Híbridos de sorgo biomassa diferem em crescimento e uso de nitrogênio em baixa saturação de bases em solo arenoso Biomass sorghum hybrids differ in growth and nitrogen use under low bases saturation in sandy soil Los híbridos de biomasa de sorgo difieren en crecimiento y uso de nitrógeno en baja saturación de bases en suelos arenosos Received: 13/07/2020 | Reviewed: 13/08/2020 | Accept: 24/08/2020 | Published: 27/08/2020 Alcindo Sousa Brignoni ORCID: https://orcid.org/0000-0002-9771-2172 Federal Institute of Education, Science and Technology Goiano, Brazil E-mail: alcindo_brignoni@hotmail.com **Higor Ferreira Silva** ORCID: https://orcid.org/0000-0003-3357-9883 Federal Institute of Education, Science and Technology Goiano, Brazil E-mail: higorfs.eng@gmail.com Jardélcio Damião Carvalho Ervilha ORCID: https://orcid.org/0000-0003-2358-3336 Nexsteppe seeds, Brazil E-mail: jardelcioervilha@gmail.com Fabiano Guimarães Silva ORCID: https://orcid.org/0000-0003-4908-2265 Federal Institute of Education, Science and Technology Goiano, Brazil E-mail: fabiano.silva@ifgoiano.edu.br **Liliane Santos Camargos** ORCID: https://orcid.org/0000-0002-0979-4447 Paulista State University, Brazil E-mail: camargos@bio.feis.unesp.br Lucas Anjos Souza ORCID: https://orcid.org/0000-0001-5377-6820 Federal Institute of Education, Science and Technology Goiano, Brazil E-mail: lucas.anjos@ifgoiano.edu.br

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Resumo

Plantas de sorgo são bem cultivadas na região central do Brasil, que é originalmente pobre em fertilidade e rica em alumínio. Estas características demandam estudos para se conhecer melhores híbridos para tal ambiente, principalmente no que se referre ao uso de nutrientes como o nitrogênio (N). Este nutriente é o mais limitante para o crescimento, desenvolvimento e produção vegetal; por isso é de grande importância compreender os efeitos da baixa saturação de bases sobre o uso de N em sorgo biomassa, ou seja, híbridos utilizados com propósitos bioenergéticos. Com o objetivo de avaliar o efeito de saturações de bases crescentes no uso de nitrogênio em híbridos de sorgo biomassa, nós instalamos um experimento quantitativo em casa de vegetação, em delineamento fatorial inteiramente casualizado utilizando dois híbridos de sorgo biomassa (PA 5L60 e PA 5D61) em cinco diferentes saturações de bases (V%) – 15, 35, 40, 50 e 60. Este delineamento experimental nos permitiu estudar cinco diferentes concentrações de alumínio no solo. O crescimento de ambos os híbridos de sorgo foi afetado apenas em V%15. A concentração e conteúdo de N, bem como os indicadores de eficiência de uso do N forma afetados em V%15 em ambos os híbridos; porém, no híbrido PA 5D61 se mostrou menos sensível que o híbrido PA 5L60 nas condições estudadas. A análise de PCA nos mostrou que o híbrido PA 5D61 se mostrou mais tolerante ao alumínio e usa o N mais eficientemente que o híbrido PA 5L60 e, portanto, argumentamos que esse híbrido pode ser utilizado em áreas marginais de baixa fertilidade como produtor de matéria prima para bioenergia.

Palavras-chaves: Bioenergia; Nutriente; Toxicidade de alumínio; Sorghum bicolor.

Abstract

Sorghum plants are well cultivated in central region of Brazil, which is originalmente low fertility and rich in aluminum content. These features demand studies to know better hybrids for such environment, mainly regarding the use of nutrients like nitrogen (N). This nutrient is the most limiting to plant growth, development, production and for this reason it is of great concern to understand the effects of low bases saturation on N use in biomass dedicated sorghum plants, that is hybrids used for bioenergy purpouses. With the aim to assess the effect of increasing liming rates on nitrogen use in biomass sorghum hybrids we set a greenhouse quantitative research as a completely randomized factorial experiment by using two sorghum hybrids (PA 5L60 and PA 5D61) under five different bases saturation (V%) – 15, 35, 40, 50 and 60. This experimental design allowed us to study five different concentrations of aluminum in this soil. The growth of both sorghum hybrid was impaired

only under at V% 15. Nitrogen concentration, content and the N use efficiency indicators were affected at V% 15 for both sorghum hybrids; however, the hybrid PA 5D61 showed to be less sensitive than PA 5L60 under the studied conditions. The PCA analysis showed that the hybrid PA 5D61 showed to be more tolerant to aluminum and uses N more efficiently than the hybrid PA 5L60 and, therefore, we can argue that this hybrid should be used in marginal, low fertility lands as a feedstock producer for bioenergy.

Keywords: Bioenergy; Nutrient; Aluminum toxicity; Sorghum bicolor.

Resumen

Las plantas de sorgo se cultivan bien en el centro de Brasil, que es pobre en fertilidad y rico en aluminio. Estas características requieren estudios para conocer los mejores híbridos para el medio ambiente, especialmente aquellos que se refieren al uso de nutrientes como el nitrógeno (N). Este nutriente es el factor más limitante para el crecimiento, desarrollo y producción de las plantas; por eso es muy importante comprender los efectos de la baja saturación de bases en el uso de N en la biomasa serológica, es decir, los híbridos utilizados con fines bioenergéticos. Para evaluar el efecto del aumento de la saturación de bases en el uso de nitrógeno en híbridos de biomasa de sorgo, instalamos un experimento cuantitativo en invernadero en diseño factorial aleatorizado utilizando dos híbridos de biomasa serológica (PA 5L60 y PA 5D61) en cinco saturaciones de bases diferentes (V%): 15, 35, 40, 50 y 60. Este diseño experimental nos estudia cinco componentes diferentes. de aluminio en el suelo. El crecimiento de ambos híbridos de sorgo se vio afectado solo por V% 15. La concentración y el contenido de N, así como los indicadores de eficiencia de uso de N se vieron afectados por V% 15 en ambos híbridos; sin embargo, ningún híbrido PA 5D61 fue menos sensible que el híbrido PA 5L60 en las condiciones estudiadas. Un análisis de la PCA que mostró que el híbrido PA 5D61 mostró más tolerancia al aluminio y usa N de manera más eficiente que el híbrido PA 5L60 y, por lo tanto, argumenta que este híbrido puede usarse en áreas marginales de baja fertilidad como producto primario para la bioenergía.

Palabra clave: Bioenergía; Nutriente; Toxicidad por alumínio; Sorghum bicolor.

1. Introduction

A great problem associated with infertile soils of tropical regions is the high availability of exchangeable aluminum (Al^{3+}). This element is the 3rd most abundant mineral, composing 8% of the earth's crust (Kochian et al. 2004) and more than 50% of the arable lands for

agricultural production are acidic. Therefore, they present high Al^{3+} content, consequently they present limitations for crops production (Piñeros et al. 2005). The high reactivity of Al^{3+} is responsible for several cellular disorders such as alteration of cell wall integrity and plasma membrane, which ultimately impairs nutrients uptake from the growth media (Bojórquezquintal et al. 2017) consequently leading to decrease in production.

In Brazil, especially in the cerrado biome, the soils present high levels of Al^{3+} due to their low pH and in this context there is a prevalence of H⁺, Al^{3+} and Mn^{2+} cations; and lower concentrations of basic cations such as Ca^{2+} , Mg^{2+} and K⁺, as well as lower availability of phosphorus and nitrogen, and therefore there i salso nutritional limitation associated with Al^{3+} availability/toxicity.

In this sense, plants that are able to develop in different types of soils and environments give the crop the indication of rusticity and relative tolerance to adverse conditions (Salvador et al. 2000). In relation to Al^{3+} , the main symptom of its toxicity is the inhibition of root development and decrease of root elongation, shortly after exposure to the mineral (Souza et al. 2016). In a nutrient solution, the phytotoxic effects caused by aluminum at pH <5.5, have a very rapid response, within 25 to 30 minutes after exposure of the root to aluminum (Justino et al. 2006), causing serious anomalies to the root system, modifying the patterns of nutrient uptake and metabolism (Salvador et al. 2000).

Regarding N nutrition, some studies revealed that nitrate uptake is impaired in the presence of Al^{3+} in commercial cultivars of sorghum (Cambraia et al. 1989). In a similar and more recent study, Souza et al. (2016) demonstrated in maize that Al^{3+} affected several pathways of N assimilation, including decreasing nitrate reductase enzyme activity. These data show that a satisfactory production is beyond just supply fertilizer. But instead, the soil condition should be adequate for root growth and functioning.

In land of low productivity, there is a tendency to cultivate plants more tolerant to adverse abiotic conditions such as drought and nutrient limitations. In this sense, the cultivation of biomass sorghum is very interesting considering the production of raw material for the production of second generation bioenergy or ethanol, thus being a friendly alternative for the environment (Silva et al. 2018). The biomass sorghum has a rapid cycle and can reach up to six meters in only four months of growth and the Palo Alto biomass sorghum hybrids, developed by NexSteppe, provide high-yield as a low-cost biomass feedstock for bioenergy, including biogas and cellulosic biofuels.

According to CONAB's report, in the 2017/2018 harvest for grain sorghum, the centralwest region produced 891 thousand tons in 295 thousand ha, corresponding to an average

yield of 3,020 kg/ha. The state of Goiás, by itself, produced 710 thousand tons in 229 thousand ha, with an average yield of 3,100 kg/ha. However, the cultivation of sorghum biomass is still not widespread in Brazil as an option for cogeneration of energy and, therefore, we do not yet have information on the production of biomass sorghum in different Brazilian regions and little studies have considered the association of aluminum stress with nitrogen use efficiency.

The use of energy crops in soils with toxic concentrations of Al^{3+} is a possibility of better utilization of less productive lands in cerrado biome. The possibility to identify the use of N by biomass sorghum in these environments is important for the optimization, economy and sustainability in the production system. Thus, the identification of nitrogen use efficient hybrids in in poor soils with different availability of aluminum can indicate sustainability in the supply of raw material as an efficient energy source (Masters et al. 2016).

Therefore, we aimed to determine the tolerance to Al^{3+} and its effect in nitrogen use in two hybrid of biomass sorghum (Palo Alto 5L60, Palo Alto 5D61).

2. Materials and Methods

The experiment has been carried out between august-2016 and December-2016 at "Instituto Federal Goiano, Campus Rio Verde, GO". We carried out a qualitative and quantitative controlled environment and laboratory research and using statistical methods to analyse all collected data (Pereira et al. 2018) The soil was homogenized in an industrial concrete mixer and sieved in a 2 mm mesh. It was used a Quartzarenic Neosol whose chemical and physical characteristics are shown in Table 1. Based on the results of the chemical analysis, 2 dm³ of soil were packed in plastic bags and then dolomitic limestone was added: 0.98; 2.12; 3.25 and 4.39 g to obtain the following base saturations (V%): 35, 40, 50 and 60 (Table 1), respectively, according to the following formula: NC = (V2 - V1) * T / PRNT. Afterward, the soil was allowed to stand for 30 days to proper limestone reaction in glasshouse.

Before transferring the soil to 2 L pots, it was sampled and analysed to confirm the characteristic after liming (Table 1 part I and part II). Soil fertilization was carried out prior to seed sowing, and the total N, P and K were, respectively, 176, 406 and 292 mg.dm⁻³.

Table 1 (Part I)	- Soil	chemical	features	after	soil	liming to	achieve	the	different	V%
treatments.										

Traat	Р	S	\mathbf{K}^+	Ca ²⁺	Mg^{2+}	Al^{3+}	H+A1	В	Na	Cu	Fe	Mn	Zn	
Heat.	mg.c	lm ⁻³			cmol _c .dn	n ⁻³				mg.dm ⁻³				
V ₁₅	2.86	3.5	0.05	0.31	0.20	0.77	2.86	0.11	3.0	0.19	95.1	9.5	0.23	
V ₃₅	2.40	3.0	0.04	0.59	0.39	0.25	1.90	0.11	2.0	0.12	78.5	6.8	0.17	
V_{40}	2.31	3.8	0.04	0.81	0.51	0.08	2.00	0.11	2.0	0.19	75.6	6.9	0.18	
V_{50}	2.40	4.5	0.04	1.08	0.70	0.02	1.71	0.11	2.0	0.16	81.4	6.9	0.19	
V_{60}	2.42	3.8	0.03	1.25	0.86	0.01	1.51	0.11	1.0	0.19	79.4	6.4	0.16	

Source: Authors (2020).

Table 1 (Part II) – Soil chemical features after soil liming to achieve the different V% treatments.

Tuest	pН	O.M	O.C	B.S.	CTC	V	m	
Treat.	-	mg.d	m ⁻³	cmol _c .	dm ⁻³	%		
V15	3.9	21.1	12.2	0.57	3.43	16.6	57.5	
V35	4.4	19.2	11.1	1.03	2.93	35.2	19.5	
V_{40}	4.8	19.3	11.2	1.37	3.37	40.7	5.5	
V ₅₀	5.2	21.0	12.2	1.83	3.54	51.7	1.1	
V ₆₀	5.7	18.5	10.7	2.14	3.65	58.6	0.5	

Treat.: Treatments; pH: CaCl₂; Ca²⁺, Mg²⁺, Al³⁺ extracted with KCl 1M; P, K⁺, Cu²⁺, Fe²⁺, Mn²⁺, Zn²⁺ extracte with Melhich; S extracted with Ca(H₂PO₄)₂. Source: Authors (2020).

In the tables above, it is clear that the treatments lead to the conditions expected, mainly concerning Al³⁺ availability according to the liming rate.

The seeds were previously treated if Cruiser 350 FS- 400 mL p.c./100 kg⁻¹ of seeds; Maxim XL-100 mL p.c./100 kg⁻¹ of seeds and Apron-200 mL p.c./100 kg⁻¹ of seeds. In the pots at glasshouse, it was sowed 6 seeds per pot and after 15 day four plants were cut off and the remaining two plants were allowed to grow for more 30 days.

At the end of the experiment, the plants were separated in roots and aerial part and the following parameters were measured: leaf leaf area (Flowmarfe[®] portable leaf area meter); root volume; number of leaves and stem length. Subsequently, aerial part and roots were packed in paper bags and kept in a forced circulation oven at 65° C for 3 days until constant mass at the moment of dry mass measurement. Subsequently, all the aerial tissue was grinded for total N quantitation.

The total nitrogen concentration was determined by distillation-titration (Kjeldahl). Based on N concentration data and shoot dry weight it was calculated the content of N and also determined the following efficiencies: N Uptake Efficiency (NUpE), N Utilization Efficiency (NUtE) and N use efficiency (NUE) according to Moll et al. 1982 and Good et al. 2004.

The experiment was set as a completely randomized design, with 2 biomass sorghum hybrids (PA5L60 and PA5D61), which were supplied by NexSteppe Sementes do Brasil; and 5 levels of V% (V15%, V35%, V40%, V50% and 60%) respectively (V16% represents the initial V% of the soil) with 4 replicates for each treatment.

The data were submitted to analysis of variance (ANOVA) and regression analysis for the growth over time and response of each hybrid in different V% by SISVAR[®] software version 5.3 (Ferreira, 2011). The data were also submitted to the Tukey test at 5% for comparison of the variables between the hybrids within each V% level.

The principal component analysis (PCA) was performed using the Past3[®] software (Hammer et al. 2001) and the correlation matrix was performed using the package Performance Analytics (Peterson *et al.* 2019) in RStudio (R Core Team, 2019).

3. Results and Discussion

Biomass sorghum hybrids 5L60 and 5D61 showed similar growth responses from V% 35 while in V% 15 the hybrids showed impaired growth (Figures 1 A and B). The impaired growth response in V% 15 is directly related to the high availability of Al^{3+} , the main stress factor, and partially related to the low availability of Ca^{2+} and Mg^{2+} (Table 1).

Therefore, these results demonstrate that crop uses Ca^{2+} and Mg^{2+} cations efficiently when they are in concentrations below the recommended levels, since no visual deficiency symptoms of these nutrients were observed.

Figure 1 – Growth pattern of biomass sorghum hybrid 5L60 (A) and 5D61 (B) under increasing V%. Measure number denotes the total measuring taken during the whole experiment and the measuring number 10 occurred at the experiment conclusion moment. ***: p<0.001.



Source: Authors (2020).

In a study carried out by Carlin & dos Santos (2010), the researchers evaluated the response of the sugarcane cultivar IAC91-5155 to acidity in combination with water deficit. In this experiment, the researchers observed that in V% of 23 and 33 there were a decrease in several growth components, even in plants without water restriction.

In the study conducted by Crusciol et al. (2012), the authors evaluated the response of two rice cultivars in three different V% (10, 40 and 70) and found that there is genetic/physiological variation in liming response; in which the cultivar Caiapó presented lower performance in relation to the cultivar Maravilha when submitted to V% 10.

Similarly, in biomass sorghum materials studied, we also observed behavior similar to that reported by Crusciol et al. (2012) (Figures 2A to 2F), in which we observed that in the V% 15 the 5L60 hybrid presented lower values for growth variables in relation to the hybrid 5D61 (Figures 2A to 2I), but in others V%, these hybrids did not differ between them. However, it is evident that for each of the hybrids, the V% 15 treatment severely affected the growth component when we compared the response of each individual hybrid within the different V% (Figures 2A to 2I), but without changing the biomass partition (Figure 3).

In acid soil conditions, the Al^{3+} is an extremely toxic element for growth (Vitorello et al. 2005). It was possible to observe the effects of its toxicity when the plants were submitted to V% 15, in which the Al^{3+} content was extremely high, 0.77 cmolc.dm⁻³ (Table 1). The Al^{3+} content corresponds to a concentration of 2.5 mM of free Al^{3+} , which is highly toxic to several organisms (Liu et al. 2014) and the main effect is the inhibition of root growth and development (Souza et al. 2015), in the work of Li-Ming et al. (2018) the researches have found that Al^{3+} at 60 μ M have caused a decrease of 40% in root growth of maize plants and, additionally, several desorders in transcripts was also observed, thus confirming the deleterious effects of Al^{3+} toxicity.

Both sorghum hybrids presented growth and root volume affected by the low V% (Figures 2A and 2B), however, in V% 35, whose m% is approximately 20%, still toxic content because it is equivalent to a concentration of 0.8 mM of Al^{3+} , the hybrids showed satisfactory growth and root development, similar to the results observed for V% greater than 35. In general, the studies show that in nutrient solution, concentrations below 200 μ M Al^{3+} are responsible for inhibiting growth of several species (Maron et al. 2008, Caniato et al. 2007).

Figure 2 – Initial growth features: root volume (A), root dry weight (B), stalk dry weight (C), leaf dry weight (D), shoot dry weight (E), total biomass (F), number of leaves (G), leaf area (H), specific leaf area (I) of two biomass sorghum hybrids (5L60 and 5D61) growing under increasing bases saturation (V%). The asterisk (*) indicates statistical difference in averages between sorghum hybrids in V% treatments by Tukey's test at 5% probability.



■5L60

□5D61

Т

15

35

40

Soil bases saturation (%)

50

60

1200

1000

400 200

0

Leaf area (cm²) 800 600



Source: Authors (2020).

As the roots were affected by Al^{3+} , there was a direct side effect on the shoot production of these materials, which was possible to observe decreased leaf area in both hybrids in V% 15. However, comparing the hybrids with each other, the hybrid 5D61 still showed higher leaf area compared to the hybrid 5L60, which suggests that this material has a better adaptation response to the condition studied (Figures 2H and 2I).

Figure 3 – Carbon partitioning in two biomass sorghum hybrids (5L60 and 5D61) growing under increasing base saturation (V%).



Source: Authors (2020).

The genetic variability of Al^{3+} tolerance in sorghum is extensive and has already been characterized in a study conducted by Caniato et al. 2007. In this study the authors explored the *Alt*_{SB} gene which is related to Al^{3+} tolerance, and its relation with levels of Al^{3+} tolerance in sorghum materials of different origins, which led the authors to conclude that there is presence of multiple alleles at the locus in question, thus explaining the existence of different tolerance levels in sorghum. Furthermore, the authors determined that allelic and non-allelic heterogeneity are important factors for the improvement of sorghum in relation to Al^{3+} tolerance.

Regarding the study conducted by Santo et al. (2018), in which 20 sorghum hybrids were evaluated for aluminum tolerance growing in soil with m% 40, identified 10 hybrids with higher average yield in low and high m% conditions, suggesting that these materials can be considered in a plant breeding program. These hybrids may be recommended for production in more restrictive environments. In a broader perspective, we can consider the production scenario in the no-tillage system, in which the identification of genotypes more adapted to develop in poor soils is of fundamental importance because the soil management techniques are different from the conventional system production techniques, mainly concerning the reaction time of correctives that are applied in soil surface instead of being incorporated (Soratto et al. 2010).

The cultivation of any crop in acid soils has limitation besides the Al³⁺ toxicity, since a nutritional concern is directly related to the productive potential of the crop (Zhao et al. 2018, Kochian et al. 2015). Soils of Brazilian cerrado have low levels of organic matter, nitrogen, phosphorous, potassium, calcium, magnesium, zinc, boron and copper (Vendrame et al. 2010), due to the high degree of weathering that these soils were subjected.

Nitrogen, in particular, is highly demanded because of its essential role as a component of proteins and nucleic acids, thus being indispensable for reaching high yields (Mokhele et al. 2014; Mariano et al. 2015). However, it was little described how sorghum plants use nitrogen under conditions of alic soil and low fertility. In this experiment, we observed that the N content was severely affected in the condition of low base saturation (V% 15) and that in base saturations from V% 35 the contents of N did not differ between sorghum hybrids nor in function of the different V% (Figures 4A and 4B).

In maize, there is evidence that Al^{3+} affects the uptake of nitrate and ammonium; in the study conducted by Purcino et al. (2003), the authors demonstrated that there was less accumulation of N in maize plants treated with Al^{3+} and related this effect to the decrease in uptake capacity of nitrate and ammonium forms available in the growth medium.

Another researches clearly demonstrate that the upatake capacity and incorporation of N in organic molecules are impaired in plants exposed to Al^{3+} ; for instance, the effect of Al^{3+} on nitrate reduction was observed by Cruz et al. (2011) and Souza et al. (2016), in sorghum and maize, respectively; and this has led to drastic decreases in plant growth capacity. This response is directly related to the role of N in plant growth; nitrate reduction is the first step of the N-NO₃⁻ pathway and takes place in the cytosol; and then, the nitrite is transported to the plastids to be reduced to ammonia that it promptly protonated to ammonium which is incorporated in carbon skeletons via GS/GOGAT pathway (Masclaux-Daubresse et al. 2010). In this sense, any factor that interferes in any of these steps significantly contributes to the decrease in growth and production of any crop, once the N available in soil will have a decreased use due to the toxicity of Al^{3+} , as observed in the treatment V% 15 (Figures 4A to 4E).

Figure 4 – Nitrogen concentration in shoots (A), nitrogen content in shoots (B), nitrogen uptake efficiency (C), nitrogen use efficiency (D) and nitrogen use efficiency (E) in two biomass sorghum hybrids (5L60 and 5D61) growing under increasing base saturation (V%). The asterisk (*) indicates statistical difference in averages between sorghum hybrids in V% treatments by Tukey's test at 5% probability.





Source: Authors (2020).

This response leads us to evaluate the impact of high availability of Al^{3+} on the uptake and utilization efficiency of N as we observed that NUpE (N uptake efficiency), NUtE (N utilization efficiency) and NUE (N use efficiency) were affected at the lowest V%, which means higher m% (Figures 4C to 4E).

The biomass sorghum hybrids differed only when compared in V% 15, and the 5D61 hybrid presented higher values of all variables than the hybrid 5L60 (Figures 4A to 4E), a fact that suggests that the hybrid 5D61 is more tolerant to the stressful condition assessed in this experiment and, thus, it can be used to deepen the studies regarding tolerance to Al^{3+} and use of N by studying the parental sorghum materials.

According to Zhao et al. (2018), the genotypes adapted to high acidity have preference for ammonium instead of nitrate and relate this characteristic to the more efficient use of N, suggesting a more rational use of nitrogen fertilizers; however the molecular mechanisms that relate the use of ammonium and the tolerance to Al^{3+} are not yet evidenced and, therefore, further studies are needed to understand how plants regulate the uptake and use of N-NH₄⁺ in alic soil conditions, it will be possible to improve the use of nitrogen fertilizers and increase productivity in a sustainable way.

By using principal componente analysis (PCA) with all the morphometric variables (LDW, SDW, SH.DW, RDW, RV and LA) and nitrogen indicators (N.C., N.Ct., NUpE; NUtE and NUE) we could evaluate the biomass sorghum hybrids in the different V% treatments. The principal component 1 (PC1) explained 94.5% of all observed variances, while principal component 2 (PC2) explained 2.89% of all variations, and together these two components explain more than 97% of all variances observed (Figure 5).

For PC1, the variable N.Cc. was least representative variable (loading plot in PCA additional information) which corroborates with the complete negative correlation of this

variable to any other (Figure 6), while the other variables had similar contribution with loadings that varied between 0.289 and 0.308; PC2 has four more significant variables that contributed for grouping: NUpE, N.Ct., N.Cc. and S.D.W; whose loadings are 0.485 for the first two, 0.262 for the third and 0.257 for the fourth (Figure 5).

Figure 5 – Principal component analysis using morphological and nitrogen traits for two biomass sorghum hybrids under increasing base saturation. (\blacksquare) represents the hybrid 5D61 and (\bullet) represents the hybrid 5L60. The number after hybrid identification represents each V% (15, 35, 40, 50 and 60) treatment. PC 1 explains 94.5% of total variation while PC2 explains 2.89% of total variation observed. The three most representative loadings for PC1 are L.D.W. (0.306), SH.D.W. (0.308) and N.U.E. (0.308); while to PC2 are N.Cc. (0.261), N. Ct. (0.485) and N.Up.E. (0.485).



Source: Authors (2020).

This approach allowed us to observe that the variables N. Ct, NUpE, NUE, S.D.W., SH.D.W., were sufficient to group the hybrid 5D61 in the quadrant with the highers loads for PC2; while the variables L.D.W., R.D.W., R.V. and NUtE were responsible for clustering the 5L60 hybrids in the quadrant with lower PC2 loads. In addition, the V% 15 treatment segregated the two hybrids in relation to the other treatments (Figures 5 and 6).

In view of such results we can observe that each hybrid has different attributes even in similar V% conditions. Regarding the genetic improvement, this identification is of fundamental importance in order to have a better selection of lineages for the development of sorghum hybrids more tolerant to Al^{3+} and more productive.

In the work carried out by Menezes et al. (2014), the authors evaluated 55 lines of grain sorghum for aluminum tolerance and found that in overall, at 20% aluminum saturation, there was a 16% drop in overall production. However, the authors identified materials with different levels of tolerance and with different combination capacities to produce hybrids more tolerant to Al^{3+} .

Figure 6 – Pearson's linear correlation matrix, plot and their significance between all variables measured in biomass sorghum under increasing V%. Variable description: Leaf dry weight (L.D.W); stalk dry weight (S.D.W.); shoot dry weight (SH.D.W.); root dry weight (R.D.W.); Root volume (V.R.); leaf area (L.A.); nitrogen concentration (N.Cc.); nitrogen content: (N.Ct.); nitrogen uptake efficiency (NUpE); nitrogen utilization efficiency (NUtE); nitrogen use efficiency (NUE).



Source: Authors (2020).

4. Conclusions and Suggestions

The hybrid 5D61 is more tolerant to conditions imposed under low bases saturation and uses N more efficiently than 5L60 hybrid. It brings us the possibility to study the lineage that were used to produce such hybrids and the possibility to use such hybrid in marginal land

areas to produce feedstock for bioenergy in low fertility lands. For next researches we will focus in determine nitrogen metabolism enzymes in order to a better understanding of how these sorghum hybrids uses available N to support their growth under stressful conditions.

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