ABSTRACT: The root system is essential for sugarcane regrowth and the vigor of ratoon cycles as it represents the unique source of carbon skeletons and energy for the initial plant development. However, root system dynamics after shoot harvesting and its role in sugarcane regrowth remains poorly known. Here, it was hypothesized that sugarcane plants with small volume of root system will accumulate less biomass after shoot harvesting than plants with larger volume and that such regrowth is dependent on root reserves. In sugarcane plants grown in nutrient solution, shoots were cut, and two root treatments were established: reference plants with the entire root system (100%); and plants with half of the root system (50%), randomly removing half of root system. After 37 days of shoot harvesting, plants with the entire root system showed higher shoot, root and total dry mass, root length, root diameter, root area and root volume, when compared with those with 50% of the root system. Sugarcane plants with the entire root system had higher root content of starch, soluble sugar and nonstructural carbohydrates as compared to plants with 50% of the root system. A significant positive correlation was found between the variation of shoot dry mass and the variation of root nonstructural carbohydrates. Interestingly, this data revealed a disproportionate effect of root system size on sugarcane regrowth, with plants with the entire root system accumulating almost three times more biomass than plants with half of the root system during regrowth.

Key words: biomass; carbohydrates; harvesting; ratoon; Saccharum spp.

INTRODUCTION

Ratoon sprouting—also known as regrowth—is started and new tillering is established just after harvesting sugarcane stalks. The regrowth is an important phase because it determines vigorous plants and then the ratoon yield (Silva et al. 2004). In fact, vigorous regrowth is a desirable characteristic, as several harvestings should be obtained in the same area to reduce costs of planting (Yadav et al. 2009). Many factors can influence sugarcane regrowth, such as soil temperature and moisture, the genetic background, plant age and health, and agricultural practices (Zambrosi et al. 2017). Sugarcane regrowth is also influenced by straw left in the field after mechanized harvesting (Bernache et al. 2020; Kroes and Harris 1996; Manhães et al. 2015).

After stalk harvesting, the old root system remains active for some time, and then it is replaced by roots of new tillers in a slow and gradual process. In addition, ratoon roots are more superficial than those of plant cane, as tillering occurs close to soil surface (Bacchi 1983). During regrowth, the reserves found in underground organs from the previous cycle are fundamental (Carneiro et al. 1995; Trivelin et al. 2002). In other words, the root system of sugarcane is essential for the regrowth and ratoon cycle as it represents the unique source of carbon skeletons and energy for the initial plant development (Casagrande 1991; Smith et al. 2005).
However, root system dynamics after harvesting and its role on sugarcane regrowth remains poorly known. While it seems obvious that sugarcane regrowth is dependent on root reserves, there is no paper addressing this theme. Here, the aim was to test the hypothesis that sugarcane plants with small root system will accumulate less biomass after shoot harvesting than plants with large root system, and that such regrowth is dependent on root reserves. The experimental strategy and proof of concept were developed with young plants grown in nutrient solution. Under controlled condition, it was possible to manage the root system, which is practically impossible under field conditions.

**MATERIALS AND METHODS**

**Plant material and growth conditions**

Sugarcane (*Saccharum* spp.) plants ‘IACSP95-5000’—a high-yielding cultivar launched by the ProCana Breeding Program, IAC (Landell et al. 2007)—were propagated by using stalk segments (with one bud) placed in 0.5 L plastic pots containing commercial substrate composed of *Sphagnum*, rice straw and perlite (7:2:1, Carolina Soil of Brazil, Vera Cruz, RS, Brazil). After 40 days, plants were transplanted to 4 L plastic boxes containing nutrient solution (Sarruge et al. 1975): 15 mmol N·L\(^{-1}\) (7% as NH\(_4^+\)); 4.8 mmol K·L\(^{-1}\); 5.0 mmol Ca·L\(^{-1}\); 2.0 mmol Mg·L\(^{-1}\); 1.0 mmol P·L\(^{-1}\); 1.2 mmol S·L\(^{-1}\); 28.0 μmol B·L\(^{-1}\); 54.0 μmol Fe·L\(^{-1}\); 5.5 μmol Mn·L\(^{-1}\); 2.1 μmol Zn·L\(^{-1}\); 1.1 μmol Cu·L\(^{-1}\); and 0.01 μmol Mo·L\(^{-1}\). Before transferring plants to the nutrient solution, roots were washed with tap water to remove any substrate. The modified Sarruge nutrient solution was diluted at ¼ strength during the first four days and then renewed by using ½ strength. After another four days, full strength solution was supplied to plants. In total, acclimation to the nutrient solution took 12 days and then the experiment began (day 0) and lasted for 67 days.

The nutrient solution was renewed every four days throughout the experimental period, maintaining its electrical conductivity between 1.2 and 1.5 mS·cm\(^{-1}\) and pH 5.9 ± 0.1. The pH was adjusted daily with 0.5 mol·L\(^{-1}\) citric acid or 0.5 mol·L\(^{-1}\) NaOH. Both variables were monitored daily using a portable conductivity meter (mCA 150P, MS Tecnopon Instrumentação, Piracicaba, SP, Brazil) and a portable pHmeter (mPA 210P, MS Tecnopon Instrumentação, Piracicaba, SP, Brazil). The volume of nutrient solution was also checked daily and completed with water when necessary. The solution inside each box was continuously aerated by an air compressor (Master Super II, Master, São Paulo, SP, Brazil).

After 30 days (from day 0), two root treatments were established: reference plants with the entire root system (100%, without root management); and plants with half of the root system (50%, randomly removing half of root system). Then, the shoots of both treatments were cut. Plants were grown under greenhouse conditions, where air temperature varied between 31.0 and 15.5 °C, and the maximum photosynthetic active radiation reached 1100 μmol·m\(^{-2}\)·s\(^{-1}\). Plants remained under those conditions for 37 days, time needed for plants to develop a reasonable leaf area and canopy.

**Leaf nutritional analysis**

Macronutrients (N, P, K, Ca, Mg and S) and micronutrients (B, Cu, Fe, Mn and Zn) were quantified in leaf samples collected at the end of experimental period. Samples were dried in an oven with forced air circulation (60 °C) until reaching constant weight. Milled samples (500 mg) were transferred to digestion glass tubes, containing 5 mL of concentrated nitric acid (65%). After mixing and left reacting overnight, the tubes were heated in a digestion block at 160 °C. After digestion, the tubes were cooled and 2 mL of perchloric acid (70%) were added. The samples were again placed in the digestion block and the temperature was gradually raised to 210 °C. After new cooling, the solution volume was completed to 50 mL with deionized water. Quantification was carried out using induced plasma atomic emission spectrometry (ICP-AES) (Bataglia et al. 1983). Leaf nutrient concentrations were compared with the sufficiency range proposed for sugarcane by Raij et al. (1997).
Sugarcane regrowth and root reserves

Soluble sugar, starch and nonstructural carbohydrate content

Roots were collected just after harvesting (before regrowth) and at the end of the experiment (after regrowth). Samples were initially dried in an oven with forced air circulation (60 °C) and total soluble sugars (SS) were assayed in 75 mg subsamples. Root samples were ground in 3 mL of extraction medium containing methanol:chloroform:water (12:5:3 v/v). After mixing in a vortex shaker (Basic K40 2810, 2800 rpm–Kasvi, São José do Pinhais, PR, Brazil), the samples were stored for 48 h at 5 °C. Then, 1.2 mL of water and 1.8 mL of chloroform were added, and samples were stored again at 5 °C for 24 h. The upper aqueous phase was collected and concentrated in a water bath at 55 °C. Afterwards, it was resuspended by adding 1.5 mL of water (Bieleski and Turner 1966). After extraction and concentration of subsamples, the SS concentration was quantified following DuBois et al. (1956): 5 μL of the extract were pipetted into test tubes and 495 μL of distilled water, 500 μL of phenol solution (5% w/v) and 2 mL of concentrated sulphuric acid (98% v/v) were added. After cooling at room temperature, the absorbance was read in a spectrophotometer (Ultrospec 1000, Pharmacia Biotech, Cambridge, UK) at 490 nm and the SS content was estimated from a standard curve using glucose (0 to 100 μg·mL−1).

The starch content was determined using the precipitate derived from the extraction of SS, following the methodology described by McCready et al. (1950): the supernatant was despised, and the precipitate was used for the quantification of starch. 1 mL of 80% ethyl alcohol, heated to 50–60 °C, was added to the precipitate and then samples were centrifugated for 15 min. The supernatant was despised again, and starch extraction was started by adding 1 mL of perchloric acid (30%) to the precipitate. Sample solution was then centrifuged at 10,000 rpm for 15 min. The starch concentration was determined by the anthrone method, as described by Yemm and Willis (1954). 10 μL of the extract were pipetted into test tubes as well as 1.5 mL of anthrone solution (2 g of anthrone in 100 mL of concentrated sulphuric acid 98%, v/v). The tubes were shaken and kept in a water bath at 100 °C for 3 min. After cooling at room temperature, the absorbance was read in a spectrophotometer (Ultrospec 1000, Pharmacia Biotech, Cambridge, UK) at 620 nm and the starch content was calculated from a standard curve using glucose (0 to 30 μg·mL−1).

The root concentration of nonstructural carbohydrates (NSC) was estimated as: NSC = SS + starch. The NSC variation (ΔNSC, mg·d−1) was calculated as the difference in NSC content between sampling times (before and after regrowth) divided by 37 days.

Biometry

Before and after the regrowth period, leaf and root dry masses were quantified and the shoot:root dry mass ratio (SDM:RDM) calculated. The shoot dry mass variation (ΔSDM, g·d−1) was calculated following the same procedure described for ΔNSC. Root morphological parameters, such as total root length, root area, root volume and root diameter, were determined using a scanner EPSON1680 and the WinRHIZO software (Regent Instruments Inc., Quebec, Canada), as done by Rampazzo et al. (2018).

Experimental design and statistical analyses

The experiment was carried out in a completely randomized design. Data were analyzed using the Bayesian ANOVA and the free available JASP software (https://jasp-stats.org/). When significant differences were detected between treatments, the mean values (n = 5) were compared using the Bayes Factor (BF₁₀), with BF₁₀ > 3 indicating positive support to the alternative hypothesis (Miranda et al. 2021).

RESULTS

Sugarcane regrowth was significantly affected by root treatment, and a large reduction in shoot, root, and total dry masses was noticed in plants with half (50%) of the root system, without any change in shoot:root ratio (Table 1, Fig. 1). Root length, diameter, area, and volume were significantly higher in plants with the entire root system, as compared to those with smaller root system (Table 1).
Table 1. Shoot (SDM), root (RDM) and total (TDM) dry mass, shoot:root ratio (SDM:RDM), total root length (RL), average root diameter (RD), total root area (RA) and total root volume (RV) of sugarcane plants with half (50%) and entire (100%) root system, grown in nutrient solution. Values indicate the means (n = 5) ± sd.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Root system*</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50%</td>
<td>100%</td>
</tr>
<tr>
<td>SDM (g)</td>
<td>1.25 ± 0.34b</td>
<td>3.70 ± 0.70a</td>
</tr>
<tr>
<td>RDM (g)</td>
<td>0.65 ± 0.07b</td>
<td>1.80 ± 0.51a</td>
</tr>
<tr>
<td>TDM (g)</td>
<td>1.90 ± 0.35b</td>
<td>5.50 ± 1.18a</td>
</tr>
<tr>
<td>SDM:RDM</td>
<td>1.93 ± 0.53a</td>
<td>2.10 ± 0.27a</td>
</tr>
<tr>
<td>RL (cm)</td>
<td>661.86 ± 15.52b</td>
<td>3068.48 ± 84.88a</td>
</tr>
<tr>
<td>RD (mm)</td>
<td>2.83 ± 0.67b</td>
<td>6.69 ± 1.29a</td>
</tr>
<tr>
<td>RA (m²)</td>
<td>0.07 ± 0.02b</td>
<td>0.28 ± 0.06a</td>
</tr>
<tr>
<td>RV (cm³)</td>
<td>5.94 ± 0.95b</td>
<td>20.45 ± 3.03a</td>
</tr>
</tbody>
</table>

*Measurements were taken 37 days after shoot cutting. Different letters indicate statistical difference between root treatments (BF10 > 3). Δ means the ratio between treatments (100%:50%).

Figure 1. Visual aspect (37 days after shoot cutting) of sugarcane plants with half (50%) and entire (100%) root system, grown in nutrient solution.

The nutritional status of leaves taken at the end of the experiment revealed no differences between root treatments, with concentrations of both macro and micronutrients within the sufficiency range for sugarcane (Table 2).

As expected, the amount of root reserves given by starch and SS was higher in plants with 100% of the root system as compared to plants with 50% of the root system before regrowth (Fig. 2a, b). At the end of regrowth period (37 days after shoot harvesting), there was a reduction in root NSC of plants from both treatments, but such reduction was more intense in plants with smaller root system (Fig. 2c). At this time, plants with the entire root system presented higher starch (+ 6.1 times), SS (+ 6.2 times) and root NSC (+ 6.2 times) than ones with 50% of the root system (Fig. 2).
Table 2. Concentration of macro and micronutrients in sugarcane leaves taken from plants with half (50%) and entire (100%) root system and grown in nutrient solution. Values indicate the means (n = 5) ± sd. Range means optimal concentration for sugarcane leaves (Raij et al. 1997).

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Root system*</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (g·kg⁻¹)</td>
<td>25.7 ± 0.5a 25.1 ± 3.6a</td>
<td>18 to 25</td>
</tr>
<tr>
<td>P (g·kg⁻¹)</td>
<td>5.0 ± 0.7a 5.2 ± 0.2a</td>
<td>1.5 to 3</td>
</tr>
<tr>
<td>K (g·kg⁻¹)</td>
<td>22.3 ± 0.9a 21.2 ± 0.5a</td>
<td>10 to 16</td>
</tr>
<tr>
<td>Ca (g·kg⁻¹)</td>
<td>5.1 ± 0.4a 5.3 ± 0.6a</td>
<td>2 to 8</td>
</tr>
<tr>
<td>Mg (g·kg⁻¹)</td>
<td>2.4 ± 0.6a 2.6 ± 0.3a</td>
<td>1 to 3</td>
</tr>
<tr>
<td>S (g·kg⁻¹)</td>
<td>1.3 ± 0.4a 2.0 ± 0.1a</td>
<td>1.5 to 3</td>
</tr>
<tr>
<td>B (mg·kg⁻¹)</td>
<td>43.1 ± 5.6a 31.5 ± 2.2a</td>
<td>10 to 30</td>
</tr>
<tr>
<td>Cu (mg·kg⁻¹)</td>
<td>7.2 ± 1.1a 9.3 ± 0.4a</td>
<td>6 to 15</td>
</tr>
<tr>
<td>Fe (mg·kg⁻¹)</td>
<td>1075 ± 179a 93.1 ± 16.3a</td>
<td>40 to 250</td>
</tr>
<tr>
<td>Mn (mg·kg⁻¹)</td>
<td>132.8 ± 9.5a 146.7 ± 0.4a</td>
<td>25 to 250</td>
</tr>
<tr>
<td>Zn (mg·kg⁻¹)</td>
<td>33.8 ± 3.5a 40.0 ± 2.3a</td>
<td>10 to 50</td>
</tr>
</tbody>
</table>

*Measurements were taken 37 days after shoot cutting. Same letters indicate nonstatistical difference between root treatments (BF₁₀ < 3).

Figure 2. Root starch (Sta, in a), soluble sugar (SS, in b) and nonstructural carbohydrate (NSC, in c) contents in sugarcane plants with half (50%) and entire (100%) root system, evaluations were done before (light blue boxes) and after (dark blue boxes) the regrowth period.

Note. Box plots show the minimum and maximum values, as well as the 25th, 50th (median) and 75th percentiles (n = 5). *Statistical difference (BF₁₀ > 3) between root treatments in a given evaluation.
The gain of shoot dry mass in plants with the entire root system was 2.9 times higher than in plants with 50% of the root system (Fig. 3a) and there was a significant correlation between the variation of shoot dry mass and the variation of root NSC during the experimental period (Fig. 3b).

![Graph showing changes in shoot dry mass (ΔSDM) due to root treatment (a) and as function of root NSC variation (ΔNSC_root) during the regrowth period (b) in sugarcane plants with half (50%, white symbols) and entire (100%, black symbols) root system.](image)

**Figure 3.** Changes in shoot dry mass (ΔSDM) due to root treatment (a) and as function of root NSC variation (ΔNSC_root) during the regrowth period (b) in sugarcane plants with half (50%, white symbols) and entire (100%, black symbols) root system.

Note. Box plots show the minimum and maximum values as well as the 25th, 50th (median) and 75th percentiles (n = 5). *Statistical difference (BF10 > 3) between root treatments in a given evaluation.

**DISCUSSION**

It was found that sugarcane plants with half of the root system accumulated less shoot biomass than plants with the entire root system during the regrowth (Table 1), which was dependent on root reserves (Fig. 3b). However, a proportional influence of root size on plant regrowth was expected, i.e., regrowth about two times higher in plants with the entire root system as compared to those with 50%, as the former had twice more root reserves (Fig. 2c). This data suggests that plants with the entire root system started to produce photoassimilates earlier than plants with 50% of the root system, which would explain the highest accumulation of biomass (Table 1, Fig. 1). In fact, shoot, root and total dry masses were almost three times higher in plants with 100% of the root system, as compared to ones with half of the root system.

Faster initial growth of plants with the entire root system would also justify the disproportionate influence of treatments on sugarcane regrowth. Accordingly, root length, diameter, area, and volume were between 2.4 and 4.6 times higher in plants with the entire root system as compared to plants with 50% of root system (Table 1). From a morphological point of view, plants with the entire root system would explore more effectively soil/substrate resources as they have longer roots, with higher volume, area and diameter than plants with only half of the root system. Interestingly, SDM:RDM ratio did not change between root treatments (Table 1), which suggests sugarcane plants maintain the pattern of biomass allocation even when there are large changes in root system size. From a practical perspective, a less vigorous and reduced root system would be a plausible cause for the decreasing trend in sugarcane biomass and yield in long-term (Esteban et al. 2019)—a hypothesis that must be tested in field conditions.

Decreases in root carbohydrate reserves were noticed in both treatments at the end of the experiment (Fig. 2). After regrowth, plants with the entire root system showed a reduction of 64, 60 and 61% in starch, SS and NSC contents, respectively. On the other hand, sugarcane plants with half of the root system showed decreases of 88, 87 and 88% in starch, SS and NSC contents, respectively. These results imply that carbohydrates were partially consumed as new biomass was produced. According to Guo et al. (2017), decreases in root weight after shoot cutting would be expected because root carbohydrate reserves are used for regrowth. As root biomass increased along the experimental period (Table 1), it is possible to argue that
root resources and root biomass were decreased at the beginning of regrowth and then new leaves started to produce and supply photoassimilates not only for the shoot, but also for root regrowth. As there are no leaves producing photoassimilates just after shoot harvesting, the persistence of the root system is critical for the initial regrowth due to the uptake of water and nutrients and supplying of carbohydrate reserves.

Here, any nutritional limitation can be ruled out as cause of variation in biomass accumulation because plants were well supplied with nutrients throughout the experiment and leaves had similar nutritional status in both root treatments, considering either macro or micronutrients (Table 2). While some authors reported that the root system quickly becomes nonfunctional and dies after harvesting (Baver et al. 1963), there is evidence that roots remain functional for long periods (Ball-Coelho et al. 1992). Glover (1968) reported the functional capacity of sugarcane roots after harvesting by applying very small amounts of $^{32}$P to the surface of apparently dead roots and finding $^{32}$P in shoots after some days. Herein, roots were darkened and seemed dead after one week of shoot harvesting. However, new roots were noticed emerging from those old roots at the end of the experimental period, as well as the appearance of roots from new tillers.

In sugarcane fields, rhizosphere conditions define root development, which is affected by plant-microbial interactions, nutrient and water availabilities (Darrah 1993), soil characteristics, root exudates and root decomposition (Singh et al. 2013), and soil pests (Dinardo-Miranda and Fracasso 2010; Dinardo-Miranda et al. 2019). Although all factors above may affect sugarcane regrowth, here the role of root system size as a source of reserves was addressed and there is no reason for assuming that this dependency is not present in field-grown plants. Although it is possible to consider the soil-plant interaction is different when comparing field conditions and the experimental design of this study, any biotic and abiotic factor can essentially affect the root system size and thus the amount of carbohydrate reserves. Therefore, the relationship between sugarcane regrowth and reserve consumption can be maintained, regardless of the growing condition. Further research is needed for revealing the sensitivity of root growth to constraining environmental factors and how such changes in root system size would affect shoot development and sugarcane yield.

**CONCLUSION**

In conclusion, this experimental approach using young plants grown in nutrient solution revealed that sugarcane regrowth is dependent on root reserves. As root carbohydrates are partially consumed for producing new leaves and these leaves are also sources of photoassimilates for regrowth, the influence of the initial root system size on sugarcane regrowth was amplified in a nonproportional way. Then, sugarcane plants with the entire root system accumulated almost three times more biomass than plants with half of the root system.

**AUTHORS’ CONTRIBUTION**


**DATA AVAILABILITY STATEMENT**

All dataset were generated or analyzed in the current study.
ACKNOWLEDGMENTS

Not applicable.

REFERENCES


Sugarcane regrowth and root reserves


