The Effect of Mycorrhizal Provision and Watering Frequency on the Nutrient and Prussic Acid Content of Sorghum (*Sorghum bicolor* (L.) Moench)

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ABSTRACT
Sorghum is a cereal plant that has the potential to be developed as animal feed due to its resistance to drought stress and regrowth ability after harvest. This study aimed to determine the response of sorghum plants given mycorrhizae with different watering frequencies to the nutrient and prussic acid content. This research was a completely randomized design with a 3x3 factorial pattern. Mycorrhizal fungi provision consisted of three levels, namely without mycorrhizal (M0), mycorrhizal 10 g/polybag (M10), and mycorrhizal 20 g/polybag (M20). The watering frequency consisted of watering every day (A1), watering every four days (A4), and watering every eight days (A8). Nutrient and prussic acid content of sorghum forage were affected by mycorrhiza ($p<0.05$). Different watering frequencies did not have a significant effect on the nutrient and prussic acid content of forage sorghum. However, mycorrhizal provision significantly increased the nutrient and prussic acid content of sorghum forage. The watering frequency did not affect the nutrient content of sorghum forage, but the watering once every eight days increased the content of sorghum forage. The best mycorrhizal treatment was 10 g/polybag.

Keywords: *Sorghum, Mycorrhizae, Watering frequency, nutrient content, prussic acid.*

1. INTRODUCTION

Sorghum is a cereal crop that has the potential to be developed as an animal feed crop. This plant has good regrowth after harvest and can be cultivated in areas with high drought rates [1]. Irawan *et al.* [2] reported that each dry weight of sorghum leaves contained 7.82% crude protein, 2.60% lipid, 28.94% crude fiber, and 11.43% ash. The development of sorghum as a forage crop in several areas of Indonesia with high drought levels such as East Nusa Tenggara (NTT) has a constraint, namely the water availability.

There are several aspects for productivity and quality of the forage i.e. planting space [3], [4], type of variety [5], [6], planting material [7], level of fertilizer [8], level of defoliation [9], and harvesting age [10]. Sorghum harvest age affects ~~to~~ the production dan prussic acid content [11]. The sorghum resistance to drought stress can be seen by the watering frequency [12]. The watering frequency of once a day resulted in sorghum's best production [13]. Sorghum requires water as much as 400 to 450 mm [14]. This plant will experience drought stress if the soil water content is less than the water requirements. It could affect the nutrient content decrease. When sorghum plant experiences drought stress, its content of prussic acid increases [15]. Sorghum plants that experience drought stress will form secondary metabolites to adapt to environmental conditions. This condition is the same with *Brachiaria sp*, on the production of oxalate in the dry season [16]. The secondary metabolic product of sorghum is prussic acid which is an anti-nutrient for ruminants. One of the efforts to overcome the impact of drought stress on sorghum plants is by the mycorrhizal provision.

Mycorrhizae are soil fungi that can perform symbiotic mutualism with plant roots. [17], [18] stated that mycorrhizae help plant roots absorb nutrients, increase plant resistance to drought stress, and increase

Table 1. Soil nutrient content as a growing media for sorghum

No	Parameter	Unit	Test Results
1	N-Total	%	2.52 ± 0.08
2	Potassium (K)	K/Kg	34.9 ± 2.80
3	Phosphate	mg/L	1.26 ± 0.01

plant root resistance to root pathogen attacks. Giving arbuscular mycorrhizal fungi as much as 10 g/plant increased the number of leaves and the degree of root infection in sweet sorghum plants and increased the production of sorghum plants [19], [20].

There is still a lack of study on the effect of mycorrhizal provision at different levels with different watering frequencies on the nutrient and prussic acid content. A study on the mycorrhizal provision and the watering frequency on the nutrient and prussic acid of sorghum plants need to be carried out to determine the effect of mycorrhizae on the nutrient content and the ability of mycorrhizae to reduce the prussic acid content of sorghum plants due to drought stress.

2. MATERIALS AND METHODS

The research was conducted in the Greenhouse of Animal Science, Universitas Gadjah Mada from May to September 2020. The materials used were polybags, sorghum, commercial mycorrhizae, and organic fertilizer. Soil nutrient content can be seen in Table 1. The tools used were hoe, machete, shovel, Camry electric scales with 5 kg capacity and the smallest scale of 1 gram, thermometer (DEA).

2.1. Research methodology

This research was a completely randomized design with a 3x3 factorial patterns with 3 repetitions for each treatment. The research factors were mycorrhizal level and watering frequency. Mycorrhizal fungi provision consisted of three levels, namely without mycorrhizal (M0), mycorrhizal 10 g/polybag (M10), and mycorrhizal 20 g/polybag (M20). The watering frequency consisted of watering every day (A1), watering every four days (A4), and watering every eight days (A8).

Media preparation included disassembling the soil and sifting with a diameter of 1 mm. The media were mixed with an organic fertilizer in a ratio of 9:1. Then put into polybags as much as 10 kg/polybag. There were 45 polybags. After preparing the planting media, the sorghum seeds were planted. Planting holes were made with a depth of ± 3 cm. After that, as many as 3 to 5 plants were given in each planting hole. Sorghum was thinned within 6 days after being planted, leaving one of the best plants. Mychorrizae was given after the plants were 7 DAP. Plant watering was done according to the research treatment. Maintenance was done by cleaning weeds, controlling pests, and watering. Harvesting was done when the plants were 70 DAP and cut at a distance of 5 cm from the soil surface.

2.2. Laboratory analysis

The forage obtained was then prepared by grinding and sieved through a 1 mm diameter sieve and then continued for proximate analysis. The dry matter analysis according to AOAC [21] samples was dried in an oven at 105ºC for 24 hours. Weight after oven at 105ºC is the dry matter weight. Organic matter analysis, the sample was put into a furnace at a temperature of 600ºC and became ash. Crude protein analysis, there are three stages in crude protein analysis, namely destruction, distillation, and titration. Protein content analysis was carried out using the Kjeldahl method. Extract ether analysis, the sample was wrapped in fat-free filter paper, then placed in an oven at 105ºc for one night. Then the hot sample was weighed. The package was put in a Soxhlet extractor and extracted with petroleum benzene for ± 16 hours. After that, the package was removed and put in an oven at 105ºC overnight [21]. Prussic acid analysis: The sample was put into a tube that has been closed with yellow picrate paper then heated. The paper captured the evaporated cyanide. Then yellow picrate paper was dissolved and observed using a spectrophotometer.

2.3. Data analysis

The study results were analysed quantitatively using the Analysis of Variance (ANOVA) based on the factorial pattern. Further testing was carried out with Duncan's New Multiple Range Test (DMRT) to data with significant differences using the SPSS version 23 application.

3. RESULTS

3.1. Nutrient content of sorghum forage

The nutrient content of sorghum forage is presented in Table 2. Mycorrhizal provision can increase dry matter, organic matter, crude protein, and extract ether. Furthermore, it reduces the content of crude fiber sorghum forage ($p<0.05$). Different watering frequencies do not increase the nutrient content of sorghum forage (dry matter, organic matter, crude fiber, crude protein, and extract ether). The highest dry matter content is found in the M10 and A1 treatments (21.98%). The highest organic matter content is found

Table 2. The nutrient content of sorghum in the treatment of mycorrhizal fungi and different watering frequency

Nutrient content (%)	Mycorrhizal lavels	Watering Frequency			Average
		A1	A4	A8	
Dry matter	M0	21.08 ± 0.17	20.35 ± 0.82	20.82 ± 1.14	20.75 ± 0.77^{a}
	M10	21.98 ± 0.25	21.67 ± 0.46	21.27 ± 0.34	21.64 ± 0.44^{b}
	M20	20.81 ± 0.67	21.04 ± 0.24	21.22 ± 0.60	21.02 ± 0.50^{a}
	Average	21.29 ± 0.64	21.02 ± 0.75	21.10 ± 0.70	21.14 ± 0.68
Organic matter	M0	83.31 ± 0.85	82.91 ± 1.10	84.04 ± 0.91	83.42 ± 0.97^{a}
	M10	86.07 ± 0.43	86.39 ± 0.98	85.22 ± 1.78	85.89 ± 1.16^{b}
	M20	83.28 ± 0.33	84.67 ± 0.91	85.12 ± 0.31	84.36 ± 0.97^{a}
	Average	84.22 ± 1.47	84.66 ± 1.74	84.79 ± 1.16	84.55 ± 1.44
Crude fiber	M0	34.70 ± 0.68	34.89 ± 1.73	34.63 ± 1.70	34.74 ± 1.27^{b}
	M10	31.75 ± 0.60	32.66 ± 0.60	33.87 ± 0.67	32.76 ± 1.07^{a}
	M20	32.80 ± 0.65	33.05 ± 0.87	31.67 ± 0.51	32.51 ± 0.88^{a}
	Average	33.08 ± 1.40	33.53 ± 1.44	33.39 ± 1.63	33.33 ± 1.45
Crude protein	M0	9.91 ± 0.15	9.95 ± 0.24	9.76 ± 0.53	9.87 ± 0.31^{a}
	M10	11.50 ± 0.50	12.41 ± 0.57	12.02 ± 0.41	11.98 ± 0.58^{c}
	M20	11.53 ± 0.43	10.40 ± 0.15	10.27 ± 1.26	10.75 ± 0.22^{b}
	Average	10.91 ± 0.83	10.87 ± 1.23	10.63 ± 1.18	10.80 ± 1.06
Extract ether	M0	5.00 ± 0.26	5.03 ± 0.23	5.16 ± 0.18	5.06 ± 0.21^{a}
	M10	6.61 ± 0.14	6.72 ± 0.13	6.73 ± 0.21	6.69 ± 0.15^{b}
	M20	7.12 ± 0.44	6.45 ± 0.36	6.41 ± 0.26	6.66 ± 0.46^{b}
	Average	6.24 ± 0.99	6.07 ± 0.82	6.10 ± 0.75	6.14 ± 0.83

M0 = without mycorrhizal, M10 = mycorrhizal 10 g/polybag and M20 = myciorrhizal 20 g/polybag
A1 = watering everyday, A4 = watering every four days, and A8 = watering every eight days
^{a,b,c} different superscripts on the same column showed significant differences (p<0.05) in the mean treatment

Table 3. The prussic acid of sorghum in the treatment of mycorrhizal fungi and different watering frequency

Prussic acid (%)	Mycorrhizal lavels	Watering Frequency			Average
		A1	A4	A8	
Prussic acid	M0	125.58 ± 5.05	145.71 ± 5.05	150.80 ± 5.00	140.70 ± 12.34^{a}
	M10	157.98 ± 2.54	163.92 ± 2.75	170.86 ± 4.96	164.25 ± 6.39^{c}
	M20	138.69 ± 10.59	156.76 ± 3.34	164.83 ± 6.61	153.43 ± 6.61^{b}
	Average	140.75 ± 15.34^{k}	155.46 ± 8.61^{l}	162.16 ± 10.14^{m}	152.79 ± 14.49

M0 = without mycorrhizal, M10 = mycorrhizal 10 g/polybag and M20 = myciorrhizal 20 g/polybag
A1 = watering everyday, A4 = watering every four days, and A8 = watering every eight days
^{a,b,c} different superscripts on the same column showed significant differences (p<0.05) in the mean treatment
^{k,l,m} different superscripts on the same line showed significant differences (p<0.05) in the mean treatment

in the M10 and A4 treatments (86.39%). The highest crude fiber is found in the M0 and A4 treatments (34.89%). The highest crude protein is found in the M10 and A4 treatments (12.41%). The highest extract ether is found in the treatment M20 and A1 (7.12%). The interaction between mycorrhizal levels and watering frequency do not affect the nutrient content of sorghum forage (dry matter, organic matter, crude fiber, crude protein, and extract ether).

3.2. Prussic acid content of sorghum forage

The prussic acid content of sorghum forage is presented in Table 3. Mycorrhizal provision significantly increased the prussic acid content of sorghum forage (p<0.05). The treatment of different watering frequencies increased the prussic acid content of sorghum forage significantly (p<0.05). The interaction between mycorrhizal levels and watering frequency do not affect ($p>0.05$) the prussic acid content of sorghum forage. The highest prussic acid content is found in the M10 and A8 treatments (170.86 ppm), while the lowest prussic acid content is found in the M0 and A1 treatments (125.58 ppm).

4. DISCUSSION

4.1. Nutrient content of sorghum forage

Mycorrhizal fungi provision to sorghum plants can affect the nutrient content of sorghum forage (dry matter, organic matter, crude fiber, crude protein, and extract ether). The use of mycorrhizae can increase the dry matter content, organic matter, crude protein, and extract ether. Furthermore, it can reduce the crude fiber content of sorghum forage. The more the amounts of mycorrhizal application to the plant, the more nutrients are available for plant growth and can improve plant nutrient quality. Mycorrhizae can effectively increase the absorption of nutrients, both macronutrients, and micronutrients. Hutauruk *et al.* [19] stated that mycorrhizae can be associated with almost 90% of plant species and can increase nutrient uptake efficiency.

The dry matter content is an important factor in determining the forage quality [22]. There are several factors to increase productivity and quality such as nitrogen source. This nitrogen source can come from urine [23]. The dry matter content of sorghum forage in this study is presented in Table 2. The dry matter content ranged from 20.35-21.98% while, Mali et al [24] reported that the dry matter content ranged from 15.9-16.8% which was harvested at 52 days after planting. The dry matter content in this study was higher, this could be influenced by a longer harvest age [25], which was 70 days after planting. The longer the harvest age has an impact on the length of the plant assimilation process so that the dry matter content of the plant increases [26]. Increased numbers and length of roots enhance and absorption that in turn increases fresh weight and dry weight[27]. Gardner *et al.* [28] reported that the longer the assimilation took place, the higher the dry matter of the plant. Mycorrhizae can absorb phosphorus and help plants absorb elements of N, K, Zn, Mg, Cu, and Ca [17], [29]. Koten *et al.* [30] stated that available N, P, and K are needed by plants in the process of photosynthesis. The higher the photosynthesis process, the higher the assimilation results stored in plant tissue. This has an impact on increasing the dry matter and organic matter content of sorghum forage.

The increase in crude protein content in this study is caused by the ability of mycorrhizae to absorb nitrogen in the soil. The crude protein content of forage sorghum is presented in Table 2. The treatment of mycorrhizae 10 grams/polybag performed the highest crude protein content. Syafria *et al.* [31] reported that the crude protein content of Kumpia grass (*Hymenachne amplexicaulis* (Rudge) Nees.) was the highest in the mycorrhizal provision treatment of 20 grams/polybag. Higher plant density resulted in higher DM and CP content [32]. Nitrogen is important for plant leaf growth because it is a component in the photosynthesis process [33]. Crude protein content in the study is higher because, in the research treatment, mycorrhizal fungi are combined with organic and inorganic fertilizers (TSP (150 kg P205/ha); KCl (100 kg K_2O/ha); $CO(NH_2)_2$ (200 kg N/ha), and $CaCO_3$ (2 tons/ha)) as basic fertilizer. Simanungkalit *et al.* [34] stated that organic fertilizer is the main source of soil nitrogen, moreover, it plays a significant role in improving the physical, chemical, and biological properties of the soil and the environment. In addition, the genetic characteristics of each different plant also affect the nutritional quality of the plant itself.

The mycorrhizae 10 grams/polybag and 20 grams/polybag treatments had the lowest crude fiber content. It is clear in Table 2 that giving mycorrhizae increased the crude protein content of sorghum forage, and decreased the crude fiber content of forage sorghum. Astuti *et al.* [26] stated that the higher the crude protein content, the lower the crude fiber content of the plant. Mycorrhizal provision can reduce the crude fiber content of sorghum forage. Sutanto *et al.* [35] reported that giving mycorrhizae 15 grams/polybag reduced the crude fiber content of forage *Indigofera zollingeriana* and increased the crude protein content of forage *Indigofera zollingeriana.* Mali *et al.* [24] stated that mycorrhizae can decompose carbohydrates and lignin so that the crude fiber of plants is reduced.

The extract ether of sorghum forage with mycorrhiza was higher than those without mycorrhizae. The highest average extract ether was found in mycorrhizae 10 grams/polybag treatment (6.69%). Mycorrhizae can decompose bound mineral compounds into available minerals. Sutanto *et al.* [36] stated the formation of these compounds from the transformation process of organic matter through the mineralization process of organic matter, including the decomposition process and the humification process. Extract ether content is also formed from humin compounds as a result of the humification process from humus which still contains lipid and wax.

The watering frequency factor in this study did not affect the nutrient content of sorghum forage. This was due to the sorghum's ability to survive in drought stress so that the nutrient content of the forage was still stable. In addition to the water factor, sunlight is crucial for plants for the photosynthesis process. Gardner *et al.* [28] stated that the photosynthesis and respiration rate in plants is also affected by light. This study placed the plants in a greenhouse so that they received the same radiation. It was suspected that the penetration of sunlight was hampered by the greenhouse.

4.2. Prussic acid content of sorghum forage

The prussic acid content increased in sorghum forage with mycorrhizal provision. The sorghum's ability to absorb N in the soil increased crude protein content and increased the prussic acid content. This is in line with the statement Sher *et al.* [37] that higher nitrogen can increase the prussic acid content of sorghum forage. Prussic acid is composed of the amino acid tyrosine so that the increase in crude protein for sorghum forage is directly proportional to the increase in prussic acid. Umami *et al.* [1] explained that prussic acid is a secondary metabolite compound of plants derived from amino acids. Prussic acid is usually found in stressed plants. In this study, there was an increase in prussic acid in sorghum with the watering frequency of once every eight days. Prussic acid content increases due to sorghum plants experiencing drought stress. Barling *et al.* [38] stated that prussic acid is formed when plant cells are damaged resulting in stunting and freezing that causes glycosides to be degraded and form free HCN. Prussic acid is formed by enzymatic reactions resulting from disturbed plant growth [1].

The highest prussic acid content in this study is 170.86 ppm. This amount is not adversely impacting ruminants. Sher *et al.* [39] reported that the maximum amount of prussic acid in plants is 300-500 ppm. Forage administration containing prussic acid of more than 500 ppm can cause poisoning to death in ruminants.

5. CONCLUSION

The mycorrhizal provision in sorghum forage significantly increases the nutrient content of sorghum forage. It also increases the prussic acid content of sorghum forage. However, the prussic acid content in the study does not harm ruminants because the amount of prussic acid is still below 300 ppm. The watering frequencies do not affect the nutrient content of forage sorghum, but the watering frequency of every eight days increases the content of prussic acid. The best mycorrhizal treatment is 10 g/polybag.

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