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An Analysis of Glucose and Bioethanol Content of Sorghum (*Sorghum bicolor* (L) Moench) Varieties

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Abstract: **Fossil energy as a fuel source in the world is decreasing. This research aims to provide bioenergy as a renewable alternative energy source, namely sweet sorghum (*Sorghum bicolor* (L.) Moench), which is cheaper, safer, and reduces air pollution. The specific objective is to increase the production of glucose content and stem bioethanol in several varieties of sorghum against panicle pruning and the dose of organic fertilizer given.**

This research examines sorghum varieties to observe the contents of glucose and bioethanol in the stems of sorghum varieties (*Sorghum bicolor* (L) Moench) in response to fertilization. The design used for the research was: RAK Split Plot, two treatment factors arranged factorially with four repetitions. The first factor was variety, namely: Kawali and Super 1. The second factor is the dose of organic fertilizer (5, 10, 15 tons/h^{-1}). The research started from September 2021 to January 2022 in the Padanggalak area, Sanur Village, Denpasar City.

The results showed that the various treatment had a very significant effect ($P<0.01$) on the variables of plant height at 77 days after planting: stem glucose content, stem juice (sap) production, bioethanol yield, leaf chlorophyll content, and leaf sucrose content. While the effect of fertilizer dose had a very significant effect ($P < 0.01$) on the variables of plant height, diameter, and stem glucose content in plants aged 77 days after planting. The interaction between the two factors had no significant effect ($P>0.05$) on all variables but had a very significant effect ($P<0.05$) on the variable of plant height 77 days after planting. It was discovered that the leading variety of sweet sorghum (Super 1) produced more glucose (12.43% brix) and bioethanol (1.03% brix) than the Kawali variety. The organic fertilizer dose of 10 tons h-1 gave a plant height of 169.46 cm higher than the other two fertilizer doses.

Keywords: **sweet sorghum, glucose contents, stem juice (sap) production, bioethanol.**

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1. INTRODUCTION

Fossil energy as the fuel source in the world is decreasing. This research aims to provide bioenergy as an alternative renewable energy source, namely sweet sorghum (*Sorghum bicolor* (L.) Moench), which is cheaper, safer, and reduces air pollution. The specific objective is to increase the production of glucose content and stem bioethanol in several varieties of sorghum against panicle pruning and the dose of organic fertilizer given.

As a preparation, efforts must be made to select leading sorghum varieties and cultivars capable of producing the most ethanol. There are several types of sorghum that are cultivated, including 1) Local grain sorghum, used for food and animal feed, as well as for raw materials for beverages and bioethanol manufacturers, 2) Broomcorn, its stalks are used for making brooms, and 3) Sweet sorghum, its stem liquid is used as raw material for making syrups (Sugiono, 2011) and raw material for ethanol making (Daniel et al., 2017).

Research by Almodares and Sepahi (1996) resulted in brix glucose levels of sweet sorghum stems ranging from 14.32 – 22.35%. Meanwhile, Noura et al. (2020) on Balnda and Chian Woua varieties found that the glucose contents (brix) ranged from 8.9 - 21.8% brix and 11.8 – 22.5% brix, as well as ethanol content of 279.5 – 3101.2 l/ha^{-1} in Chad and the United

States. The high glucose content in sorghum stalks can be utilized as a raw material for ethanol. Mangena et al. (2018) analyzed the feasibility of sorghum ethanol production. They concluded that sweet sorghum could be used as an ethanol feedstock if all cellulosic glucoses could be efficiently hydrolyzed and converted to ethanol. According to Almodares and Sepahi (1996), the rate of glucose accumulation in sorghum stems varies between cultivars.

The response of glucose and ethanol contents to panicle pruning in sorghum is still sporadic; no one may have studied it. The stem glucose contents produced by the KCS 105 variety was 2.6 tons/ha, the ethanol content of sorghum stems was 94.1% (Agung et al., 2013), and the relatively high ethanol productivity of 1.44g/l (Rolz et al., 2019). Pruning male flowers in corn plants can increase seed yield and seed quality. The interaction of leaf pruning and male flower pruning may also affect the distribution of assimilates between reproductive and vegetative organs (Heidari, 2013).

The results of Surtinah's research (2005) showed that male flowers on pruned corn plants tended to produce higher yields than those that were not pruned. The increase in yield due to male flower pruning is caused by the loss of the top of the plant, so the phytohormones available will be directed to the growth of the stem and cob, which is a modification of the corn plant stem. The increase can also be caused by the cessation of assimilate delivery to male flowers (because male flowers no longer exist) so that the existing assimilate is sent only to the generative parts that need it, namely seeds, and stored in the stems of the corn plant. Based on the research on corn, it is suspected that by pruning the panicles, the stem glucose content in sweet sorghum will increase.

2. METHOD AND MATERIALS

This research was conducted on marginal/arid land in the Padanggalak area, Sanur Village, South Denpasar District, Denpasar City, from September 2021 to January 2022. This study used leading sorghum varieties and doses of organic fertilizer. The design used was a Split Plot Randomized Group Design with two treatment factors arranged factorially. The first factor, 'sorghum variety' (V), consisted of 2 levels, namely: Kawali (V_1) and Super 1 (V_2). The second factor, 'organic fertilizer' (K), consisted of 3 levels, namely: 5 tons/ha (K_1), 10 tons/ha (K_2), and 15 tons/ha (K_3).

The materials used in this study were seeds of sweet sorghum varieties Kawali and Super 1, organic fertilizers, and pesticides. The tools used were tractors/hoes for cultivating the soil, hand sprayers for spraying plants, measuring tapes for measuring planting distance and plant height, wooden planks & bamboos for execution board, plastics, raffia strings, papers for plant samples, refractometer, spectrophotometer, Whatman no. 42 filter papers, glucosecane squeezer, gas chromatography, and labels.

The spacing used was 70 cm x 20 cm, resulting in 5 x 6 = 30 plants per plot. The size of the area per plot was 3.5 m x 1.5 m or 5.25 m^2. Three sample plants were taken from each plot. The distance between plots is 0.5 m, and the distance between each replication is 1 m. Pest and disease control will be carried out if there is an attack. Harvesting is done at the age of 105 days or physiologically ripe. The determination of harvest days is also adjusted accordingly to the harvest age of each variety. The data collected were then analyzed statistically with variance analysis (Anova) using SAS-26 computer software, which was used to compare the mean values of variables. If there is significantly different data, it will be continued with Duncan's Multiple Range Test.

3. RESULTS AND DISCUSSION

In this study, various treatments showed that the variable of stem bioethanol production gave the highest results in the Super 1 variety at 1.03%, which was significantly different from Kawali at 0.43% (Table 1). This was also supported by the dominance of leading sorghum Super 1 over Kawali on various variables: plant height at 77 days after planting at 185.49 cm, stem glucose content at 12.43% brix, and stem juice (sap) production at 539.26 ml/kg. Bioethanol production variable with brix glucose content showed a positive correlation (r = 52%), with plant height (r = 72%) having a solid relationship with bioethanol yield. This is in line with the results of Kumar et al. (2012) and Mangena et al. (2018), where they found a high positive correlation between plant height, brix glucose content, days to flowering, and ethanol production (r = 0.83%). Pabendon et al. (2012) found a moderate positive correlation between brix glucose content and ethanol yield (0.76%). This shows that leading sorghum varieties are indeed able to adapt to widely varying climatic and soil conditions. It can be said that the leading sorghum variety is one of the most efficient crops in converting CO^2 into glucose when compared to glucosecane and corn, making this crop a promising source of bioenergy. This is because the plant contains high glucose content that can produce ethanol through fermentation (FAO, 2002). This is also in line with the results of research by Noura et al. (2020) on Balnda and Chian Woua varieties which found brix glucose yields ranging from 8.9 - 21.8% brix and 11.8 - 22.5% brix and ethanol content of 279.5 - 3101.2 l ha-1 in Chad and the United States. In general, the gene characters of leading and grain sorghums are the same, only distinguished by several genes that control plant height and control of *stay green* leaves so that plants can maintain their stems and leaves remain green despite limited water supply (Borrell et al., 2006) and stem juice (sap) contents (Justice et al., 2018).

The high glucose accumulation in the stem of the Super 1 variety, with a stem glucose content of 12.43% brix, was significantly different from the Kawali variety, which was 9.88% brix. Sorghum that has a high brix glucose content in the stem is classified as sweet (leading) sorghum (Reddy & Sanjana, 2003). The glucose content of sweet sorghum stems ranges from 8.9 - 21.8% brix (Noura et al., 2020). Sweet sorghum is a C4 crop. In high irradiation and hot temperatures, they can photosynthesize faster so as to produce more biomass compared to C3 plants (Salisbury & Ross, 1992). Pabendon et al. (2012) also found that the Super 1 variety, which is directed for bioethanol production, has a glucose content of 10.8 - 14.1% brix, sap volume of 198 - 242 ml/kg stem, plant height of 197 - 232 cm, 50% flowering at 56-60 days, and harvest age of 105-120 days. Stem glucose content is inherited quantitatively. The rate of glucose accumulation in the stem varies between genotypes (Almodares & Sepahi, 1996). To maintain and increase the high glucose content in the stem, panicles are cut when the seeds are physiologically mature (Bitzer, 2009).

The content of stem juice (sap) in the Super 1 variety amounted to 539.26 ml/kg, significantly different from the stem juice production of the Kawali variety, which amounted to 285.09 ml/kg (Table 1). Because of panicle pruning, most of the sweet sorghum (leading) photosynthate is distributed on the stem so that relatively little is distributed to the panicle. The results of Surtinah's research (2005) showed that trimmed male flowers of corn plants gave higher production compared to those that were not trimmed. The increase in yield due to male flower pruning is due to the loss of the top of the plant so that the phytohormones will be directed to the growth of the stem and cob, which is a modification of the corn plant stem. Based on the research on corn plants, it is suspected that the glucose content and juice (sap) production in sorghum stems will increase by pruning panicles on sorghum plants.

The correlation results of the two variables, namely the glucose content in the stem ($^{\%}$brix) with stem juice (sap) production (ml/kg), have a positive correlation (r = 53%) and have a very high and genuine relationship. In contrast, the results of research by Clerget et al. (2008) showed potentially negative interactions between stem development and sweet sorghum seed yield, which is in contrast to grain sorghum accessions where carbohydrate accumulation is for grain, while sweet sorghum accessions are characterized by carbohydrate accumulation in their weak stems.

According to Elangowan et al. (2013), some crucial characteristics in identifying sweet sorghum leading genotypes for bioethanol production are glucose's $^{\%}$brix, sap volume, and high total soluble glucose. The stem juice (sap) percentage varied among varieties, ranging from 51.2 - 80.0%. To optimize ethanol yield, the extraction rate of stem sap is standardized to at least 50% of the total stem weight. Pabendon et al. (2012) produced stem sap from the tested sweet sorghum hope genotypes of 300 - 458 ml of sweet sorghum stems. This is in line with the research of Murray et al. (2008), which showed that the yield of stem sap had a more significant influence than the concentration of glucose content in determining the total glucose yield.

The variable leaf sucrose content obtained in the Kawali variety was 0.93%, significantly different from the leaf sucrose content of the Super 1 variety of 0.48%. In this study, panicle pruning was carried out. Sucrose, as the main product of photosynthesis and the most significant component in storage, is accumulated and distributed as a carbohydrate source. Sucrose that is primarily accumulated in the stem will later be fermented into alcohol/ethanol. This is in accordance with the opinion of Sepahi (1996) and Almodares et al. (1997), who stated that the duration of sucrose accumulation in the stem varies among cultivars. Stem juice (sap) production in sweet sorghum also varies among varieties (Almodares et al., 1994; Naoura et al., 2020).

The treatment of organic fertilizer doses of 10 tons ha^{-1} showed a very significant effect on plant height (169.46 cm), different from 15 tons ha^{-1} (150.25 cm) and 5 tons ha^{-1} (138.33 cm) (Table 2). The interaction between varieties and doses of organic fertilizer (V x K) also showed an interaction of the average plant height of 175.64 cm in the Super 1 variety, significantly different from the average plant height of the Kawali variety, which was 129.72 cm. The results of plant height variables with varieties and doses of organic fertilizer obtained a positive relationship (r = 86%), and this is in line with the research of Naoura et al. (2020), which found a positive relationship between plant height and days to flowering, that taller plants tend to flower later. Nutrients transported by sweet sorghum plants in biomass, especially if stems and leaves are not left on the soil surface, if followed by fertilization with a balanced dose of organic fertilizer, will be able to increase yield (Rego et al., 2003). Proper application can also increase sucrose and the overall growth rate of sweet sorghum (Tsialtas & Maslaris, 2005).

This is in accordance with the research of Suarni (2004), that sweet sorghum has potential as bioethanol biofuel because it has a starch composition of 70-80%. Sweet sorghum starch can be converted into bioethanol through hydrolysis and fermentation processes. Liquification and fermentation are two important processes in the conversion of sorghum into bioethanol. Liquification is the process of converting starch into complex glucose (dextrin), while saccharification converts dextrin into simple glucose (glucose). Berlinda (2011) used *Scheffersomyces stipites* yeast in the fermentation process after hydrolysis of sweet sorghum starch. The selection of yeast in the fermentation process also affects the fermentation results. The low result of bioethanol content in this study can be caused by the occurrence or still an increase in pH due to fermentation which not only produces ethanol, but also produces other compounds such as acetic acid, levulinic acid and formic acid. Acetic acid can be produced by bacterial contaminants that live with yeast, namely acetobacter. Lactobacillus can also contaminate and convert glucose into lactic acid so that it will reduce the yield of ethanol content and inhibit yeast growth (Samsuri et al. 2007). Based on the results of this study, polysaccharides are broken down into monosaccharides or simple glucoses so that yeast will more easily ferment into bioethanol. The monosaccharides formed will be converted by yeast into alcohol and carbon dioxide (CO2). In general, the reaction equation can be written as follows:

$$3\ C_5H_{10}O_5 \longrightarrow 5\ C_2H_5OH + 5\ CO_2$$

Saccharides *yeast* ethanol gas

The bioethanol yield obtained in this study was supported by plant height. Plant height was significantly correlated with stem biomass weight. The correlation coefficient value is also high between the weight of stem biomass with ethanol yield per unit area of 0.98 (Pabendon et al., 2012). This is in line with the research of Murray et al. (2008), which showed that the yield of stem juice (sap) had a more significant influence than the concentration of glucose content in determining the total glucose yield.

In the treatment of organic fertilizer doses, almost all the observed variables have not shown a significantly different effect (except for the variables of plant height, stem diameter, and %brix glucose content) (Table 1). This may be because organic matter needs time to decompose and release the nutrients it contains to be available to plants. Some studies show that it takes four to five years or even more to show the effect of their interaction in increasing crop yields (Agung et al., 2013). Another possibility is that the added organic fertilizer has not sufficiently increased the soil's organic matter and C-organic content.

Research on the study and analysis of glucose content and bioethanol in several sorghum plants can be said to have provided new research on the leading variety of sweet sorghum Super 1 with the results of the stem glucose content of 12.43% brix and stem bioethanol yield of 1.03%. Meanwhile, by giving a dose of organic fertilizer of 10 tons h^{-1}, the yield of stem glucose content increased by 11.55% brix in sweet sorghum stems as a result of panicle pruning treatment and organic fertilizer application. Thus, sorghum varieties with high stem glucose contents can be considered raw materials for bioethanol.

4. CONCLUSION

Sweet sorghum leading variety Super 1 gave the highest glucose content production of 12.43% brix and bioethanol content of 1.03% compared to the Kawali variety. Organic fertilization treatment at a dose of 10 tons h^{-1} can increase the glucose content of sorghum stems.

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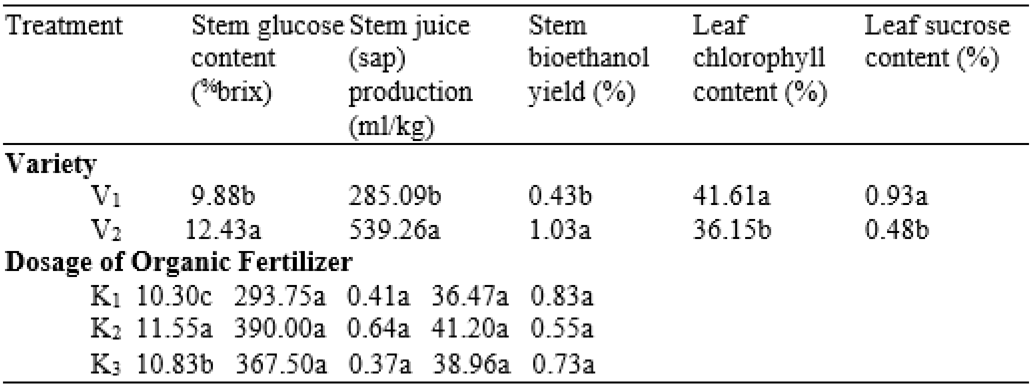
APPENDICES – A

Table 1: Effect of variety treatment and organic fertilizer dose on stem glucose content, stem juice (sap) production, bioethanol yield, leaf chlorophyll content, and leaf sucrose content



Treatment	Stem glucose content (%brix)	Stem juice (sap) production (ml/kg)	Stem bioethanol yield (%)	Leaf chlorophyll content (%)	Leaf sucrose content (%)
Variety					
V_1	9.88b	285.09b	0.43b	41.61a	0.93a
V_2	12.43a	539.26a	1.03a	36.15b	0.48b
Dosage of Organic Fertilizer					
K_1	10.30c	293.75a	0.41a	36.47a	0.83a
K_2	11.55a	390.00a	0.64a	41.20a	0.55a
K_3	10.83b	367.50a	0.37a	38.96a	0.73a

Note: numbers followed by the same letter in each factor are not significantly different at Duncan's 5% Multiple Range Test.

Table 2: Duncan's 5% Multiple Range Test on the interaction of varieties (V) and doses of organic fertilizer (K) on plant height at 77 days after planting

Variety / Fertilizer Dose	V_1	V_2	Mean
K_1	127.33a	149.33c	138.33b
K_2	133.42a	205.50a	169.46a
K_3	128.42a	172.08b	150.25b
Mean	129.72	175.64	
LSR 5% V	**13,83**		
LSR 5% K	**16,94**	**17.88**	