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Irrigation strategies of sugarcane seedlings from micropropagation and biofactory methods

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Abstract. The sugarcane industry has been suffering from unstable productivity on commercial fields. The major factors causing this problem are mechanized harvesting damage to cane clumps in the field and the slow process of releasing and adopting new sugarcane cultivars. By utilizing new micropropagation processes involving the extraction of apical meristem from new cultivars and biofactory methods for multiplying the material, it is possible to produce an extraordinary number of sugarcane seedlings to provide nurseries rapidly with new cultivars for planting on commercial fields. The goal of this study was to evaluate several irrigation strategies (IS) to determine the best one for supplying the biofactory sugarcane seedlings water requirements, under conditions of different volumes of substrate (VS): 56, 73, 93 and 125 cm^3. The irrigation management experiment comprised eight IS based on different periods of accumulated reference evapotranspiration (ET_o). We found that the irrigation application must occur at intervals below 30 mm of accumulated ET_o. IS1 (maintenance of soil moisture at field capacity) results in a larger number of tillers, longer extension of the primary stalks, and enhanced dry matter (DM) yield independent of VS. The VS factor accounted for statistical differences in sugarcane survival rate and morphological characteristics, but only for low initial soil moisture conditions. The intermediate VS of 73 cm^3 was the best option for plants to thrive in the field; larger VS (93 and 125 cm^3) produced young plants with many leaves, which transpire a lot in the field, increasing the chances of early death under water stress after planting; the smaller VS (56 cm^3) resulted in young plants with small root systems and minimal water reservoirs, resulting in lower survival under drought conditions.

Keywords: *Saccharum* spp., soil moisture, volume of substrate, agricultural water management.

HIGHLIGHTS

- Different irrigation strategies (IS) for the planting and management of micropropagated sugarcane seedlings in different volumes of substrate (VS) were evaluated.
- IS1 (maintenance of soil moisture at field capacity) resulted in a larger number of tillers, longer extensions of primary stalks, and enhanced dry matter (DM) yield independent of VS.
- VS accounted for differences in sugarcane survival rate and morphological characteristics, but only for low initial soil moisture conditions.
- The intermediate VS of 73 cm^3 was the best option for plants to thrive in the field.
- Larger VS (93 and 125 cm^3) and smaller VS (56 cm^3) resulted in young plants with similar problems surviving in the field under drought conditions.

1. INTRODUCTION

The sugarcane (*Saccharum* spp.) planting operation is defined according to the multiplication technique that is used (Rocha and Sparovek, 2021; Santos et al., 2022). When the material multiplication is carried out through the plant's own vegetative structures, a previously cultivated sugarcane field, called a nursery, is used as the source of planting material. From this nursery, seedlings can be removed manually or by mechanical harvesters adapted to the task. Other sugarcane multiplication techniques are known and used by growers to provide higher proliferation rates. The tissue culture method of in vitro micropropagation stands out as very favorable (Silva et al., 2018; Matoso et al., 2021). In addition to improving the quality of the product, it enables the propagation of plants free of viruses and other diseases, promotes the maintenance of the characteristics of the matrix plant and the optimization of productivity, while providing rapid, large-scale multiplication of seedlings in so-called biofactories (Peloia et al., 2019).

This biotechnological process needs to be well defined and to operate with appropriate technology, for production on a commercial scale. When planted in the field, micropropagation seedlings already have a good leaf area and root system contained in a substrate, while sugarcane seedlings produced vegetatively (stalks with leaf buds) can only generate new plants if conditions are favorable (Castro et al., 2019; Hu et al., 2022). The presence of leaves results in evapotranspiration that can cause stress from moisture loss. If the water available in the soil or in the substrate is too little for survival of the seedlings, there will be irreversible losses in the plant population and subsequent financial losses (Tavares et al., 2018; Maldaner et al., 2020).

Thus, whether considering nurseries for micropropagation of sugarcane seedlings or another methodology in which the plants already have aerial parts, a type of irrigation must be adopted that guarantees vegetative development of the plants and growth of the stalks, leaving sufficient water available in the soil during periods of higher temperature and longer photoperiod (Aquino et al., 2018; Farias-Ramírez et al., 2024). Given this need of the production sector to renew areas with reduced productivity by introducing new cultivars with higher productive potential (Sanches et al., 2019; Poudial et al., 2022; Barbosa et al., 2024), this research aimed to evaluate different irrigation strategies (IS) for the planting and management of micropropagated sugarcane seedlings in different volumes of substrate (VS).

2. MATERIAL AND METHODS

2.1. Location and characterization of the experimental area

The study was conducted in 2013 at the University of São Paulo, located in the municipality of Piracicaba, São Paulo State, Brazil (22°46'39" S, 47°17'45" W) at an altitude of 570 m a.s.l. The experimental units (plots) were distributed in a rain-out shelter with an area of approximately 160 m^2 containing 96 asbestos-cement boxes of 100 L each (Cherri et al., 2019), with dimensions of 0.60 x 0.40 x 0.45 m (Figure 1A), distributed in four strips spaced 0.80 m between rows and 0.50 m between boxes (Figure 1B). The soil used to fill the boxes was classified as an Oxisol (Typic Ustox) with a sandy loam texture, the predominant soil in sugarcane growing areas in Brazil. The physical-water characteristics of the soil are shown in Table 1.

The irrigation system consisted of the following components: (i) a five-hundred-liter reservoir, (ii) polyethylene pipes, (iii) a KSB-C500N motorized pump, (iv) two disk filters, and (v) four control heads with eight registers, responsible for controlling the flow of the experimental units. Two self-compensating button surface drippers of 8 L h^{-1} each were installed in each asbestos-cement box, and for better distribution of the flow of each dripper and standardization of the wetted area, a discharge divider with two rods was connected to each dripper (Figure 1C).

The following climatic elements were monitored: global solar radiation (MJ m^{-2} day^{-1}) using a pyranometer (LP02-L12, Campbell Scientific), air temperature (°C) and relative humidity (%), using sensors (Vaissala

Table 1. Physical and hydric characterization of soil in four layers.

Layers m	Granulometric fractions			U_{FC}	U_{PWP}	D_s	D_p	AWC mm	TP %
	Sand	Silt	Clay						
	%			g g^{-1}		g cm^{-3}			
0 - 0.10	75.1	7.8	17.1	0.148	0.069	1.53	2.65	12.11	42.3
0.10 - 0.20	74.5	8.0	17.5	0.151	0.065	1.50	2.65	12.81	43.4
0.20 - 0.30	74.5	8.0	17.5	0.151	0.065	1.50	2.65	12.81	43.4
0.30 - 0.40	74.4	8.6	17.0	0.143	0.078	1.69	2.64	10.88	36.0

U_{FC}: moisture at field capacity (corresponding to a matric potential of -4.85 kPa, according to Tapparo et al., 2019); U_{PWP}: moisture at the permanent wilting point (corresponding to a matric potential of -1500 kPa); AWC: available water capacity; D_s: soil bulk density. D_p: soil particle density; TP: total soil porosity.

Figure 1. Photos showing details of the experimental area. Asbestos-cement boxes (100 L) with dimensions of 0.60 x 0.40 x 0.45 m (A). Distribution of asbestos-cement boxes in four rows spaced 0.80 m between rows and 0.50 m between boxes (B). Self-compensating button surface drippers and discharge divider with two rods (C). Sensors installed in the center of the experimental area and above the crop canopy (D). Tensiometers installed at depths of 0.10, 0.20, 0.30 and 0.40 m (E). Digital vacuometer used in the experiment (F).

HMP45C-L12, Campbell Scientific). Three sensors were installed in the center of the experimental area and above the crop canopy (Figure 1D). Data were monitored by a data acquisition system (datalogger), with averages stored every 15 minutes.

2.2. Experimental design, treatments and irrigation management

A randomized block design was used in a factorial scheme (4 x 8), in three blocks, for the 96 boxes available for the experiment. Each experimental plot was represented by a box containing four micropropagated sugarcane seedlings approximately 60 days old. The treatments were four VS, 56, 73, 93 and 125 cm^3 for seedling production (Figure 2) and eight IS.

For the IS, eight treatments were defined from IS1 to IS8. In four of them, the seedlings were planted with the soil initially moist at an initial irrigation depth of 30 mm (from IS1 to IS4). The 30 mm depth was adopted because most sugarcane mills have winding reels for irrigation of the micropropagated seedling nursery. This equipment performs the application at a fixed irrigation depth and, in order to supply sufficient water to the plants, a depth of 30 mm is recommended since a large part of the water is lost through evaporation. For the other IS (from IS5 to

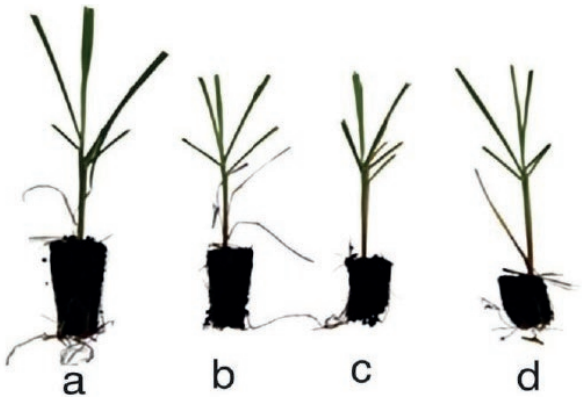

Figure 2. Seedlings produced in each type of container with different volumes of substrate (VS). Seedlings with 125 (a), 93 (b), 73 (c) and 56 cm^3 (d) of VS.

IS8), seedlings were planted in dry soil, without setting an irrigation depth after soil stabilization in the experimental unit. Subsequently, IS1 was maintained with the soil at field capacity throughout the entire experiment. The 30 mm irrigation depth applied in each pre-fixed strategy for different periods of accumulated reference evapotranspiration (ET_o) are detailed in Table 2.

As a more suitable parameter for the IS treatments based on the water demand in each period, the ET_o was estimated using the standard method (Penman-Monteith) proposed by the FAO (Allen et al., 1998). To define the irrigation dates for the treatments over time, the accumulated ET_o in mm was calculated and the fixed irrigation depth (ID_{30}) was achieved through measurements of the accumulated ET_o during different periods. Therefore, IS2 received seven ID_{30} spaced every 30 mm of accumulated ET_o, starting with 15 mm of accumulated ET_o plus the initial ID_{30}. IS3 received two ID_{30} spaced every 95 mm of accumulated ET_o plus the initial ID_{30}. IS4 received two ID_{30}, initial and another with 130 mm of accumulated ET_o. IS5 received seven ID_{30} spaced every 30 mm of accumulated ET_o and starting with 10 mm of accumulated ET_o. IS6 received five ID_{30} spaced every 40 mm of accumulated ET_o and starting with 15 mm of ET_o accumulated. IS7 received three ID_{30} spaced every 70 mm of accumulated ET_o and starting with 30 mm of accumulated ET_o. IS8 received two ID_{30} spaced every 100 mm of accumulated ET_o and starting with 40 mm of accumulated ET_o.

The irrigation management for IS1 was carried out based on soil moisture data obtained from tensiometer readings. Tensiometers were installed for all IS1 treatments (12 boxes) at depths of 0.10, 0.20, 0.30 and 0.40 m, totaling 48 tensiometers (Figure 1E). Readings were taken within a maximum interval of three days using a digital vacuometer (Figure 1F). Based on the mean readings obtained from the soil water matric potential, the irrigation necessary to bring the soil moisture to field capacity was calculated using the van Genuchten approach (van Genuchten, 1980), according to Equation 1:

$$\theta(\Psi_m) = \theta_r + \frac{(\theta_s - \theta_r)}{(1 + (\alpha \times \Psi_m)^n)^m} \quad (1)$$

where θ (ψm) is the soil volumetric water content (cm^3 cm^{-3}), θ_r is the soil residual volumetric water con-

Table 2. Irrigation strategies (IS) used in the study with applications of irrigation depths during different periods of accumulated reference evapotranspiration (ET_o).

Accumulated ET_o mm	Days after planting (DAP)	IS1	IS2	IS3	IS4	IS5	IS6	IS7	IS8
0	0	ID_{30}	ID_{30}	ID_{30}	ID_{30}				
10	4	ID_{FC}				ID_{30}			
15	6	ID_{FC}	ID_{30}				ID_{30}		
30	12	ID_{FC}						ID_{30}	
40	18	ID_{FC}				ID_{30}			ID_{30}
45	22	ID_{FC}	ID_{30}						
55	26	ID_{FC}					ID_{30}		
70	36	ID_{FC}				ID_{30}			
75	39	ID_{FC}	ID_{30}						
95	50	ID_{FC}		ID_{30}			ID_{30}		
100	53	ID_{FC}				ID_{30}		ID_{30}	
105	57	ID_{FC}	ID_{30}						
130	80	ID_{FC}			ID_{30}	ID_{30}			
135	83	ID_{FC}	ID_{30}				ID_{30}		
140	86	ID_{FC}							ID_{30}
160	100	ID_{FC}				ID_{30}			
165	104	ID_{FC}	ID_{30}						
170	107	ID_{FC}						ID_{30}	
175	110	ID_{FC}					ID_{30}		
190	121	ID_{FC}		ID_{30}		ID_{30}			
195	124	ID_{FC}	ID_{30}						

ID_{FC}: irrigation depth to maintain soil moisture at field capacity; ID_{30}: 30 mm fixed irrigation depth.

tent ($cm^3\,cm^{-3}$), θ_s is the volumetric water content of the saturated soil ($cm^3\,cm^{-3}$), m and n are the regression parameters of equation (dimensionless), α is the parameter with dimension equal to the inverse of the tension (kPa^{-1}) and ψ_m is the function of the matric potential (kPa).

2.3. Planting, crop management, and evaluated features

The sugarcane cultivar selected for the experiment was RB93509 due to its ready availability in the market. According to the Technical Bulletin of the Interinstitutional Network for the Development of the Sugar and Alcohol Sector (RIDESA), this cultivar has medium resistance to drought, good tillering in plant cane and ratoon cane. At the end of the micropropagation procedures, the seedlings were transplanted into four models of trays filled with substrate, based on pine bark and coconut fiber, into which a solution of a hydroretentive polymer was mixed with the substrate at a concentration of 5 g L^{-1}. The trays used had four VS options, 56, 73, 93 and 125 cm^3 (according the treatments).

The seedlings were transplanted with an approximately similar leaf area; pruning was performed, to reduce all plants to ~20 cm in height. This procedure of reducing the leaf area is carried out on commercial sugarcane plantations to reduce transpiration. The upper surface of each box was divided into four quadrants of equal area and a hole was made in the center of each quadrant slightly larger than the root system of the seedlings, for the seedlings to be planted.

The experiment ran from April to August, ending 141 DAP. The tillering intensity in all treatments was measured by counting the number of tillers (NT), with a complete tiller being considered the sprout formed from the planted seedling, including the primary stalk. This evaluation was done at 141 DAP. For determining the NT, the average of the four plants in the experimental unit was used. Using a measuring tape, the maximum extension of the primary stalk (MEPS) from the soil surface to the tip of the highest leaf (stretched manually) was determined at 141 DAP, this measure being representative of the total growth of the plant, both stalk and leaves. For plant survival analysis, the percentage of live clumps was determined at 30, 50, 80 and 120 DAP. A dead clump was one that did not show any green leaves, not even the cartridge leaves, or on both the tillers and the primary stalk, and no emission of new tillers. Plants were collected for dry weight determination at the end of the experiment. To measure the dry matter (DM) of the clumps, all tillers present in the experimental plot were cut. To obtain the dry weight, after the collection procedure, the material was dried in an oven with a forced hot air circulation system at a temperature of 65°C until the moisture level reached a constant value. To calculate the total DM weight, the total weight of each plant in the experimental unit was determined.

2.4. Data analysis

For the analysis of variance, we verified whether the statistical assumptions of the main effects were additive, and the independent, normally distributed errors and the homogeneous variances were satisfied. The evaluated parameters were checked for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The data were statistically analyzed using analysis of variance, splitting the analyses whenever the interaction was significant based on Tukey's test at a 5% probability level.

3. RESULTS AND DISCUSSION

3.1 Meteorological data and irrigation water applied

During the experimental period, the average temperature was 20.7°C and relative humidity was 72.9%. The maximum temperature was 39.7°C at 140 DAP and the minimum of 4.3°C occurred at 78 DAP. The maximum relative humidity was 98.1% at 76 DAP and the minimum was 15.2% at 127 DAP. The maximum and minimum values of global solar radiation occurred at 3 DAP (11.4 MJ m^{-2} day^{-1}) and 59 DAP (0.6 MJ m^{-2} day^{-1}), respectively. The transparent plastic cover (diffuser film), and a black screen on the sides intercepted 30% of the incident radiation as reported by Costa et al. (2015) and Chaves et al. (2021). The daily ET_o calculated by using the Penman-Monteith method ranged between 0.6 mm day^{-1} (59 DAP) and 2.7 mm day^{-1} (1 DAP). The vapor pressure deficit (VPD) fell between 0.2 kPa (59 DAP) and 2.6 kPa (118 DAP) (Figure 3).

The total amount of irrigation water applied to IS1 was 180 mm. For treatments that received fixed irrigation depth of 30 mm, the total amount of irrigation water applied was 240, 90, 60, 210, 150, 90 and 60 mm for IS2, IS3, IS4, IS5, IS6, IS7 and IS8, respectively (Figure 4). IS2 and IS5 received amounts of irrigation water greater than the treatment with irrigation management and maintenance of soil moisture at field capacity (IS1). The other treatments (IS3, IS4, IS6, IS7 and IS8) received a smaller amount of irrigation water when compared to IS1.

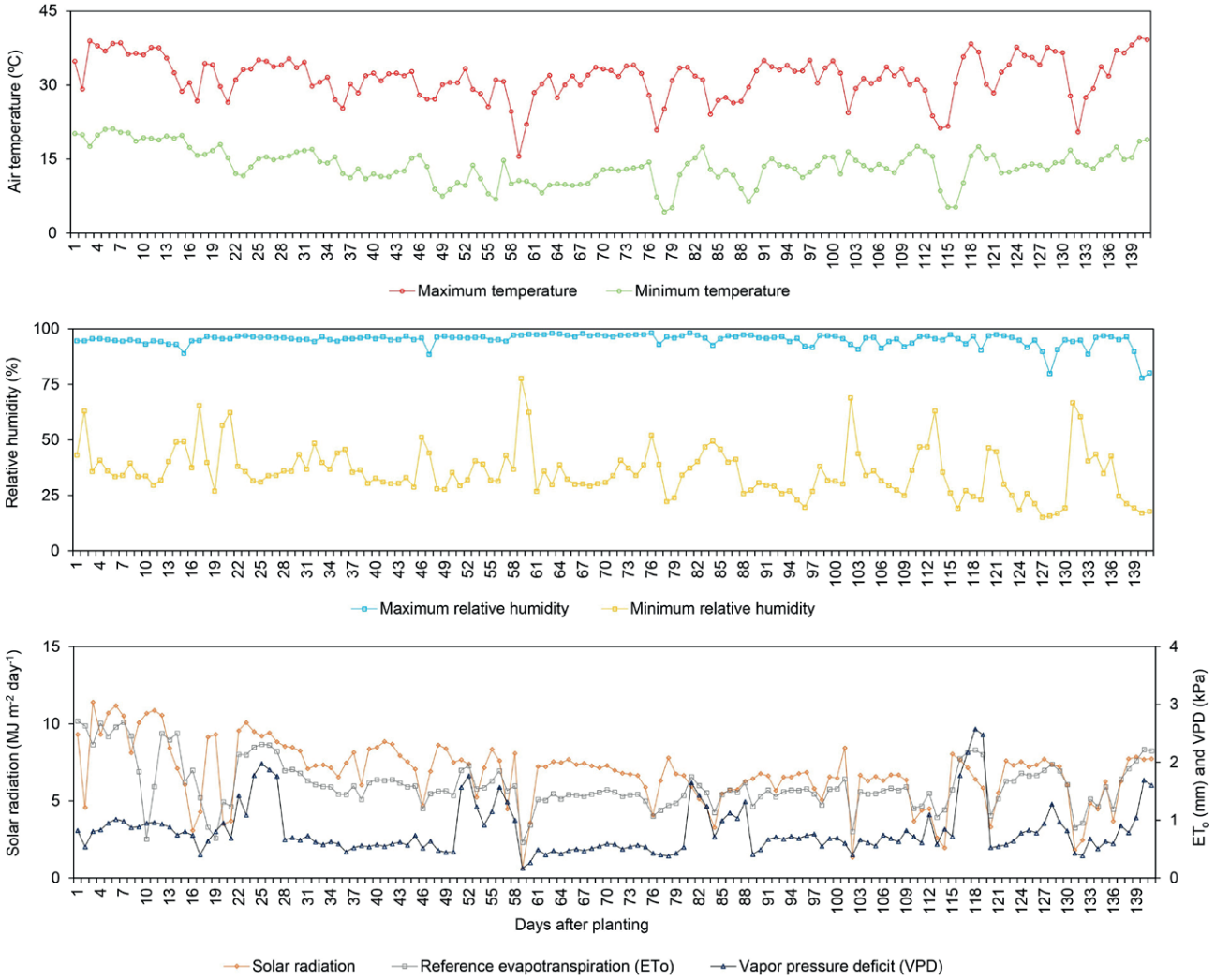

Figure 3. Daily data on meteorological variables air temperature, relative humidity, solar radiation, reference evapotranspiration (ET_o) and vapor pressure deficit (VPD).

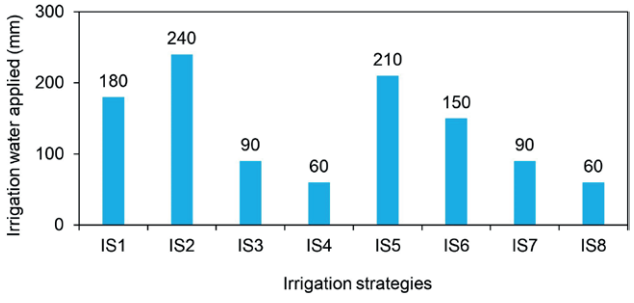

Figure 4. Irrigation water applied (mm) as a function of irrigation strategies (IS).

3.2. Responses related to plant development

Regarding the percentage of dead plants, it was found that for the interaction IS and VS there was a difference for four accumulated ET_o periods (30, 50, 80 and 120 DAP). The IS and VS factors alone showed no difference. IS3, IS4, IS7 and IS8 resulted in the highest percentages of dead plants especially in the periods of 80 and 120 DAP. IS1, IS2 and IS5 resulted in the lowest percentages of dead plants in all VS tested and in the four periods evaluated (Table 3). It can be seen that there was no difference in IS1, IS2, IS4 and IS8 for the tested VS, during the four evaluated periods. As for IS3, IS5 and IS6, there were differences in some of the periods, and the size of the VS influenced the survival of the plants.

Table 3. Percentage of dead plants (%) as a function of irrigation strategies (IS) with a specific volume of substrate (VS) for the evaluation periods of 30, 50, 80 and 120 days after planting (DAP).

Irrigation strategies	Evaluation period							
	30 DAP				50 DAP			
	Volume of substrate (cm^3)							
	56	73	93	125	56	73	93	125
IS1	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
IS2	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
IS3	0 a	0 a	0 a	0 a	58 bc	0 a	67 c	8 b
IS4	0 a	0 a	0 a	0 a	67 a	100 a	100 a	100 a
IS5	50 b	0 a	0 a	0 a	50 a	0 a	0 a	0 a
IS6	75 a	67 a	33 a	75 a	75 a	67 a	33 a	75 a
IS7	75 bc	17 a	100 c	42 ab	75 ab	25 a	100 b	50 ab
IS8	58 a	100 a	100 a	100 a	58 a	100 a	100 a	100 a
Irrigation strategies	Evaluation period							
	80 DAP				120 DAP			
	Volume of substrate (cm^3)							
	56	73	93	125	56	73	93	125
IS1	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
IS2	0 a	0 a	0 a	0 a	0 a	0 a	8 a	0 a
IS3	58 a	33 a	67 a	25 a	92 a	100 a	100 a	100 a
IS4	67 a	100 a	100 a	100 a	83 a	100 a	100 a	100 a
IS5	50 a	0 a	0 a	0 a	50 b	0 a	0 a	0 a
IS6	75 a	67 a	33 a	75 a	75 b	67 b	33 a	75 b
IS7	75 ab	25 a	100 b	50 ab	100 b	50 a	100 b	100 b
IS8	58 a	100 a	10 a	100 a	92 a	100 a	100 a	100 a

Mean values followed by the same letter in the row within the same evaluation period do not differ significantly by Tukey's test at 5% probability.

For planting meristem or pre-twinned seedlings, the density per linear meter was two to three plants. However, the percentage of dead plants constitutes more relevant information for decision makers to choose a certain IS and VS to be adopted in a planting nursery. Maintaining conditions to ensure the survival of all plants after planting helps to guarantee an ideal final stand in the nursery (Poja et al., 2020). On the other hand, planting gaps between 0.30 and 0.50 m do not always affect the quality of the sugarcane stand, since, under these conditions, tillering can be stimulated by greater solar radiation, thus compensating for the lower NT in the crop. However, when the failure rate is >50%, it is recommended to replant the area. This is valid for commercial stands in which 12 to 15 buds per linear meter are recommended for manual planting or 20 to 25 buds for mechanical planting. It is understood that each bud is likely to germinate and originate a new plant (Li et al., 2020; Otto et al., 2021; Rocha et al., 2022).

Throughout the entire experiment there was no increase in plant survival time with increasing VS. In IS6, for example, the highest survival rate (67%) was observed with the 93 cm^3 tray. As the VS factor is imposed at planting, plants exhibit different rates of development depending on this factor and plants with a VS of 125 cm^3 showed greater initial development. In this case, however, the plants' water consumption was higher, which increased the water deficit in the soil and caused critical water stress compared to the treatment in the 93 cm^3 tray; the same result occurred in IS7. The best performance was observed for the treatment with the 73 cm^3 tray, and this result was maintained for all evaluations.

The VS for the NT showed significance, however, the VS for total DM and MEPS was not significant. With respect to the IS factor, there was significance for total DM, MEPS and NT. For NT, it was found that treatments IS1, IS2 and IS5 presented the highest averages, with 8.3, 6.6 and 6.8 tillers, respectively, considering the four VS.

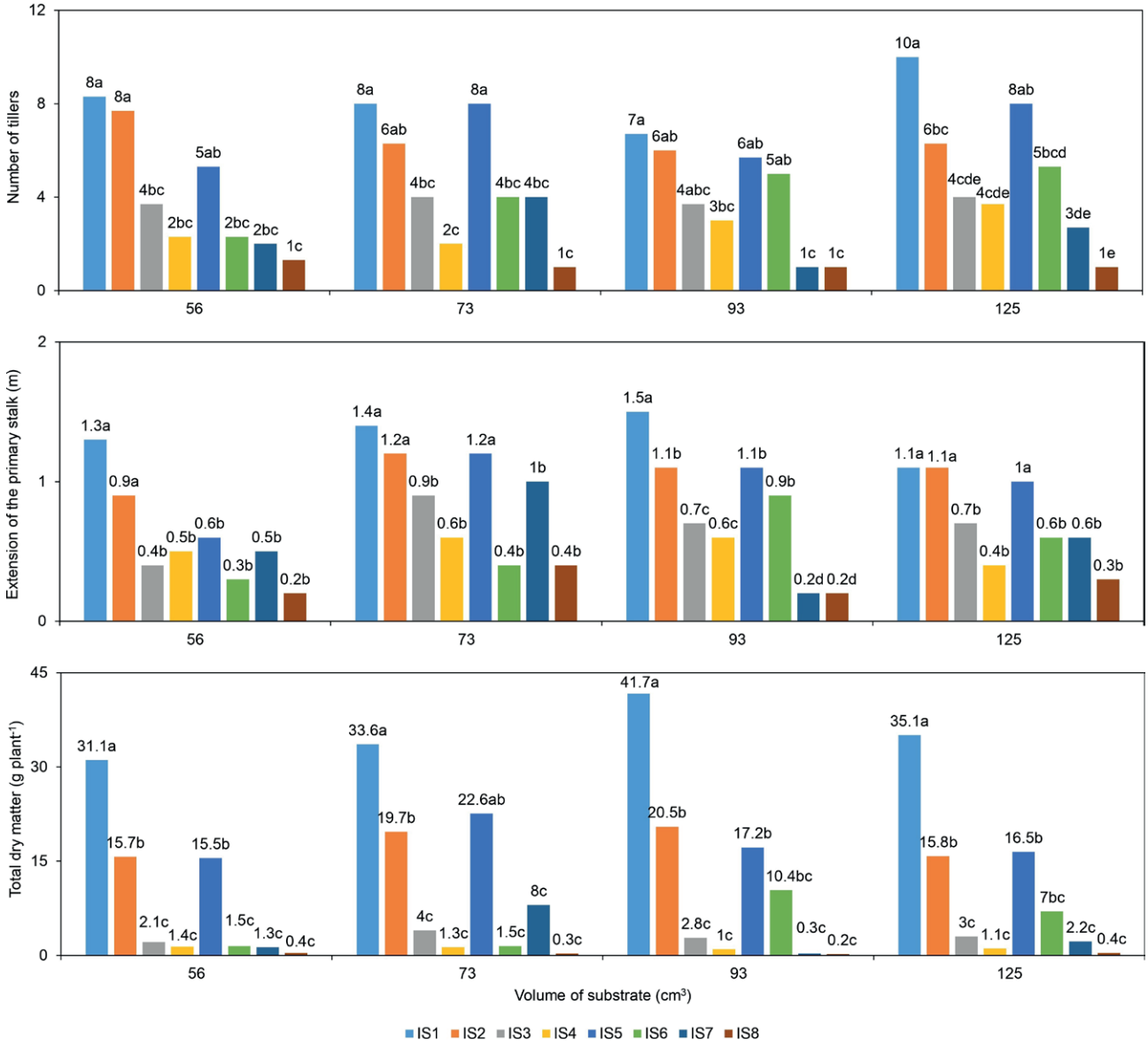

Figure 5. Number of tillers (NT), maximum extension of the primary stalk (MEPS) and total dry matter (DM) as a function of irrigation strategies (IS) with a specific volume of substrate (VS). Mean values followed by the same letter within each VS do not differ significantly by Tukey's test at 5% probability.

Treatments IS7 and IS8 had the lowest averages, 2.4 and 1.1 tillers, respectively, considering the four VS (Figure 5).

We observed that plants with larger stalk diameters generally had smaller NTs. However, because of size differences, the total weight may be the same although the NT is different. The NTs in the vegetative stage is usually higher than at the end of the crop cycle, as some tillers in the clump are lost due to competition during development (Lal et al., 2015; Santos et al., 2019). Because of this, the total DM weight and the NTs are presented and discussed together. They are considered to be related characteristics for assessing a stand of a sugarcane in a nursery area, even taking into account the vegetative phenological stage.

For MEPS, it was found that treatments IS1 and IS2 yielded the highest averages of 1.3 and 1.1 meters, respectively, for the four VS conditions, while IS6 and IS8 had the lowest averages at 0.5 and 0.3 meters, respectively. As for total DM, it was observed that IS1 and IS2 presented mean values much higher than the

other treatments, for the four VS, 35.6 and 18 g plant^{-1}, respectively. IS6, IS7 and IS8 had the lowest average values of total DM, 5.1, 2.9 and 0.3 g plant^{-1}, respectively (Figure 5).

With respect to the IS factor, there was significance for total DM and NT, which highlights the importance of an adequate water supply for the plants to establish good growth. The greater the availability of water in the soil for the plants, the more tillering occurred, and consequently there was a greater accumulation of biomass, reflected in higher DM yield.

It should be noted that there was a greater difference between the treatments for the 93 cm³ VS, in four average groups, unlike the other VS, which presented only two groups. Thus, for a VS of 93 cm³, under the conditions of the experiment, the IS with the lowest water deficit (IS1, IS2 and IS5) promoted cell growth in the plants. In contrast, the IS7 and IS8 had more severe water deficits, and the plants with the 93 cm³ VS stopped growing; IS7 showed the lowest MEPS value of 0.22 m and the largest difference between groups.

4. CONCLUSIONS

It was concluded that for planting biofactory seedlings in moist soil with a frequency of irrigation every 30 mm of accumulated ET_o, the volume of substrate is not important, as no plant death was observed with any of the strategies analyzed in this study. For planting biofactory seedlings in dry soil with irrigation frequency every 30 mm of accumulated ET_o, the minimum reserve substrate volume must be 73 cm³ (plus 20% gel), as the lower volume of 56 cm³ resulted in the death of 50% of the plants. For biofactory planting in dry soil with irrigation frequency every 30 mm of accumulated ET_o, volumes of substrate greater than 93 or 125 cm³ caused a greater initial water consumption by the plants resulting in a water deficit and a reduction in the stand.

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