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Short Communication

EFFECTS OF PHOSPHATE-SOLUBILIZING BACTERIA FROM PROBIOTIC AND PHOSPHORUS FERTILIZATION ON JUICE CHARACTERISTICS OF BROWN MIDRIB SORGHUM (*Sorghum bicolor* L. MOENCH)

Riesi Sriagtula[1*], Mardhiyetti[1], Gusri Yanti[2], and James Hellyward[1]

[1]Faculty of Animal Science, Andalas University, Indonesia, West Sumatra, Padang 25163.

[2]Faculty of Science, Social and Education, Prima Nusantara University, Bukittinggi 26122, West Sumatra, Indonesia

ARTICLE HIGLIGHTS

- *Bacillus* sp. probiotics can replace 100% inorganic phosphate fertilizer.
- Sorghum stem diameter strongly correlates with juice volume.
- Juice yield was not affected by phosphorus dose or *Bacillus* sp. colony size.
- Sorghum juice sugar content reached 11.77–12.75% Brix.
- Phosphate-solubilizing bacteria improved phosphorus availability in ultisol.

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***Corresponding author, e-mail:**
riesisriagtula@ansci.unand.ac.id

ABSTRACT

This study was conducted to observe the effects of phosphate-solubilizing bacteria (PSB) combined with different phosphorus fertilizer levels on the juice characteristics of the BMR sorghum mutant line. The research was carried out at the Edufarm of the Faculty of Animal Science, Andalas University, Padang, using a randomized block design (RBD) with four treatments and four replications. The treatments were as follows: P0 = Urea + KCl + 0% TSP + PSB; P1 = Urea + KCl + 50% TSP + PSB; P2 = Urea + KCl + 75% TSP + PSB; and P3 = Urea + KCl + 100% TSP (control). The PSB used was obtained from Waretha Probiotics, consisting of *Bacillus* sp. culture at a dose of 10 mL per planting hole, equivalent to 10^7 cfu/g. The parameters measured were sugar content (%Brix), volume of stem juice (mL), and the correlation of various parameters with stem juice volume. The results showed that the application of probiotic *Bacillus* sp. and different dosages of phosphorus fertilizer did not result in significant differences for all parameters ($P > 0.05$). Sugar content and juice volume ranged from 11.78 to 12.75 (%Brix) and 188.75 to 218.5 (mL/stem), respectively. The correlation coefficient analysis indicated significant positive correlations between stem diameter ($r = 0.575^*$) and fresh stem weight ($r = 0.504^*$) with the volume of sorghum stem juice. It was concluded that the application of PSB from *Bacillus* sp. can replace phosphorus fertilizer, producing sugar content and juice yield comparable to the 100% P fertilizer treatment. A correlation was observed between stem diameter and fresh stem weight with stem juice volume. PSB was found to play a crucial role in increasing phosphate availability to plants. The implications of this research include the potential production of forage sorghum with high Brix content for ruminant energy and Water-Soluble Carbohydrates (WSC) for silage production.

Keywords: *Bacillus* sp., brown midrib (BMR), fertilizer, phosphate-solubilizing bacteria, sorghum

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is considered to have potential for development as a commodity to increase the productivity of marginal land, as it is characterized by wide adaptability and low input requirements for growth (Sriagtula *et al.* 2019). A mixture of sorghum harvested at the mid-dough stage with concentrate has been reported to be suitable for use in dairy heifers at a ratio of 65:35 (Kljak *et al.* 2016). Sorghum plants are recognized to have advantages over cereal crops in general, including high production potential and the ability to be ratooned, thereby increasing efficiency in seed and tillage costs. Breeding programs for sorghum have been conducted to produce varieties suitable for forage, resulting in the development of brown midrib (BMR) mutant sorghum strains.

BMR sorghum is characterized by a lower lignin content than non-BMR sorghum, allowing it to be potentially developed as forage for ruminants. As an industrial raw material, BMR sorghum is more easily fermented for bioethanol production. Its nutritional composition has been reported to contain 8.10% crude protein (CP), 20.01% crude fiber (CF), 3.31% crude fat (EE), and 4.91% ash (Sriagtula *et al.* 2019). BMR sorghum is classified as a sweet sorghum due to its high stem sugar content, which ranges from 10% to 15% (Siddique *et al.* 2018). The sugar content in sorghum stalks has been found to be positively correlated with ethanol yield, and a correlation has also been observed between sugar concentration (sucrose) in Brix units and total sugar content. The water requirement of sorghum has been reported to be lower than that of maize, enabling intensive cultivation on dry lands (Roby *et al.* 2017).

Drylands are characterized by low water availability, low fertility, and low productivity. In Indonesia, drylands cover 144.47 million ha, 82% of which are classified as suboptimal. Acidic dryland has been identified as one of the most dominant suboptimal dryland types, with an area of 107.36 million ha (74.3% of the total dryland area) and the widest distribution found on the islands of Sumatra, Kalimantan, Java, and Papua (Rachman *et al.* 2021). The main limiting factor of acidic drylands is a soil pH below 5.5. Ultisol soil has been described as acidic soil with very low phosphorus (P) availability due to fixation by Al and Fe (Hasanudin *et al.* 2021). Phosphate has been recognized as a limiting factor for plant growth and yield, and one approach to increasing phosphate fertilization efficiency in overcoming low soil phosphate availability is through the utilization of soil microorganism groups.

Soil organisms are recognized to play a role in recycling nutrients within the soil, making them available to plants and increasing crop yields (Rajper *et al.* 2016). Microbial-based inoculants have been widely applied to enhance soil microbial activity. Through the addition of microbial-based inoculants, nutrient supply is increased, thereby improving crop productivity and maintaining soil fertility. Probiotic bacteria, which are known for their use as feed additives, have also been applied as providers of phosphate-solubilizing bacteria (PSB) to increase crop production (Menendez & Fraile 2017). Probiotics have been shown to increase the solubility of phosphorus in soils and phosphate fertilizers, thereby increasing the availability of P to plants. The probiotic bacterial strain *Bacillus* sp. has been identified as one of the soil microbes functioning as PSB (Prakash & Arora 2019). The application of probiotics in agriculture has been reported to improve plant growth and yield by enhancing photosynthesis, producing bioactive substances such as plant growth regulators and enzymes, stimulating the decomposition of organic matter, and releasing inorganic nutrients that can be absorbed by plants. Previous research has indicated that the addition of microbial-based inoculants is more efficient and cost-effective because it reduces the use of chemical fertilizers and pesticides (Rajper *et al.* 2016). Furthermore, microbial-based inoculant technology has been reported to result in plant growth equal to or superior to that achieved through conventional agricultural practices (Shahwar *et al.* 2023).

Numerous studies have examined the effect of phosphorus on sugar content. Phosphorus has been reported to be required for increasing sugar content in sorghum stems (Ali & Anjum 2017; Maw *et al.* 2016). In contrast, other studies have concluded that phosphate fertilization does not influence the sugar concentration in the juice (Brix) of sugarcane (Caione *et al.* 2015). An increase in sucrose content in sugarcane plants was observed by Aye *et al.* (2021) with the addition of phosphate-solubilizing bacteria (PSB). However, the application of probiotics as PSB in agriculture remains limited; therefore, this study was conducted to examine the effect of PSB derived from probiotics and the reduction of inorganic phosphorus fertilizer dosage on the stem juice characteristics of BMR mutant sorghum grown in ultisol soil.

MATERIALS AND METHODS

The tools used in this research included a plow machine, sprayer, scales, and hand refractometer. The materials used comprised BMR sorghum mutant line BIOTROP seeds obtained from the Silviculture Laboratory of SEAMEO-BIOTROP, Bogor; manure; urea; TSP; KCl; and *Bacillus* sp. culture from Waretha Probiotic (a probiotic produced by Universitas Andalas, Indonesia). The research was conducted at the Edufarm of the Faculty of Animal Science, Universitas Andalas, Padang, Indonesia (-0.912535, 100.467847), using a Randomized Block Design (RBD) consisting of four treatments and four replications. The treatments were as follows:

P0 = Urea + KCl + 0% TSP + Probiotic PSB;

P1 = Urea + KCl + 50% TSP + Probiotic PSB;

P2 = Urea + KCl + 75% TSP + Probiotic PSB;

P3 = Urea + KCl + 100% TSP (control).

Land Preparation

Each experimental plot measured 4 × 5 m. The results of soil analysis prior to the study indicated a pH of 4.5 (acidic) and a low available phosphorus content of 9.61 ppm. Rainfall during the study period ranged from 226 mm to 492 mm. The field planting design is presented in Figure 1.

Probiotic Preparation

The *Bacillus* sp. strain from Waretha Probiotic was cultivated on nutrient broth (NB) medium, producing a bacterial culture population density of 10^7 cfu/g. The bacterial count was determined using the Total Plate Count Method (Cundell 2015). A total of 10 g of Waretha Probiotic was dissolved in 90 mL of sterile distilled water. Special media for *Bacillus* sp. were prepared in 100 mL volumes and heated to boiling before being used as agar media for the bacterial culture procedure, which was conducted using the surface method. A 1 mL aliquot from the 10^7 dilution was pipetted onto the agar surface in a sterile Petri dish and spread evenly after the medium had cooled to 47 - 50 °C. The plates were then incubated at 36 °C for 24 hours.

Planting Seeds and Fertilizing

The fertilizers used were urea, TSP, and KCl at a ratio of 2:3:2 (v/v), corresponding to application rates of 60 kg/ha, 90 kg/ha, and 60 kg/ha, respectively (Sriagtula *et al.* 2016; Wahyono *et al.* 2019). The first fertilizer application was conducted at 14 days after sowing (DAS) with two-thirds of the total dosage, while the remaining one-third was applied at 40 DAS. Manure was applied at a rate of 10 tonnes/ha (Sriagtula *et al.* 2016).

The probiotic PSB from Waretha Probiotics, with a density of 10^7 cfu/g, was applied at a dose of 10 mL per planting hole. The application was performed twice, first at 14 days after sowing (DAS) and again at 40 DAS. Sorghum was harvested at the soft dough stage (93 DAS) by cutting the stems 10 cm above the soil surface.

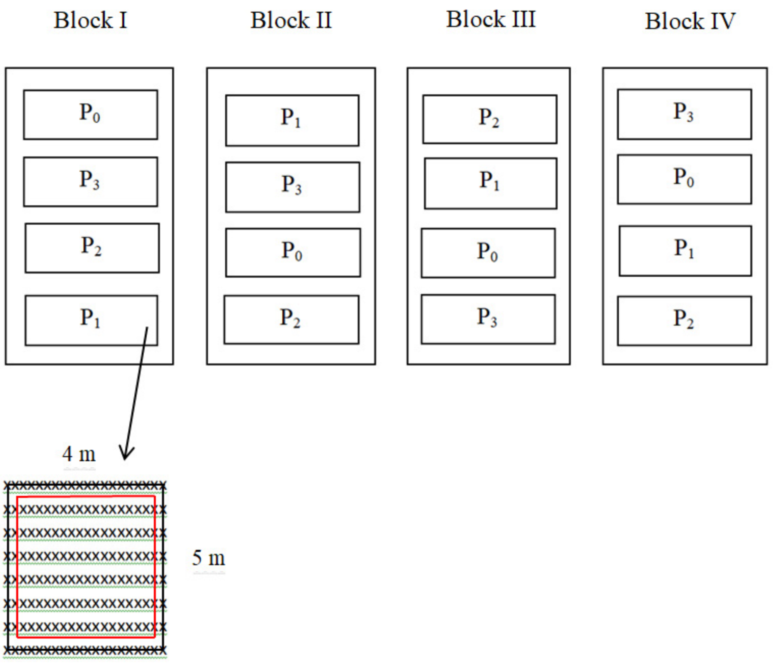

Figure 1 Field planting design

Estimation of Soil *Bacillus* sp. Population

One gram of soil sample was suspended in 9 mL of distilled water in a test tube and vortexed to obtain a 10^{-1} dilution. Subsequently, 1 mL of this suspension was transferred into a new test tube containing 9 mL of distilled water to prepare serial dilutions up to 10^{-8}. One milliliter of the appropriate dilution was then pipetted into a Petri dish, and 7 - 10 mL of *Bacillus* selective media was added. The mixture was homogenized by gently rotating the dish in a figure-eight pattern and then incubated at 37 °C for 24 hours. Observations were conducted on the second day after planting, and bacterial colonies were counted. Colony counts were expressed as colony-forming units (cfu) per milliliter. The plate counting method was employed for enumeration (Cundell 2015).

Phosphate-Solubilizing Bacteria Assay

Approximately 1 mL of each serial dilution (10^{-1}, 10^{-2}, and 10^{-8}cfu/g soil) was inoculated onto Pikovskaya medium. The plates were incubated at room temperature for 3 to 5 days. Phosphate-solubilizing bacteria (PSB) were identified by the formation of clear zones around colonies, indicating their ability to solubilize phosphorus on the Pikovskaya medium.

Observations and Measurements

Observations were conducted on 10 plants randomly selected from each plot. The parameters measured included stem height, which was recorded after harvest using a measuring tape. Stem sugar content (%Brix) was determined from juice extracted from the lower stem using a refractometer by placing stem juice droplets on the instrument. Juice volume was measured by pressing sweet sorghum stems with a sugarcane squeezer, and the extracted juice was collected and quantified using a measuring cup. Correlation coefficients between various parameters and the brown midrib mutant sorghum stem juice volume were analyzed.

Data Analysis

The data were analyzed using analysis of variance (ANOVA) in SPSS version 20. Multiple linear regression analysis was employed to evaluate the parameters influencing stem juice yield. Additionally, linear regression and Pearson correlation analyses were conducted to determine the relationships between parameters.

RESULTS AND DISCUSSION

Sugar Content, Juice Volume, Stem Height and Total Colony

The average sugar content (%Brix) of BMR mutant sorghum stem juice following the application of probiotic *Bacillus* sp. and varying doses of phosphorus fertilizer is presented in Table 1. Statistical analysis showed that the treatments did not have a significant effect ($P > 0.05$) on the sugar content of BMR mutant sorghum stem juice. This result is consistent with Kansaye *et al.* (2023), who reported minimal variation in sugar concentration under high or low phosphorus fertilization. In contrast, Oliveira *et al.* (2022) found that available phosphorus strongly influenced the sucrose percentage in sugarcane juice. Additionally, Ariefin *et al.* (2021) noted that sorghum variety significantly affects stem sugar content and juice volume. Since this study utilized the same sorghum line, i.e., BMR sorghum from BIOTROP, the Brix level and juice volume showed no significant differences.

The average sugar content in this study ranged from 11.77% to 12.75% Brix. Lestari *et al.* (2019) reported that sugar content in sweet sorghum stem juice during the maturity phase ranged from 7% to 18% Brix. However, the findings of the present study do not align with those of Sriagtula *et al.* (2019), who observed sugar content ranging from 14.57% to 18.75% Brix. This variation is attributed to differences in sorghum varieties and average rainfall during the study period. Climate factors, particularly precipitation, also influence stem sugar content. Monthly rainfall during the study ranged from 226 mm to 492 mm, representing moderate intensity. Latupapua *et al.* (2018) classified monthly rainfall between 200 mm and 500 mm as moderate to heavy, which has been associated with lower %Brix in sorghum stem juice. Muñoz and Trujillo (2020) reported that %Brix in sorghum juice is affected by precipitation levels, as excessive water can inhibit photosynthesis by reducing stomatal conductivity, leading to decreased water absorption by roots and stomatal closure, thereby reducing sugar synthesis.

The results indicated that the application of PSB (Phosphate Solubilizing Bacteria) and different doses of phosphorus fertilizer did not have a significant effect ($P > 0.05$) on the stem juice volume of BMR mutant sorghum. This finding contrasts with that of Munawwarah and Sulaeman (2023), who reported that biofertilizer application increased stem juice volume. The planting season has been identified as a primary factor influencing stem juice quantity. Pabendon *et al.* (2017) reported that sorghum planted during the rainy season produced higher fresh juice yields compared to planting during the dry season. Munawwarah and Sulaeman (2023) also found that harvesting time, rainfall, variety, and soil fertility affected juice volume. Other factors influencing sorghum stem juice volume include genotype and harvest timing. In this study, sorghum plants were harvested at the soft dough stage, at 93 days after sowing (DAS). Mursyid *et al.* (2017) found that harvesting before physiological maturity resulted in optimal juice volume. Based on the treatments applied, the average stem juice volume ranged from 184.25 to 206.97 mL per stem, exceeding the 101.93 to 136.48 mL per stem reported by Lestari *et al.* (2019) for sweet sorghum mutant harvested at 80 DAS.

No significant differences were observed in stem height growth or the total colony count of PSB *Bacillus* sp. in the soil among all treatments compared to the control (P3), indicating that the phosphate solubilization capacity was assumed to be similar across treatments (Table 1). This finding aligns with Djuuna *et al.* (2022), who reported a direct correlation between PSB population density and soil phosphorus availability, with larger bacterial populations associated with enhanced nutrient uptake to support plant growth. The application of PSB did not significantly affect total colony counts in any treatment. This outcome may be explained by the PSB concentration used in this study (10^7 cfu/g), which was lower than the recommended 10^9 cfu/g. Li *et al.* (2024) demonstrated that the soil microbial population is significantly altered when phosphate-solubilizing bacterial inoculants are applied at concentrations of 10^9 cfu/g, promoting beneficial microbial interactions that enhance soil health, nutrient cycling, soil fertility, and phosphorus availability.

Bacteria cultivated on Pikovskaya agar medium produced clear zones surrounding their colonies, indicating phosphate solubilization. Insoluble phosphate ($Ca_3(PO_4)_2$) is present in Pikovskaya medium, and bacteria bind to this molecule, releasing $H_2PO_4^-$ ions. The release of these ions results in the formation of clear zones around the colonies. This phenomenon is attributed to phosphatase enzyme activity, which causes a localized decrease in medium pH (Fig. 1). Additionally, these microorganisms produce organic acids that lower pH and react with phosphorus-binding agents such as Al^{3+}, Fe^{3+}, Ca^{2+}, and Mg^{2+} to form organic chelates, thereby releasing free phosphate ions (PO_4^{3-}) (Fatimah *et al.* 2021). However, the area of the clear zones was not measured in this study.

Significant relationships were observed between juice volume and stem weight, stem diameter, stem height, and %Brix, with R^2 values of 0.5669, 0.5621, 0.432, and 0.0691, respectively (Fig. 2). These results indicated that juice volume increased with increasing stem weight, stem diameter, stem height, and %Brix. Therefore, stem weight, stem diameter, stem height, and %Brix were identified as important parameters influencing juice volume. Conversely, juice volume exhibited a negative relationship with %panicle, described by the equation $y = -21.464x + 826.1$ and an R^2 value of 0.2416.

Table 1 Average BMR mutant sorghum stem juice sugar content

Treatment	Sugar content (%Brix)	Juice volume (mL)	Steam height (cm)	Colony of *Bacillus* sp. (1x108 cfu/mL)
P0	12.75±1.87	206.97±54.22	222.32±40.24	30.00±1.60
P1	12.42±2.06	188.75±51.43	219.53±31.09	29.00±1.08
P2	11.77±1.76	205.13±34.83	227.30±34.91	31.25±2.53
P3	12.10±.2.03	184.25±47.19	214.23±29.47	34.25±3.86
Mean	12.06±1.98	189.10±53.32	220.85±34.19	31.12±2.27

Notes: The effect of treatments is not significantly different (P > 0.05); P0 = Urea + KCl + 0% TSP + Probiotic PSB; P1 = Urea + KCl + 50% TSP + Probiotic PSB; P2 = Urea + KCl + 75% TSP + Probiotic PSB; P3 = Urea + KCl + 100% TSP + without Probiotic PSB.

Figure 1 The results for PSB (Phosphate Solubilizing Bacteria) test
Note: Red arrow indicated the formation of the clear zone.

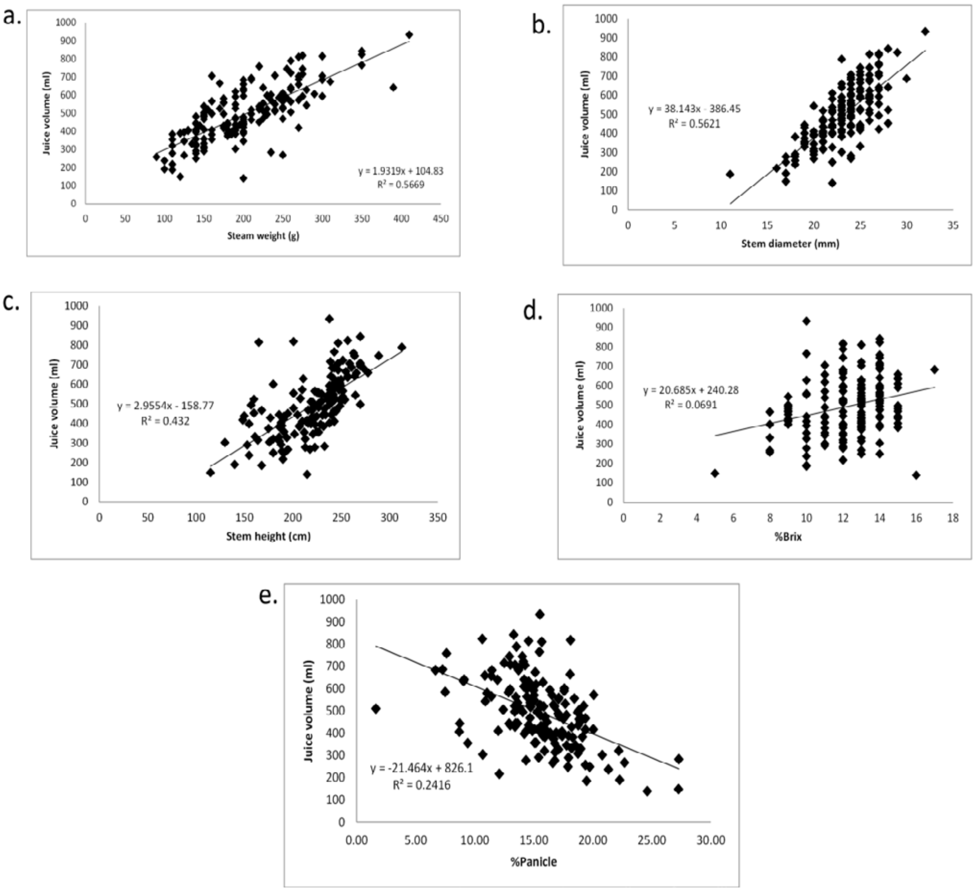

Figure 2 Relationships between juice volume and stem, sugar content, and panicle
Notes: a = relationship between juice volume with stem weight; b = relationship between juice volume and stem diameter; c = relationship between juice volume and stem height; d = relationship between juice volume and sugar content (%Brix); e = relationship between juice volume and %panicle.

Correlation Coefficient of Various Parameters on the Volume of Sorghum Stem Juice

The correlation coefficient values between various parameters and the juice volume of BMR sorghum stems are presented in Table 2. Analysis revealed a significant relationship between stem diameter and juice volume, with a correlation coefficient of 0.515**, indicating a strong positive association. An increase in stem diameter corresponded to a higher juice volume, as larger stem diameters allow for greater storage of photosynthates in the stem. Oliveira *et al.* (2021) similarly reported a positive correlation between stem diameter and juice volume (r = 0.03*). Thus, stem size was found to significantly affect juice productivity.

Correlation coefficient analysis revealed a significant relationship between stem weight and juice volume, with a correlation coefficient of 0.481**, indicating a strong positive association. An increase in fresh stem weight corresponded to a higher juice volume. This result is consistent with findings by Mursyid (2017), who reported a significant correlation between stem weight and juice volume (r = 0.858**). These findings suggest that optimal juice volume is associated with higher fresh stem weight.

Correlation coefficient analysis showed no significant relationship between sugar content and juice volume (r = 0.154, not significant). This finding contrasts with earlier results reported by Suwarti *et al.* (2018), who observed a significant correlation between sugar content and juice volume (r = 0.40). These results suggest that sorghum genotypes with high sugar content do not necessarily exhibit high juice volume potential. Additionally, juice volume was not significantly correlated with dry matter content (r = 0.182, not significant) in BMR mutant sorghum according to the correlation analysis.

Table 2 .Correlation coefficient values of various parameters on the volume of juice of BMR mutant sorghum stems (n=160)

	%Brix	**Juice volume (mL)**	**Stem weight (g)**	**Stem diameter (mm)**	**Stem height (cm)**	**%Panicle**	**Dry matter (%)**
%Brix	1	.154	.263**	.176*	.337**	.420**	.182*
Juice volume (mL)	.154	1	.481**	.515**	.003	.010	.135
Stem weight (g)	.263**	.481**	1	.750**	.657**	.492**	.22 1
Stem diameter (mm)	.176*	.515**	.750**	1	.333*	.256**	.043**
Stem height (cm)	.337**	.232**	.657**	.333*	1	.514**	.330**
%Panicle	.420**	.202*	.492**	.256**	.514**	1	.271**
Dry matter (%)	.182*	.119	.221	.043**	.330**	.271**	1

CONCLUSION

Phosphate-solubilizing bacteria (PSB) application using probiotic *Bacillus* sp. was shown to replace phosphorus fertilizer, resulting in sugar Brix values and juice production comparable to those obtained with 100% phosphorus fertilizer treatment.

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