



Article

Effects of Planting Pre-Germinated Buds on Stand Establishment in Sugarcane

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Abstract: Sugarcane (a complex hybrid of *Saccharum* spp.) is propagated vegetatively by using stem pieces of mature cane with healthy buds. Abiotic and biotic stress may cause pre-germination of these buds, which may have an impact on both emergence and plant cane stand establishment. There is very limited information available in the literature. A greenhouse study was conducted with single-budded seed pieces of three levels of bud germination (ungerminated buds, Pop-eyes, and Lalas) from three different cultivars (CP 96-1252, CPCL 05-1201, and CPCL 02-0926) planted in pots and repeated over time. Data on growth parameters (tiller count, primary shoot height, SPAD, and dry biomass of shoots and roots) at early growth showed that Lalas produced more tillers and higher shoot dry biomass than Pop-eyes and ungerminated buds. Both Lalas and Pop-eyes produced higher root dry biomass than ungerminated buds in one of the two experiments. The cultivar had a significant effect on primary shoot height and SPAD. A small plot field experiment was conducted with cultivar CP 96-1252 to validate the results of greenhouse experiments, and similar results were reported for tiller count. The results indicate that pre-germinated buds may have a neutral or positive effect on early sugarcane growth and establishment. Further on-farm research needs to be conducted to confirm these results before using pre-germinated buds as a potential seed source for the late season planting of sugarcane.



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Keywords: sugarcane; ungerminated buds; Pop-eyes; Lalas; top visible dewlap leaf (TVD); SPAD (Soil Plant Analysis Development)

1. Introduction

Sugarcane (*Saccharum* spp.) is an important row crop in Florida, with approximately 160,458 ha grown near Lake Okeechobee in southern Florida [1]. Sugarcane is vegetatively propagated using the mature stems (also called stalks) with healthy buds from previous commercial crops or grown specifically for seed cane. Commercial sugarcane in Florida is commonly propagated by laying whole stalks horizontally in the furrow and then chopping each stalk into three to four pieces (billets) with two to three buds on each billet. Buds, also called eyes, sprout when planted in damp soil and, under ideal conditions, primary shoots emerge within 2 to 3 weeks after planting [2]. Hence, seed cane quality (especially healthy buds) is critical to achieving a good crop stand in plant cane. Multiple biotic and abiotic conditions can impact buds and, thus, seed cane quality.

Bud germination before planting (also called pre-germination) occurs especially when the apical growing point dies because of biotic (e.g., insect pest, disease) or abiotic stress factors (e.g., freeze, lodging). This situation ceases the apical dominance and promotes the growth of lateral buds [3]. Based on the level of germination, axillary buds are divided into three categories: ungerminated buds, Pop-eyes, and Lalas (Figure 1). Ungerminated buds do not show any signs of lateral shoot growth or an emergence of green leaf tissue. Some buds only grow bigger and project buds, while others grow 2 to 3 cm, which are known as

Pop-eyes. Pop-eye is a term used to describe the bulging buds on the cane stalk that have the potential to grow into a full plant when planted in soil. Certain axillary or lateral buds may continue to grow into full-fledged Lalas even if the apical bud continues to operate at a very slow rate of growth but has temporarily lost dominance over the lateral buds. At each node, Lalas are pale green side shoots or branches with wiry adventitious roots growing from the buds. At this point, the buds connected with the oldest green leaves are typically fully mature, and their development is blocked by hormones, such as auxins produced by the meristematic tip, which renders the stem's lateral buds dormant [4]. Sugarcane cultivars deal with bud dormancy in different ways. When withered and decaying leaves are plucked off growing cane, the buds continue to grow into side shoots called Lalas and are never dormant [5]. If the leaves are not removed until they die naturally, buds become dormant and likely would not germinate again.



Figure 1. Sugarcane bud germination levels used for the experiment.

There is little understanding of the effects of planting pre-germinated buds on sugarcane stand establishment and early growth, and whether it varies among cultivars. However, some efforts have been made in other countries, such as Brazil, with promising results using pre-germinated buds as a novel method to establish sugarcane [6–9]. We hypothesized that pre-germinated buds may have some negative effect on stand establishment and the effect may vary with genetics. Therefore, a greenhouse study was conducted to evaluate three levels of bud germination for their effect on the early growth of three sugarcane cultivars.

2. Materials and Methods

2.1. Greenhouse Experiment Setup

Two greenhouse experiments were conducted at the University of Florida, Everglades Research and Education Center (EREC) in Belle Glade, Florida, between January 2021 and June 2021 to determine the effect of different levels of bud germination on early sugarcane growth. The soil used for this study came from a field at EREC with a long history (almost 20 years) of sugarcane production. Soil type was Histosol or Dania series muck (Euic, hyperthermic, shallow Lithic Haplosaprist) with pH of 7.0, 80% of organic matter content, and 0.66 Mg m^{-3} of bulk density. To eliminate large clods, topsoil (0 to 10 cm soil layer) was excavated from a stubble-free and weed-free area and put through a sieve of mesh 5 (4-mm holes). The soil was air dried for 24 h before filling into the pots. A total of 54 plastic pots of 6.28-L capacity (20 cm diameter \times 20 cm depth) were then filled with 3.85 kg

of soil. Single-budded seed pieces (with a 2-cm of stalk on either side) were obtained from the middle of mature stalks of three sugarcane cultivars, CPCL 02-0926 [10], CP 96-1252 [11], CPCL 05-1201 [12], and were planted in pots and placed in a greenhouse at EREC. Two seed pieces were planted per pot, widely apart in such a way that the buds on the nodal section of the ungerminated seed piece faced upwards, and the Lalas were kept above the soil surface, and this was conducted similarly for the Pop-eyes. The planted seed pieces were covered with a 3 to 4 cm soil layer. The pots were watered until field capacity (indicated by the drainage of excess water from the pots) and watered at regular intervals (once a week throughout the experiment period). Fertilizers were not applied in this experiment because the soil had enough concentration of all available or mobile nutrients, as reported in lab tests (99.84 mg kg^{-1} P, 470.4 mg kg^{-1} K, $19,036 \text{ mg kg}^{-1}$ Ca, $3028.7 \text{ mg kg}^{-1}$ Mg, and $113.96 \text{ mg kg}^{-1}$ Si). The 54 pots were arranged in groups using a factorial design with sugarcane cultivars and the level of bud germination as two factors. There were six replications of each combination of the two factors. Identical experimental and data-collection procedures were used in both experiments, with the second experiment (March 2021) being a repetition of the first one (January 2021).

2.2. Field Experiment Setup

A field experiment was established in organic soils at EREC (Belle Glade, FL) in March 2022. The experimental design was a randomized complete block design with three treatments (bud germination levels) and four replications. The experimental area comprised of 4 blocks with 3 plots, and each plot was 4.5 m long and 3 rows wide. A 3-row wide alley and 6.09 m long gap was maintained between the blocks and each plot, respectively. With 30.2% of Florida's total sugarcane land under cultivation, CP 96-1252 is the state's most popular commercial sugarcane cultivar and, thus, was used for the field experimental trail [13]. At the time of planting, the sugarcane whole stalks with 3 levels of bud germination (Ungerminated, Pop-eyes, and Lalas) were harvested manually and these stalks were dropped in 15–20 cm deep furrows as pairs. This was followed by chopping the cane stalks into billets (60–90 cm long with 3–4 buds/billet). The furrows were then covered, and other management practices were conducted consistent with the standard commercial sugarcane cultivation in the organic soils of Florida. Data on plant population or tiller count were collected until 95 days after planting to determine the effect of bud germination level on emergence, early growth, and establishment.

2.3. Meteorological Conditions

Whiteman et al. [14] previously documented the environmental impacts on the germination stage in sugarcane. Increases in leaf area were directly associated with the increases in temperature. For sugarcane crop, in the early stages, a temperature of $\sim 30^\circ\text{C}$ is ideal for plant and stalk growth [15]. Since climatic conditions can influence the crop growth rate, for this experiment, average temperatures and daily precipitation were obtained from the EREC weather station positioned 500 m or less from the experimental location (FAWN weather data). Average temperature and daily precipitation data are provided from January to October 2022 to cover the experimental period (March–June) (Figure 2).

2.4. Growth Measurements

In greenhouse studies, the length of the primary shoot was measured from the base of the plant to the base of the top visible dewlap (TVD) leaf to estimate plant height. Starting at 30 days after planting (DAP), data on primary shoot height was collected every week for 10 weeks. The total number of primary, secondary, and tertiary shoots (known as tillers) was counted at 30, 60, and 96 DAP. The leaf Soil Plant Analysis Development (SPAD) readings were recorded at 70 DAP by using a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan). SPAD was measured at three spots (at the base of leaf, in the middle, and at the tip of leaf) on the TVD in each pot and the average value was calculated. During harvest at 100 DAP, the plants were uprooted, and the above ground biomass (stems and

leaves) was cut from the base using sharp scissors. Similarly, roots were separated from seed pieces and washed thoroughly on a sieve of mesh 10 (2-mm holes) to remove adhering soil particles. The shoots and roots were air dried to remove excessive surface moisture and were weighed on a precision balance (Mettler Toledo Balance XPR204S) to collect data on fresh biomass. To estimate dry biomass, the shoots and roots were maintained in a drying room at 60 °C for 10 days until a constant weight was reached. The dry biomass of shoots and roots was measured using the same precision balance. For the field study, tillers (plant population) were counted at 36, 43, 53, and 95 DAP to determine emergence and early season crop establishment. There were no other data collection in this field experiment.

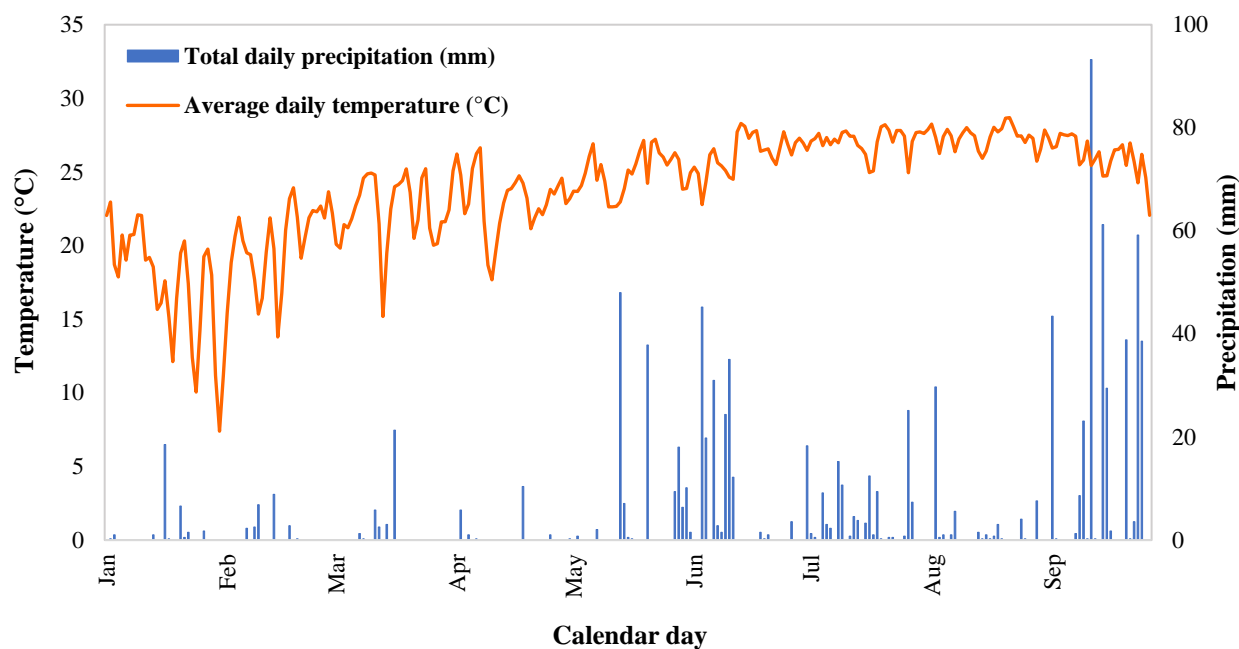


Figure 2. Average temperatures and daily precipitation during the experimental year 2022 (FAWN, 2022).

2.5. Statistical Analysis

Greenhouse data were analyzed using the Proc mixed model in SAS v 9.4 (SAS Institute, Cary, NC, USA). Sugarcane cultivar, bud germination level, and their interactions were considered as fixed effects. Replication and its interaction with other fixed factors were considered as random effects. Means were separated using Tukey's HSD test when treatments and interactions were significant at $p \leq 0.05$. Field data were analyzed using 2-way ANOVA using R programming language (R.4.0.0). Bud germination level treatment was considered as a fixed factor and replication was considered as a random factor. Means were separated using Least Common Difference (LSD) when treatments and interactions were significant at $p \leq 0.05$.

3. Results and Discussion

There was a significant experimental effect on most of the parameters measured in this study. Therefore, data from the first and second greenhouse experiments were analyzed separately. In the first greenhouse experiment, bud germination level showed a significant effect on the number of tillers (primary, secondary, and tertiary shoots) and shoot dry biomass. Cultivar had a significant effect on primary shoot height, SPAD, and root dry biomass, whereas the interaction between the bud germination level and the tested cultivars presented significant effect for SPAD values (Table 1). In the second greenhouse experiment, the bud germination level had a significant effect only on root dry biomass, and cultivar had a significant effect on primary shoot height and root dry biomass. However, there was no significant effect of the interaction cultivar and the bud germination level for any of the measured parameters (Table 1).

Table 1. ANOVA *p*-values for number of tillers and primary shoot height at 96 DAP, SPAD, shoot dry biomass, and root dry biomass in response to sugarcane cultivar and bud germination level.

First Greenhouse Experiment					
Fixed Effects	Number of Tillers	Primary Shoot Height (cm)	SPAD	Shoot Dry Biomass (g)	Root Dry Biomass (g)
Cultivar	0.1378	0.0012 *	0.0002 *	0.0598	0.0452 *
BD level	0.0100 *	0.1325	0.3501	0.0001 *	0.1393
Cultivar × BD level	0.9154	0.5797	0.0584 *	0.1185	0.9582
Second Greenhouse Experiment					
Cultivar	0.1687	<0.0001 *	0.8773	0.1521	<0.0001 *
BD level	0.1012	0.9216	0.9224	0.1942	0.0033 *
Cultivar × BD level	0.9022	0.8500	0.9240	0.3438	0.7147

Asterisk (*) denotes significant differences at $p \leq 0.05$, SPAD: soil plant analysis development, BD level: bud germination level.

3.1. Tiller Count

Lalas produced more tillers per pot (4.61) than Pop-eyes (3.21) and ungerminated buds (2.94) in the first greenhouse experiment, with no significant differences between Pop-eyes and ungerminated buds (Table 2). In the second greenhouse experiment, there was a trend toward increased tiller production with Lalas, but both bud germination level and cultivar had no significant effect on the number of tillers (Table 1). Tillering is primordial for sugarcane as it determines the number of millable canes (NMC). Among the parameters associated with sugarcane yield, the population of stalks present the highest correlation. Therefore, the profitability of the crop depends primarily on the tillers from which the stalks are formed, determining the final number of harvestable stalks [16]. Tillers are functional units, shoots with roots and stem, and leaves that become independent of the mother-shoot and may produce their own tillers, too [17]. Tillering is also influenced by genetic and environmental factors [18]. A higher tillering response in the Lalas and Pop-eye bud germination levels can be attributed to the already existing root and shoot structures. The developed leaves in Lalas and the protruding shoot structures in Pop-eyes can establish faster when planted in soil, and can have a head start with photosynthesis. This might have helped produce more tillers in the given period. In contrast, the ungerminated bud took longer to establish and then produce the primary shoot and tillers within the same time lapse. The genetic makeup of the cultivar also determines its tillering ability. Botanical traits such as bud length, leaf length, and leaf width are higher in CPCL 05-1201, and it has the tendency to produce more stalks (tillers) [12]. Similarly, the sugarcane cultivar CPCL 02-0926 is known to produce more tonnage and is a high yielding cultivar in muck soils [19]. These characteristics support the results where the cultivars CPCL 05-1201 and CPCL 02-0926 reported higher tiller production than CP 96-1252.

Table 2. Mean tiller production and shoot dry biomass in response to different bud germination levels in first greenhouse experiment ^a.

Effect	Treatment	Tillers Pot ^{−1}	Shoot Dry Biomass (g)
Bud germination level	Lalas	4.61 ^a	27.47 ^a
	Pop-eyes	3.21 ^b	19.80 ^b
	Ungerminated	2.94 ^b	17.74 ^b

^a Means followed by different letters in a column are significantly different according to Tukey's HSD at $p \leq 0.05$.

In the field study, the bud germination level had a significant effect on the emergence and tiller production at the dates of data collection. Lalas had significantly higher tiller counts compared to Pop-eyes and ungerminated treatments (Table 3). However, there was no significant difference between Pop-eyes and ungerminated buds. Similar results in the field study validate the results of greenhouse studies. In this sense, Lalas present

an interesting alternative for sugarcane establishment and production, given that profuse tillering is considered as a good crop establishment feature that counteracts eventual hydric stresses and cumulative shooting and tillering failures in the following ratoons [16]. A high number of tillers present a “physiologic compensatory continuum”, which imparts the sugarcane plant an ability to overcome biotic and abiotic stresses [20].

Table 3. Mean number of tillers per hectare (Tillers ha^{−1}) in response to bud germination levels in field experiment ^a.

Treatment	36 DAP	43 DAP	53 DAP	95 DAP
Lalas	82,075 ^a	103,872 ^a	113,021 ^a	240,304 ^a
Pop-eyes	37,135 ^b	47,630 ^b	52,474 ^b	128,620 ^b
Ungerminated	29,332 ^b	41,172 ^b	47,092 ^b	157,960 ^b

^a Means followed by different letters in a column are significantly different, DAP: days after planting.

3.2. Primary Shoot Height

Based on the primary shoot height data collected just before harvest, only cultivar had a significant effect, where CPCL 05-1201 reported taller primary shoots than the other two cultivars in both greenhouse experiments (Figure 3). The temporal variation in the primary shoot height shows that CPCL 05-1201 was similar to the other two cultivars early in the season (first couple of months), and then CPCL 05-1201 outpaced them (Figure 3). Primary shoot height is an affordable visual observation parameter that could be easily used to characterize the variations in crop growth stages, especially in the grass family [21]. Plant height was significantly affected by cultivar. CPCL 05-1201 reported in the second greenhouse experiment the highest values compared to CPCL 02-0926 and CP 96-1252. This could be attributed to genetic differences in plant height and some cultivars may slow in early growth compared to others. Similar findings were reported by Edmé [12] for CPCL 05-1201 cultivar, which reported a higher height in field plantings compared to the reference cultivars CP 78-1628 and CP 89-2143.

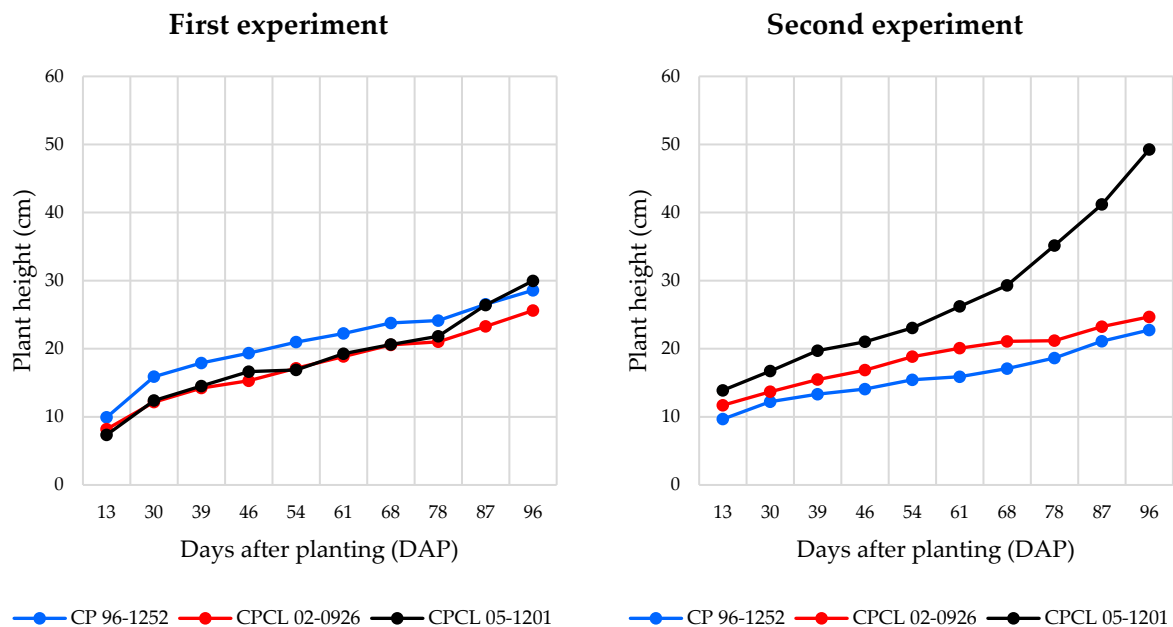


Figure 3. Primary shoot height of three sugarcane cultivars at different days after planting in the first and second greenhouse experiment.

3.3. SPAD

Cultivar and its interaction with the bud germination level showed a significant effect on SPAD values in the first greenhouse experiment (Table 1). SPAD is used to indicate relative

chlorophyll content in the leaves of several crops, including sugarcane [22–24]. Among cultivars, CPCL 02-0926 had statistically significant higher SPAD values (38.11) than CPCL 05-1201 (33.36) and CP 96-1252 (31.98) (Table 4), which can be attributed to genetic differences, suggesting that this cultivar might be more tolerant to heat stress [25] and especially to water stress, according to several studies that have found a high correlation between a high SPAD index and drought tolerance in sugarcane [26–29]. Moreover, CPCL 02-0926 showed an adequate Nitrogen (N) leaf concentration compared with the other two cultivars.

Table 4. Mean SPAD in different sugarcane cultivars in first greenhouse experiment ^a.

Effect	Treatment	SPAD
Cultivar	CP 96-1252	31.98 ^b
	CPCL 05-1201	33.36 ^b
	CPCL 02-0926	38.11 ^a

^a Means followed by different letters in a column are significantly different according to Tukey's HSD at $p \leq 0.05$. CP: Canal Point, CPCL: Canal Point and Clewiston, SPAD: soil plant analysis development.

Among the cultivars and bud germination interactions, Lalas had higher SPAD values (36.18) than Pop-eyes (30.43) in CPCL 05-1201, but there was no significant difference between the three bud germination levels in the other two cultivars (Table 5). In the present study, SPAD readings showed a particular trend for the effects of the cultivars and their interaction with the bud germination level. Interestingly, it was found that Lalas presented an enhanced SPAD readings in cultivars CP 96-1252 and CP 05-1201, and did not significantly affect this parameter in CPCL 02-0926 (Table 5), being the last cultivar with the highest N concentration (Table 4). Taking into account that CP 96-1252 and CP 05-1201 reported SPAD readings below 34 (Table 4), Lalas interaction with these cultivars reached SPAD readings above 34, which is considered the suitable N concentration for sugarcane [30]. In other words, Lalas might represent a convenient effect for sugarcane establishment in terms of more efficient N uptake and therefore better tolerance to water stress.

Table 5. ANOVA p -values for SPAD in response to the interaction between cultivar and bud germination level in first greenhouse experiment ^a.

Cultivar	Bud Germination Level	SPAD
CP 96-1252	Lalas	34.67 ^{bcd}
	Pop-eyes	30.23 ^d
	Ungerminated	31.04 ^d
CPCL 05-1201	Lalas	36.18 ^{abc}
	Pop-eyes	30.43 ^d
	Ungerminated	33.48 ^{cd}
CPCL 02-0926	Lalas	35.86 ^{abc}
	Pop-eyes	39.90 ^a
	Ungerminated	38.57 ^{ab}

^a Means followed by different letters in a column are significantly different according to Tukey's HSD at $p \leq 0.05$. CP: Canal Point, SPAD: soil plant analysis development.

3.4. Shoot Dry Biomass

Shoot dry biomass was higher in Lalas than Pop-eyes and ungerminated buds in the first greenhouse experiment with no significant difference in the second greenhouse experiment. All cultivars produced similar shoot dry biomass in both experiments (Table 1). Higher shoot dry biomass in Lalas (27.47 g) is attributed to higher tiller count and plant height compared to the other bud germination levels (Table 6). Higher shoot dry biomass may eventually result in higher sugarcane yield at maturity [31]. Sugarcane physiology is poorly understood, but root–shoot relationships have the ultimate effect on the yield [32]. Milligan et al. [33] reported a positive correlation between cane yields with stalk characteristics (stalk number and stalk weight). In the present study, the positive response of

shoot dry biomass for the Lalas bud germination level was observed. This statement can be supported by the fact that initial shoot growth of Lalas had a positive response on the total biomass production and accumulation compared to Pop-eye and ungerminated bud levels. Much of the energy produced from photosynthetic activity in Lalas is also consumed in developing new structures, such as new leaves, which contribute for the increased plant height and more biomass in each period compared to ungerminated bud level [17].

Table 6. Shoot dry biomass in response to different bud germination levels in the 2021 first greenhouse experiment ^a.

Effect	Treatment	Shoot Dry Biomass (g)
Bud germination level	Lalas	27.47 ^a
	Pop-eyes	19.80 ^b
	Ungerminated	17.74 ^b

^a Means followed by different letters in a column are significantly different according to Tukey's HSD at $p \leq 0.05$.

3.5. Root Dry Biomass

Root dry biomass was not significantly affected by the bud germination level in the first greenhouse experiment (Table 1), but in the second greenhouse experiment, both Lalas and Pop-eyes had a higher biomass than ungerminated buds (Table 7). The higher root dry biomass values for the bud germination level of Lalas over the other two germination levels (Table 7) were mostly attributed to the presence of pre-existing sett roots at the time of planting. From the discussion above, it is understood that Lalas's germination level is highly efficient in developing new shoot structures within a given period over the other two germination levels. This was similar in developing root structures. The developed sett roots in the first weeks of germination supply water and nutrients to the growing shoot and lead to the production of shoot roots in the later stages [34].

Table 7. Root dry biomass in different sugarcane bud germination levels in second greenhouse experiment ^a.

Effect	Treatment	Root Dry Biomass (g)
Bud germination level	Lalas	13.40 ^a
	Pop-eyes	11.78 ^a
	Ungerminated	8.78 ^b

^a Means followed by different letters in a column are significantly different according to Tukey's HSD at $p \leq 0.05$.

Among cultivars, CPCL 02-0926 had a higher root dry biomass than CP 96-1252 in the first greenhouse experiment. In the second greenhouse experiment, the trend was similar, but CPCL 02-0926 had a significantly higher root dry biomass (14.85 g) compared to the other two cultivars, and even CPCL 05-1201 (12.03 g) had a significantly higher root dry biomass than CP 96-1252 (7.06 g) (Table 8). The data collected from both the trails indicated that sugarcane cultivars had a significant effect on root biomass. In both the trials, CPCL 02-0926 had higher root biomass, which can be attributed to the efficiency of the cultivar to establish itself in the muck soils.

Table 8. Root dry biomass in different sugarcane cultivars in first and second greenhouse experiments ^a.

Effect	Treatment	Root Dry Biomass (g)	
		1st Experiment	2nd Experiment
Cultivar	CP 96-1252	15.74 ^b	17.06 ^c
	CPCL 05-1201	19.63 ^{ab}	12.03 ^b
	CPCL 02-0926	10.51 ^a	14.85 ^a

^a Means followed by different letters in a column are significantly different according to Tukey's HSD at $p \leq 0.05$.

4. Conclusions

Sugarcane planting late in the season often encounters the challenge of poor seedcane quality, which may have been caused by environmental factors such as freeze, lodging due to a hurricane, etc. In the case of freeze damage to the growing point, lateral buds may start germinating into Pop-eyes and Lalas. In the current study, planting of pre-germinated buds (Pop-eyes and Lalas) represent an interesting alternative for efficient sugarcane propagation, as they may provide a head start in early sugarcane growth due to the already existing root and shoot structures. Planting seed cane with Lalas and Pop-eyes may not have any negative effect on yield. Moreover, Lalas show promising performance for a better sugarcane establishment given their higher tillering and shoot dry biomass at early growth (at 3 to 4 months after planting). However, it is important to consider that seedcane used in current greenhouse and field studies was cut manually and handled carefully to avoid any damage to pre-germinated buds. This may not be the case in commercial cane planting in which seedcane is cut mechanically, and mechanical cutting may cause greater damage to pre-germinated buds than ungerminated buds. In greenhouse studies, the Lalas were also kept out of the soil at planting in the pots. Therefore, further on-farm research needs to be conducted to confirm these results before using pre-germinated buds as potential seed source for late season planting of sugarcane.

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