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ORIGINAL PAPER

# How does early defoliation influence the morphophysiology and biochemical characteristics of maize?

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## Abstract

Defoliation is a type of mechanical stress, and few studies have investigated this process in the early stages of maize development. Pest attacks, hail and machinery traffic have increased in recent decades, thus increasing this stress and potentially leading to losses. Furthermore, there are corn production systems in Brazil where early defoliation naturally occurs. Thus, the objective of this study was to determine the morphophysiological and biochemical changes in maize subjected to early defoliation and their effects on recovery from this stress. The experiment was performed in pots, and the plants were subjected to two treatments at the four fully expanded leaf stage: without defoliation (control) and with defoliation. Morphometric parameters, such as gas exchange, leaf pigment and biomolecule content, phytohormone content, root morphology and leaf anatomy, were evaluated at seven and fourteen days after defoliation. Compared with the control plants, the defoliated corn plants were shorter in height, stem diameter, length, surface area, root diameter and volume, dry biomass and leaf anatomy. However, photosynthesis, chlorophyll content and nutrient content were similar in both treatments. After seven days of treatment, the amino acid content increased in the defoliated plants, and after fourteen days, the reducing sugars, amino acids and proteins decreased in these plants. The levels of gibberellins and salicylic acid were greater in plants subjected to defoliation. The reestablishment of corn plants after defoliation occurred through the action of gibberellins and salicylic acid, which promoted the growth of aboveground biomass, maintenance of chlorophylls and gas exchange. The reallocation of amino acids and reducing sugars also contributes to the formation of new leaf primordia in defoliated plants.

**Keywords** Sugars · Phytohormones · Photosynthesis · Leaf anatomy · *Zea mays* L

## Introduction

Maize (*Zea mays* L.) is one of the most important crops in the world. This cereal is the raw material of a series of products that are used both for human consumption and mainly for animal consumption. Furthermore, maize is a commodity that can be used as an energy matrix for ethanol production. Brazil is the 3rd largest maize producer in the world (Carvalho et al. 2022), occupying a cultivation area of 19.09 million hectares (Barbosa et al. 2022).

Maize yield is affected by several biotic and abiotic factors. Among the abiotic factors, mechanical stress due to defoliation is still little explored in maize research, especially at the initial stages of growth, despite its ability to reduce the leaf area of plants. Hail, wind and machinery traffic are examples of factors that can lead to maize defoliation (Silva et al. 2021). The reduction in the photosynthetically active area due to defoliation can modify the source–sink relationship of the plant, thus leading to possible reductions in productivity. Defoliation stress can also generate several

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morphophysiological, biochemical and molecular changes in plants.

There is evidence in the literature that, depending on the phenological stage of maize, defoliation has no effect on productivity. Experimental results in the field and greenhouse settings have demonstrated that the initial loss of leaves (early defoliation) did not lead to a loss of yield in maize plants (Silva and Dalchiavon 2020; Karam et al. 2020). This scenario is plausible due to the small size of the leaves in the initial stages, in addition to the location of the apical meristematic region of the stem, which is below the ground until the stage of six fully expanded leaves (V6), thus allowing for the regrowth of defoliated plants.

Blanco et al. (2023) described the ability of corn to compensate for defoliation in the vegetative phase. The same authors highlighted the need to reevaluate recommendations for the application of insecticides against corn defoliating insect pests. This is especially relevant before the development of the seventh leaf, as there are no losses in productivity and reduced costs in corn crop management (Blanco et al. 2023). Furthermore, in areas with frequent lodging, the defoliation of corn seedlings can be a profitable agronomic practice. The defoliation of plants reduces the height of the plants and the insertion of ears, thus resulting in less lodging and consequent improvements in grain yield (Xu et al. 2023). However, few studies have explored the effects of defoliation associated with the biochemical and morphophysiological mechanisms by which plants respond, resume growth and avoid changes in grain yield.

Despite being a mechanical stress, early defoliation has been gaining prominence in production systems in Brazil, such as the *Tecnologia Antecipe*. *Anticipe* is a soy/maize production system that involves the mechanized sowing of maize between soybean rows from the R5 stage (the beginning of grain filling) of the legume (Karam et al. 2020). The strategy of this system is to anticipate the maize harvest by twenty days and to reduce the climate risk in the second harvest (*safrinha*) in the country. However, with the soybean harvest, there is early defoliation in maize at the V4 stage due to machinery traffic. Despite defoliation, maize regenerates from this stress, thus preserving and/or increasing its productivity (Karam et al. 2020).

In the literature, studies on corn defoliation that investigate losses in productivity and the effects on plant morphology (such as leaf area, height, and number of leaves) generally do not focus on earlier stages of development of this crop and have limited physiological approaches.

To date, no studies have investigated maize in which morphophysiological and biochemical characteristics are evaluated as a function of defoliation in the initial stages, such as in V4 (four fully expanded leaves), when considering physiological parameters related to gas exchange, phytohormones and biomolecules (sugar content, proteins and amino acids). Knowledge of how early defoliation affects the morphophysiological and biochemical characteristics of maize plants can improve *Anticipe Technology*, in addition to providing information on damage and the responses involved in this process promoted by physical stress (mainly regarding plant recovery), without compromising productivity.

Therefore, the hypotheses of this study were that maize plants with defoliation in V4 (four fully expanded leaves) would: (i) modify gas exchange and pigment content for rapid reestablishment of buds; (ii) increase the levels of the phytohormones gibberellins, auxins, salicylic acid, cytokinin, abscisic acid and jasmonic acid; iii alter the amino acids and sugars in the aboveground biomass to promote the formation of new leaves; iv. reduce the root system, thus affecting growth, volume, and other morphological attributes; and v. alter the thickness of leaf tissues. In this context, this study aimed to determine the morphophysiological and biochemical changes in maize subjected to early defoliation and their effects on the recovery of this mechanical stress in the early stages of development.

## Materials and methods

### Plant material and growth

The experiment was conducted from November 2021 to December 2021 in the greenhouse of the Santa Clara Education Department of the Federal University of Alfenas (UNIFAL-MG), in the city of Alfenas, in the state of Minas Gerais, Brazil, at an altitude of 818 m and geographic coordinates of 21° 25’ 20’’S 45° 59’00’’ W. The minimum and maximum temperatures in the greenhouse during the experiment were 17.8 °C (minimum) and 31.1 °C (maximum).

The experiment was performed in a completely randomized design (DIC) consisting of two treatments, including control (without mechanical defoliation damage) and defoliation (with mechanical defoliation damage at stage V4)—with 30 replications each, totaling 60 pots in the entire experiment. Each experimental plot consisted of a vase with a capacity of 20 L that contained two plants.

A single hybrid BRS 1055 developed by the *Embrapa Milho e Sorgo* maize breeding program was used. The selected hybrid has a semiearly cycle and is indicated for medium-investment areas. The soil that was used was classified as a typical dystrophic Latosol (Embrapa 2006), and the results of the chemical analysis in the 0–20 cm layer before the experiment are presented in Table 1.

Based on the results of the soil analysis and identification of the nutritional needs of maize according to Ribeiro et al.

**Table 1** Chemical attributes of the soil used for maize cultivation in the defoliation experiment

| pH | P | K | Al | Ca | Mg | $H^+Al$ | SB* | T* | V* | M* | O.M. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $H_2O$ | ------mg $dm^{-3}$------- | | | ----------cmolc $dm^{-3}$---------- | | | | | ---%--- | | dag $kg^{-1}$ |
| 5.9 | 5.5 | 35.1 | 0.0 | 2.3 | 0.6 | 1.7 | 3.0 | 4.7 | 64.1 | 0.0 | 1.8 |

* Sum of Bases (SB); cation exchange capacity – potential CTC (T); base saturation index (V); aluminium saturation (M)

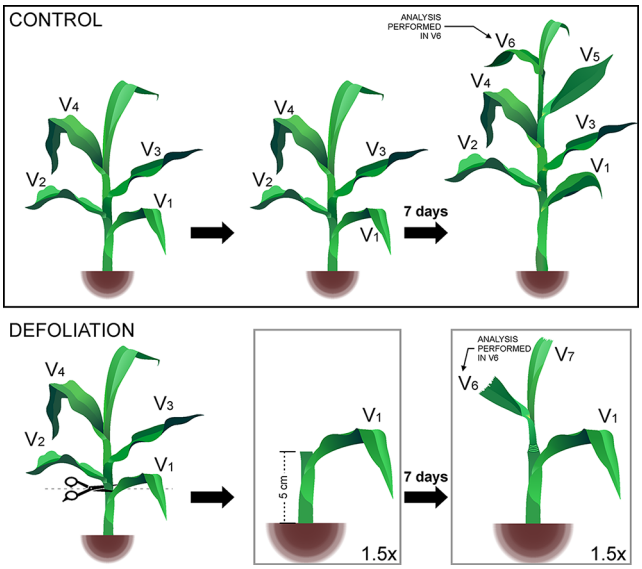


**Fig. 1** Scheme showing defoliation on the stalk of maize plants 5 cm from the ground. Morphometric, physiological, phytohormone, biomolecular and leaf anatomy analyses were performed on leaf V6 plants in both treatment groups

(1999), 40 kg $ha^{-1}$ of nitrogen, 120 kg $ha^{-1}$ of phosphorus and 40 kg $ha^{-1}$ of potassium were added and homogenized to the soil, which corresponded to seeding fertilization. The nutrient sources that were used were single superphosphate (18% of P2O5) and a formulation with NPK (20:5:20). Four maize seeds were sown in each pot, and after emergence, thinning was performed, with only two plants per pot remaining. When the plants reached the V3 stage, cover fertilization was applied, and 120 kg $ha^{-1}$ of nitrogen, 40 kg $ha^{-1}$ of potassium, 4 kg $ha^{-1}$ of boron and 4 kg $ha^{-1}$ of zinc were added. The utilized nutrient sources were urea (45% N), NPK (20:5:20), boric acid (P.A.) and zinc sulfate (P.A.). Daily irrigation was applied to maintain the soil close to 80% of the maximum water-holding capacity during the entire experimental period.

## Defoliation conditions

When the plants reached the stage of four developed leaves and the collar was visible (V4; 15 days after seeding), the treatments were applied. Defoliation was performed randomly with the aid of scissors, adopting a cutting height of 5 cm above the soil surface, thus eliminating all of the shoots of the plants in the defoliation treatment. In the control treatment, the plants were grown without damage (Fig. 1).

Physiological analyses (gas exchange and pigment content), quantification of phytohormones and anatomical evaluations in the control treatment were standardized on V6, which corresponds to the sixth fully expanded leaf. In the defoliation treatment, the first leaf that expanded after mechanical damage by defoliation was standardized, which also corresponded to V6 (Fig. 1). The first leaf that expanded after mechanical damage was defined as V6 after successive desiccations of the defoliated plant in previous experiments.

## Morphometric and physiological analyses

Morphometric analyses were performed on ten plants per treatment at one, seven and fourteen days after the imposition of defoliation. Plant height was measured by using a graduated ruler, and stem diameter was measured by using a digital caliper. For the dry biomass of shoots, four replicates of each treatment were collected, placed in paper bags, and placed in a forced air circulation oven at 65 °C until a constant weight was obtained. After this phase, the percentage of accumulated shoot biomass was calculated over the fourteen days in the control and defoliation treatments.

Gas exchange was evaluated in eight plants per treatment. The evaluations occurred in the morning (between 8 and 10 am) at seven and fourteen days after the imposition of defoliation. The net $CO_2$ assimilation rate (A), stomatal conductance (gs), intercellular $CO_2$ concentration (Ci) and transpiration (E) were evaluated. An infrared gas analyzer (IRGA, model LI 6400, LI-COR, Lincoln, NE, USA) was used. Measurements were performed on a sheet area of 6 $cm^2$, and the airflow in the chamber had a $CO_2$ concentration of 380 mmol $mol^{-1}$. A photon flux density (PPFD) of 1,500 µmol $m^{-2}$ $s^{-1}$ was used, and the chamber temperature was 28 °C.

For the determination of chlorophyll *a* and *b*, as well as for total and carotenoids, five replicates of each treatment were collected at seven and fourteen days after the imposition of damage by defoliation. Subsequently, 0.1 g of leaf tissue was fragmented into pieces of approximately 3 mm and immersed in 20 ml of 80% acetone (v $v^{-1}$) for 24 h in a light-protected environment for the complete extraction of the pigments. After this period of time, the pigments were determined according to Lichtenthaler and Buschmann (2001) based on spectrophotometric readings.

## Analysis of biomolecules and phytohormones

The analyses of phytohormones and biomolecules were performed in the sixth completely expanded leaf at seven and fourteen days after the imposition of the treatments. For the analysis of phytohormones, fresh biomass was used, whereas for the determination of biomolecules, dry biomass was used.

For the extraction of biomolecules, 0.2 g of dry biomass from leaves was homogenized in four replicates with 5 mL of 0.1 M potassium phosphate buffer (pH 7.0), followed by incubation in a water bath for 30 min at 40 °C and centrifugation at 10,000 × g for 20 min. This process was repeated once, and the supernatants were combined to obtain a final extraction volume of 10 mL. Aliquots of the supernatant were used for the analysis of total soluble sugars, reducing sugars, amino acids, and proteins. The resulting pellet was solubilized in 1.5 mL of 30% perchloric acid (m $v^{-1}$), and the mixture was incubated for 24 h and centrifuged at 10,000 × g for 20 min. The supernatant was used for starch quantification.

The quantification of reducing sugars followed the methodology of Miller (1959), and that of total soluble sugars and starch was performed according to the methodology described by Yemm and Willis (1954). The quantification of proteins followed the method proposed by Bradford (1976), and the quantification of amino acids was performed according to Yemm and Cocking (1955).

The determination of phytohormones was performed on one leaf per treatment, with five replications. For this purpose, the sixth completely expanded leaf of each repetition was collected, which was conditioned in liquid nitrogen (-80 °C) immediately after collection and maintained during transport from the greenhouse to the laboratory. Samples were removed from liquid nitrogen and added to 10 mL of extraction solution (acetonitrile: Milli-Q water 1:1). After addition, the tubes containing the solutions were briefly vortexed, stirred for 30 min on a shaker, and subsequently centrifuged at 16,000 g and 4 °C for 5 min. The supernatant was transferred to a new microcentrifuge tube (1.5 mL) and dried at VCA speed. After drying, 100 µL of methyl alcohol was added to each of the samples, which were then centrifuged again at 16,000 g and 4 °C for 10 min. The supernatant was analyzed via high-performance liquid chromatography coupled to mass spectrometry (HPLC/MS), as described by Trapp et al. (2014). The levels of the phytohormones gibberellins (GA3; GA), auxins (indoleacetic acid [IAA] and indolebutyric acid [IBA]), salicylic acid (AS) and cytokinin (zeatin trans riboside and zeatin) were quantified. Abscisic acid (ABA) and jasmonic acid (JA) were also analyzed; however, both showed values below the detection limit in samples from both treatments.

## Root morphology and nutrients

For root morphology analysis, samples were collected at seven and fourteen days after the imposition of treatments, with three replications. The roots were collected, washed in running water and then stored in 70% ethanol until analysis. For the analysis of the morphology of the root system, the WinRHIZO Pro 2007a image analysis system (Regent Instruments, Sainte-Foy, QC, Canada) coupled to a professional scanner (Epson, Expression 10,000 XL, Epson America, Inc., USA) equipped with an additional light unit (TPU) was used. Images were obtained according to Souza et al. (2012). The following parameters were measured: root length (cm), root surface area ($cm^2$), root mean diameter (mm) and root volume ($cm^3$). Root length, root surface area and root volume were also analyzed by diameter class (0 to 4.5 millimeters) using the same software. Afterwards, the roots were stored in paper bags and transported to a forced air circulation oven at 65 °C until a constant weight was obtained. In this phase, the percentage of root biomass accumulation over the fourteen days in the control and defoliation treatments was calculated.

Other attributes involving morphological and dry mass data were determined: the relationship between root dry mass and shoot dry mass (RDW/SDW g $g^{-1}$), specific root length (SRL cm $g^{-1}$), root fineness (RF cm $cm^{-3}$) and root tissue density (RMDe g $cm^{-3}$).

The determination of nutrient content in the shoots of maize plants was performed after obtaining the dry biomass of the shoots. For this purpose, five samples from each treatment were ground in a rotor-type mill (Pulverizette 14 classic line model, Fritsch GmbH, Germany) at 16,000 rpm. The ground material was used to determine the nutrient contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), sulfur (S) and boron (B) according to the methodology proposed by Silva (2009).

## Leaf anatomy

For leaf anatomy, five replicate samples were collected from the middle third of each leaf at seven and fourteen days after treatment. The samples were stored in a 70% ethanol solution until the anatomical sections were taken. Transverse cuts were made freehand with a razor blade, and five washes were performed, the first with 50% HCl for eight minutes to lighten the sections, the second with 1% acidified water for five minutes to remove excess HCl and subsequent three consecutive washes in distilled water for five minutes each to rehydrate the material. After the washes, for the staining procedure, safrablau (safranin + astrablau) was used. The slides were mounted with 50% glycerin and sealed with

enamel. The results were recorded by using an Axio Scope Al photomicroscope (Carl Zeiss, Oberkochen, Germany), and measurements were performed by using ImageTool Version 3.0 software (University of Texas, San Antonio, TX, USA). Photomicrographs were used to measure the following parameters for the leaves: thickness of the epidermis abaxial (BET) and adaxial (DET), thickness of the mesophyll (MPT), area of the vascular bundle (VBA), diameter of the metaxylems (DMV) and area of the phloem (PA).

To perform the paradermal cuts, a thin layer of glue (Super Bonder®, Loctite, São Paulo – SP) was spread over the surface of the sheet. After drying, the film formed by the glue, which contained the impression of the leaf surface, was removed, and the slides, as well as the cross sections, were mounted for imaging. This procedure was performed on the adaxial and abaxial surfaces of the leaf. Measurements were also performed by using ImageTool software. The parameters that were analyzed on the adaxial face included stomatal density (SD), polar diameter (PD) and equatorial diameter (ED) of the stomata and the number of epidermal cells (NE).

### Data analysis

For all of the analyzed parameters, the means and the standard error of the mean of the repetitions were calculated. For the statistical analysis of the results, analysis of variance (ANOVA) and Student's t test at a 5% probability level were used, and the Sisvar program version 4.3 (Federal University of Lavras, Lavras, Brazil) was utilized for the analysis.

## Results

### Morphometric and physiological response

Up to the V4 stage (cutting day), all of the plants had similar and standardized heights and stem diameters. After the treatments, the maize plants in the control treatment group were taller and taller than those in the defoliated treatment group at seven and fourteen days after defoliation ($p < 0.05$) (Fig. 2).

There were no significant differences in leaf gas exchange, net $CO_2$ assimilation rate (A), stomatal conductance ($g_s$), intercellular $CO_2$ concentration (Ci) or transpiration (E) between the defoliation treatment group and the control group at seven days after defoliation ($p < 0.05$) (Fig. 3). The same pattern was observed in the fourteen-day evaluation for A (Fig. 3a). However, plants subjected to defoliation had greater gs, Ci and E than did the control plants at fourteen days after treatment ($p < 0.05$) (Fig. 3b-d).

There were no significant differences in the Chl a, Chl b, total Chl or carotenoid contents among the maize plants at seven or fourteen days after defoliation (Fig. 4).

### Biomolecule and phytohormone response

There were no significant differences in the contents of starch, total soluble sugars (TSS), reducing sugars (RS) or protein (PTN) at seven days after defoliation (Table 2). However, the amino acid (AA) content was 19% greater in the defoliated plants (137.5) than in the control plants (115.8) ($p < 0.05$) (Table 2). Fourteen days after defoliation, the RS, AA and PTN contents were greater in the control plants than in the defoliated plants (Table 2). In contrast, starch and TSS did not significantly differ between treatments at fourteen days after the imposition of defoliation (Table 2).

For phytohormones, gibberellic acid (GA3), indoleacetic acid (IAA) and trans zeatin riboside (zeatin) did not differ between treatments at seven days after defoliation (Table 3). However, compared with those under the control treatment, the plants under defoliation treatment showed increases in the concentrations of 79% GA (1.72) and 210% SA (0.0128) at the same time of evaluation ($p < 0.05$) (Table 3). However, the concentration of indole butyric acid (IBA) was greater in the control plants than in the plants under defoliation (Table 3). Fourteen days after defoliation, the levels of AIA, IBA and zeatin were lower in the plants in the defoliation treatment than in the control plants (Table 3). The concentrations of GA and AS in maize leaves were 55% and 25%, respectively, which were greater in the defoliation treatment group than in the control group at fourteen days after the imposition of the treatments ($p < 0.05$) (Table 3). For GA3,

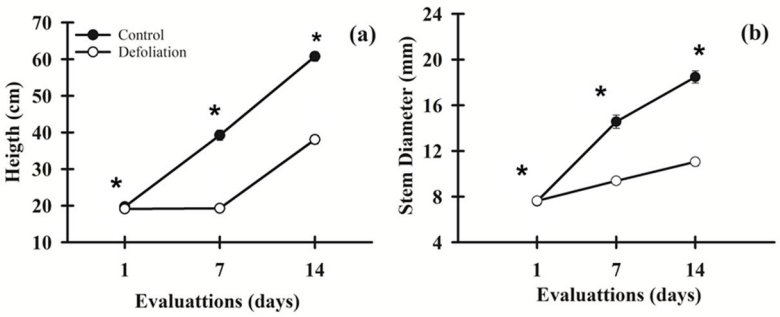


**Fig. 2** Height (**a**) and stem diameter (**b**) of maize plants under defoliation at 1, 7 and 14 h after treatment. * Indicates a significant difference in the defoliation treatment according to Student's t test at the 5% probability level ($p < 0.05$). Each value indicates the treatment mean ± standard error of the mean

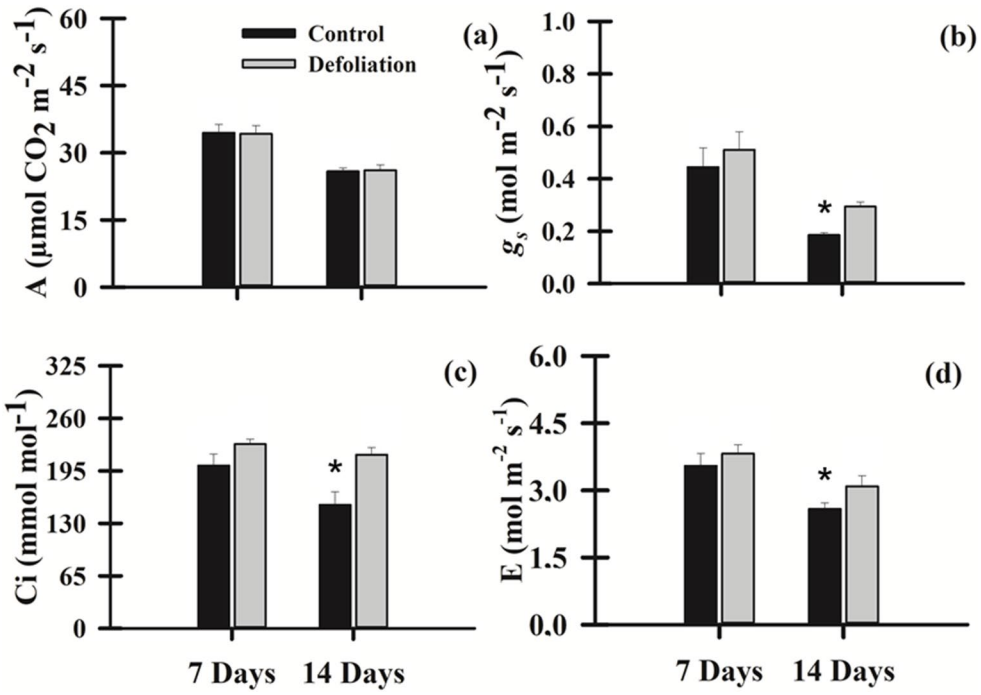


**Fig. 3** Net $CO_2$ assimilation rate (**A**, **a**), stomatal conductance ($g_s$, **b**), intercellular $CO_2$ concentration (Ci, **c**) and transpiration (**E**, **d**) of maize plants in the control and defoliation treatments at seven and fourteen days after treatment. * Indicates a significant difference in the defoliation treatment according to Student's t test at the 5% probability level ($p < 0.05$). Each value indicates the treatment mean ± standard error of the mean

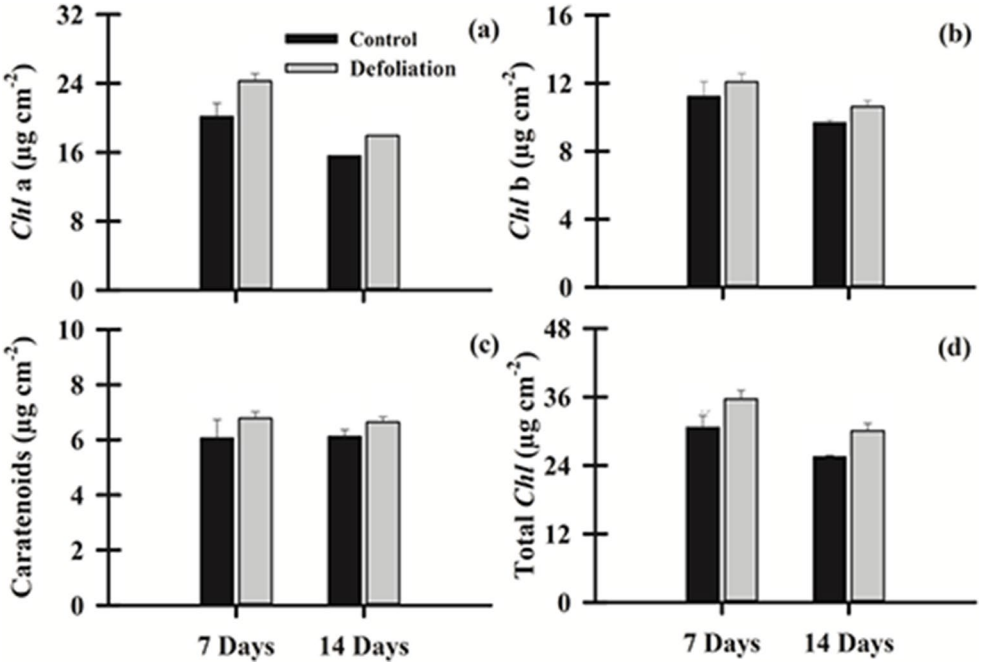


**Fig. 4** Chlorophyll *a* content (Chl a, **a**), chlorophyll b content (Chl b, **b**), carotenoid content (**c**), and total chlorophyll content (total Chl, **d**) of maize plants in the control and defoliation treatments at seven and fourteen days after treatment. * Indicates a significant difference in the defoliation treatment according to Student's t test at the 5% probability level ($p < 0.05$). Each value indicates the treatment mean ± standard error of the mean

there was no significant difference between treatments at the same evaluation time (Table 3).

## Root morphology and nutrients

For the root morphology variables, such as length, surface area, mean diameter and root volume, the control plants showed greater values than did the plants with defoliation at seven and fourteen days after the implementation of the treatments ($p < 0.05$) (Fig. 5). The same trend was observed for length, surface area and root volume by root diameter class at both evaluation times ($p < 0.05$) (Fig. 6).

The shoot dry biomass (SDW), root dry mass (RDW), RDW/SDW ratio and specific root length (SRL) of the control maize plants were greater than those of the control maize plants at both seven and fourteen days after defoliation ($p < 0.05$) (Fig. 7). However, when comparing the percentage of shoot and root growth over 14 days, it was verified that the control plants showed 243% and 346% increases in the dry biomass of shoots and roots, respectively, in the

**Table 2** Starch, total soluble sugars (TSS), reducing sugars (RS), amino acids (AA) and protein (PTN) contents in maize leaves from the control and defoliation treatments at seven and fourteen days after the imposition of the treatments

| Treatments | Starch | TSS | RS | AA | PTN |
|---|---|---|---|---|---|
| **7 days** | | | | | |
| Control | 0.8 ± 0.1 | 3.4 ± 0.2 | 18.1 ± 7.1 | 115.8 ± 6.1 | 30.5 ± 0.2 |
| Defoliation | 1.1 ± 0.4 | 3.3 ± 1.4 | 17.8 ± 6.6 | 137.5 ± 3.4 * | 26.2 ± 1.9 |
| CV (%) | 22.4 | 9.1 | 28.5 | 4.8 | 10.6 |
| **14 days** | | | | | |
| Controle | 1.5 ± 2.4 | 2.4 ± 5.4 | 44.1 ± 2.2 * | 89.5 ± 6.1 * | 21.1 ± 2.4 * |
| Desfolha | 0.9 ± 3.6 | 2.1 ± 3.3 | 23.9 ± 6.4 | 56.8 ± 4.5 | 17.1 ± 1.3 |
| CV (%) | 21.0 | 11.1 | 7.9 | 15.3 | 12.0 |

* Indicates a significant difference between treatments using the Student's t-test at a 5% probability level ($p < 0.05$). Each value indicates the treatment mean ± standard error of the mean. CV: coefficient of variation

**Table 3** Gibberellic acid (GA3), indoleacetic acid (IAA), indole butyric acid (IAB), salicylic acid (AS), trans zeatin riboside (zeatin) and gibberellin (GA) in maize leaves from control and defoliation plants at seven and fourteen days after the imposition of treatments

| Treatments | GA3 | GA | AIA | AIB | AS | Zeatina |
|---|---|---|---|---|---|---|
| | ---------- mg $kg^{-1}$ ---------- | | | | | |
| **7 days** | | | | | | |
| Control | 0.0134 ± 0.004 | 0.9614 ± 0.4 | 0.0258 ± 0.01 | 0.0622 ± 0.01 * | 0.0058 ± 0.0 | 0.0020 ± 0.0 |
| Defoliation | 0.0126 ± 0.01 | 1.7204 ± 0.21 * | 0.0180 ± 0.01 | 0.0262 ± 0.01 | 0.0128 ± 0.01 * | 0.0022 ± 0.001 |
| CV (%) | 24.7 | 11.7 | 32.8 | 14.6 | 12.9 | 18.2 |
| **14 days** | | | | | | |
| Control | 0.0146 ± 0.002 | 0.6546 ± 0.22 | 0.0442 ± 0.004 * | 0.0622 ± 0.003 * | 0.0036 ± 0.002 | 0.0016 ± 0.001 * |
| Defoliation | 0.0150 ± 0.01 | 1.0056 ± 0.05 * | 0.0220 ± 0.002 | 0.0492 ± 0.007 | 0.0054 ± 0.001 * | 0.0000 ± 0.00 |
| CV (%) | 29.6 | 7.7 | 16.9 | 12.5 | 12.2 | 28.4 |

* Indicates a significant difference between treatments using the Student's t-test at a 5% probability level ($p < 0.05$). Each value indicates the treatment mean ± standard error of the mean. CV: coefficient of variation

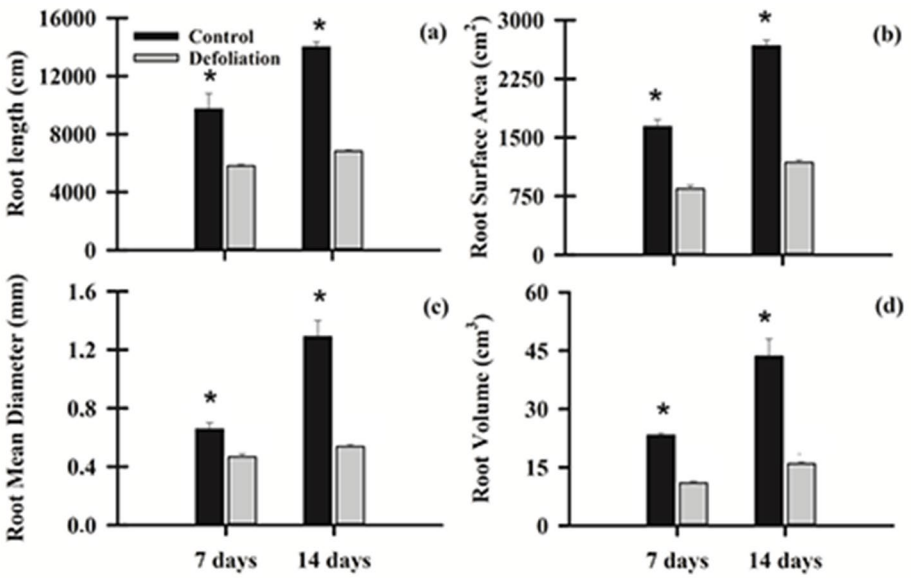


**Fig. 5** Root system length (**a**), surface area (**b**), mean diameter (**c**) and root volume (**d**) of the control treatment and defoliation of maize plants at seven and fourteen days after treatment. * Indicates a significant difference in the defoliation treatment according to Student's t test at the 5% probability level ($p < 0.05$). Each value indicates the treatment mean ± standard error of the mean

two evaluated time periods (Fig. 8). In contrast, plants under defoliation showed increases of 766% and 305% in the dry biomass of shoots and roots, respectively (Fig. 8). Compared with those of the plants in the defoliation treatment, the root fineness (RF) at both evaluation times was lower in the control treatment ($p < 0.05$) (Fig. 7e). For root tissue density (RMDe) at seven days after defoliation, the control treatment plants expressed higher values than did the defoliated plants (Fig. 7f). Nevertheless, at fourteen days, the RMDe of the defoliated maize plants was greater than that of the control plants ($p < 0.05$) (Fig. 7f).

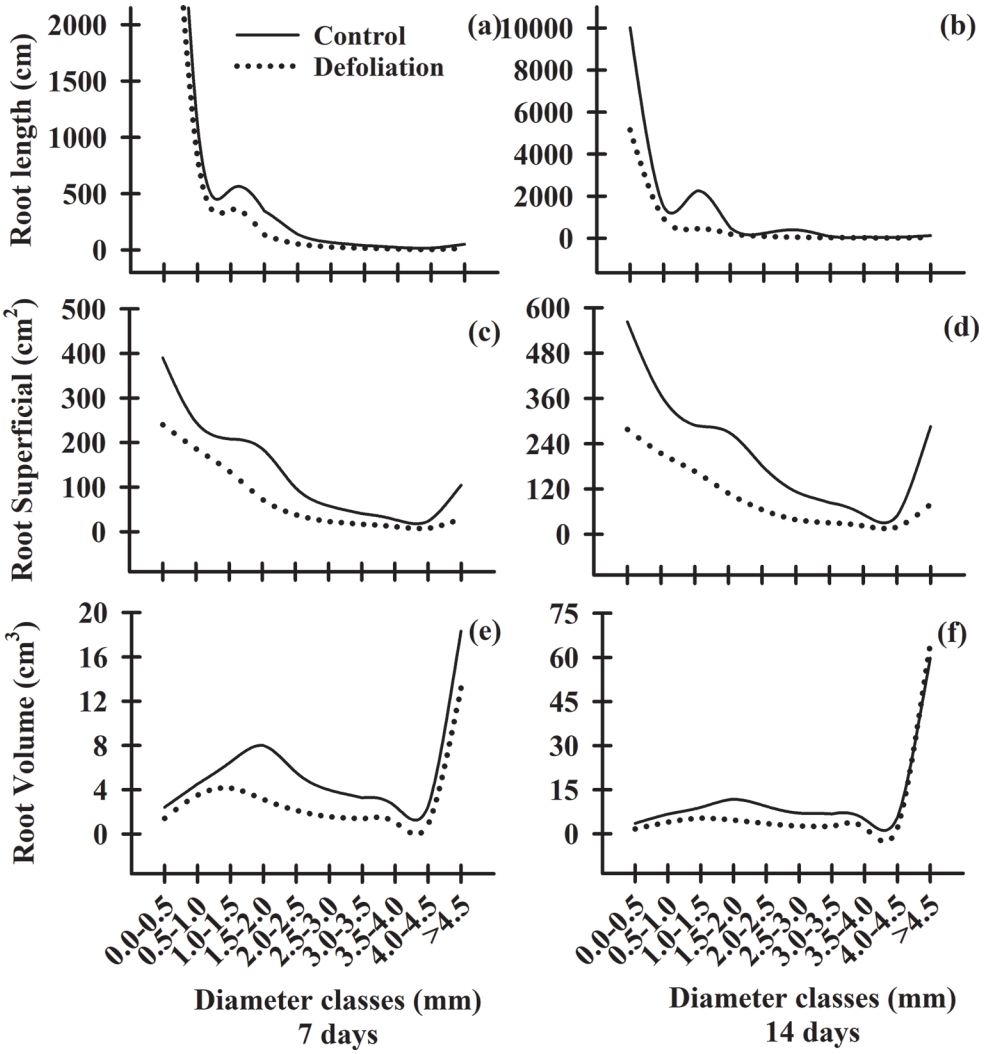


**Fig. 6** Length (root length), surface area (root superficial), and volume (root volume) of the root system in the different diameter classes of maize plants at seven (**a**, **c**, **e**) and fourteen days (**b**, **d**, **f**) after defoliation

According to the leaf analysis, only the copper content was greater in the defoliated maize plants than in the control plants ($p < 0.05$) (Table 4). The other nutrients, including N, P, K, Ca, Mg, S, B, Fe and Mn, did not significantly differ among the treatments (Table 4).

### Leaf anatomy

In terms of leaf anatomy, the maize plants in the control treatment had greater abaxial epidermis thickness (BET), adaxial epidermis thickness (DET), mesophyll thickness (MPT), vascular bundle area (VBA), metaxylem vessel diameter (DMV) and phloem area (PA) than those in the defoliation treatment at seven and fourteen days after defoliation ($p < 0.05$) (Table 5).

In the paradermal section, the plants from the defoliation treatment had lower stomatal density (SD), polar diameter (PD), equatorial diameter (ED) and number of epidermal cells (NE) than those in the control treatment, both at seven and fourteen days after defoliation ($p < 0.05$) (Table 6).

## Discussion

### Morphometric, phytohormones and biomolecules

The maize plants had their leaves removed, and a minimum leaf area was maintained, which led to a reduction in their biometric measurements. The intensity of defoliation interferes with damage to plant structure; thus, the removal of the total leaf area reduces the vegetative growth of plants (Rezende et al. 2015). In addition, decreases in height, diameter and even root morphology and biomass occurred because when the leaf area decreased, the availability of photoassimilates to be distributed to the drains decreased until the re-establishment of new leaves and the resumption of growth.

In the adult vegetative stage that begins after V4, the demand for photoassimilates increases, and resources are directed toward the intense development of the shoots and roots observed in the control plants between 7 and 14 days after the imposition of the treatments (Jans et al. 2010). This

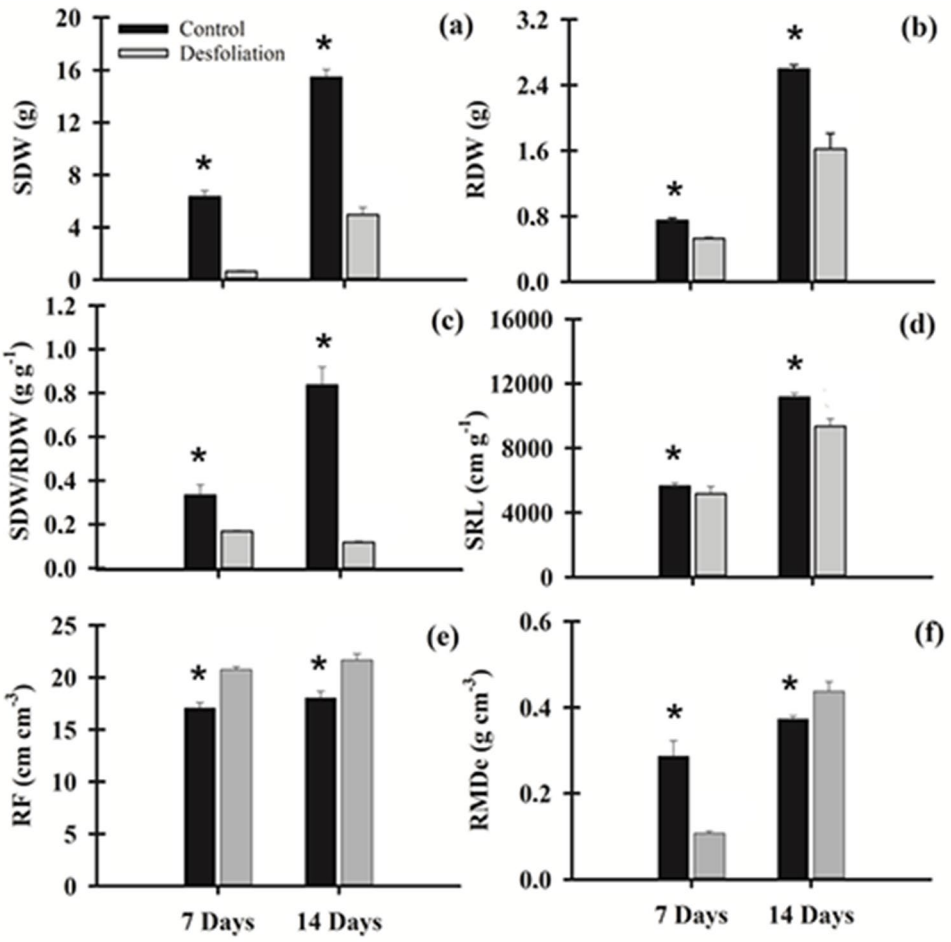


**Fig. 7** Attributes of dry biomass and morphology of maize plants with and without defoliation. Dry biomass of shoots (SDW, **a**), root dry biomass (RDW, **b**), the relationship between root dry biomass and shoot dry biomass (RDW/SDW, **c**), root-specific length (SRL, **d**), root fineness (RF, **e**), and root tissue density (RMDe, **f**) at seven and fourteen days after defoliation. * Indicates a significant difference in the defoliation treatment according to Student's t test at the 5% probability level ($p < 0.05$). Each value indicates the treatment mean ± standard error of the mean

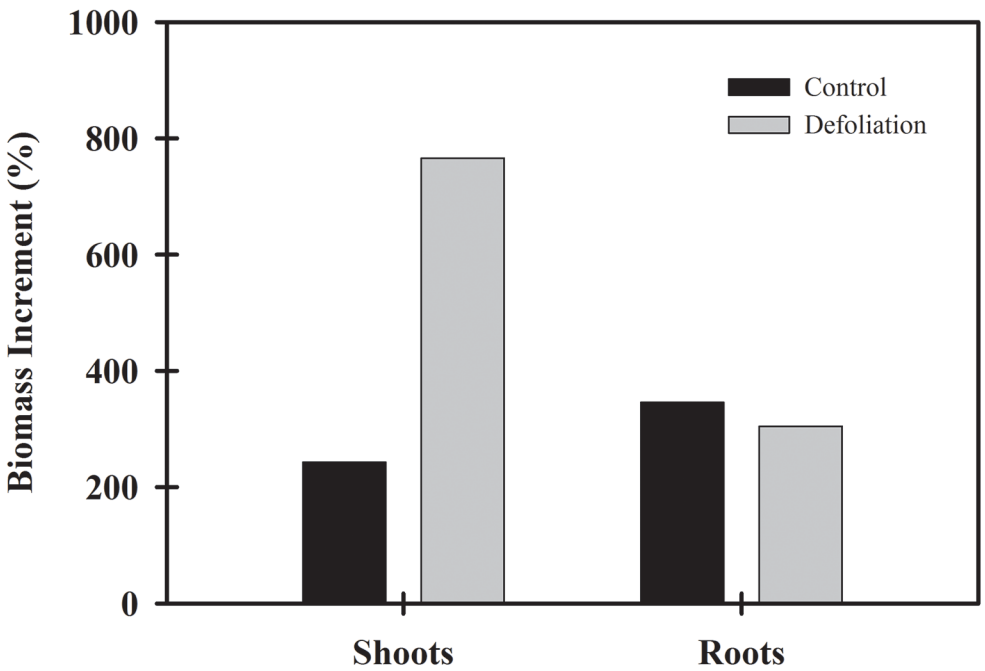


**Fig. 8** The percentage of shoot biomass accumulation calculated over the fourteen days in the control and defoliation treatments

**Table 4** Concentration of macro and micronutrients in maize leaves in the control and defoliation treatments after fourteen days of treatment imposition

| Treatments | N | P | K | Ca | Mg | S | B | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | g $kg^{-1}$ | | | | | | mg $kg^{-1}$ | | | | |
| Control | 26.7 ± 1.5 | 2.9 ± 0.5 | 22.0 ± 6.4 | 7.4 ± 2.5 | 2.9 ± 1.3 | 1.2 ± 0.4 | 17 ± 2.5 | 9.5 ± 1.2 | 98.7 ± 6.5 | 46.7 ± 1.3 | 6.5 ± 1.4 |
| Defoliation | 29.9 ± 3.7 | 3.4 ± 0.2 | 27.4 ± 1.0 | 9.8 ± 1.1 | 4.5 ± 0.3 | 1.1 ± 0.1 | 20.6 ± 4 | 30.2 ± 5.8 * | 79.7 ± 5.2 | 48.7 ± 4.1 | 5.6 ± 0.3 |
| CV (%) | 10.0 | 12.3 | 18.1 | 12.5 | 25.4 | 9.9 | 19.3 | 21.3 | 17.7 | 11.4 | 16.1 |

* Indicates a significant difference between treatments using the Student's t-test at a 5% probability level ($p < 0.05$). Each value indicates the treatment mean ± standard error of the mean. CV: coefficient of variation

**Table 5** Abaxial epidermal thickness (BET), adaxial epidermal thickness (DET), mesophyll thickness (MPT), vascular bundle area (VBA), metaxylem vessel diameter (DMV) and phloem area (PA) of maize plants control and defoliation at seven and fourteen days after defoliation

| Treatments | BET | DET | MPT | VBA | DMV | PA |
|---|---|---|---|---|---|---|
| **7 days** | | | | | | |
| Control | 3.0 ± 1.4 * | 2.9 ± 0.9 * | 19.9 ± 4.7 * | 24871.1 ± 29.8 * | 49.8 ± 3.4 * | 5019.9 ± 80.6 * |
| Defoliation | 1.9 ± 0.4 | 2.0 ± 0.7 | 17.4 ± 3.6 | 20189.5 ± 32.4 | 36.1 ± 7.2 | 3176.7 ± 61.1 |
| CV (%) | 1.0 | 7.9 | 1.9 | 3.6 | 7.2 | 8.0 |
| **14 days** | | | | | | |
| Controle | 3.9 ± 0.2 * | 4.1 ± 1.1 * | 59.3 ± 15.6 * | 43882.4 ± 46.20 * | 60.3 ± 8.4 * | 8620.8 ± 81.7 * |
| Desfolha | 2.8 ± 0.9 | 2.9 ± 0.6 | 43.3 ± 11.6 | 26072.8 ± 55.8 | 35.9 ± 3.2 | 4712.5 ± 54.9 |
| CV (%) | 7.2 | 12.0 | 13.5 | 4.2 | 3.6 | 5.4 |

* Indicates a significant difference between treatments using the Student's t-test at a 5% probability level ($p < 0.05$). Each value indicates the treatment mean ± standard error of the mean. CV: coefficient of variation

**Table 6** Anatomical analyses of paradermal leaf sections from the adaxial surface of the leaves of control and defoliated maize plants at seven and fourteen days after defoliation. Stomatal density (SD), polar diameter (PD), equatorial diameter (ED) and the number of epidermal cells (NE).

| Treatments | SD | PD | ED | NE |
|---|---|---|---|---|
| **7 Days** | | | | |
| Control | 175 ± 21 * | 21 ± 10 * | 27 ± 7 * | 276 ± 24 * |
| Defoliation | 152 ± 14 | 16 ± 6 | 20 ± 2 | 254 ± 19 |
| CV (%) | 4.09 | 9.45 | 2.24 | 3.67 |
| **14 Days** | | | | |
| Controle | 182 ± 12 * | 22 ± 3 * | 29 ± 5 * | 294 ± 26 * |
| Desfolha | 163 ± 19 | 18 ± 1 | 24 ± 6 | 264 ± 17 |
| CV (%) | 6.41 | 2.08 | 1.47 | 3.28 |

* Indicates a significant difference between treatments using the Student's t-test at a 5% probability level ($p < 0.05$). Each value indicates the treatment mean ± standard error of the mean. CV: coefficient of variation

is also reflected when observing the levels of starch, sugars, and proteins in the control plants. In defoliated plants, there was a reduction in the levels of reducing sugars, amino acids and proteins compared to those in the control, thus explaining the lower development of stressed plants. However, in defoliated plants, foliar reducing sugars increased after 14 days of defoliation, which is consistent with the re-establishment of shoot development.

Despite the reduction in biometric measurements compared with those of the control, the defoliated maize plants showed a greater percentage of biomass accumulation than did the control plants between seven and fourteen days after defoliation. The 766% increase in shoot dry mass in the plants in the defoliation treatment group can be attributed to the action of phytohormones, such as gibberellin (GA) and salicylic acid (SA), at seven and fourteen days after defoliation. Gibberellins play roles in stem cell elongation, leaf expansion, the development of new leaf primordia, and plant adaptation to biotic and abiotic stresses (Gao and Chu 2020; Amin et al. 2022). In addition, in agricultural practices, the manipulation of GA levels is often used to optimize maize growth and productivity, even under conditions of environmental stress (Stutts et al. 2018).

The action of gibberellin may have induced the growth of the shoots of maize plants under early defoliation. Gibberellin increases stem growth and the length/thickness ratio of maize leaves (Sprangers et al. 2020). An increase in the concentration of GA in maize plants subjected to defoliation induced an increase in plant height but reduced the thickness of epidermal and mesophyll cells, as well as in the vascular bundle. Conversely, we observed that after 14 days of defoliation, there was an increase in the thickness of the leaf blade compared to that at 7 days, thus indicating a possible re-establishment of leaf growth, especially in the thickness of the mesophyll and in the phloem area.

Furthermore, the increase in the concentration of salicylic acid (SA) in the defoliated plants may also have helped in

the recovery of maize plants. SA plays an active role in the response of maize to various abiotic stresses, such as water deficits, salinity, heavy metals, high temperature and cold (Kaur et al. 2019; Naz et al. 2021; Sultan et al. 2021; Zhang et al. 2021). SA also enhances the biotic stress response of maize, whereby it is a key component in the hypersensitivity response and induced and acquired systemic defenses (Chen et al. 2020; Feng et al. 2022; Ziemann et al. 2018). SA can cause most of the deleterious effects of stress while supporting plant defenses (Sultan et al. 2021). The increase in AS concentration in plants under defoliation may have contributed to the maintenance of chlorophyll content and an increase in stomatal conductance (Zhang et al. 2021), carbohydrate (Kaur et al. 2019) and protein contents (Zanganeh et al. 2019). Together, these effects contributed to the resumption of plant development after defoliation.

However, the levels of the phytohormones indoleacetic acid (IAA), indolebutyric acid (IBA) and zeatin decreased in the defoliated plants, which was mainly observed at fourteen days. Auxins are mainly produced in shoots and play a key role in regulating plant root development, shaping the root system in response to variations in the environment and conferring an adaptive advantage (Xiao and Zhang 2020). In maize, auxins induce the formation of primary, seminal, lateral, and adventitious roots (Viana et al. 2022). Conversely, cytokinin is a known repressor of rhizogenesis, and the increase in its degradation in maize plants contributes to root development and nutrient uptake efficiency (Ramireddy et al. 2021).

The hormonal pattern expressed in the control plants between 7 and 14 days involved a reduction in the levels of gibberellins and salicylic acid but an increase in the levels of auxins. This result may indicate a prioritization of shoot growth during the maize juvenile vegetative phase (up to V4), followed by the intensification of root growth at the beginning of the adult vegetative phase, to prioritize water and nutrient uptake that will support rapid growth relating to plant development (Jans et al. 2010). However, in the defoliated plants, the auxin levels were lower than those in the control plants, thus explaining the reduced root growth and indicating that the plants subjected to defoliation were preferentially colonized by the shoots.

Although we have observed these indications of plant recovery under defoliation, the increase in gibberellin levels and the reduction in auxins induced the development of thin leaves and less development of the root system, which are characteristics that lead to greater sensitivity to environmental stress. In contrast, an increase in SA can improve tolerance to subsequent environmental stresses.

## Physiological characteristics

It is also worth noting that maize under defoliation, despite having decreased leaf area, exhibited good physiological recovery, due to the fact that gas exchange, as well as pigment and nutrient contents, were not affected compared with those of the control plants. Furthermore, greater stomatal conductance ($g_s$), transpiration (E) and intercellular $CO_2$ concentration (Ci) were observed in defoliated maize at fourteen days after the beginning of treatment, thus suggesting a positive response of these plants after this mechanical stress, because these plants did not experience any other limiting factor. High $g_s$ increases $CO_2$ availability to chloroplasts and can mitigate photosynthetic limitations and compensate for leaf area loss. Although there was a reduction in the leaf area and the number and size of the stomata, the net $CO_2$ assimilation rate (A) was also similar in the two treatments at both evaluation times, thus indicating that although the leaf area was smaller, the maize maintained efficient carbon fixation for the recovery of these plants. These results may indicate greater efficiency in the stomatal control of plants under defoliation. This may also be related to the maintenance of chlorophyll contents, due to the fact that plants with high chlorophyll contents exhibit a high growth rate after defoliation (Raineri et al. 2022). In addition, these results may indicate that even under defoliation stress, maize plants maintain photosynthesis and photoassimilate accumulation, thus promoting rapid growth. In this manner, plants continue to develop, thus initiating new leaf primordia (Khaliliaqdam et al. 2012).

With a goal of promoting compensatory growth after defoliation, plants reallocate assimilates from the root system to the shoot (Quentin et al. 2011). Thus, when analyzing the results of the biomolecules, significant differences were observed, which were likely due to this reallocation of resources from the root to the shoot, which aimed to compensate for the tissues after defoliation (Liu et al. 2007). These results, combined with less root development, demonstrate the reallocation of resources to recompose the shoots. When this defoliation process occurs, there is a very high energy expenditure for the plant to be able to regrow and restore its leaves, which are sources of photoassimilates. Chen et al. (2009) reported that after defoliation in rice seedlings, the remaining aboveground tissues used anaerobic respiration as an emergency measure for energy/substrate supply in response to the sudden loss of photosynthetic leaves.

Thus, after suffering defoliation, plants exhibit an imbalance in the development and allocation of carbon reserves, where root growth is reduced, whereas the regeneration of shoots is maintained by an increase in the allocation of reserves from the root to the stem (Barbosa et al. 2019). At seven days after defoliation, the higher content of amino

acids in the defoliated plants may be related to the ability of maize to take advantage of the available nitrogen for the formation of new leaf primordia. Thus, the allocation of carbon from roots, together with the availability of free amino acids, supports the synthesis of new molecules aimed at the maintenance and growth of new leaves. This balance between carbon and nitrogen metabolism is fundamental for plant homeostasis and the synthesis of new structures and other fundamental compounds. Fourteen days after defoliation, there was a decrease in reducing sugars and amino acids in the plants under defoliation, which may be related to the high use of these molecules by cellular metabolism aimed at reconstituting leaf tissues, which consequently required high energy expenditure.

Maize plants that were defoliated at the V4 stage showed potential for growth recovery up to the V7 stage. Carbohydrate metabolism did not change at seven days after defoliation. This fact may be related to the early occurrence of changes in metabolism in the first hours after defoliation, which is aimed at the rapid recovery of the shoots of the plants. Conversely, after the establishment of the leaves at fourteen days, a high consumption of reducing sugars in the leaves was evident, thus indicating high metabolic activity. It is also necessary to consider that some of the carbohydrates reallocated from the roots may have been quickly used to supply energy or carbon skeletons for the synthesis of defense compounds aimed at protecting the photosynthetic apparatus against excess radiation and temperature (Batista 2015).

## Root morphology and nutrients

With defoliation, there is a reduction in the development of the root system caused by the relocation of assimilates and nitrogenous compounds to the shoots under reconstruction. After the establishment of the leaves, which resume their role as source organs, there is a reconstitution of metabolism in search of the stabilization of all plant mechanisms (Iqbal et al. 2012). This may be related to the greater levels of amino acids in the leaves at seven days, followed by a decrease at fourteen days after defoliation. Initially, all of the resources were directed to the shoots, which aimed to restore the leaves. As foliar metabolism was enhanced, there was a rapid consumption of amino acids and reducing sugars, the latter effect being closely related to the supply of energy for cellular processes.

The increase in metabolism of the defoliated plants in this study can be attributed to the greater percentage of shoot growth (766%) in comparison to that of the control plants (243%) after seven days. First, plants with the C4 mechanism can accumulate more carbohydrates in their tissues, which can be used for regeneration after defoliation stress (Long et al. 2006). In addition, the good recovery of Poaceae after defoliation stress is due to the existence of a leaf meristematic zone below ground level up to the V6 vegetative stage (with six fully expanded leaves), which ensures prompt recovery (Lestienne et al. 2006). The phytohormone response helped the plants develop again in search of recovery. Finally, growing plants under ideal conditions contributes to this recovery after this mechanical stress (Karam et al. 2020).

In this study, the only stress to which the maize plants were subjected to was defoliation; therefore, good irrigation and ideal nutrient conditions may have contributed to the good recovery of the plants, which produced good results in terms of gas exchange, chlorophyll content and nutrient content up to fourteen days after defoliation. No symptoms of nutritional deficiencies were observed in the plants; in addition, except for Zn, all of the nutrients were found in concentrations compatible with those determined for the crop (Ribeiro et al. 1999). This suggests that nutrient supply did not limit plant performance, allowing enzymatic reactions, ionic exchanges, and other metabolic processes to function correctly. Most micronutrients are related to the activity of different enzymes, and macronutrients are extremely important for the formation of new cells and leaf expansion (Kirkby and Romheld 2007). For example, nitrogen is one of the essential elements for plants and is closely related to the development of new leaf primordia (Malavolta et al. 1997), with marked effects on biomass accumulation and crop productivity (Seaver 2022), in addition to being directly related to the chlorophyll content in the leaves.

When analyzing the high growth of shoots at the expense of root growth, it is possible to infer the reallocation of reserves. Specifically, concerning the length, surface area, diameter and volume of the root system, lower values were observed in plants subjected to defoliation stress. Defoliation led to a decrease in morphological variables, which was reflected both in the reduction in root biomass and in the greater investment of resources for shoot growth.

The root surface area is the one variable that is most related to the absorption of nutrients (Imada et al. 2008), whereas the root volume provides greater efficiency of absorption of these nutrients, thus favoring good development of the plant (Marques et al. 2018). Despite the decrease in surface area and root volume, the plants were in ideal growing conditions, which possibly favored poststress recovery. Furthermore, there were no differences in nutrient values between control and defoliated plants, thus suggesting that the roots were efficient at absorbing nutrients in defoliated plants.

Defoliation also led to changes in root morphology in relation to diameter class. Thin roots are highly important for the absorption of water and nutrients; thus, plants with

thin roots exhibit greater vigor (Magalhães 2021). Compared with the control treatment, defoliation did not severely impact the amount of fine or very fine roots. This may have been decisive for the defoliated plants to be able to absorb the nutrients that were made available, thus contributing to the maintenance of their metabolism.

The relationship between shoot and root biomass was lower in plants with defoliation. This can be attributed to the disadvantage of the shoots and the allocation of resources from the roots to the formation of new leaves. In addition, it was also possible to observe a decrease in the specific length of the roots (SRL), thus suggesting that in the initial postdefoliation recovery, the energy investment was specific for the shoots. Despite the ability of SRL to reflect on greater exploration and acquisition of water and nutrients in the soil per unit of carbon invested (Bouma et al. 2001), the plants were not severely impacted due to good management conditions.

Roots with high amounts of dead and fibrous mass (root tissue density, or RMDe) are very common in plants subjected to stress conditions (Cruz et al. 2021). In this study, at seven and fourteen days after treatment, defoliation resulted in greater root tissue density, thus showing that the plants used their energy for the development of new leaf primordia.

### Leaf anatomy

Defoliation led to a decrease in leaf anatomy variables. This can be justified as representing a plant strategy to reduce energy expenditure. Specifically, although it is possible to reallocate resources from the roots to the shoot, the plants likely reduced the investment in tissue formation, with the goal of eliciting energy savings. In this poststress phase, resources are invested in the production of more leaf primordia but in smaller leaves. This action may also be related to the action of phytohormones as an initial response to stress. Furthermore, it is likely that at later stages, maize plants will similarly re-establish tissue growth to control plants.

However, it is worth mentioning that further studies with maize in more advanced vegetative stages are important to understand at which stage the plant re-establishes growth that is equal to that of the control. In addition, we aimed to understand how hormonal patterns and biomolecules act during the recovery of defoliated plants over time.

## Conclusions

Early defoliation in maize modifies the morphological characteristics of the plants, with a reduction in the development of the shoots and the root system.

Maize plants that are defoliated at the V4 stage have a high capacity for resuming growth and biomass accumulation. This re-establishment of maize plants after defoliation involves the action of the phytohormones gibberellin and salicylic acid through the induction of shoot growth, maintenance of chlorophyll content, increase in stomatal conductance and greater efficiency in carbon fixation.

After mechanical stress, maize plants exhibit thinner leaves and less development of the root system, which are characteristics that reinforce the prioritization of shoot growth. In addition, in plants undergoing defoliation, there is a reallocation of amino acids and reducing sugars, which helps in the formation of new leaf primordia.

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