Original Research Article

Development and gas exchange of pre-sprouted sugarcane seedlings in different substrates

ABSTRACT

Objective: Evaluate the development and gas exchange of pre-sprouted sugarcane seedlings in different substrates.
Study design: A randomized block design was used with two genotypes, three types of substrate (2 x 3), four replicates and five plants were treatment, totaling 120 plants.
Setting and duration of study: Department of Crop Sciences of the Center for Agrarian Sciences, *Universidade Federal de Paraíba* in the city of Areia, Brazil, between August and October 2018.
Methods: The following were the treatments: T1 – genotype 1 in commercial substrate; T2 – genotype 1 in commercial substrate + bovine manure; T3 – genotype 1 in bovine manure + sand; T4 – genotype 2 in commercial substrate; T5 – genotype 2 in commercial substrate + bovine manure; T6 – genotype 2 in bovine manure + sand. Variables analyzed: height, diameter, dry mass of shoot and root and physiological response of seedlings.
Results: A significant difference at the 1% level was found for the dry matter of the seedling roots among treatments with difference substrates. A significant difference at the 1% level was also found for shoot length and a significant interaction at the 5% level was found between genotype 1nd substrate for this characteristic. Significant effects were found with regard to the rate of photosynthesis, stomatal conductance, transpiration and carboxylation efficiency.
Conclusion: The substrate with manure + sand favored the accumulation of root dry matter and shoot length. Genotype 1 exhibited its best performance with regard to photosynthesis and stomatal conduction in the treatment with the commercial substrate and demonstrated greater carboxylation efficiency than genotype 2, independently of the substrates. The pre-sprouted seedling production system needs to be explored further to obtain greater information regarding the nutritional requirements and characteristics of the genotypes.

Keywords: Fertilization; Physiological behavior; Bovine manure; Propagation; Saccharum spp.

1. INTRODUCTION

Figuring prominently in the Brazilian economy, sugarcane is the raw material for the production of ethanol and bioenergy and is responsible for more than half of the sugar sold throughout the world [1].

Using pre-sprouted seedlings when planting sugarcane stands out among the new techniques that favor the growth of the sugar-alcohol industry [2]. This method enables a reduction in the volume of stalks per hectare, the uniformity of the crop and the use of a

smaller volume of material in the field, which increases the productivity, longevity and quality of sugarcane fields [2].

The substrate is a fundamental aspect of the formation and quality of seedlings as well as the productivity of the crop. According to Kratz et al. (2013) [3], besides giving sustenance to the seedling, the substrate should enable the adequate functioning of the root system and supply the nutritional requirements necessary to the initial development of the plant.

The use of organic waste from rural properties in the form of substrates for seedling production is a viable way to reduce the costs of a production system [4]. Moreover, using organic waste as substrate benefits the recycling of nutrients, thereby enhancing the productivity of the crop and making the farming system more sustainable [5].

Vegetative growth depends on the capacity of the renewal of air from the substrate through the input of O_2 and the output of CO_2 so that there is no accumulation of toxic gas near the roots [6]. Therefore, knowledge on the physiological behavior of sugarcane in the seedling formation phase can contribute to the adequate management of the plant during the nursery phase.

Due to the potential of the pre-sprouted seedling production system and the scarcity of studies on this topic, the aim of the present study was to evaluate the development and gas exchange of pre-sprouted sugarcane seedlings in different substrates.

2. MATERIALS AND METHODS

This study was conducted at the Department of Crop Science of the Center for Agrarian Sciences, *Universidade Federal de Paraíba* (UFPB) in the city of Areia, Brazil (6°58‟12‟‟S and 35º 42‟ 15‟‟‟W; altitude: 619 m) between August and October 2018.

The experiment was conduct in two environments. The buds were kept for 30 days in a protected environment and subsequently taken to remain another 30 days in direct sunlight. The cultivars used were genotype 1, which stands semi-erect, has good recovery after periods of drought and is quite responsive to irrigation, and genotype 2, which has semi-decumbent growth and rapid development and is tolerant to water stress. Both genotypes are well adapted to northeastern Brazil. The buds employed for the production of seedlings were acquired from UFPB.

A randomized block design was used with two genotypes, three types of substrate (2 x 3), four replicates and five plants were treatment, totaling 120 plants. The following were the treatments: T1 – genotype 1 in commercial substrate; T2 – genotype 1 in commercial substrate + bovine manure; T3 – genotype 1 in bovine manure + sand; T4 – genotype 2 in commercial substrate; T5 – genotype 2 in commercial substrate + bovine manure; T6 – genotype 2 in bovine manure + sand.

According to the manufacturer's specifications, the commercial substrate is composed of turf, corrective elements, vermiculite, vegetal coal and pine bark; has a solid nature (with no granulometric specification), 50% humidity p/p, 150% p/p water retention capacity, density (wet base) of 350 kg/m³, pH 5.8 and carboxylation efficiency of 25 mS/cm. Chart 1 displays the components and chemical characteristics of the bovine manure used.

Chart 1. Chemical analysis of bovine manure acquired from UFPB and cured for one month, Areia, Brazil.

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TC	OC	N	P	K	Ca	Mg
---------- % ----------		---------- g kg-1 ----------				
-	-	17.68	5.52	12.68	18.27	16.31
S	Na	Cu	Zn	Fe	Mn	B
---------- g kg-1 ----------		---------- mg kg-1 ----------				
1.69	-	7.53	53.32	1120.31	83.00	35.70

TC- total carbon; OC- oxidized carbon; N- nitrogen; P- phosphorus; K- potassium; Ca- calcium; Mg- magnesium; S- sulfur; Na- sodium; Cu- copper; Zn- zinc; Fe- iron; Mn- manganese; B- boron. Source: Plant Tissue Analysis Laboratory (UFPB).

Planting was performed in perforated polyethylene bags, each with 400 g of substrate. The buds were planted at a depth of 2 cm and turned upward. In a protected environment, height and diameter were measured weekly using a ruler and calipers, respectively. After being transferred to an environment exposed to direct sunlight, these measurements were taken every two weeks.

At the end of the experiment, shoot length and root length were measured with a metric tape and the dry mass of the shoot and root was determined. For such, the root system and shoot were separated, dried in a forced-air oven at 65 ºC until reaching a constant mass and weighed on a semi-analytical scale for the determination of the mass (g) of the root dry matter (RDM) and shoot dry matter (SDM).

An infrared gas analyzer (Model Li-COR® 6400 XT] was used for the determination of the internal concentration of CO_2 (*Ci*) (µmolm^{-2} s^{-1}), stomatal conductance (*g*s) (mol m^{-2}s^{-1}), transpiration (*E*) (mmol m^{-2} s^{-1}), rate of photosynthesis (*A*) (µmol m^{-2} s^{-1}) and leaf temperature (LT) (°C). The instantaneous water uptake efficiency (A/E) and instantaneous carboxylation efficiency (A/C_i) were then determined. The gas analysis was performed at the end of the experiment prior to the last determination of shoot and root length and dry matter.

For the statistical analysis, root dry matter and height were transformed by $\sqrt{Y+1}$ to meet the premises of analysis of variance (ANOVA). The data were then analyzed using ANOVA and the means of the qualitative data were compared using Tukey's test ($P < 0.05$). When significant, the quantitative data were submitted to polynomial regression analysis ($P \leq 0.05$) using the SISVAR 4.3 statistical package [7].

3. RESULTS AND DISCUSSION

Table 1 displays the ANOVA results for seedling growth (height and diameter) of genotypes 1 and 2 developed in different substrates. A significant difference was found between the two genotypes only with regard to height, whereas the substrates exerted no influence on seedling development in terms of height and diameter. The genotype (G) x substrate (S) x time (T) interactions (G x S, G x T, S x T and G x S x T) were also non-significant. However, time favored the seedling development in terms of height and diameter (1% significance level).

Table 1. ANOVA results for growth characteristics of pre-sprouted sugarcane seedlings (G1 and G2) produced in different substrates (S1- commercial substrate; S2- commercial substrate + bovine manure, S3- bovine manure + washed sand)

Source of variance	Height	Diameter

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Genotype (G)	4.86*	1.21ns
Substrate (S)	0.412ns	0.527ns
Time (T)	83.053**	49.98**
Block	1.668ns	0.703ns
G x S	0.036ns	0.209ns
G x T	1.814ns	0.224ns
S x T	0.691ns	0.367ns
G x S x T	1.40ns	1.016ns
Error	2.187	1.2
Coefficient of variance (%)	23.86	13.98
Regression		
Linear	300.768**	193.409**
Quadratic	3.560ns	3.037ns
Cubic	18.951**	2.555ns

***significant at 1%, *significant at 5%, ns - non-significant, both with F test.*

Table 2 displays the ANOVA results for the final characteristics of seedling development: shoot length, root length, shoot dry matter and root dry matter. No significant differences were found between the two genotypes. In contrast, a significant difference was found among the different substrates with regard to root dry matter and a significant G x S interaction was found among the blocks with regard to shoot length.

Table 2. ANOVA results for growth final characteristics of pre-sprouted sugarcane seedlings (G1 and G2) produced in different substrates (S1- commercial substrate; S2- commercial substrate + bovine manure, S3- bovine manure + washed sand)

Source of variance	SL	RL	SDM	RDM
Genotype (G)	2.337ns	0.423ns	1.458ns	0.474ns
Substrate (S)	0.612ns	0.183ns	0.27ns	6.447**
Bloco	7.554**	0.844ns	2.763ns	0.59ns
G x S	4.619*	0.599ns	1.751ns	0.078ns
Error	95.81	42.99	7.845	0.057
Coefficient of variance (%)	8.03	19.15	24.86	13.24

***significant at 1%, *significant at 5%, ns - non-significant, both with F test, SL: shoot length, RL: root length, SDM: shoot dry matter, RDM: root dry matter of pre-sprouted seedlings of Saccharum officinarum produced in different organic substrates.*

In the analysis of the different genotypes and substrates, genotype 1 had a taller final height (shoot length) compared to genotype 2 only in substrate 3 (bovine manure + sand) (Figure 1-C).

According to Table 1, no significant interaction was found between substrate and genotype with regard to growth during the development of the seedlings (genotype growth was independent of the type of substrate). However, the group of genotype 2 was significantly higher compared to genotype 1 during the establishment period of the seedlings (Figure 1-D). This was likely associated with the genetic characteristics of each genotype, meaning that the genetic characteristics of genotype 2 were more favorable to plant growth than the characteristics of genotype 1.

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Although the physical characteristics and some chemical characteristics of substrates exert an influence on the formation and initial growth of plants, Gazola et al. (2017) [8] point out that there are gaps in the pre-sprouted seedling method that need to be filled and the complex interactions between the substrate and genotype further hamper the obtainment of this information.

Root dry matter was significantly higher when substrate 3 was used in comparison to substrates 1 and 2, whereas no significant difference was found between the latter two substrates (Figure 1-E). It is likely that the bovine manure + sand composition improved the characteristics of substrate 3. Although sand is free of organic matter, it may have improved the physical conditions of the composition by creating porous spaces and increasing the granulation of the substrate, thereby regulating water retention and favoring root development as well as the absorption of the available nutrients [9, 10].

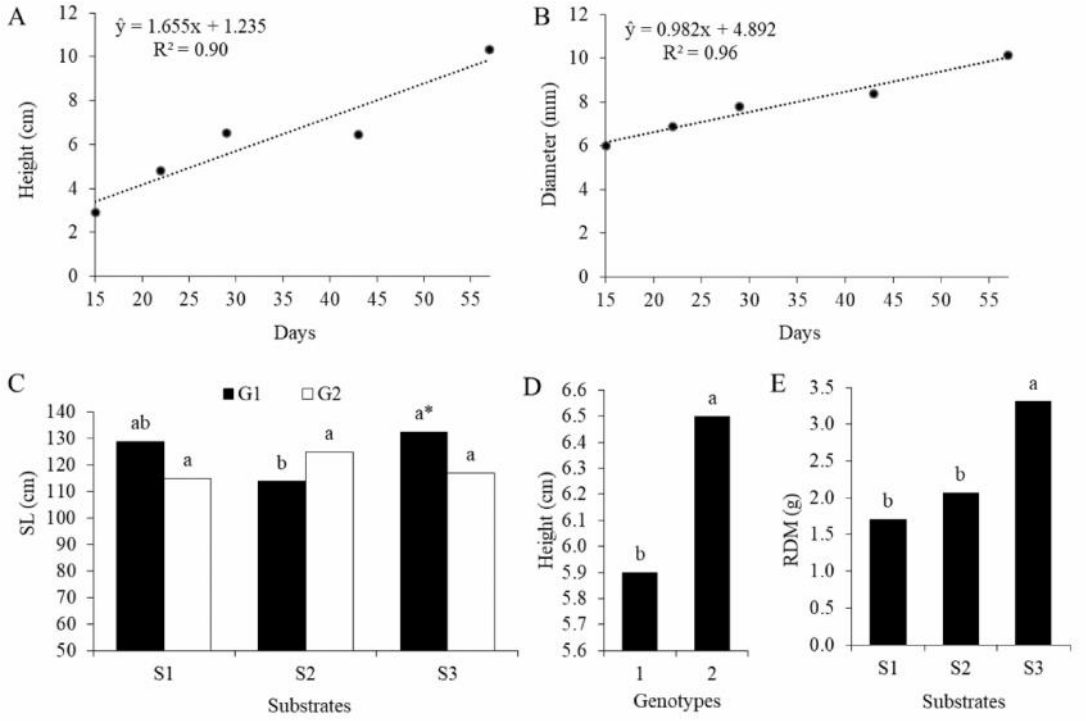

Figure 1. Growth variables of sugarcane seedlings (G1 and G2) produced in different substrates (S1- commercial substrate; S2- commercial substrate + bovine manure, S3- bovine manure + washed sand)

SL: shoot length, RDM: root dry matter

Table 3 displays the ANOVA results for physiological characteristics of the seedlings. Significant differences between genotypes and among the substrates were found with regard to the rate of photosynthesis (A) and a significant G x S interaction was also found for this variable. Significant G x S interactions were found with regard to stomatal conductance, transpiration and leaf temperature. A significant difference between genotypes was found for carboxylation efficiency. In contrast, no significant differences were found among the treatments with regard to internal CO_2 concentration or water uptake efficiency (Table 3).

Table 3. ANOVA results for physiological characteristics of pre-sprouted sugarcane seedlings (G1 and G2) produced in different substrates (S1- commercial substrate; S2- commercial substrate + bovine manure, S3- bovine manure + washed sand)

Source of variance	A	Gs	Ci	E	LT	A/E	A/Ci
Genotype(G)	43.80^{**}	0.20^{ns}	4.02^{ns}	0.5^{ns}	0.71^{ns}	4.96^{ns}	11.47^{**}
Substrate (S)	16.82^{**}	0.71^{ns}	0.610^{ns}	0.974^{ns}	0.87^{ns}	1.31^{ns}	4.23^{ns}
Block	35.38^{**}	1.92^{ns}	0.82^{ns}	3.75^{*}	11.77^{**}	1.75^{ns}	6.72^{**}
G x S	21.44^{**}	4.21^{*}	0.57^{ns}	5.319^{**}	0.25^{ns}	0.33^{ns}	1.89^{ns}
Error	2.03	0.001	2753.09	0.53	0.35	0.089	0.0005
Coeff. of variance (%)	12.51	42.12	20.77	12.82	1.79	20.19	33.4

**significant at 1%, *significant at 5%, ns - non-significant, both by F test, A: rate of photosynthesis, Gs: stomatal conductance, Ci: internal CO_2 concentration, E: transpiration, LT: leaf temperature, A/E: water uptake efficiency, A/Ci: carboxylation efficiency of pre-sprouted *Saccharum officinarum* seedlings produced in different organic substrates

Genotype 1 only exhibited an increase in the rate of photosynthesis when substrate 1 was used, with a significantly higher increase compared to genotype 2 (Figure 2-A). The rate of photosynthesis was significantly higher in genotype 2 in the treatments with the other substrates. Plants with a higher rate of photosynthesis have a greater ability to produce their own food and redistribute it to non-photosynthesizing parts of the plant. By enabling growth, the substrate exerts a direct effect on water retention capacity, porosity, cationic exchange and translocation of water in the soil-plant-atmosphere system, thereby influencing plant development and the rate of photosynthesis [11].

A significant difference between genotypes in stomatal conductance was only found in substrate 1, with genotype 1 exhibiting significantly greater conductance than genotype 2 (Figure 2-B). A reduction in stomatal conductance leads to a reduction in the loss of water through the stomata, but restricts the uptake of CO_2, causing reductions in photosynthesis and respiration [12].

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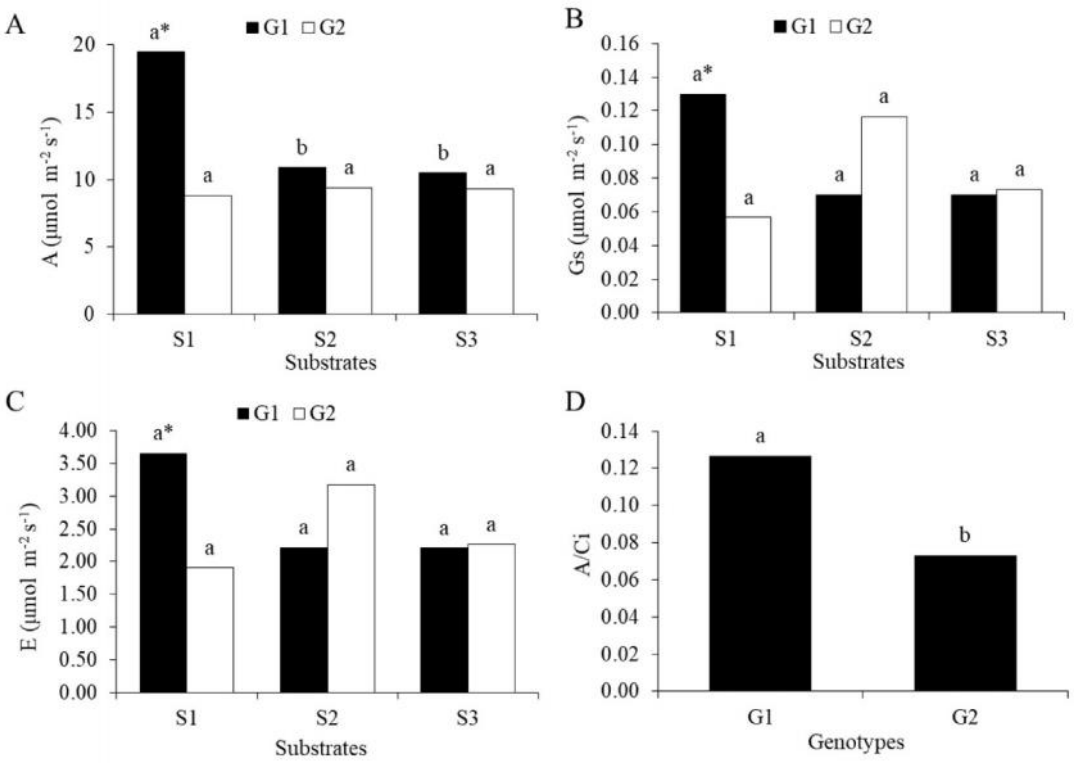

Figure 2. Physiological variables of sugarcane seedlings (Genotypes 1 and 2) produced in different substrates (S1- commercial substrate; S2- commercial substrate + bovine manure, S3- bovine manure + washed sand)

A: photosynthesis rate, Gs: stomatal conductance, E: transpiration, A/Ci: carboxylation efficiency

In the treatment with substrate 1, transpiration (E) (Figure 2-C) was significantly lower in genotype 2 compared to genotype 1, whereas the difference in transpiration was non-significant in the other substrates. Transpiration is influenced by diverse factors, such as climate, species, age and type of soil. Therefore, the physical and chemical characteristics of the commercial substrate may have exerted an influence on transpiration. A reduction in transpiration is a strategy to cope with limited water availability by avoiding the loss of water during the dry season [13]. According to Abad, Martinez and Martinez (1993) [14], an ideal substrate should have a volumetric or apparent density less than 400 g L^{-1}. In the present study, the commercial substrate had this characteristic, which may have contributed to its influence on transpiration.

The carboxylation efficiency of the seedlings was significantly higher in genotype 1 than genotype 2. High internal concentrations of CO_2 indicate an increase in instantaneous carboxylation efficiency due to the availability of ATP and NADPH from the substrate to the RuBisCO. Thus, A/Ci depends on several factors in order for photosynthesis to occur, such as the availability of CO_2 in the leaf mesophyll, amount of light, temperature and enzyme activity [15]. As these factors are related to the genotype, each genotype has greater or lower carboxylation efficiency during seedling development depending on its intrinsic characteristics.

4. CONCLUSION

The substrate formulated with a mixture of bovine manure and sand favored the accumulation of root dry mass and shoot length of the pre-sprouted sugarcane seedlings, which demonstrates the potential of this material for use as an alternative to the commercial substrate in the production of seedlings.

Regarding physiological behavior, genotype 1 exhibited a better performance in the commercial substrate with regard to photosynthesis and stomatal conductance and also demonstrated greater carboxylation efficiency compared to genotype 2, independently of the substrate.

The pre-sprouted seedlings production system has potential in the sugar-alcohol industry, but needs to be explored further to enable greater information on the nutritional requirements of the different genotypes used in this system.

COMPETING INTERESTS

The authors declare no conflicts of interest.

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