples collection these samples were packed in paper bags and dried in an oven and ground in a Willey mill with a 1 mm mesh sieve, after which crude protein, mineral matter and crude fiber were determi-

ned using the analytical methods of the Association of Official Analytical Chemists (AOAC 1995).

Agronomical traits

Concomitant to the feed analysis, three representative plants from each plot to determine the biometric variables: plant height, height of ear insertion, number of ears per plant and stem diameter were collected. Plant height was measured to the base of the tassel, and height of ear insertion was measured to the insertion of the highest viable ear. Stem diameter was measured at the second internode from the base of the plant. Also, after harvesting, a plant from each plot was separated into stem, leaves and ears, and the fresh matter of each component was measured using a precision scale. The material was then packed in paper bags and placed in a forced ventilation oven at 65°C for 72 hours, so as to estimate the dry matter production in t ha^{-1} , and the ear percentage, stem and leaf in the dry matter. The remaining plants harvested from the plot were weighed to determine the green matter production in t ha^{-1} .

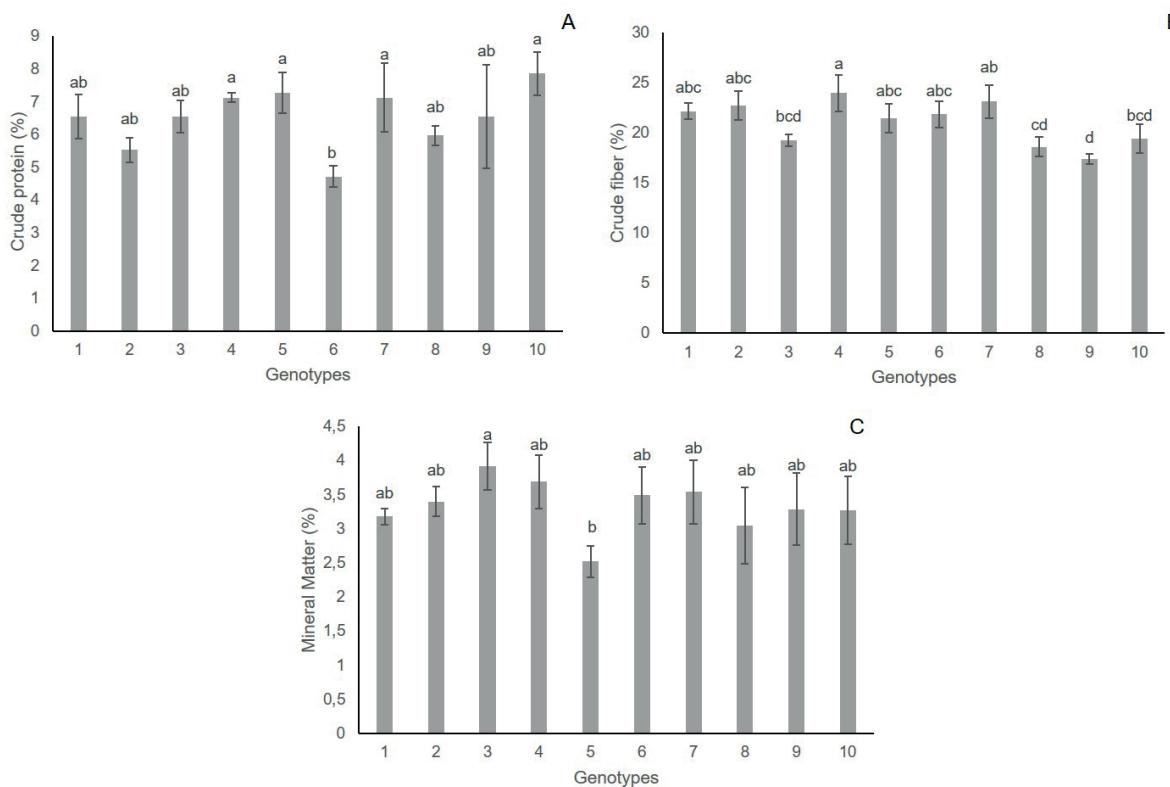


Fig. 4 -Analysis of: A - Crude protein (%), B - Crude fiber (%) and C - Mineral Matter (%) of *Zea mays L* genotypes (1 - MG 110 Milho Amarelão; 2 - MS 043 Crioulo; 3 - MG 083 Milho Branco; 4 - RN 013 Milho Metro; 5 - MG 073 Milho Vermelhinho; 6 - MS 016 Palha Roxa 90; 7 - MG 079 Milho Cunha; 8 - RR 040 Crioulo de Roraima; 9 - AC 015 Milho Boliviano and 10 - Pioneer P4285). Averages followed by the same letter in the column do not differ statistically from one another by the Duncan test with 95% probability, and the bars indicate de \pm SEM.

Statistical Analysis

The data were submitted to an analysis of variance, using the procedure PROC GLM of the program Statistical Analysis System (SAS, 2003), and the means of the treatments were compared through the Duncan test. The level of significance was set at 95%.

Results and discussion

The maize landrace varieties are important to small-holders, who use them for animal feed (Carpentieri-Pípolo et al, 2010), for this reason the physiological understanding of plants can lead to improvements in crop production. The data showed that these unimproved genotypes had inherent physiological and feed characteristics, which were different from those found in hybrid plants. All of the varieties showed a stable photosynthetic behavior throughout the whole cycle of plant growth. However, there was a greater variation in the photosynthetic parameters during the vegetative stage, verified in the first experiment (Table 1), and more homogeneous values during the reproductive stages (Tables 2 and 3). The observed photosynthesis

rates may relate to the ability of the plants to interact with the environment, leading inevitably to changes in this physiological process (Hayano-Kanashiro et al, 2009), or to genetic regulation, resulting from plant breeding (Magalhães et al, 2002). This second hypothesis corroborates our results, in which the hybrid P4285 showed a high physiological adaptation to the conditions of the field trial, resulting in the highest rates of photosynthesis in all three evaluations

For stomatal conductance and transpiration analysis, it was also found significative differences between genotypes evaluated, our observations indicate that, at the end of the vegetative phase, the hybrid P4285 showed the highest results for stomatal conductance and transpiration, being superior in comparison to the unimproved maize: MS - 043 Crioulo, MG 083 - Milho Branco, MG 073 - Milho Vermelhinho, and AC 015 - Milho Boliviano (Table 1), while for gas exchange analyses during the initial reproductive phase, the strain MG 079 - Milho Cunha showed the lowest values of stomatal conductance and transpiration, without being statistically different from the hybrid P4285. The phenological development of maize plants led to changes in stoma-

tal regulation and control in the loss of water (Tables 1, 2 and 3), for evaluations of stomatal conductance and transpiration, were observed in the genotypes that

All varieties showed very homogeneous values of the efficient use of water through the different phenological stages (Tables 1, 2 and 3), proving that all genotypes

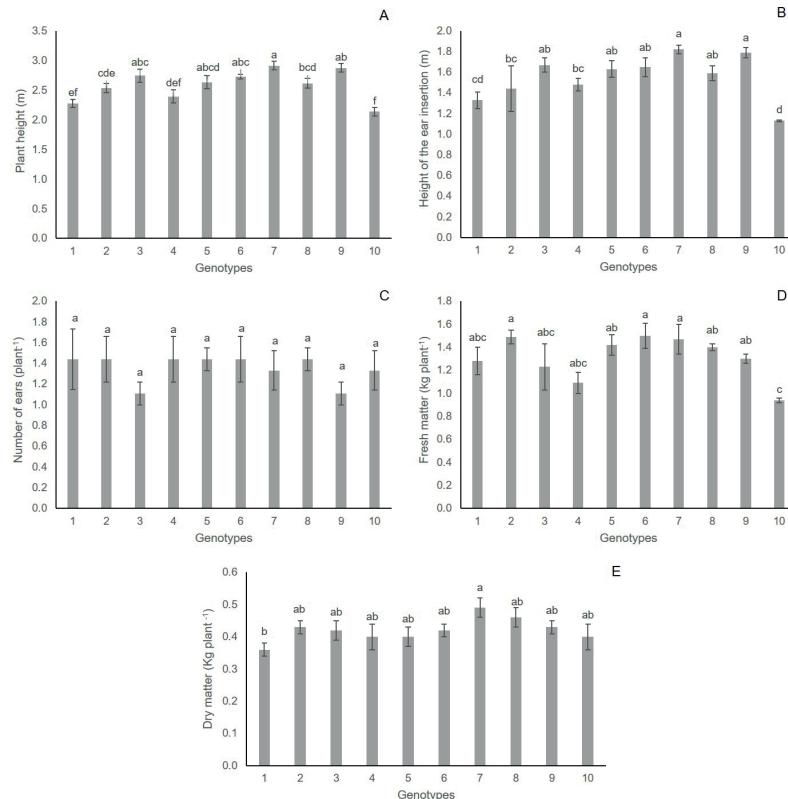


Fig. 5 -Analysis of: A - Plant height (m), B - Height of the ear insertion (m), C - Number of ears per plant, D - Fresh matter of the plant (kg) and E - Dry matter of the plant (kg) of *Zea mays L.* genotypes (1 - MG 110 Milho Amarelão; 2 - MS 043 Crioulo; 3 - MG 083 Milho Branco; 4 - RN 013 Milho Metro; 5 - MG 073 Milho Vermelhinho; 6 - MS 016 Palha Roxa 90; 7 - MG 079 Milho Cunha; 8 - RR 040 Crioulo de Roraima; 9 - AC 015 Milho Boliviano and 10 - Pioneer P4285). Averages followed by the same letter in the column do not differ statistically from one another by the Duncan test with 95% probability, and the bars indicate de \pm SEM.

presented greater stomatal conductance, also presented worse rates of transpiration, which led to greater water losses. Under unfavorable conditions, the stomata close to reduce water loss and consequently the photosynthetic rate reduces: however, different varieties have been shown to behave differently (Hayano-Kanashiro et al, 2009).

There were no statistically significant differences between the varieties in the internal concentration of CO_2 at the phenological stages V7-V8 (Table 1). the observed values of the internal concentration of CO_2 among the varieties at the reproductive stage, possibly resulting from their different stages of maturity, which reflect the increase or decrease in CO_2 carboxylation inside the plant (Flexas et al, 2008). It is possible to assert, that each variety showed a different physiological behavior with respect to gas exchanges to maintain its good status (Hayano-Kanashiro et al, 2009).

have the ability to regulate the flow of gas exchange, in the Cerrado conditions.

After the physiological analyses, information on nutritional parameters were collected; the hybrid variety showed the highest crude protein content, while the unimproved varieties presented higher crude fiber contents (Table 4). Among the unimproved strains, the variety AC 015 – Milho Boliviano showed a nutritional quality similar to the hybrid material, while the varieties RN 013 – Milho Metro, MG 073 – Milho Vermelhinho and MG 079 – Milho Cunha showed promising results regarding protein content.

The feed quality evaluation showed that the mineral content was within the expected range for maize plants used to produce silage, according Domingues et al (2012) the average of crude protein in silage from maize hybrids presents variation according to crop management, but presents a range of 7.27 and 8.88%. On

the other hand, the variety Milho Palha Roxa has no promising features for silage purposes because, according to Deminicis et al, (2009), the balance between grains and fibrous matter will determine the quality of the material to be ensiled. Moreover, the digestibility of the material and its voluntary consumption by livestock are inversely proportional to the fibrous fraction content of the plants (Cruz et al, 2010).

For the biometric parameters plant height (PH), height of ear insertion (HE), fresh matter (FM) and dry matter (DM) (Table 5), the variety MG 079 Milho Cunha presented the highest averages compared to the other genotypes, and no significant differences were observed for number of ears per plant, among genotypes (Table 5). According the results presented about plant biometrics the genotype MG 079 Milho Cunha showing high ability to allocate photoassimilates in plant biomass, a predicted response considering that landrace genotypes have a longer phenological cycle, with late maturation and greater heights of ear insertion (Ferreira et al, 2009), this is a phenotype opposite to that observed in the hybrid P4285. These allocation responses of the photoassimilated products by the plant are genetically controlled, but they are also strongly influenced by the environment, which will determine the relative increase in vegetative or reproductive matter of maize plants (Durães et al, 2005).

We did not notice difference to number of ears per plant, and consequently, the amount of grain in the total dry matter of the silage, indicated that the hybrid maize Pioneer P4285, had a good potential to provide soluble carbohydrates, favoring digestibility and being a predominant factor for the quality of fibrous feed (Santos et al, 2010). Finally, we consider that the genetic improvement shown by the hybrid P4285 had resulted in a variety highly adapted to the local conditions: however, many of the unimproved materials had promising characteristics for future selection studies, especially regarding animal feeding.

Conclusions

The hybrid maize Pioneer P4285 showed the best physiological and nutritional performance among all of the evaluated samples. Among the maize from the Germplasm Bank, the MG 110 – Milho Amarelão and MG 015 – Milho Boliviano stand out for their physiological adaptation to the Cerrado of Minas Gerais, as well as showing characteristics suitable for use in the silage process.

Funding

This work was supported by the National Council for Scientific and Technological Development (CNPq), for

provide Scientific Initiation Grants for undergrad students (first and second authors).

Disclosure statement

No potential conflict of interest was reported by the authors.

Acknowledgments

To the Germplasm Bank (BAG Milho) of Embrapa Maize and Sorghum of Sete Lagoas – MG, for the availability of maize varieties used in this experiment, and CNPQ for granting scientific initiation scholarships to the first and second authors. The authors are grateful to PhD Jeff Pursglove for language corrections and suggestions in this manuscript.

References

A.O.A.C. Association of Official Analytical Chemists. 1995. Official methods of analysis. Association of Official Analytical Chemists, Washington (DC).

Carpentieri-Pípolo V, Souza A, Silva DA, Barreto TP, Garbuglio DD, Ferreira JM, 2010. Avaliação de cultivares de milho crioulo em sistema de baixo nível tecnológico. *Acta Sci-Agron* 32:229-233. doi:10.4025/actasciagron.v32i2.430.

Cruz JC, Pereira Filho IA, Gontijo Neto MM, 2011. Milho para silagem. Agência Embrapa de Informação Tecnológica, Sete Lagoas (BR).

Cruz PG, Figueiredo MP, Pereira LGR, Bergamaschi KB, Rodrigues CS, Rech CLS, 2010. Fracionamento e cinética da fermentação ruminal in vitro dos carboidratos de cinco variedades de cana-de-açúcar. *Cienc Anim Bras* 11:784-793. doi: 10.5216/CAB.V11I4.4808

Deminicis BB, Vieira HD, Jardim JG, Araújo SAC, Neto AC, Oliveira VC, Lima ES, 2009. Silagem de milho: características agronômicas e considerações. *Redvet* 10:1-18.

Domingues AN, Abreu JG, Cabral LS, Galati RL, Oliveira MA, Reis, RHP, 2012. Valor nutritivo da silagem de híbridos de milho no Estado do Mato Grosso, Brasil. *Acta Sci-Anim Sci.* 34(2):117-122. doi:10.4025/actascianimsci.v33i3.9890

Dubreuil V, Fante KP, Planchon O, Neto JLS, 2018. Os tipos de climas anuais no Brasil: uma aplicação da classificação de Köppen de 1961 a 2015. *Confins* 37. doi: 10.4000/confins.15738

Durães FOM, Magalhães PC, Gama EEG, Oliveira AC, 2005. Caracterização fenotípica de linhagens de milho quanto ao rendimento e à eficiência fotossintética. *Rev Bras Milho Sorgo.* 4:355-361. doi: 10.18512/1980-6477/rbms.

v4n3p355-361

Ferreira JM, Moreira RMP, Hidalgo JAF, 2009. Capacidade combinatória e heterose de em populações de milho crioulo. *Cienc Rural* 39:332-339. doi: 10.1590/S0103-84782008005000058.

Flexas J, Ribas-Carbó M, Diaz-Espejo A, Galmés J, Medrano H. 2008, Mesophyll conductance to CO₂: current knowledge and future prospects. *Plant Cell Environ* 31:602-621. doi: 10.1111/j.1365-3040.2007.01757.x.

Hallauer AR, Carena MJ, Miranda Filho JB, 1988. Quantitative genetics in maize breeding. Iowa University Press, Ames (IA).

Hayano-Kanashiro C, Calderón-Vázquez C, Ibarra-Laclette E, Herrera-Estrella L, Simpson J, 2009. Analysis of gene expression and physiological responses in three Mexican maize landraces under drought stress and recovery irrigation. *Plos One* 4:7531. doi: 10.1371/journal.pone.00007531.

Magalhães PC, Durães FOM, Carneiro NP, 2002. Fisiologia do Milho. Embrapa Milho e Sorgo (Bag Milho), Sete Lagoas.

Miranda, GV, Souza LV, Santos IC, Mendes FF. 2007. Resgate de variedades crioulas de milho na região de Viçosa-MG. *Rev. Bras. Agroecol.* 2: 1145-1148.

Nass LL, Paterniani E, 2000. Pre-breeding: a link between genetic resources and maize breeding. *Sci Agr.* 57:581-587. doi: 10.1590/S0103-90162000000300035.

Ritchie S, Hanway JJ, 1989. How a maize plant develops (Special Report, 48). Iowa University Press, Ames (IA).

Santos RD, Pereira LGR, Neves ALA, Azevêdo JAG, Moraes AS, Costa CTF, 2010. Características agronômicas de variedades de milho para produção de silagem. *Acta Sci-Anim Sci* 32:367-373. doi:10.4025/actascianimsci.v32i4.9299.

SAS, 2003. Institute Inc. Statistical Analysis System user's guide. Version 9.1. Statistical Analysis System Institute, Cary (NC).

Tagliari OS, 2001. Milho crioulo avança no Oeste catarinense. *Agropec Catar* 14:27-32.