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Study of interaction between genetic source, harvest time and storage time in some mutant varieties of sweet sorghum to support future bioindustry development

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Abstract. This research aimed to find out interaction of genetic source×harvest time×storage time in 10 mutant varieties of sweet sorghum to support sorghum bioindustry. The study was carried arranged in split-split plot design with 3 replications. Main plot was variety which consists of 10 mutant varieties with 2 control varieties (Numbu and Super 1). Whereas subplot was harvest time consisted of 3 levels (75, 90 and 105 days after planting/DAP). Sub-subplot was storage times (0, 4, 8 days after harvest/DAH). Mutant varieties influenced juice volume and sugar yield. Harvest time influenced brix content and juice volume. Storage time, interaction of variety×harvest time, variety×storage time, and harvest time×storage time had significant effects on all observed traits. Interaction between variety×harvest time×storage time) affect the brix content. High brix content can be gained at harvest time of 90 DAP and with storage time of stalk at 4 DAH. There were 4 mutant varieties of sweet sorghum with high brix content, namely GMS-1 (23,05%), GMS-3 (22,88%), GMS-8 (22,75%), GMS-2 (22,62%) and GMS-6 (22,08%). Mutant variety of GMS-5 had the highest juice volume in all harvest times at 0 DAP. The mutant variety GMS-9 had stable sugar yield in all three harvest and storage times.

1. Introduction

Currently, main raw material for world sugar production processed and derived from sugar cane plant. Sugar cane advantages in the context of sugar raw material provision are that it has simpler processes and lower energy consumption, which can reduce production cost at 20-60% [1]. However, sugar cane plants have relatively high cultivation requirements, such as higher demand for water irrigation, requires suitable temperature, and long planting season (9 to 12 months). These constraints resulted in limited planting areas that are suitable for production to be optimum [2]. Sweet stalk sorghum (*Sorghum bicolor* (L) Moench), poses as analogue to sugar cane with high accumulation of sucrose, but has more adaptability in various environments and agronomical stability to temperature fluctuation. It also has lower water requirement, early harvest time (3 to 5 month) and higher tolerant to salinity, alkalinity and aridity [3]. Furthermore, sorghum is multifunctional crop that has the potential to become alternative source of food and feed at remote areas [4].



Sweet sorghum has large potential in fulfilling the sugar industry needs to become as an alternative in future development of sugar and energy diversification [5]. The reasons to utilize sorghum as raw material for sugar industry are its raw material availability, continuity, quality and readiness in sugar making technology to be mass produce. However, lack of excellent genetic source, lack of information in agronomical principles and technology in determining proper harvest and storage time become obstacles in developing sorghum as raw material for sugar industry.

The studies on single effect of genetic source to sorghum liquid sugar production have been conducted. Diverse genetic materials have different potency in juice producing [6,7,8]. The brix content in sweet sorghum is controlled by recessive genes expression at locus D, and mutation is a method that frequently used to acquire recessive genes in plants. The research on harvest time in sorghum showed that the best harvest time to obtain high content of sugar from sorghum is started when panicles begin to sprout, and the sugar content in sorghum is rapidly increased after flowering [9,10]. The study on sorghum stalk storage time is still finite. This study aims to get on the interaction between genetic materials, harvest time and storage time in mutant varieties of sorghum.

2. Materials and methods

The research was conducted at Maros experimental farm, Indonesian Cereals Research Institute (ICERI), from May to September 2017. The study was arranged in split-split plot RCBD design with 3 replications. The main plot was variety consist of 10 genetic materials of sweet sorghum mutants and 2 control varieties (Numbu and Super 1). The subplot was harvest times (75 DAP, 90 DAP and 105 DAP) and sub-subplot was stalk storage times after harvesting (0 DAH, 4 DAH and 8 DAH). Land plowing was performed to make loose soil, and then the plots were made in square measured at 5 m x 4 m, where each mutant variety of sweet sorghum was planted in 4 rows/plot with 20 cm plant spacing in row and 70 cm between rows, to make a total of 100 plants/plot. The planting process was performed by using dibber, 2 to 3 seeds in each plant hole. To avoid pest attack, the seeds were treated with Karbaril insecticide and added with carbofuran in planting hole. The first fertilization was performed when the plants reached 10 to 15 DAP with dosage of 150 kg/ha urea, 100 kg/ha SP36 and 75 kg/ha KCl. The second fertilization was done when they reached 30 to 35 DAPs with dosage of 150 kg/ha urea. Plant cares include water sprinkling, weeding, straightening the plant and covering the panicles. Pest and pathogen controlling were performed when the plants were infected by pests and illnesses.

The research was focused to find out the interaction between variety, harvest time and storage time, so that the first treatment was performed when plant's age reached 75 DAPs, by harvesting 45 samples of sorghum stalks. Furthermore, five stalk samples were directly squeezed, 5 other stalk samples were squeezed after had been storage for 4 days, and 5 next stalk samples were squeezed after had been storage for 8 days. Treatment II was performed when plants were 90 DAP by harvesting 45 samples of sorghum stalk, then 5 stalk samples were directly squeezed, 5 stalks were squeezed after it had been stored for 4 days, 5 stalk samples were squeezed after it had been stored for 8 days. Treatment III was done when plants were at 105 DAP (the last harvesting/physiological mature) by harvesting 45 samples of sorghum stalks. Furthermore 5 stalk samples were directly squeezed, 5 stalk samples were squeezed after it had been stored for 4 days, and 5 stalk samples were squeezed after it had been stored for 8 days with 3 replications for all treatments. The observed variables were brix sugar content measured by using refractometer (%), juice volume (ml) and sugar yield (%).

Collected data were then tabulated and analyzed by using anova based on split plot design by means of SAS Portable 9.1 software. If the result of anova showed a significant/highly significant influence, then it was further analyzed by an advanced test, the least significant difference test (LSD 0.05).

3. Results and discussions

Based on variance analysis results (**Table 1**), it is showed that the mutant varieties of sweet sorghum had no significant effect on the trait of brix sugar content in the stalk, but it was highly significant toward trait of juice volume and sugar yield. Harvest and storage time treatments had highly significant effect on the percentage of brix sugar content, juice volume and sugar yield. A strong significant interaction was observed between variety x harvest time in the percentage of brix sugar content and juice volume, while there was no significant interaction in sugar yield trait. Furthermore, the interaction was also highly significant between variety x storage time, harvest time x storage time in all of the three traits observed. Whereas, interaction of all three treatments, variety x harvest time x storage time was found to be highly significant only in the percentage of brix sugar content, and there was no significant effect on juice volume and sugar yield.

The mutant varieties tested appeared to have no significant effect on brix sugar content trait. It means variation occurred in juice volume, which could be due to different biomass within varieties. These differences will eventually lead to variation in sugar yield that was obtained in correlation to the amount of juice produced. Some of research stated that the brix sugar content is influenced by genetic factor determined by recessive genes that codes for leaf vein characters at locus D, and the mutation mostly influences the leaf vein color [11]. Sorghum traits such as juice volume and brix sugar content are highly influenced by genetic and environment factors which in this research were harvest time and storage time. A study by Dewi [12] showed that variation in harvest time did not influence total content of dissolved solid and viscosity of sorghum juice, while significant difference of density was observed in the plant at age 114 days. The effect of sorghum variety on nutrition content and its concentration is not significantly different [13,14]. However, there was an effect of interaction of variety on juice content of sorghum stalk (ml), level of juice sweetness (brix), and juice sugar content of stalk (%) [15]. Information of optimum harvest and storage time becomes crucial in improving brix value of sorghum plant, because these traits were more dominant rather than genetic factor of varieties.

Table 1. Analysis of 10 sweet sorghum mutant lines and 2 control varieties at different harvest time and stalk storage time before juice extraction.

| Source of variation | Mean Square | | |
|------------------------------------|----------------------|------------------------|----------------------|
| | Brix Sugar Content | Juice Volume | Sugar Yield |
| Variety | 12.01 ^{ns} | 12562.64 ^{**} | 372.20 ^{**} |
| Harvest time (days) | 826.35 ^{**} | 72467.30 ^{**} | 266.03 [*] |
| Variety×harvest time | 9.61 ^{**} | 2344.72 ^{**} | 96.28 ^{ns} |
| Storage time (days) | 194.90 ^{**} | 75933.86 ^{**} | 657.38 ^{**} |
| Variety×storage time | 6.21 ^{**} | 4551.88 ^{**} | 124.11 ^{**} |
| Harvest time×storage time | 98.09 ^{**} | 15167.90 ^{**} | 420.42 ^{**} |
| Variety×harvest time ×storage time | 6.34 ^{**} | 1251.52 ^{ns} | 43.77 ^{ns} |
| Coefficient of variability (%) | 9.99 | 18.32 | 21.89 |

^{ns} Not significant; ^{*} Significant at $p < 0.05$; ^{**} Significant at $p < 0.01$

The rate of brix sugar accumulates highest when it was harvested at 90 DAP, which then decreased at maturity (**Figure 1**). This means that the optimum time to obtain highest brix sugar was not at seed physiological maturity. Average value of brix at 90 DAP and stored for 4 days was 17.2 %, which is considered high for sweet sorghum and almost similar to brix yield in sugar cane. Compared to 90 DAP at 4 and 8 days, brix value at 105 DAP was slightly decreasing in value. Harvesting at 105 DAP regardless storage days appeared to had similar values.

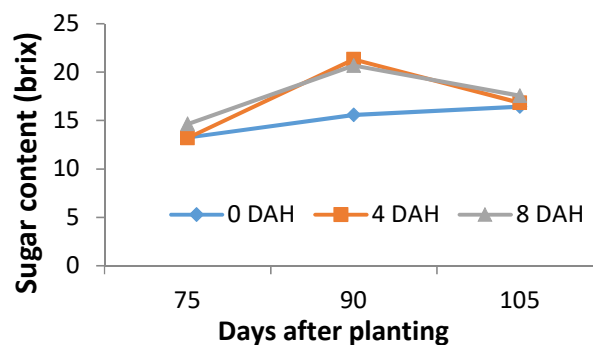


Figure 1. The effect of harvest and storage time on brix sugar content

The highest volume of stalk juice was obtained when it was collected after flowering stage (75 DAP), on the average at 195,8 ml/kg (**Figure 2**). The juice content continued to decreased 27 to 34% when approaching physiological maturity phase (95 DAP) ranged at 128-136 ml/kg until the plants were physiologically matured (105 DAP) (**Figure 2**). The effect of harvest storage on the juice content caused significant decreased of juice content in stalk. Percentage decrease in juice content due to storage for 4 to 8 days after harvesting at 75 DAP was 18 to 19%, and it continued to decrease to 35-44% when it was harvested at 95 DAP. The highest decrease was at 45-47% due to storage was when it was harvested at physiological mature stage (105 DAP).

The juice volume was reduced with longer harvesting time. It was due to increment in sugar formation while water content was reduced. The juice volume appeared to be decreasing at physiological maturity phase because the stalk begins to harden, because the old stalk lacks their capacity to hold water and evaporation occurred. The reduction of juice volume when approaching physiological mature was also accompanied by the increment of brix sugar content.

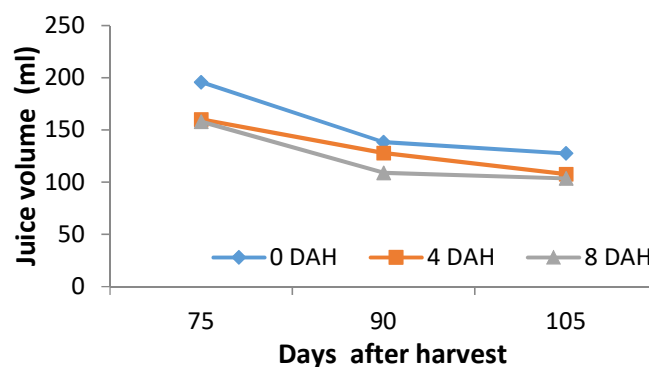


Figure 2. The effect of harvest and storage time on stalk juice volume

The amount of sugar yield resulted was related to the quantity of juice yield and percentage of juice brix sugar. On the average, high sugar yield was obtained after flowering phase (75 DAP) and at physiological mature phase (105 DAP) (**Figure 3**). The quantity of sugar yield when harvested at 75 DAP was due to high quantity of juice, despite of lower sugar content (**Figure 2**). On the other hand, at physiological mature phase, the brix sugar content was increased while the juice quantity was decreased. The effect of storage was significant when harvested at 75 DAP, with the reduction of sugar yield at 17 to 30% when stored for 4 to 8 days. However, if harvesting was conducted at physiological maturity phase, shelf life can still be tolerated until 4 days. It can be explained that when the plants reached 75 DAP, they will direct the photosynthate product storage mostly to form panicles

and seeds, which make the storage in stalk will not be maximal. It is also due to high water content in young stalk which are consisted of young parenchyma tissues.

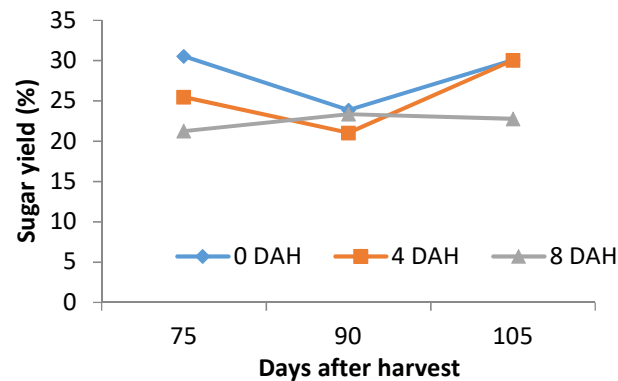


Figure 3. The effect of harvest and storage time on sugar yield

3.1. The contribution of the varieties to the brix sugar content during harvest time and storage time.

Generally, all varieties of sweet sorghum encountered reduction of brix sugar percentage of the juice when harvest is stored for 4 to 8 days. The Numbu variety consistently had the highest brix sugar content when harvested at 75 to 105 DAP, with 4 to 8 days at storage (**Figure 4**). A study by Satheesh K., *et al.* (2018) [16] showed that quantity of non-reducing sugar (such as sucrose) continued to increase from flag leaf stage, and it peaked at hard dough stage, and further continued to decrease until after physiological mature stage. The highest amount of reducing sugar was obtained when the plants were harvested at hard dough stage that was at 90 to 100 DAP.

The research on sugar cane plants by Septyan *et al.* [17] demonstrated that storage time influenced total sugar and brix of sugar cane, where the longer storage the less total sugar and more brix content obtained. A study conducted by Destianty *et al.* [18] showed that the storage of sugar cane juice at room temperature could significantly reduce juice brix content. The cane sugar content could be reduced and had damaged with milling delay for 3 days at storage [19,20]. In sweet sorghum, harvesting time at morning, noon and afternoon influenced juice and brix sugar content, and the best result was obtained at morning harvesting. Planting management and plant variety choices are crucial to gain results of stalk and juice of sweet sorghum [21]. The best time for harvesting storage duration became crucial to produce sorghum sugar with best quality and quantities. However, there are always deviations between the literature studies and the actual results at field [22], so that, it requires a dynamic and synergistic field study and research.

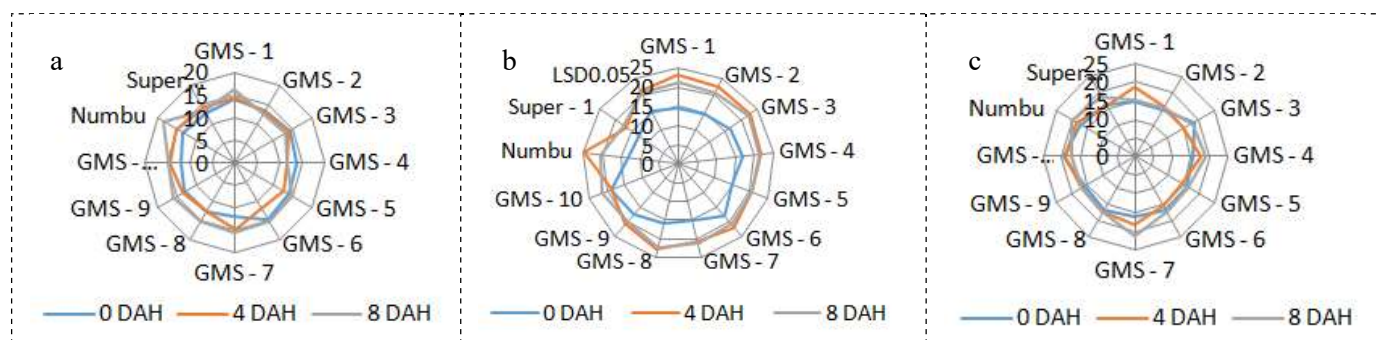


Figure 4. The contribution of sweet sorghum variety on the percentage of brix sugar at harvest time 75 DAP (a), 90 DAP (b) and 105 DAP (c) and harvest storage.

Results from this study demonstrated that harvest time at 75 DAP and 105 DAP, all varieties did not undergo significant reduction in brix sugar content during storage. And the best storage time when harvested at 90 DAP was 4 DAH. The varieties with slightly reduced brix content at harvest time 75 DAP were GSM 1, 2, 3 and 5, whereas at the harvest time 90 DAP, the varieties with slightly improved brix sugar were GSM-9 and 10. Other results were that the variety GM-5 had a quite high content of juice, when harvested at 75 to 105 DAP. And variety GM-10 had a relatively high content of juice when harvested at 105 DAP. At harvesting time 90 DAP, there were an increase of brix value during storage for 4 to 8 days observed in all varieties. The reason was due to raining with a quite high intensity occurred for few days before harvesting, and this led to high water content in stem and reduce the brix sugar content.

3.2. The contribution of the varieties to juice volume during harvest time and storage time.

The juice volume of stalk was reduced during storage. The most reduction was observed when it harvested at 105 DAP. The varieties GSM-2, GSM-4 and GSM-5 had significant reduction of juice volume in all harvesting time (Figure 5). While for GSM-8 the reduction was observed only when harvested at 105 DAP. Some varieties were moderately stable with less reduction of juice volume, they were varieties GSM-6, GSM-7, GSM-9 and GSM-10. The juice volume is related to plant capability to keep water and it is highly influenced by plant stem tissue condition. The juice volume was also highly influenced by environmental factors at planting, harvesting, and storage. The stalk storage condition that is quite dried and with enough heat will speed up stalk drying, therefore it can significantly reduce juice volume and vice versa.

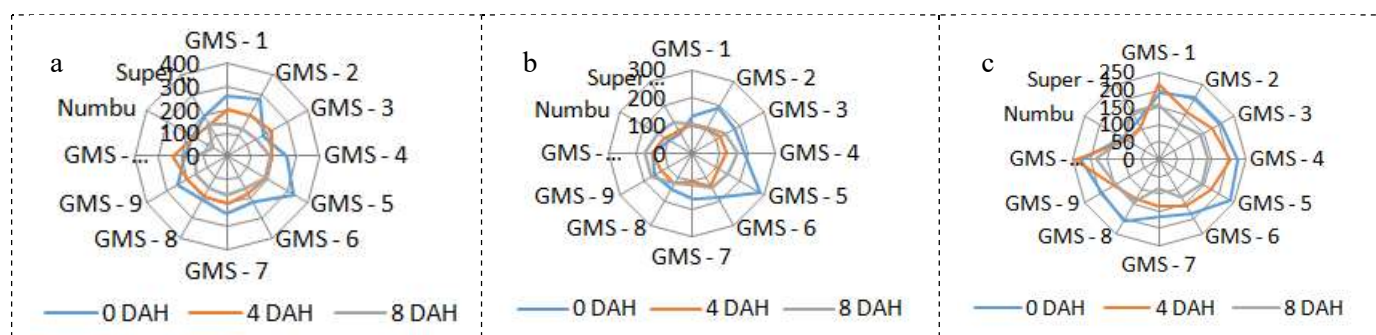


Figure 5. The contribution of sweet sorghum variety on juice volume /kg at harvest time 75 DAP (a), 90 DAP (b) and 105 DAP (c) and harvest storage duration.

3.3. The contribution of varieties to sugar yield during harvest time and storage time.

Sugar yield is a crucial character in producing sugar from sorghum. Varieties with high sugar yield become a priority in sugar syrup industry [23]. Varieties with observed high sugar yield were GMS-1, GMS-2, GMS-5, GMS-8 and GMS-10 (**Figure 6**). Varieties with stable sugar yield on all harvest time were GMS-5 and GMS-9. Variety with stable results on all harvest and storage time was GMS-9. Furthermore, variety GMS-5 had quite high yield of sugar when harvested at 75 to 105 DAP, whereas variety GMS-10 had a relative high yield of sugar when harvested at 105 DAP. The variety GMS-9 had a stable sugar yield at 3 harvest times and 3 storage times.

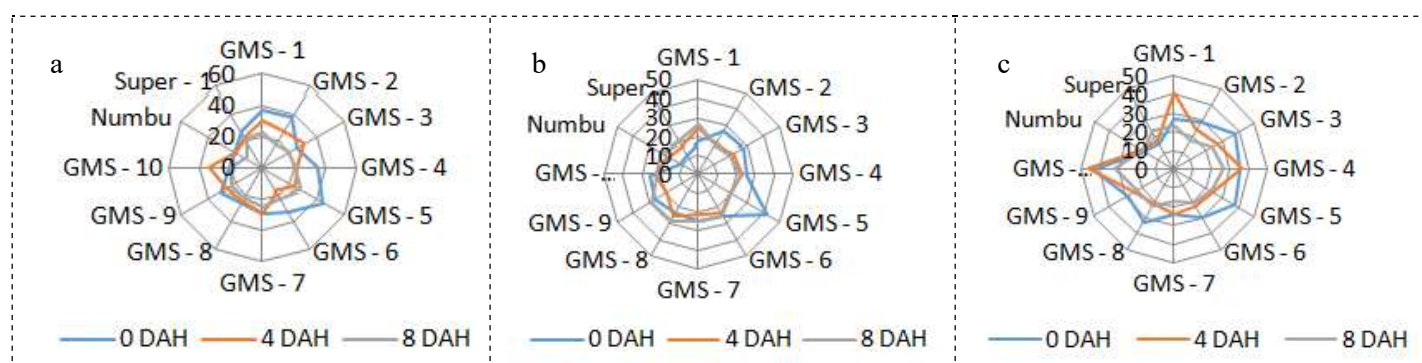


Figure 6. The contribution of sweet sorghum variety on Sugar Yield at harvest time 75 DAP (a), 90 DAP (b) and 105 Dap (c) and harvest storage duration.

The correlation value between all treatments and observed traits indicates whether the relation between treatments and observed traits are significant or not (**Table 2**). The trait of sugar yield was negatively correlated to variety and storage time. While trait of brix sugar content was positively correlated to harvest time and storage time. This study also found that trait of juice volume was negatively correlated to varieties, harvest time, storage time and sugar brix, but positively correlated to sugar yield. Improvement of varieties based on correlation results is by indirect selection of traits that positively correlated to sugar yield. Thus, juice volume was one significant trait that could be use as selection parameter to determine potential varieties suitable for sugar production.

Table 2. Correlation coefficient values between treatments and observed traits.

| Traits | | Variety | Harvest Time | Storage Time | Sugar Yield | Brix Sugar Content |
|--------------------|-----|----------|----------------------|--------------|-------------|--------------------|
| Sugar yield | R | -0.2225 | 0.0205 | -0.2512 | | |
| | Sig | 0.0001** | 0.7138 ^{ns} | 0** | | |
| Brix sugar content | R | -0.0086 | 0.4148 | 0.2923 | 0.0508 | |
| | Sig | 0.8769 | 0** | 0** | 0.3621 | |
| Juice volume | R | -0.1819 | -0.2219 | -0.3725 | 0.7484 | -0.445 |
| | Sig | 0.001** | 0.0001** | 0** | 0** | 0** |

^{ns} Not significant; * Significant at $p < 0.05$; ** Significant at $p < 0.01$

4. Conclusions

Based on the result of research, it is concluded as follows are the interaction between varieties, harvest time and storage time had a significant influence to the sugar brix content of juice. There was interaction between harvest time and storage time that influenced sugar brix of juice, juice volume and

sugar yield. The highest brix value was obtained at harvest time 90 DAP and storage time 4 to 8 DAH. The highest juice volume was obtained at harvest time 75 DAP with storage 0 DAH, while the highest sugar yield was obtained when harvested at 75 DAP with storage time 0 DAH, and when harvested at 105 DAP with storage time 0 and 4 DAH. The variety GM – 5 had a quite high percentage of sugar brix when harvested at 75 – 105 DAP with storage time 4 DAH and with a relative high juice volume. The sugar yield was negatively correlated to varieties and storage time, and positively correlated to juice volume. The varieties with the highest sugar yield were GMS-3, GMS-5 and GMS-8 with the best harvest time at 75 and 105 DAP and storage time 0 DAH.

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