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Influence of the bud position in the *Saccharum officinarum* stalk on the initial growth of sprouts

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ABSTRACT

The use of buds in different positions on the thatch for planting can become a problem, as each part has different concentrations of reserves and hormonal balances, acting differently on the sprouts. This study aimed to evaluate the initial growth of seedlings from sugarcane buds selected from different parts of the plant stalk. The experiment was conducted in a greenhouse using a randomized block design arranged in a 2 x 3 factorial scheme, consisting of two sugarcane varieties and three bud collection positions, with four replications. Buds from the base, middle, and apex of the sugarcane stalk were used. The sprouting speed index for the RB922579 variety, for buds from the base, middle, and apex, was 115, 367, and 339% higher than the SP803280 variety. Similarly, the values for the sprouting coefficient were 67.8, 78.3, and 191.6%, respectively. The apex buds sprouted 65% more than the base buds. The RB922579 variety was superior to the SP803280 variety regarding sprouting and biometric traits, with only the opposite occurring for sprouting speed. Buds from the apex of the stem provide greater sprouting, sprouting speed index, and sprouting speed coefficient than buds from the base and middle of the stem. Sprouting was not correlated with the biometric traits, which were positively correlated.

Keywords: *Saccharum officinarum,* Sugarcane billets, Pre-sprouted seedlings, Bud formation.

Influência da posição da gema no colmo de *Saccharum officinarum* no crescimento inicial de brotações

RESUMO

A utilização de gemas em diferentes posições no colmo para o plantio pode se tornar um problema, devido cada parte possuir diferentes concentrações de reservas e balanços hormonais, agindo de forma diferente nas brotações. Assim, esse estudo teve por objetivo avaliar o crescimento inicial de mudas provenientes de gemas de cana-de-açúcar selecionadas de diferentes partes do colmo da planta. O experimento foi conduzido em casa de vegetação utilizando o delineamento experimental em blocos casualizados, em esquema fatorial 2 x 3, consistindo em duas variedades de cana-de-açúcar e três posições de coleta das gemas, com quatro repetições. Foram utilizadas gemas da base, do meio e da ponta do colmo da cana-de-açúcar. O índice de velocidade de brotação para a cultivar RB922579, para as gemas da base, meio e ponta foram 115, 367 e 339% superior a cultivar SP803280, respectivamente. Da mesma forma, os valores para o coeficiente de brotação foram de 67,8, 78,3 e 191,6%, respectivamente. A brotação das gemas da ponta foram 65% superior ao observado nas gemas da base. A cultivar RB922579 foi superior a cultivar SP803280 nas características de brotação e biométricas, sendo que apenas na velocidade de brotação ocorreu inversão. Gemas provenientes do ápice do colmo proporcionam maior brotação, índice de velocidade de brotação e coeficiente de velocidade de brotação em relação às gemas da base e do meio do colmo. A brotação não se correlacionou com as caraterísticas biométricas, que apresentam correlação positiva entre si.

Palavras-chave: *Saccharum officinarum*, Toletes de cana-de-açúcar, Mudas pré-brotadas, Formação de gemas.

1. Introduction

Brazil is the world's largest producer of sugarcane (*Saccharum officinarum*), with an estimated growth of 4.4% compared to the previous crop season, with production expected to reach 637.1 million tons for the 2023/24 crop season in a planted area of approximately 8,410.3 thousand hectares (Conab, 2023). Sugarcane stands out for its role as a raw material for producing sugar and ethanol. It is a semi-perennial crop, which means it can be cut several times without the need for new planting, and in commercial plantations, it is propagated vegetatively asexually (Pinto et al., 2016; Jesus et al., 2019).

Asexual propagation uses stalks with axillary buds and root primordia in the nodal regions, which will give rise to new plants (Segato et al., 2006; Thomas, 2016). Normally, the stalks are fractionated into parts containing between three and four buds at the time of planting, placed base to apex, as the sprouting process can vary depending on the position of the origin of the bud in the stalk due to the different concentrations of hormones and energy (reserve sugars) (Manhães et al., 2015; May; Ramos, 2019; Sousa et al., 2020).

In mechanized planting of sugarcane fields, under favorable conditions, the bud can develop, but if these conditions do not occur, the bud may not sprout, resulting in failures in the sugarcane field and, consequently, a reduction in gain and yield (Oliveira et al., 2018). With this in mind, the Agronomical Institute of Campinas (Instituto Agronômico de Campinas – IAC) has developed a planting method using pre-sprouted seedlings (PSS) of sugarcane from individualized buds. The PSS system enables increased efficiency and economic gains when setting up nurseries, replanting commercial areas, and expanding and renewing sugarcane plantations (Jesus et al., 2019; Pinto et al., 2016).

In the conventional system, approximately 18 to 20 tons of seedlings are needed to plant one hectare of sugarcane. When using the PSS technology system, this amount decreases to up to 2 tons per hectare (Oliveira et al., 2018). The objective of pre-sprouted seedlings (PSS) is the rapid multiplication of seedlings, which is associated with a standard of phytosanitary, vigor, and uniformity of planting, and also aims to produce at a lower cost, reducing the use of raw materials (Jesus et al., 2019; Pinto et al., 2016).

May and Ramos (2019) also report that the PSS method allows for the mechanization of the bud extraction process when the buds are kept in the greenhouse and reinforces the up to 10-fold reduction in the volume of substrate required, reduces seedling formation time, and achieves uniform buds in size and physiological stage, as well as ensuring a reduction in the final cost of the seedling produced by automating the extraction and tray preparation process and the total production time.

To obtain a seedling of optimum quality at the time of planting, it is necessary to look for buds that have favorable characteristics for sprouting and to take into account in this process the position in which the bud is removed from the stalks because as the accumulation of assimilates is not uniform in the plant, this can affect the quality of the bud and consequently the quality of the seedling. This research evaluated seedlings sprouting and initial growth from sugarcane buds selected from different parts of the plant stalk.

2. Material and Methods

The experiment was conducted in a greenhouse at the Federal University of Mato Grosso do Sul, campus of Chapadão do Sul-MS, at 18°46'44" S and 52°36'59" W, and an altitude of 810 m. The greenhouse, which is 16 m long and 6 m wide, has micro sprinkler irrigation and is covered with black shade (50%) on the sides and 150 micron polyethylene film on the roof. The floor was covered in gravel, and there was a cultivation bench 1.40 m high where the containers with the sugarcane seedlings were placed. The average temperature inside the greenhouse during the experiment was 26.9°C (± 2°C), and the relative air humidity was 68% (± 4%).

The experimental design used was randomized blocks in a 2 x 3 factorial scheme, consisting of two varieties of sugarcane (SP803280 and RB922579) and three positions for collecting the buds (upper, middle, and lower third, considered as the apex, middle, and base), defined by dividing the plant's stalk into three parts. A total of 240 tubs with a volume of 290 cm³ were used, all filled with a mixture of soil taken from the layer below 0.20 m deep, together with ash and filter cake, both of which come from waste from the sugar alcohol industry.

The soil used to fill the tubes consisted of a Latossolo Vermelho distrófico (LVd) with clay texture (Santos et al., 2018). The ash and filter cake used in the substrate mixture came from the sugar alcohol industry in a 2:1:1 ratio of soil:ash:filter cake. The chemical compositions of the substrate components are shown in Table 1. The tubes were filled until the substrate completely covered the mini billets. Planting occurred on April 25, 2015, using one mini billet per tube, with the bud directed upwards. Stalks from first year nurseries, 11 months old, were used. The mini billets were cut three days before planting, with two centimeters on each side of the bud in each position defined for the treatments. Damaged or poorly formed buds were discarded. These buds did not undergo any treatment.

Table 1. Chemical analysis results for the cake, ash, and soil samples used in the experiment. Chapadão do Sul, MS, 2015

Material	Parameters							
	pH	Humidity	O.M.	OC	N	P_2O_5	K_2O	MgO
		%						
Filter cake	7.0	29.8	62.5	36.3	1.7	2.8	0.4	0.5
Ash	8.8	24.1	18.4	10.7	0.2	0.9	0.6	0.6
Soil	Ca	Mg	K	Al	H+Al	BS		
	$mmol_c\ dm^{-3}$							
	5	1	0.1	1	19	6.1		
	pH ($CaCl_2$)	O.M. (g dm^{-3})	S (mg dm^{-3})	P (resin) (mg dm^{-3})	V (%)	CEC ($mmol_c\ dm^{-3}$)		
	4.6	8	2	4	24	25.1		

O.M. = Organic Matter; OC = Organic carbon; V = Base Saturation; CEC= Cation Exchange Capacity; B.S. = Sum of Bases.

Once the sprouting process was complete, the total height of the plant, the diameter of the stalk, and the number of leaves per plant were determined. Plant height was determined using a ruler, measuring the distance from the substrate to the last bud where leaf growth begins. The diameter of the stalk was measured with a caliper, and all fully expanded leaves were counted.

The number of sprouts was observed daily until 30 days after the first mini sprout. The indices for determining the speed of emergence, sprouting speed, and sprouting speed coefficient were determined according to the follow equations: a) Sprouting speed index (Maguire, 1962):

$$SSI = \frac{G1}{N1!} + \frac{\text{G2}}{N2!} + \cdots + \frac{Gn}{Gn},$$

Where:

SSI = Sprouting speed index; G= number of sprouts in each count; N= number of days from planting to each count;

b) Sprouting speed (Edmond and Drapala 1958):

$$\text{SS} = \frac{(N1\ G1)(N2\ G2) + \cdots + (Nn\ Gn)}{G1 + G2 + \cdots + Gn},$$

SS = sprouting speed (days); G and N= have the same meaning as the previous formula; c) Sprouting speed coefficient (Furbeck et al. 1993):

$$\text{SSC} = \frac{G1 + G2 + \cdots + Gn}{(N1\ G1)(N2\ G2) + \cdots + (Nn} x100,$$

Where:

SSC = sprouting speed coefficient; G and N= have the same meaning as the previous formula.

The assumptions of normal distribution and homogeneity of variance were checked for the data. They were then subjected to analysis of variance, and the means were compared using the Tukey test at 5% probability. The statistical analysis was done using Sisvar software (Ferreira, 2011). Pearson correlation analysis was also conducted among all the variables studied.

3. Results and Discussion

All the variables studied were influenced by the treatments used, except for the diameter of the stalk, which was not influenced by the bud position. The factors affected the variables when used in isolation (variety or bud position) or the interaction between the two (Table 2). Evaluating sprouts, such as those observed in this study, when working with vegetative propagation is of the utmost importance, as sprouting is a biological procedure that consumes energy from the plant to be conducted (Carvalho et al., 2015).

This energy comes from the degradation of reserve substances in the stalk. The buds sprout once this process is complete, and the shoots emerge. This phase can be influenced by plant regulators such as auxins, cytokinins, and gibberellins, which can enhance the growth and development of sugarcane PSS (Rosseto, 2015). The higher values found for the sprouting speed index (Figure 1A) in variety RB922579 indicate greater physiological potential for sprouting this material than the SP803280 variety. For both varieties, the apex was the position that provided the highest SSI.

The RB922579 variety showed a difference in SSI for the three positions, while in the SP803280 variety, there was no difference in SSI between buds from the base and the middle. However, the differences in SSI between the varieties are very pronounced for the different positions. The SSI observed in the RB922579 variety for buds from the base, middle, and apex were 115, 367, and 339% higher than those observed in the SP803280 variety, respectively.

Baracat Neto et al. (2017) and Sousa et al. (2020) also observed a higher percentage and speed of sprouting in sugarcane buds from the apex region compared to the basal region. They attributed this result to the buds being probably younger and more vigorous. According to Sousa et al. (2020), the apex buds were probably influenced by apical dominance or benefited from the greater availability of reducing sugars readily available in glucose.

Table 2. Summary of the analysis of variance for sprouting speed index (SSI), sprouting speed (SS), sprouting speed coefficient (SSC), sprouting (S), height (HE), diameter (D), and number of leaves (NL) according to the varieties and bud positions.

SV	DF	Mean square of the residual			
		SSI	SS	SSC	S
Block	3	0.0013	0.3303	0.1902	11.4444
Variety (V)	1	2.6070**	497.7704**	233.7504**	2816.6667**
Position (P)	2	1.8992**	67.6866**	47.09114**	6466.6667**
VxP	2	0.8345**	19.0541**	33.9903**	466.6667**
Error	15	0.0014	0.4298	0.1468	9.4444
		HE	D	NL	
Block	3	0.0080	0.0035	0.0663	
Variety (V)	1	22.5131**	23.9667**	0.5450**	
Position (P)	2	0.5392**	0.0845ns	0.4439**	
VxP	2	5.7106**	0.7261**	0.0346*	
Error	15	0.0321	0.0254	0.0085	

**, *, and ns = significant at 1%, 5%, and not significant, respectively, by the F test. SV = sources of variation; DF = degree of freedom.

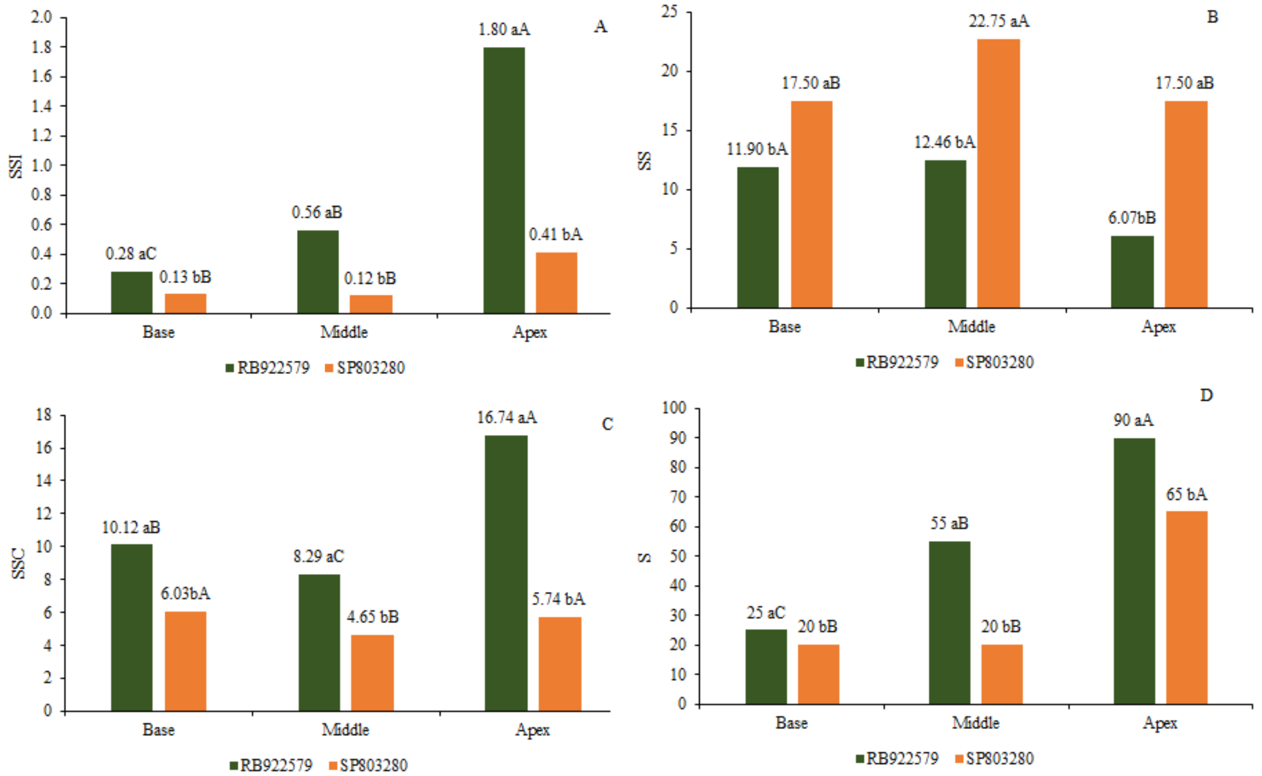

Figure 1. Sprouting speed index (SSI), sprouting speed (SS), sprouting speed coefficient (SSC), and sprouting (S) according to the varieties and bud positions. Means followed by the same lowercase letters for sugarcane varieties and uppercase letters for bud position do not differ statistically by the Tukey test at 5%.

Because they take longer to form, buds in the basal region of the plant probably have more lignin deposition, a natural plant barrier against water loss, i.e., tissue dehydration (Freire et al., 2014) and the action of biotic and abiotic factors (Meschede et al., 2012), which can inhibit or delay sprouting, as well as requiring a greater amount of energy to break through this barrier. Due to the characteristics of the availability of reserves and auxin production in these buds, there is a decreasing gradient of sprouting in buds from the apical to the basal region, i.e., from younger to older buds (Baracat Neto et al., 2017).

The lower sprouting speed values (Figure 1B) indicate that the material took longer to finish sprouting. The RB922579 variety showed lower SS for all positions when compared to the SP803280 variety, with a variation of 47.0, 82.6, and 188.0%, respectively, reinforcing that the RB922579 variety, as well as having greater physiological potential for sprouting, manages to do so in less time than the SP803280 variety.

Regarding SS for the positions (Figure 1B), variety RB922579 showed the lowest value for the apex, and there was no difference between the base and the middle. Those from the middle of the stem took the longest to sprout. For variety SP803280, the lowest SS value was found in buds from the base and apex, with no difference between them. This is because the buds at the base are located in a region rich in sucrose and must be transformed into glucose to start the process. According to Manhães et al. (2015), the older the stem, the smaller the glucose reserve and nutrients for bud growth.

The highest values for the sprouting speed coefficient (Figure 1C) were also observed for the RB922579 variety. In this variety, the buds at the apex had the highest SSC, followed by the buds at the base and finally in the middle. In the SP803280 variety, the base buds had the highest SSC but were no different from the apex buds. The SSC of the base, middle, and apex buds of the RB922579 variety was 67.8, 78.3, and 191.6% higher than that of the SP803280 variety, respectively.

Sprouting was also higher in the RB922579 variety when compared to the SP803280 variety in all three stalk bud collection positions. In the first variety, the sprouting of the apex buds was 65% higher than that observed in the base buds. For the SP803280 variety, there was no difference in sprouting between the base and middle buds; however, the apex buds were 45% superior to the apex buds. Baracat Neto et al. (2017) found greater sprouting of buds from the apex of the sugarcane stalk compared to buds from the base and middle of the stalk, due to their being younger and healthier.

The differences in the sprouting of the base, middle, and apex buds were 5, 35, and 25% in favor of the RB922579 variety, respectively. The greater sprouting of the apex buds may be due to the large amount of non-reducing sugar (sucrose) present in the stalks (Nascimento et al., 2021). The buds at the apex of the stalks are also younger and have a higher concentration of glucose, nitrogen, and water, which benefits the emergence of the primary tiller due to the availability of glucose and nitrogen in the cell division of the latent tissue (Baracat Neto et al., 2017).

Figure 2A shows that the highest plant height at 35 days after planting was obtained with the RB922579 variety for the bud position at the base of the stalk. In the SP803280 variety, the highest plant height was obtained with buds from the apex of the stalk. Like any metabolic activity, plant development is also regulated by the specific genetic programming of each variety. If the environmental conditions are ideal, growth will be dictated by the plant genotype. And as the results show, each variety grows differently (Machado et al., 1982).

Greater plant height benefits the crop when accompanied by greater stalk diameter, which provides a good height/diameter ratio. This is important because the quality of seedlings is related to the adequate partitioning of photoassimilates (dry matter) between the different plant organs (leaves, stems, and roots) (Franco et al., 2020).

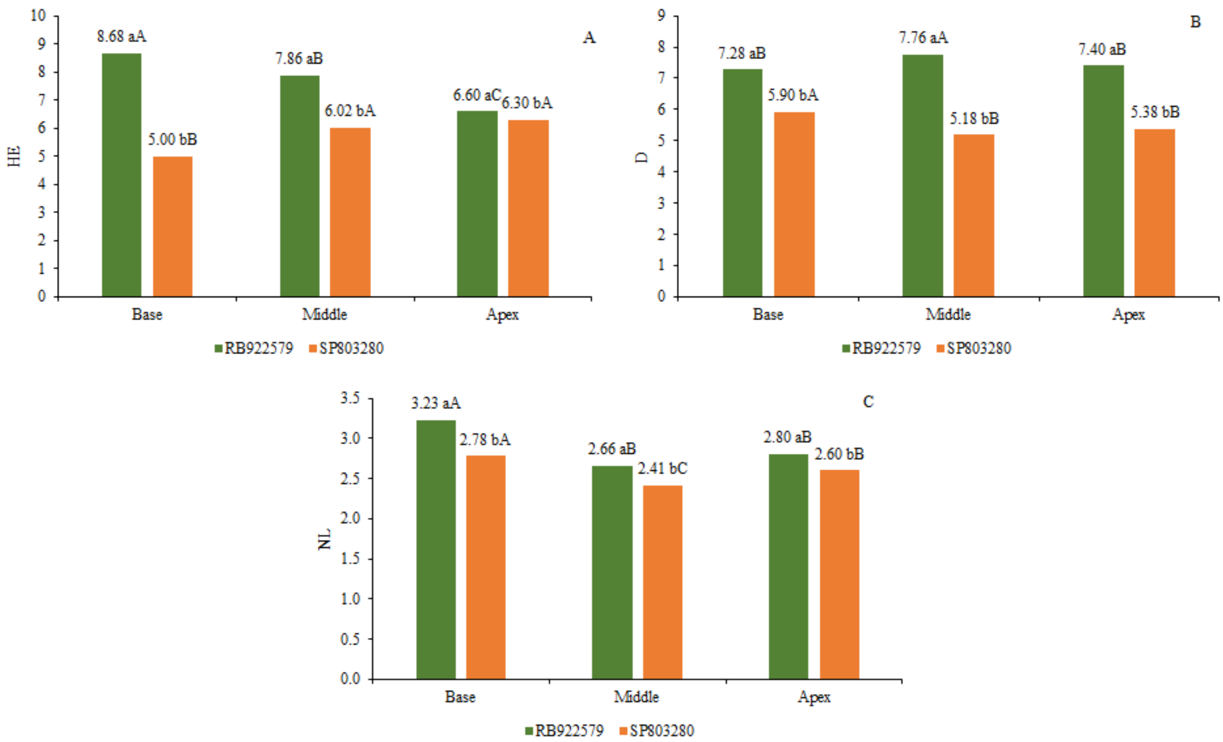

Figure 2. Height (HE), diameter (D), and number of leaves (NL) at 35 DAP in RB 922579 and SP 803280 sugarcane varieties (Chapadão do Sul, MS, 2015). Means followed by the same lowercase letters for sugarcane varieties and uppercase letters for bud position do not differ statistically by the Tukey test at 5%.

Stalk diameter is related to the characteristics of each variety, tillering, spacing, and environmental conditions (Oliveira et al., 2016). Stalks with larger diameters are positively related to the accumulation of reserves and are formed during periods of water deficiency, which coincides with sucrose accumulation (Maia Junior et al., 2018). In the RB922579 variety, the highest values for stalk diameter were obtained from buds from the middle of the stem, while there was no difference in stalk diameter between buds from the base and apex of the stem.

In the SP803280 variety, the largest stalk diameter was obtained by buds from the base of the stalk, while the middle and apex buds showed no difference in stalk diameter. It should be noted that the stalk diameter is an important variable in assessing the survival and growth potential of the seedlings after planting, as it contains the reserves that will affect the final value of the crop raw material. In all positions, the highest values for stalk diameter were found for the RB922579 variety. According to Landell (2012), the reserves of the stalks are essential for developing the sprout for 60 days after planting, and the need for reserves gradually decreases with the development of the roots and shoots of the growing plant.

The highest number of leaves for both varieties was observed in the buds from the base of the stem. For both varieties, there was no difference in the number of leaves for buds from the middle and apex of the stem. There were no differences between the two varieties when it came to using the buds from the apex of the stem, but buds obtained from the base and middle of the stem showed higher values in the RB922579 variety.

According to Taiz et al. (2017), the number of leaves on seedlings is an important parameter and entirely linked to the development of the plant, as leaves are the main organ where photosynthesis occurs and enables plant growth. In this way, the yield increases of PSS planting can be up to 18% (16 t ha^{-1}) higher when compared to conventional cultivation (Mohanty et al., 2015), a fact associated with the greater tillering of the varieties. Although this study was conducted up to 35 DAP, which is approximately half the time for the complete maturation of a seedling ready to be placed in the field, it brings important results that can be reflected at the end of its cycle. Similar results to this one, showing better performance of buds from the apex of the stem, can be seen in the work by Sousa et al. (2020).

It can be seen in Table 3 that SS showed a significant correlation with all the variables, although they were negative, indicating that higher sprouting speed is dependent on lower values for the other variables. SSI had a significant positive correlation with SSC, S, D, and NL, while SSC correlated positively with S, D, and NL. Of the biometric variables, height correlated positively with D and NL, and D correlated positively with NL.

Table 3 shows that the higher the values for the sprouting index and speed coefficient, the greater the number of sprouts will be formed, but the higher the sprouting speed, the fewer sprouts will appear. With the sprouts already formed, it can be seen that there is a dependence between the formation of the number of leaves, the stalk diameter, and the plant height.

Table 3. Pearson correlation analysis among sprouting speed index (SSI), sprouting speed (SS), sprouting speed coefficient (SSC), sprouting (S), height (H), diameter (D), and number of leaves (NL) as a function of varieties and bud positions.

		SS	SSI	SSC	S	HE	D	NL
SS	R of Pearson p-value	—						
SSI	R of Pearson	-0.815 ***	—					
	p-value	< .001	—					
SSC	R of Pearson	-0.935 ***	0.911 ***	—				
	p-value	< .001	< .001	—				
S	R of Pearson	-0.673 ***	0.874 ***	0.675 ***	—			
	p-value	< .001	< .001	< .001	—			
HE	R of Pearson	-0.481 *	0.098	0.338	0.094	—		
	p-value	0.017	0.648	0.106	0.661	—		
D	R of Pearson	-0.853 ***	0.525 **	0.711 ***	0.380	0.705 ***	—	
	p-value	< .001	0.008	< .001	0.067	< .001	—	
NL	R of Pearson	-0.536 **	0.097	0.425 *	-0.066	0.539 **	0.526 **	—
	p-value	0.007	0.651	0.038	0.759	0.007	0.008	—

* p < .05, ** p < .01, *** p < .001

4. Conclusions

The RB922579 variety was superior to the SP803280 variety regarding sprouting and biometric traits. The SP803280 variety outperforms the RB922579 only regarding sprouting speed. Buds from the apex of the stem provide greater sprouting, sprouting speed index, and sprouting speed coefficient than buds from the base and middle of the stalk.

The base buds promoted greater plant height and number of leaves, while the middle buds favored greater sugarcane stalk diameter. There was a negative correlation between sprouting speed and all the other variables. Sprouting was not correlated with the biometric traits, which were positively correlated.

Authors' Contribution

Charles Fabian Dias, Sebastião Ferreira de Lima, and Vitória Carolina Dantas Alves: conceptualization, data curation and writing-original draft. Janaína Jacinto de Oliveira and Túlio Russino Castro: writing-review and formal analysis. Eduardo Pradi Vendruscolo: writing review and editing.

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