Growth of pre-sprouted sugarcane seedlings submitted to supplementary lighting

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ABSTRACT: Brazil is the largest producer and exporter of sugarcane in the world, and the sugar and ethanol sector has invested in the development of new technologies and methods of planting to meet the market demand. Thus, the aim of this study was to evaluate the performance of sugarcane at the acclimation phase and under field conditions, from pre-sprouted seedlings exposed to supplementary lighting, through light-emitting diode, in the period of acclimation in a greenhouse. The experiment was carried out in Carpina, PE, Brazil, from January to September 2017. The experimental plots were composed of pre-sprouted seedlings acclimated in greenhouse, exposed to the supplementary lighting systems, 90% Red + 10% Blue (R:B 90/10), 80% Red + 20% Blue (R:B 80/20), 70% Red + 30% Blue (R:B 70/30) and a control. The evaluation of seedling growth was performed through the measurement of biometric indicators: plant height (cm); stem length (cm); stem diameter (cm) and number of tillers. The biometric indicators showed no significant differences, but plants under lighting systems with a greater proportion of the red wavelength (R:B 80/20 and R:B 90/10) showed higher percentage of survival in the field.

Key words: Sacharum officinarum, light-emitting diode, radiation quality

Crescimento de mudas pré-brotadas de cana-de-açúcar submetidas a iluminação suplementar

RESUMO: O Brasil é o maior produtor e exportador de cana-de-açúcar do mundo e, o setor sucoenergético tem investido no desenvolvimento de novas tecnologias e métodos de plantio de cana-de-açúcar para atender à demanda do mercado. Dessa forma, objetivou-se com esta pesquisa, avaliar o desempenho de cana-açúcar no final da fase de aclimatação e em campo, proveniente de mudas pré-brotadas expostas à iluminação suplementar à base de diodo emissor de luz no período de aclimatação em ambiente protegido. O experimento foi realizado em Carpina, PE, de janeiro a setembro de 2017. As parcelas experimentais foram compostas por mudas pré-brotadas em ambiente protegido, expostas aos sistemas de iluminação suplementar, 90% vermelho + 10% azul (V:A 90/10), 80% vermelho + 20% azul (V:A 80/20), 70% vermelho + 30% azul (V:A 70/30) e o controle. A avaliação do crescimento das mudas foi realizada por meio de indicadores biométricos: altura da planta (cm); comprimento do colmo (cm); diâmetro do colmo (cm) e o número de perfilhos. As variáveis biométricas não apresentaram diferenças significativas, no entanto, as plantas submetidas aos sistemas de iluminação com maior proporção do comprimento de onda vermelho (V:A 80/20 e V:A 90/10), apresentaram maior percentual de sobrevivência no campo.

Palavras-chave: Sacharum officinarum, diodo emissor de luz, qualidade de radiação
**Introduction**

The increase in demand and reduction in sugar stock in the global market should sustain the production of sugarcane for the next decade, which will continue to be the main crop used for sugar manufacturing, especially in developing countries of Africa, Asia and South America.

In order to meet this growing demand, in search of higher yield, the sugar-energy sector has invested in the development of new technologies and methods of planting.

According to Gazola et al. (2017), for the sugar-energy sector to continue to advance in a sustainable manner, new seedling production techniques should be studied to make the process faster. An example is the planting of sugarcane performed with pre-sprouted seedlings, from treated mini-cuttings, replacing conventional planting using stems.

Throughout the growth period, the seedlings underwent the acclimation phase, in which environmental variables can be managed to obtain more resistant and vigorous seedlings. Among these variables, solar radiation has a direct influence on seedling development.

According to Souza et al. (2011), plants grown under intense radiation develop mesophilic cells rich in chloroplast, higher dry matter and higher production. However, these authors state that in addition to intensity, the quality of radiation is also determinant for these cells to develop.

In view of the above, the objective of this study was to evaluate sugarcane performance in the final phase of acclimation and in the field, grown from pre-sprouted seedlings exposed to supplementary lighting with light-emitting diode (LED) in the acclimation period in a protected environment.

**Material and Methods**

The experiment was carried out at the Estação Experimental de Cana-de-açúcar do Carpina (EECAC), Universidade Federal Rural de Pernambuco, in the municipality of Carpina, PE, Brazil (7º 51' 3" S, 35º 15' 17" W and altitude of 184 m), from January to September 2017. The climate is characterized as, with rainfall in the winter and dry season in the summer, according to Köppen’s classification (Alvares et al., 2013). The predominant soil class is Ultisol, with sandy-clay texture.

The experiment was conducted using pre-sprouted seedlings of the sugarcane variety RB92579, acclimated in a protected environment and subjected to supplementary LED-based lighting, with light proportions of 90% red + 10% blue (RB 90/10), 80% red + 20% blue (RB 80/20), 70% red + 30% blue (RB 70/30), and a control, with seedlings exposed to natural solar radiation.

The experimental design adopted in the acclimation phase was the completely randomized, composed of three supplementary lighting systems and a control, which had only natural solar radiation, with five repetitions, each composed of 200 trays of 15 cells, totaling 300 seedlings.

The supplementary lighting system in the protected environment consisted of LED RGB bars, SYM-MT24RGB-S-BAR-24-0.52M model, in red (640 nm) and blue (470 nm) colors, installed 0.5 m above the benches with trays, in wire frame, with light flux intensity adjusted to 500 lux. The bars remained turned on for 16 h (12 h of natural solar radiation + 4 h of supplementary lighting).

At the end of the acclimation phase (40 days), the seedlings were transplanted to PVC boxes, where they remained on cultivation benches under full sun for 15 days, a process known as rustication. At this stage, irrigation was performed every day, by hand, using a plastic watering can, with a water depth of 4 mm per day. In addition, the aerial part was pruned twice every seven days.

Soil tillage for planting the seedlings in the field was carried out 15 days before planting and consisted of harrowing (plowing harrow and leveling harrow) to break soil clods, elimination of crop residues, incorporation of limestone, systematization of the area and subsequent opening of the planting furrows. Fertilization was performed according to the management employed by EECAC, applying 12 kg ha⁻¹ of N, 24 kg ha⁻¹ of P₂O₅ and 18 kg ha⁻¹ of K₂O (Cavalcanti, 2008). Sugarcane plants were grown under rainfed regime. Planting was carried out manually, with spacing of 1.5 m between rows and 0.5 m between seedlings.

In the field, the experimental plots were composed of the pre-sprouted seedlings acclimated in a protected environment, which were exposed to supplementary lighting systems, using a randomized block design with five repetitions. Each experimental plot consisted of 4 rows, with 75 plants per row.

Plant biometric analyses were performed at the end of acclimation (40 days) and at 227 days after planting in the field. Data were collected by sampling, 32 plants per treatment.

In the phase of acclimation in a protected environment, the evaluation of seedling growth considered the following biometric variables: emergence (%), sprouting speed index; leaf area index; plant height (cm); stem length (cm); stem diameter (cm); and root length (cm).

Root volume (mm³) was obtained by the displacement of water volume in a graduated cylinder. The root system was dried in an oven at 60 °C until reaching constant weight, then root dry matter (g) was determined.

The contents of total soluble carbohydrates and sucrose were determined according to the methodology described by Bezerra Neto & Barreto (2011), in which one curve was obtained by standard glucose solution for the total soluble carbohydrate content and another curve was obtained by standard sucrose solution for sucrose content, at the respective concentrations of 25, 50, 100 and 200 mg L⁻¹.

Measurements were taken as follows: stem diameter (cm) with a digital caliper at 20 cm height from the base; plant height (m) with a tape measure from the collar to the inflection point of the +1 leaf; stem length (cm) with a tape measure from the collar of the plant to the last visible stem, and number of tillers per plant, by counting (Oliveira et al., 2016). The number of dead plants was counted and the survival percentages of seedlings (45 DAP) and plants until the beginning of maturation (227 DAP) were calculated.

The experimental period was meteorologically characterized using data of air temperature (°C), air relative humidity (%), rainfall (mm) and global solar radiation (MJ m⁻² d⁻¹), from the meteorological station of the Agência Pernambucana de Águas e Clima (APAC), installed at the EECAC.

The data were subjected to analysis of variance and the means were compared by Tukey test (p ≤ 0.05), using SISVAR software, version 5.6 (Ferreira, 2011).
Results and Discussion

Higher values of emergence percentage and sprouting speed index were found in seedlings subjected to the control (64.00% and 2.96), compared to the supplementary lighting system R:B 80/20 (61.87% and 2.82), but these treatments did not show significant difference (Table 1). In addition to the environmental factors, variety, age, health of buds and management adopted in the planting can affect the sprouting process (Manhães et al., 2015). In this stage of seedling development, the effect of solar radiation has low influence on the development of sugarcane buds; therefore, the different LED lighting systems had little influence during this period, due to the low photosynthetic activity of the seedlings.

In regard to the biometric variables plant height and stem length, higher mean values were observed in the R:B 80/20 system, equal to 48.68 and 12.59 cm, respectively, being significantly higher compared to R:B 90/10. Elia (2016) found pre-sprouted seedling height of 39.4 cm at 51 days after planting in the field. Silva et al. (2014), evaluating different wavelength combinations in in-vitro cultivation of sugarcane under LED-based lighting, observed that seedlings grown under a higher proportion of red were taller.

For stem diameter, there was no significant response between the lighting systems studied, with values ranging from 0.43 (R:B 90/10) to 0.47 cm (R:B 80/20). Cristofoletti Junior (2012) evaluated the emergence of mini-cuttings of nine sugarcane varieties and observed values of plant diameter ranging from 4.45 to 4.89 cm, at 48 DAP. The LAI of the seedlings subjected to supplementary lighting systems (R:B 80/20, R:B 90/10 and R:B 70/30) were higher (4.73, 3.72 and 4.14), but only the R:B 80/20 system differed significantly from the control (3.34).

The highest content of total carbohydrates ($CH_{total}$) was observed in the control (130.29 mg L$^{-1}$), which showed significant difference when compared to the supplementary lighting systems (R:B 80/20, R:B 90/10 and R:B 70/30). There was lower $CH_{total}$ in the R:B 90/10 system (86.43 mg L$^{-1}$). For sucrose, supplementary lighting did not show significant difference.

The use of supplementary lighting caused no significant effect on root dry matter (RDM), root length (RL) and root volume (RV). These results are similar to those observed by Ferreira et al. (2017), who did not find significant differences for root length after the acclimation period of sugarcane exposed to LED lighting in in-vitro cultivation.

Elia (2016) obtained root length of 11.3 cm for pre-sprouted sugarcane seedlings cultivated without irrigation, at 51 days after planting in the field. According to Silva et al. (2016), the effects of light spectrum quality on root dry matter production, in general, are still contradictory and inconclusive.

Figure 1 shows the daily mean variation of rainfall and global solar radiation (Rg) along the period. The total rainfall in the period was 932.2 mm. The highest values of monthly rainfall were observed in January and February.

Table 1. Values of the biometric variables of the seedlings at the end of the acclimation phase (40 days after emergence, DAE), for different supplementary lighting systems

| Biometric variables                  | R:B 80/20 | R:B 90/10 | R:B 70/30 | Control | CV (%) |
|--------------------------------------|-----------|-----------|-----------|---------|--------|
| Emergence (%)                        | 61.87 a   | 42.40 b   | 44.00 b   | 64.00 a | 14.40  |
| Sprouting speed index                | 2.82 a    | 1.91 b    | 2.06 b    | 2.96 a  | 13.72  |
| Plant height (cm)                    | 48.68 a   | 44.20 b   | 45.62 ab  | 47.26 a | 4.16   |
| Stem length (cm)                     | 12.59 a   | 11.32 b   | 11.51 ab  | 12.01 ab| 5.08   |
| Stem diameter (cm)                   | 0.47 a    | 0.43 a    | 0.44 a    | 0.45 a  | 5.65   |
| Leaf area index – LAI                | 4.73 a    | 3.72 ab   | 4.14 ab   | 3.54 b  | 14.06  |
| Total carbohydrates – $CH_{total}$ (mg L$^{-1}$) | 103.44 b | 86.43 c   | 109.78 b  | 130.29 a| 8.69   |
| Sucrose – SUC (mg L$^{-1}$)          | 178.38 a  | 143.97 a  | 187.71 a  | 206.36 a| 24.22  |
| Root dry matter – RDM (g)            | 0.157 a   | 0.210 a   | 0.229 a   | 0.235 a | 18.25  |
| Root length – RL (cm)                | 15.18 a   | 14.16 a   | 14.27 a   | 15.32 a | 8.78   |
| Root volume – RV (cm$^3$)            | 7.0 a     | 10.0 a    | 8.0 a     | 7.0 a   | 30.19  |

R:B 80/20 - 80% Red + 20% Blue; R:B 90/10 - 90% Red + 10% Blue; R:B 70/30 - 70% Red + 30% Blue; Control - Seedling exposed to natural solar radiation; Means followed by the same letter in the row do not differ by Tukey test (p ≤ 0.05)

Figure 1. Daily variation of rainfall and global solar radiation (Rg) along the experimental period.
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Rainfall occurred between May and July, which corresponded to 65.4% of the total accumulated along the period evaluated. The lowest monthly rainfall occurred in February (33.5 mm).

According to Meneses & Resende (2016), one of the main factors affecting sugarcane yield in the Coastal Tablelands of Northeast Brazil, consists of the irregular distribution of rainfall, so it is necessary to manage the crop so that the periods of highest water demand, such as the vegetative growth stage, coincide with the highest availability of water in the soil. In the present study, the period of planting the seedlings coincided with the month of lowest rainfall. The period of sugarcane planting is one of the most relevant aspects to have good final yield or good stand of seedlings.

The average global solar radiation (Rg) was 25.11 MJ m$^{-2}$ d$^{-1}$, with minimum of 8.44 MJ m$^{-2}$ d$^{-1}$ and maximum of 37.85 MJ m$^{-2}$ d$^{-1}$. The month of February had the highest monthly average of Rg (29.18 MJ m$^{-2}$ d$^{-1}$) (Figure 1). In the vegetative development stage, processes that depend on solar radiation, such as tilling, are favored by intense solar radiation (Marin et al., 2009).

The lowest monthly average values of Rg were observed in May and July, with values of 22.55 and 18.95 MJ m$^{-2}$ d$^{-1}$, respectively. For good growth and development of the crop, days with average global solar radiation above 18 MJ m$^{-2}$ d$^{-1}$ are necessary. Solar radiation is the main environmental variable influencing dry matter accumulation, when the requirements of water, nutrients and health are met (Ferreira Júnior et al., 2014).

The average daily air relative humidity (RH, %) observed in the period ranged from 80.7% to 94.6% (Figure 2).

Table 2 shows the percentage of survival (%S) of seedlings in the field at 45 and 227 days after planting (DAP). Plants grown from seedlings subjected to supplementary LED lighting systems R:B 80/20 and R:B 90/10 had higher survival percentage (p ≤ 0.05) compared to the R:B 70/30 system and the control, at 45 DAP. Rocha et al. (2013) found that seedlings grown in vitro under red LEDs showed higher percentage of survival when transplanted to the acclimation phase. Among the supplementary lighting systems in the present study, the R:B 80/20 stood out, as it had survival percentage of 90.91%.

At the end of the experimental period (227 DAP), there was a reduction in the percentage of survival of the plants at the beginning of maturation, formed from seedlings subjected to supplementary lighting systems, among which the R:B 80/20 system showed higher survival percentage (72.87%), followed by R:B 90/10 (67.83%) and R:B 70/30 (62.40%). The control (natural solar radiation) had a survival percentage of 51.84%.

The average air temperature (Tair, °C) along the experimental period ranged from 21.2 to 27.7 °C, with daily average of 24.6 °C (Figure 2). Oliveira et al. (2011), in a study conducted in the municipality of Carpina, PE, Brazil, found an average annual temperature higher than 25 °C. The highest values of Tair were observed in February and March (26.6 and 26.5 °C). Conversely, the lowest value was recorded in July, with an average Tair of 22.2 °C. It can be noted that, during the studied period, Tair was not a restrictive factor for sugarcane growth and remained within the range (20-30 °C) considered optimal for its development in the field; with values below 16 °C and above 38 °C, its growth would become null (Marin et al., 2009).

Table 2. Values of the number of dead plants (NDP) and percentage of survival (%S) at 45 and 227 days after planting (DAP)

| System   | 45 DAP | 227 DAP |
|----------|--------|---------|
|          | NDP    | %S      | NDP    | %S      |
| R:B 80/20| 27.27 a| 90.91 a | 81.30 a| 72.87 a |
| R:B 90/10| 36.84 a| 87.72 a | 96.51 a| 67.83 a |
| R:B 70/30| 71.79 a| 76.07 b | 112.80 a| 62.40 a |
| Control  | 51.84 a| 82.72 b | 112.14 a| 62.62 a |
| CV       | 9.06   | 11.92   | 10.35  | 13.40   |

R:B 80/20: 80% Red + 20% Blue; R:B 90/10: 90% Red + 10% Blue; R:B 70/30: 70% Red + 30% Blue; Control: Seedling exposed to natural solar radiation; Means followed by the same letter in the column do not differ significantly by Tukey test at p ≤ 0.05

Figure 2. Daily variation of air relative humidity (RH) and air temperature (Tair) along the experimental period.
by R:B 90/10 (67.83%), with the lowest values in R:B 70/30 (62.40%) and in the control (62.62) (Table 2). Elia (2016) found total mortality of 37.9% at 156 DAP in pre-sprouted seedlings cultivated without irrigation. The author emphasizes the importance of irrigation immediately after planting to ensure the establishment of seedlings.

Plants subjected to lighting systems with higher proportion of red wavelength (R:B 80/20 and R:B 90/10) had the highest %S. Ferreira et al. (2017), when studying the effects of LED lighting on sugarcane acclimation in in-vitro cultivation, found that plants grown under LEDs (82% red and 18% blue) increased the percentage of survival after being transplanted. The spectrum in the red range is close to the peaks of maximum light absorption of chlorophyll and phytochromes; for this reason, it becomes important for the photosynthetic apparatus for accumulation of carbohydrates and increase in the development of shoots (Rocha et al., 2010).

Table 3 shows data of biometric variables evaluated at 227 DAP. For all variables studied, there were no significant differences between plants grown from seedlings associated with supplementary LED lighting systems and the control. The values of PH and SD obtained in this study are higher than those found by Oliveira et al. (2014), 224.67 and 2.65 cm, respectively, for sugarcane cut at 11 months.

Kim et al. (2004) point out that morphological or physiological adaptations are likely to occur in leaves when exposed to different types of lighting along growth, and such adaptations confuse the interpretation of single spectral effects.

The effects that each specific wavelength causes on sugarcane development, as well as its interaction with other meteorological variables, are complex and vary according to the cultivation environment, development stage and variety.

Although in the acclimation phase the supplementary lighting system R:B 80/20 resulted in greater growth of the seedlings, there were no significant differences in the field. Oliveira et al. (2014) in a study with different sugarcane cultivars concluded that the biometric variables plant height and diameter were the ones that most influenced the calculation of yield (TCH, t ha⁻¹). For Lin et al. (2013), the yield and quality of the plants are the result of interactions between various environmental factors under which plants are grown.

### Table 3. Values of sugarcane biometric variables: plant height (PH), stem length (SL), stem diameter (SD) and number of tillers (NT) at 227 days after planting (DAP)

|        | 227 DAP |
|--------|---------|
|        | PH      | SL      | SD      | NT      |
| R:B 80/20 | 327.26 a | 214.46 a | 2.73 a  | 8.2 a   |
| R:B 90/10 | 316.66 a | 214.86 a | 2.60 a  | 7.5 a   |
| R:B 70/30 | 289.26 a | 183.80 a | 2.50 a  | 7.2 a   |
| Control   | 299.33 a | 191.46 a | 2.55 a  | 6.9 a   |
| CV        | 9.33    | 15.57   | 5.94    | 20.14   |

*R:B 80/20 - 80% Red + 20% Blue; R:B 90/10 - 90% Red + 10% Blue; R:B 70/30 - 70% Red + 30% Blue; Control - Seedling exposed to natural solar radiation*

### Conclusions

1. The dichromatic LED-based supplementary lighting systems 80% red + 20% blue and 90% red + 10% blue led to seedlings with higher percentage of plant survival in the field, but without significant responses in terms of biometric variables.

2. The biometric responses of the seedlings at the end of the acclimation phase did not result in plant growth at 227 days after planting.

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