



Morphophysiological characterization in sugarcane mini setts as a function of age, position in the stalk and air temperature

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Grace Karina Kleber Romani¹, Marcela Maria Zanatta¹, Anabel Aparecida Oliarski¹, Lucas Wagner¹, João Carlos Bespalhok Filho¹ and Bruno Portela Brasileiro¹

¹Department of Plant Science and Plant Health, Federal University of Paraná, Curitiba, Paraná, Brazil.

Author for correspondence: Grace Karina Kleber Romani – grace.kromani@gmail.com

Abstract: This study evaluated the morphophysiological characteristics of sugarcane buds from different stalk positions of the Havaianinha variety under varying air temperatures, aiming to understand biotic and abiotic factors affecting seedling production via the pre-sprouted seedling (PSS) system. Two experiments were conducted at the Micropropagation Laboratory of the Federal University of Paraná, using a completely randomized design (CRD) in a 4×3 factorial scheme (four temperature levels: 15, 20, 25, and 30 °C; three bud positions: apical, median, and basal) with three replications. Each experimental unit contained 20 mini setts. The sprouting speed index (SSI), sprouting percentage (SP), and sprouting height (SH) were evaluated. Data were analyzed using ANOVA, Pearson correlation, Scott-Knott test (5%), and regression analysis for temperature. SSI, SP, and SH were influenced by mini sett age, temperature, and bud position. The highest values for all parameters occurred at 30 °C, using apical buds from 18-month-old culms or buds from any position on 6-month-old culms. Temperatures below 20 °C negatively affected all variables. Positive correlations among SSI, SP, and SH were observed. For optimal PSS seedling production, younger culms should be used to maximize bud utilization.

Keywords: *Saccharum* spp.; bud sprouting; seedling production; biotic factors; abiotic factors.

1. Introduction

Sugarcane production has great economic importance, being the oldest agro-cultural activity in Brazil. The culture is explored throughout the national territory, however, the main production centers are located in the Southeast, Center-West, Northeast and South of the country. The five largest producers, in descending order, are the states of São Paulo, Goiás, Minas Gerais, Mato Grosso do Sul and Paraná (Conab, 2023). Brazilian production aims to meet the demand for sugar and ethanol in the domestic and foreign markets (Bonassa et al., 2015).

Producers have been seeking improvements in cultural practices used in sugarcane cultivation, with the aim of increasing crop productivity. The method of renewing sugarcane fields adopted by producers has been the same for several decades, which requires a large amount of plant material, around 20 tons of stalks to plant just one hectare in the mechanized planting system, and around 7 to 10 tons in conventional planting (Civiero et al., 2016; May e Ramos, 2019).

The Pre-Sprouted Seedlings (PSS) system can be used as an alternative to reduce the volume of plant material, rapid production of seedling with quality and vigor control, high phytosanitary standards, improvements in the spatial distribution of intraspecific competition (Landell et al., 2012; Xavier et al., 2014a). Thus, the PSS system has proven to be a promising alternative for increasing sugar and alcohol productivity in areas already cultivated, reducing the pressure to open new areas for cultivation and, consequently, preserving natural resources in areas of native vegetation (Santos et al., 2020).

To be successful with this seedling production system, a high bud sprouting rate of the mini setts is essential. There are numerous factors that can influence the sprouting of sugarcane buds, which can be classified as: environmental factors (temperature, humidity and soil), genetic, physiological (variety, age, size and health of buds) and phytotechnical (agricultural practices carried out in the field).

Havaianinha is the most widespread variety regionally, as it has satisfactory agronomic and productive characteristics for small producers. In addition, varieties can be found such as: soft White, hard White, unidentified RB clones, and other varieties unknown or not identified by producers, which comprise planting with a mixture of varieties, due to the lack of selection and control of the seedling used for planting and renewing the areas (Trento Filho et al., 2008).

Therefore, the objective of this work was to evaluate the morphophysiological characteristics of sugarcane buds from different positions on the stem of the Havaianinha variety under different air temperatures, aiming to understand the biotic and abiotic factors that can interfere with the success of seedling production via the system of pre-sprouted seedlings (PSS).

2. Material and Methods

In the municipality of Morretes – PR (Lat: -25.479, Lon: -48.8317 25° 28' 44" South, 48° 49' 54" West, Alt: 8.48 m), culms of the Havaianinha variety with six months of age were collected to conduct the first experiment and, for the second experiment, 18-month-old culms were used. After collection, the culms were sectioned into mini setts approximately 4 cm long containing an axillary bud and separated according to the position on the culm (apical, median and basal).

The average, maximum and minimum temperature of the municipality of Morretes – PR, where the Havaianinha variety is grown, is represented in Fig. 1. It can be observed that the highest average temperatures occur between the months of September and April, a favorable period (average temperature with 20 °C) to the development of sugarcane.



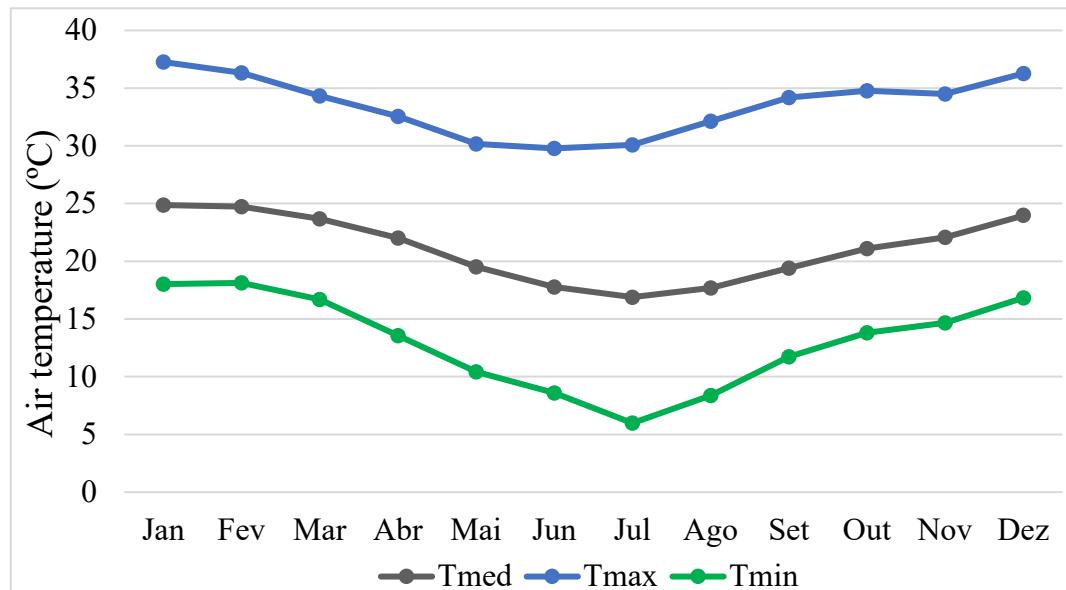


Figure 1 – Average, maximum and minimum temperature for the months of January to December from 2012 to 2022 in Morretes, PR. Source: INMET 2023.

Two experiments were conducted in the Micropropagation Laboratory of the Department of Phytotechnics and Plant Health of the Agricultural Sciences Sector of the Federal University of Paraná. A completely randomized design arranged in a 4 x 3 factorial scheme was adopted (4 levels of the temperature factor and 3 levels for the bud position factor), with three replications. The temperatures adopted were: 15, 20, 25 and 30 °C; and the bud positions were: apical, median and basal buds. Each Plot was made up of 20 mini setts.

The mini setts were placed in plastic trays with a layer of approximately 3 cm in height of Carolina Soil II class V CE 0.7 substrate, moistened with 100 ml of distilled water, and covered with a layer of approximately 2 cm of height of the same substrate. After this process, the trays were identified and taken to a B.O.D (biological oxygen demand) incubator oven of the Eletrolab model ELP202/3 at the constant temperature corresponding to each treatment, kept there for 10 days.

Next, the following variables were evaluated: sprouting speed index (SSI), sprouting percentage (SB, %) and sprouting height (SH, cm). The first count of sprouted buds to calculate SSI and SB was carried out 3 days after planting the mini setts in the trays, and the last count was carried out at 10 days. Seven days after planting, in addition to counting buds sprouted, the height of 5 representative shoots from each plot was measured with the aid of a digital caliper.

The SSI was calculated as follows: $SSI = (G1/N1) + (G2+G1/N2) + \dots + (Gn+Gn/Nn)$, where: SSI = sprouting speed index; G = number of sprouted buds; N = number of days from planting to the 1st, 2nd, (...) and 7th evaluation. The SB was calculated as follows: $SB = ((NSB/ 20) *100)$, where PS = percentage of sprouting; NSB = number of sprouted buds.

Data for all variables were subjected to analysis of variance (ANOVA) and Pearson correlation analysis. Upon finding significant differences between the positions of the bud, the average levels of this factor were grouped using the Scott-Knott test at 5% probability, and regression equations were adjusted for the temperature factor, using the statistical software R.

3. Results and Discussion

3.1. Experiment 1: Six-Month-old Culms

The analysis of variance revealed a significant interaction between temperature and bud position (T x P) and a significant difference between bud positions (P) and between temperatures (T) ($p<0.05$) for all variables analyzed (SSI, SP and SH) (Table 1). The significant T x P interaction reveals that temperature interferes with the variables differently depending on the position of the bud on the stem and vice-versa and that is why the interaction breakdowns were carried out, analyzing the performance of the positions within the temperatures and vice-versa (Table 1).

Table 1 – Mean squares of the analysis of variance for the variables sprouting speed index (SSI), sprouting percentage (SP) and sprouting height (SH) analyzed in six-month-old sugarcane buds.

SV	DF	SSI	SP	SH
Position (P)	2	2.254 *	238.194 *	0.903 *
Temperature (T)	3	45.76 *	15426.6 *	86.892 *
T x P	6	0.118 *	64.12 *	1.191 *
Residue	24	0.044	13.888	0.199
Position/15°C	2	0.173 *	219.444 *	0.001 ns
Position/20°C	2	0.914 *	144.444 *	0.006 ns
Position/25°C	2	0.518 *	58.333 ns	0.082 ns
Position/30°C	2	1.273 *	8.333 ns	4.388 *
Temperature/Apical	3	14.328 *	6244.44 *	27.37 *
Temperature/Mediana	3	17.494 *	4280.56 *	41.18 *
Temperature/Basal	3	14.174 *	5029.86 *	20.71 *
Average		3.12	67.36	3.01
CV (%)		6.74	5.53	14.83

For position unfolding within temperature, significant differences ($p<0.05$) were verified between the bud positions at all temperatures for the SSI variable, between the bud positions within 15 and 20 °C for the SP variable, and only between the bud positions within 30 °C in the SH variable (Table 1). For the breakdown of temperature within bud position, significant differences ($p<0.05$) were observed between temperatures in all bud positions in the three variables analyzed (SSI, SP and SH) (Table 1).

For the variable SSI, the means varied from 0.13 to 4.75 between temperatures for the apical position, from 0.61 to 6.03 for the median position and from 0.31 to 5.15 for the basal position. It is worth mentioning that the maximum SSI value, if all buds had sprouted on the third day after planting, would be 6.67 (Table 2). Furthermore, it is possible to observe that the buds in the median position presented the highest SSI averages, regardless of the air temperature (Table 2).

For the SP variable, the means varied from 1.66 to 98.33% between temperatures for the apical position, from 18.33 to 100% for the median position and from 6.66 to 96.66% for the basal position. (Table 2). The median position presented the highest averages at temperatures of 15 and 20 °C. At 25 °C, the average buds in the apical and median positions belong to the same group according to the Scott-Knott test, while the average basal bud belongs to another group. At 30 °C, the buds from the three positions of the stalk showed a high percentage of sprouting, not statistically different from each other (Table 2).

Table 2 – Grouping of means for the variables sprouting speed index (SSI), sprouting percentage (SP) and sprouting height (SH) analyzed in six-month-old sugarcane buds considering position within temperature and temperature within position.

Variable	Temperature (C°)	Position		
		Apical	Median	Basal
SSI	15	0.13 Ad	0.61 Ad	0.31 Ad
	20	1.72 Bc	2.82 Ac	2.24 Bc
	25	4.30 Bb	5.03 Ab	4.32 Bb
	30	4.75 Ba	6.03 Aa	5.15 Aa
SP	15	1.66 Bc	18.33 Ac	6.66 Bd
	20	61.66 Bb	75.00 Ab	65.00 Bc
	25	98.33 Aa	96.66 Aa	90.00 Bb
	30	98.33 Aa	100.00 Aa	96.66 Aa
SH	15	0.20 Ad	0.20 Ad	0.24 Ad
	20	1.30 Ac	1.37 Ac	1.28 Ac
	25	3.18 Ab	3.12 Ab	3.43 Ab
	30	7.08 Ba	8.58 Aa	6.18 Ca

Means followed by the same uppercase letter in the rows and lowercase letters in the columns belong to the same group according to the Skott Knott test at 5% probability.

Considering the SH variable, the averages varied from 0.20 to 7.08 cm between temperatures in the apical position, from 0.20 to 8.58 cm in the median position and from 0.24 to 6.18 cm in the basal position. At 30 °C, the median buds presented the highest means for this variable, differing statistically from the others, followed by the apical and basal buds, respectively (Table 2). At other temperatures there was no statistical difference between the positions of buds on the stalk.

Due to the significant differences between temperatures at different bud positions (Table 1), regression analyzes were carried out to evaluate the influence of air temperature on the sprouting speed index (SSI). For the mini sets of the three positions, first degree equations were adjusted to better explain the biological responses (Fig. 2).

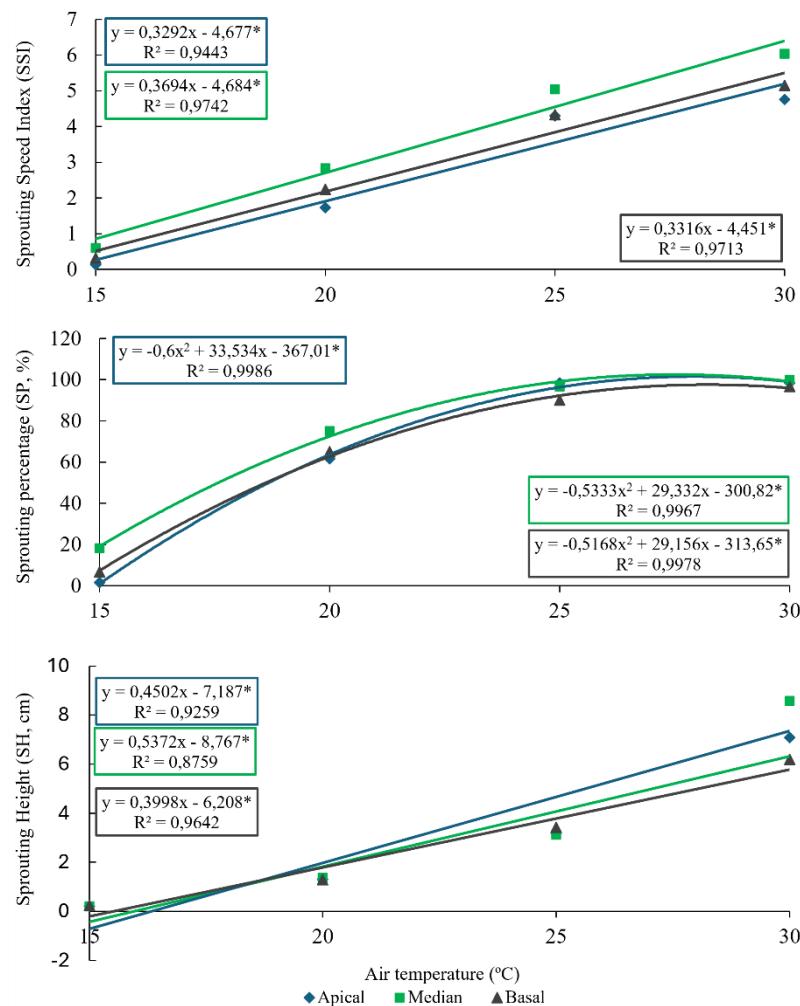


Figure 2 – Sprouting Speed Index (SSI), Sprouting Percentage (SP) and Sprouting Height (SH) of six-month-old sugar cane buds, from different stem position (apical, median and basal) under effect of air temperature (15, 20, 25, 30 °C).

In buds from the apical, median and basal positions there was an increase in SSI as the temperature increased, for each unit of temperature there was an increase of 0.33 in buds from the apical position, 0.37 for the median position and of 0.33 for the basal position (Fig. 2). These values present high reliability since the coefficients of determination (R^2) of the three equations were greater than 0.94. It is worth highlighting that R^2 corresponds to the adjustment measure of the statistical model, that is, in this case more than 94% of the variation in SSI can be explained by air temperature.

Second-degree equations were adjusted to evaluate the influence of air temperature on the sprouting percentage (SP) of buds from the three stalk positions (Fig. 2). According to the equations, there was an increase in the averages as the temperature increased to the limits of 27.9; 27.5 and 28.2 °C, values correspond to the maximum inflection point of the regression curve of the apical, median and basal position, respectively. It is worth noting that the three regression equations explained more than 99% of the variation in the SP variable (Fig. 2).

First-degree equations were adjusted to evaluate the influence of temperature on the bud height (SH) of the buds from the three stalk positions (Fig. 2). As represented in Fig. 2, buds in the apical, median and basal positions showed an increase in SH as the temperature increased, for each unit of temperature there was an increase of 0.45 cm in SH in buds in the apical position, 0.53 cm for the median position and 0.40 cm for the basal position. These values present a good fit to the sample since the coefficients of determination (R^2) of the three equations were greater than 0.87.

3.2. Experiment 2: Eighteen-Month-Old Culms

The results of the analysis of variance (ANOVA) of the second experiment are presented in Table 3. Significant differences ($p < 0.05$) were verified between the levels of the factors position of the bud on the stalk (P) and air temperature (T), in addition to a significant interaction between T x P, for all variables analyzed (SSI, SP and SH).

Considering the unfolding of the bud position within each temperature, significant differences ($p < 0.05$) were found in all situations, except within the temperature of 15 °C, in the three variables (Table 3), this was due to the absence of bud germination at this temperature. For the breakdown of temperature within position, significant differences ($p < 0.05$) were observed between the air temperatures in the three bud positions on the stalk, in all variables analyzed (Table 3).

Table 3 – Mean squares of the analysis of variance for the variables sprouting speed index (SSI), sprouting percentage (SP) and sprouting height (SH) analyzed in 18-month-old sugarcane buds.

SV	DF	SSI	SP	SH
Position (P)	2	12.499 *	3.493.750 *	34.407 *
Temperature (T)	3	14.658 *	8.082.410 *	47.944 *
T x P	6	1.636 *	417.824 *	9.462 *
Residue	24	0.100	47.222	0.053
Position/15°C	2	0.001 ns	0.001 ns	0.001 ns
Position/20°C	2	3.267 *	1769.44 *	2.406 *
Position/25°C	2	5.628 *	1219.44 *	15.845 *
Position/30°C	2	8.510 *	1.758.330 *	44.540 *
Temperature/Apical	3	13.127 *	5.208.330 *	46.206 *
Temperature/Median	3	3.289 *	2.413.190 *	19.355 *
Temperature/Basal	3	1.512 *	1.296.520 *	1.305 *
Average		1.72	42.5	2.27
CV (%)		18.4	16.17	10.14

ns, *: non-significant and significant at 5% probability, respectively.

Considering the SSI variable, the means ranged from 2.75 to 4.80 for the apical position, from 1.23 to 2.50 for the median position and from 0.75 to 1.52 for the basal position. It is worth noting that if all the buds had sprouted on the third day after planting, the SSI would be 6.67 and that there was no bud sprouting at a temperature of 15 °C, regardless of the position of the bud on the stem (Table 4). It was possible to observe that the buds in the apical position of the stem presented the highest averages, followed by the buds in the median and basal positions, in that order.

The SP averages varied from 0 to 93.33% between temperatures for buds in the apical position, from 0 to 66.66% in the median position and from 0 to 45% for buds in the basal position (Table 4).

Table 4 – Grouping of means for the variables sprouting speed index (SSI), sprouting percentage (SP) and sprouting height (SH) analyzed in 18-month-old sugarcane buds considering position within temperature and temperature within position.

Variable	Temperature (°C)	Position		
		Apical	Median	Basal
SSI	15	0.00 Ad	0.00 Ac	0.00 Ac
	20	2.75 Ac	1.23 Bb	0.75 Bb
	25	3.93 Ab	1.70 Bb	1.44 Ba
	30	4.80 Aa	2.50 Ba	1.52 Ca
SP	15	0.00 Ac	0.00 Ac	0.00 Ac
	20	70.00 Ab	41.66 Bb	21.66 Cb
	25	80.00 Ab	50.00 Bb	41.66 Ba
	30	93.33 Aa	66.66 Ba	45.00 Ca
SH	15	0.00 Ad	0.00 Ac	0.00 Ac
	20	2.15 Ac	0.86 Bb	0.43 Cb
	25	5.22 Ab	1.28 Bb	1.20 Ba
	30	9.03 Aa	5.66 Ba	1.34 Ca

Means followed by the same uppercase letter in the rows and lowercase letters in the columns belong to the same group according to the Skott Knott test at 5% probability

Considering the SH variable, the averages ranged from 2.15 to 9.03 cm for the buds in the apical position between temperatures of 20 to 30 °C, given that there was no sprouting of buds from any position on the stalk when subjected to temperature. 15°C air. For the median position, the averages ranged from 0.86 to 5.66 cm and in the basal position it ranged from 0.43 to 1.34 cm (Table 4).

Due to the significant differences between temperatures within the different bud positions (Table 3), regression analyzes were carried out to evaluate the influence of air temperature on the sprouting speed index (SSI). For all positions, the best responses were obtained by adjusting first degree equations (Fig. 3).

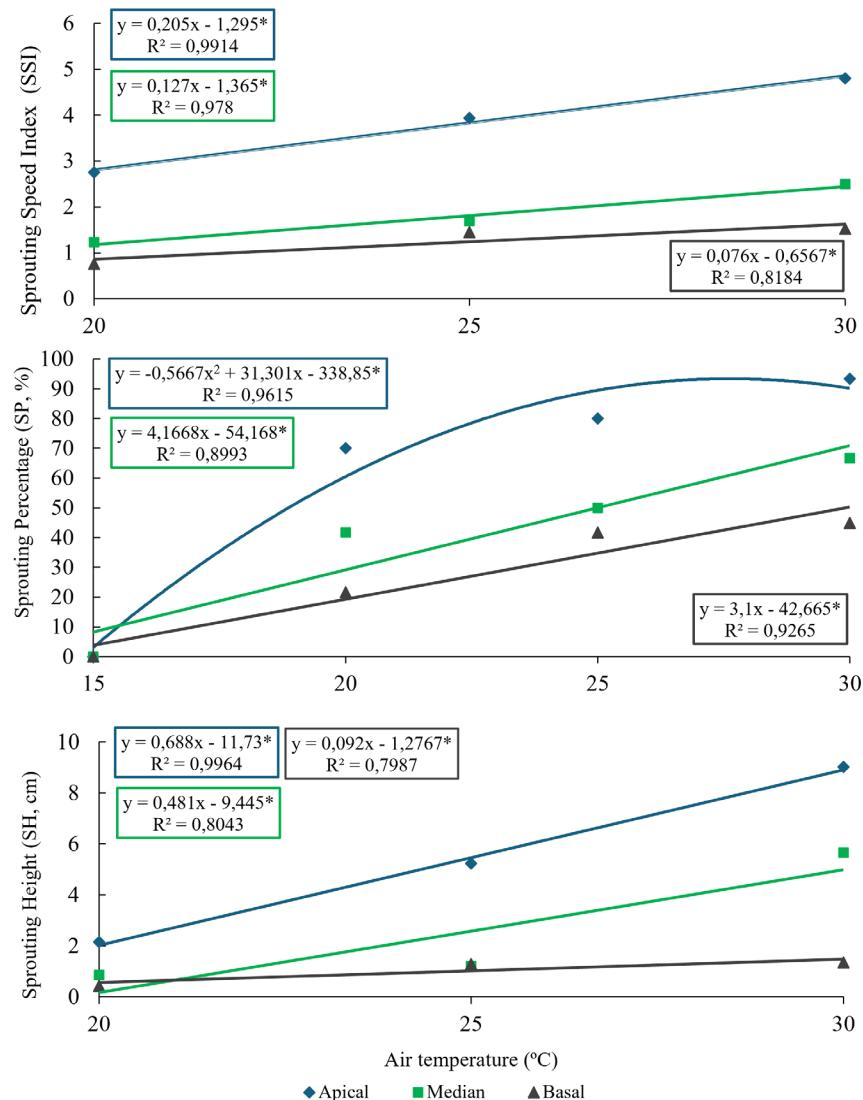


Figure 3 – Sprouting Speed Index (SSI), Sprouting Percentage (SP) and Sprouting Height (SH) of eighteen-month-old sugarcane buds, from different stem position (apical, median and basal) under effect of air temperature (15, 20, 25, 30 °C).

The buds in all positions showed an increase in SSI as the temperature increased, for each unit of temperature there was an increase of 0.20 in the buds in the apical position, 0.12 in the buds in the median position and 0.07 for the basal position. These values present high reliability since the coefficients of determination (R^2) of the three equations were greater than 0.81 (Fig. 3).

The second degree equation was adjusted to evaluate the influence of air temperature on the sprouting percentage (SP) of buds in the apical position (Fig. 3). The buds in the apical position showed an increase in SP as the temperature increased to 27.6 °C, a value corresponding to the maximum inflection point of the regression curve. For the median and basal buds, first-degree equations were adjusted, given that for each temperature unit there was an increase of 4.16% in sprouting in buds in the median position and 3.1% for buds in the basal position. These values show high reliability since the coefficients of determination (R^2) of the three equations were greater than 0.89 (Fig. 3).

First degree equations were adjusted to explain the effect of temperature on the budding height (SH) of the apical, median and basal buds (Fig. 3). The buds from all positions showed an increase in SH as the temperature increased, for each unit of temperature there was an increase of 0.68 cm in the buds in the apical position, 0.48 cm in the buds in the median position and 0.09 cm for the basal position. These values present high reliability since the coefficients of determination (R^2) of the three equations were greater than 0.79.

Furthermore, it was possible to observe that the variables are correlated with each other, that is, buds that presented high SSI also presented high SP and higher SH (Table 5). In six-month-old, it was still possible to observe a positive correlation of medium magnitude (0.76) between SP and SH. In turn, in eighteen-month-old, positive correlations of high magnitude were observed between all variables. These results reveal that mini sets with high SSI indirectly also provide desirable responses in the SP and SH variables, under the temperature conditions evaluated in the two experiments.

Table 5 – Pearson correlations between the variables sprouting speed index (SSI), sprouting percentage (SP) and sprouting height (SH) analyzed in six-month-old (correlations above the main diagonal) and eighteen-month-old (correlations below the diagonal main).

Variable	SSI	SP	SH
SSI	-	0.95*	0.88*
SP	0.97*	-	0.76*
SH	0.90*	0.84*	-

*significant at 5% probability according to the t test.

In experiment 1, where six-month-old sugarcane stalks were used, regardless of the position of the buds on the stalk, at a temperature of 30 °C the highest averages were observed in all variables evaluated (SSI, SP and SH). On the other hand, the best results, that is, the highest means of the variables, were observed in the buds in the median position (Table 2). In experiment 2, 18-month-old culms were used, at a temperature of 30 °C, buds from the apical position presented the highest averages for SSI, SP and SH (Table 4).

Therefore, it can be concluded that younger culms should be prioritized for seedling production using the PSS system, however, it is worth highlighting that in six-month-old culms the best results were obtained in buds with a medium position. According to Manhães et al., 2015, apical buds, with younger tissues, have greater metabolic activity compared to median and basal buds, therefore, they sprout quickly. Aude, 1993, states that younger buds have a higher content of water, nitrogen and glucose (they sprout faster), in contrast to older buds, which have higher levels of minerals and sucrose, which must first be transformed into glucose, demanding time and energy, thus delaying the sprouting of older buds. According to Kakde, 1985, older buds (basal) take relatively longer to sprout, which is consistent with the results obtained in the present work. Landell et al., 2012 state that for the production of seedlings using the MPB system, culms with a physiological age between 6 and 10 months must be used, which confirms the results found in this work.

Carlin et al., 2004 studied the influence of the position of the buds in the sugarcane stalk, concluding that the buds in the apical and median position of the stalk sprouted faster and had a higher sprouting percentage than the buds in the basal position. The authors attributed these responses to the age of the buds, since those located in the apical and median positions were younger and therefore had greater metabolic activity than those located in the basal portion of the stem.

The temperature during sprouting interferes with the speed of biochemical reactions and the action of phytohormones involved in the processes of cell division, differentiation and growth. Whitman et al., 1963, evaluated the effects of light, temperature and water on sugarcane sprouting. The authors emphasized that the optimum temperature for sugarcane sprouting was approximately 30 °C, at 22 °C there was a significant drop in sprouting and between 10 and 16 °C there was no sprouting.

Xavier et al., 2014a stated that temperature interferes with the speed of biochemical reactions and the action of plant hormones involved in cell division, differentiation and growth, therefore, it is one of the factors that most interfere with sprouting. May e Ramos, 2019 ensure that sugarcane buds do not have a good sprouting rate when placed at low temperatures.

Thus, Xavier et al., 2014b recommend a budding temperature of around 30 °C, whereas at temperatures below 15 °C there are problems with speed and percentage of sprouting, in addition to delaying the vegetative growth of the sprouts. Casagrande, 1991 states that vegetative development is favored by air temperatures between 25 and 35 °C.

According to Almeida et al., 2008, air temperature affects sugarcane growth. At temperatures above 20 °C, there is an increase in the crop's growth rate, the range from 25 to 33 °C is the most favorable for vegetative development. Bachi e Souza, 1978 observed in the South/Southeast of Brazil that temperatures between 18 and 20 °C are critical for crop growth.

4. Conclusion

The morphophysiological characters sprouting speed index (SSI), sprouting percentage (SP) and sprouting height (SH) are influenced by air temperature and the position of the bud on the stalk and the age of the seedling. The highest averages for SSI, SP and SH were obtained at 30 °C (ideal temperature) using buds from any position on 6-month-old culms and buds from the apical position on 18-month-old culms. Temperatures below 20 °C, regardless of the position of the bud on the stem, negatively affect the morphophysiological characteristics. For the production of seedlings via the pre-sprouted seedling system, in order to make greater use of the buds, younger culms should preferably be used.

The appropriate temperature for planting sugarcane in a conventional system (setts) or in an PSS system without temperature control is restricted to the months of the year in which the average monthly temperatures are above 20 °C, in colder months, with temperatures below 20 °C, it becomes necessary to adopt artificial temperature control. In the PSS system, it is possible to produce sugarcane seedlings all year round, as long as it is in a controlled environment. Furthermore, it is still possible to standardize the seedlings and implement the sugarcane field during the period of the year when the climatic conditions are most suitable for the crop.

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