Whole shoot gas exchange and stomatal traits in maize under water drought as related to its growth

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Abstract: Leaf area is a functional characteristic that can be used as a key indicator of responses to global environmental changes. Thus, this study aimed to evaluate the effects of drought on the growth, leaf area, gas exchange, and leaf anatomy of *Zea mays* L. The experiments were conducted in a greenhouse with a controlled temperature. The plants were exposed to three water conditions: field capacity (FC), 75% of FC, and 50% of FC, using 10 replicates of one plant each. Drought reduced the dry mass of vegetative organs, which slowed plant growth. This helped the water potential remain unchanged, indicating the stability of the partial water pressure. In addition, the number of leaves, leaf width, leaf thickness, and mesophyll area were reduced by drought and severely decreased the leaf area. No significant changes were detected in gas exchange per unit area, but gas exchange in the whole plant was lower indicating the necessity to evaluate this trait in maize. Drought reduced the stomatal index on the adaxial side and the total number of stomata and stomatal pore area on both the abaxial and adaxial sides also indicating that the responses of whole leaf area are important to maize studies under drought.

Keywords: plant productivity, leaf anatomy, leaf expansion, water stress, photosynthesis, dry mass.

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1 Introduction

Agricultural productivity is a crucial economic factor in developing countries, especially in regard to food. Maize is considered the most important basic crop in the world. However, the water deficit has affected its productivity (Song et al., 2018). Drought is a stressor for plants and can last for long periods (Cai et al., 2020). Since water is essential to a plant's growth, yield, and physiological processes, water deficit significantly reduces plant height, biomass gain, photosynthetic rate, and water content (Cowan et al., 2020) and can even modify the allocation of dry mass to different vegetative organs (Zhou et al., 2020). Due to the negative effects caused by water deficit, plants modify their anatomical and physiological characteristics to survive under these conditions.

Although the water deficit promotes several changes in plants, the changes to leaf area are particularly noteworthy. This is considered a limiting

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factor for plant species because it has a direct relationship with growth. Under drought conditions, a lower leaf area promotes lesser plant growth (Hsie et al., 2015; Widuri et al., 2020). This may be related to water conservation in the plant. In fact, a reduction in leaf area is a common response to drought because it allows plants to reduce their transpiration and increase water use efficiency (Hsie et al., 2015; Wyka et al., 2019). Water deficit also increases the occurrence of leaf senescence and abscission, which promotes the reduction in leaf area. Low turgor pressure promotes a reduction in the number of leaves and in the total leaf area (Zia et al., 2021). Therefore, the leaf area modulates the plant growth responses related to water conservation under drought conditions.

Drought promotes changes in the mesophyll, decreasing leaf expansion. In a study with barley (*Hordeum vulgare* L.), the leaves produced under drought conditions showed a lower mesophyll thickness (Wyka et al., 2019). This may be related to the meristematic activities of the leaves. In fact, lack of water inhibits cell elongation, affecting cell division, which limits leaf growth (Avramova et al., 2015). In this context, the reduction in leaf area can also lead to fewer leaf stomata, limiting gas exchange, especially the photosynthetic rate. This affects biomass gain and plant growth under drought conditions. In fact, stomatal adjustment and reduction of leaf area are considered common strategies to avoid drought and stomatal limitation can directly affect gas exchange (Cai et al., 2020). Therefore, decreasing leaf expansion may lower the number of stomata in the leaf and may limit gas exchange and plant growth.

Drought promotes reduced plant growth (Hsie et al., 2015; Song et al., 2018; Zhou et al., 2020). Decreasing growth promotes a reduction in plant productivity (Song et al., 2018). The rate of plant growth is usually related to the photosynthetic rate per unit area (Liu et al., 2019). Nevertheless, the total leaf area does not stand out as a modulator of the photosynthetic responses in the whole plant. In fact, the total leaf area can have different relationships with growth and the photosynthetic rate per unit area, since the lack of water can also reduce growth and the photosynthetic rate (Liu et al., 2019), or plant growth can be slowed without changes in the photosynthetic rate in response to a mild to moderate drought (Sapeta et al., 2013). In this context, some species decrease their growth but maintain the photosynthetic rate per unit area under drought conditions. Therefore, the analysis of the total leaf area is extremely important because it can be used to adjust some physiological parameters that are calculated in a localized manner, for example, the photosynthetic rate calculated per unit area. This allows us to measure parameters that reflect a more real-world scenario.

In this context, the total leaf area actually has a direct relationship with plant growth. In addition, associating the total leaf area with gas exchange parameters is essential to obtain more realistic values, as corroborated by Cruz et al. (2019). In fact, drought can reduce plant growth by maintaining or decreasing photosynthesis per unit area. Therefore, the leaf area may influence the reduction in growth under drought conditions, since it is not the fixed photosynthetic rate per area that may be causing the growth reduction. Plant growth may also be influenced by stomatal changes, since reduced stomatal activity may limit leaf gas exchange, which is also correlated with leaf area.

The hypothesis of this study is that whole shoot modifications in gas exchanges and stomatal traits influences are defined by the leaf area and modulate maize growth under drought conditions. Therefore, the objective of this study was to evaluate the growth, leaf area, gas exchange, and leaf anatomy of *Zea mays* L. (Poaceae) under drought conditions.

2 Materials and methods

2.1 Plant material and experimental design

The experiment was conducted in a greenhouse located at the Federal University of Lavras (UFLA), Minas Gerais state, Brazil (21°13′17″ S and 44°57′47″ W). *Zea mays* L. plants were obtained from seeds provided by EMBRAPA Maize and Sorghum, Sete Lagoas, Minas Gerais (MG), Brazil.

The seeds were sown in 5.0 L plastic pots containing 2.0 L of sand and 800 mL of 40%-strength nutrient solution (Hoagland and Arnon, 1950). Then, they were placed in a germination chamber with constant light at 25°C for approximately 7 days until the seedlings had three leaves and had reached 10 cm in height.

Seedlings in good phytosanitary conditions and with similar sizes were transplanted separately to 5.0-L plastic pots containing 3.0 L of sand and 40%-strength nutrient solution (Hoagland and Arnon, 1950). The pots were kept in a greenhouse at 25°C±2°C, 50% relative humidity, and a 12-hour photoperiod.

The plants were then subjected to three water conditions, adapting the method used by Díaz et al. (2018). The water conditions used in the experiments were (1) field capacity (FC), (2) 75% of field capacity (75% FC), and (3) 50% of field capacity (50% FC). The field capacity was considered the maximum volume of water retained by 1.0 L of sand without becoming flooded. The volume of water applied to reach FC was 310.0 ml and for 75% FC and 50% FC, the amounts of water in the substrate were kept at 232.5 and 155.0 ml, respectively. The plants remained under these conditions for 60 days. The water lost by evapotranspiration was monitored by the daily difference in the weight of each pot and then replenished. The nutrient solution was replenished weekly. The experimental design was completely randomized with three treatments and 10 replicates of one plant each. For the variables whose data were obtained from multiple analyses, the mean of each replicate was calculated.

2.2 Analysis of plant growth and water content

Plant height and number of leaves were evaluated at 10-day intervals. The plants were sampled at the end of the experiment and separated into leaves, stems and roots. Leaf area, length, and width were measured with a CI-203 handheld leaf area meter (CID Bioscience, Camas, WA, USA). Then, the fresh mass of each part was measured on an AY220 analytical balance (Shimadzu, Kyoto, Japan). Roots, leaves, and stems were dried in an oven at 60°C to constant weight, and the dry mass was evaluated on an analytical scale. The biomass allocated to each organ was calculated using the following equation (Santos et al., 2015): $AL = (ODM/PDM) \times 100$, where AL is the biomass allocation to a given organ (%), ODM is the organ dry mass (g), and PDM is the total plant dry mass (g). The water mass in each plant organ was calculated by the difference between the fresh and dry masses.

All water applied in the experiment was measured for each replicate. Water retention in plants was estimated using the following equation: $WR = (PWM/WEX) \times 100$, where WR is water retention (%), PWM is the plant water mass (g), and WEX is the total water mass applied in the experiment (g). The water loss by evapotranspiration ($EVAP$) was estimated by the equation: $EVAP\ (\%) = 100 - WR$.

Leaf water potential was measured at the end of the experiment with a portable Scholander pressure chamber (PMS Instrument Company, Corvallis, OR, USA). N_2 gas was used to apply the necessary pressure. The measurements were performed at dawn between 4:00 am and 6:00 am on the first fully developed leaf of the apex.

2.3 Physiological analyses

At the end of the experiment, leaf gas exchange was assessed with an LI-6400XT model infrared gas analyzer (LI-COR Biosciences, Lincoln, USA) coupled to an LI-6400-02B cuvette with red/blue LED light source (LI-COR, Lincoln, USA). Measurements were taken for two fully developed leaves per plant from 08:00 to 10:00 a.m., with the photon flux density fixed at 1000 μmol m^{-2} s^{-1}, and a mean temperature of 28.1ºC; CO_2 was obtained from the atmosphere, its mean concentration was 420.1 μmol mol^{-1} air, and the pump flow was 500 μmol s^{-1}. The net photosynthesis (A) (μmol CO_2 m^{-2} s^{-1}), transpiration rate (E) (mmol H_2O m^{-2} s^{-1}), stomatal conductance of water (g_{sw}) (mol H_2O m^{-2} s^{-1}) and CO_2 (g_{tc}) (mol CO_2 m^{-2} s^{-1}) were assessed. The whole-plant photosynthesis (A_{wp}) (µmol CO_2 s^{-1}), whole-plant transpiration (E_{wp}) (mmol H_2O s^{-1}), and whole-plant conductance (g_{swp}) (mol H_2O s^{-1}) were calculated with the following equations: $A_{wp} = A\times(leaf\ area)$; $E_{wp} = E\times(leaf\ area)$; and $g_{swp} = g_s\times(leaf\ area)$. The parameters obtained directly by the analysis of the IRGA are given in moles (CO_2 or water) per square meter per second thus, by multiplying by the real leaf area of the plant (much lower than one square meter at the sampling age), the calculated parameters are given in moles (CO_2 or water) per second.

The chlorophyll content was estimated with a SPAD-502 chlorophyll meter (Konica-Minolta, Tokyo, Japan). The base, middle and tip of one leaf per plant were evaluated, and the transformed values of these parameters were then averaged. At the end of the experiment, two types of water-use efficiency (*WUE*) were calculated: (1) instantaneous (WUE_i) and (2) accumulated (WUE_a). The WUE_i (µmol CO_2 $mmol^{-1}$ H_2O) was calculated as the ratio of photosynthesis (*A*) to transpiration (*E*) values obtained from the IRGA analyses. The WUE_a (mg dry-mass g^{-1} H_2O) was calculated as the ratio of the total plant dry mass (*MSt*) to the total water consumed during the period (*MSt/total water consumed*).

2.4 Anatomical analysis

Cross-sections were made using steel blades in the regions of the base, middle, and apex of the leaf, and all data were subsequently calculated. The sections were cleared with 50% sodium hypochlorite and washed twice in distilled water for 10 min. In addition, the sections were stained with safranine-astra blue solution (1% safranin: 0.1% astra blue at a ratio of 1:7) and mounted on slides with 50% glycerol (Johansen, 1940). Paradermal impressions of the abaxial and adaxial sides of leaves were obtained using a cyanoacrylate resin and then mounted on slides. The paradermal impressions were taken in the morning, from 6 am to 8 am, when the stomata were expected to be open. The slides were photographed under a Cx31 optical microscope (Olympus, Tokyo, Japan). One slide per leaf was formed, three sections and four fields were analyzed for each slide, and the data were averaged for each replicate. The images were analyzed using ImageJ software (Wayne Rasband National Institutes of Health, USA).

The following anatomical characteristics were evaluated in cross-sections: leaf thickness, total leaf area, mesophyll area, area of cells in the mesophyll, and vascular bundle area. The percentages of cells in the mesophyll and vascular bundles were calculated as follows: $A\% = (TA/total\ leaf\ area) \times 100$, where $A\%$ is the proportion of a given tissue and *TA* is the measured area of the tissue.

For the paradermal sections, the following structures were analyzed: section area, number of stomata, number of regular epidermal cells, stomatal pore area, number of open stomata, and number of closed stomata. The stomatal density (*SD*) was calculated as follows: $SD = number\ of\ stomata \times (10^6/section\ area)$. The stomatal index (*SI*) was calculated as follows: $SI = [number\ of\ stomata/(number\ of\ stomata + number\ of\ regular\ epidermal\ cells)] \times 100$. The percentage of open stomata (*OS*) was calculated as follows: $OS = (number\ of\ open\ stomata/total\ number\ of\ stomata) \times 100$. The percentage of closed stomata (*CS*) was calculated as follows: $CS = 100 - OS$. The stomatal pore area per plant (*SP*) was calculated as follows: $SP = stomatal\ pore\ area \times stomatal\ density \times leaf\ area$. The number of stomata per plant (*NSP*) was calculated as follows: $NSP = stomatal\ density \times leaf\ area$. All areas were measured in mm^2.

2.5 Statistical analysis

The data were analyzed by one-way ANOVA, and the means were compared using the Scott-Knott test at $p<0.05$ or regression analysis using Sisvar 5.0 software (Ferreira, 2011). Before the parametric analysis, the data were tested for normality using the Shapiro-Wilk test, and all variables had a normal distribution.

3 Results

Drought promoted significant differences in leaf area (Figure 1A), width (Figure 1B), and length/width ratio (Figure 1D) but did not have a significant effect on leaf length (Figure 1C). The water deficit promoted a reduction in root biomass, stem biomass, leaf dry mass, and total dry mass (Figure 2A). No significant changes were found in the percentage of biomass allocated to the roots, stems, or leaves (Figure 2B). The water mass in the roots, stems, and leaves was significantly lower under drought conditions (Figure 2C). The leaf water potential was not affected by the treatments (Figure 2D). The treatment with 50% FC decreased water retention and increased water loss by evapotranspiration (Figure 2E and F).

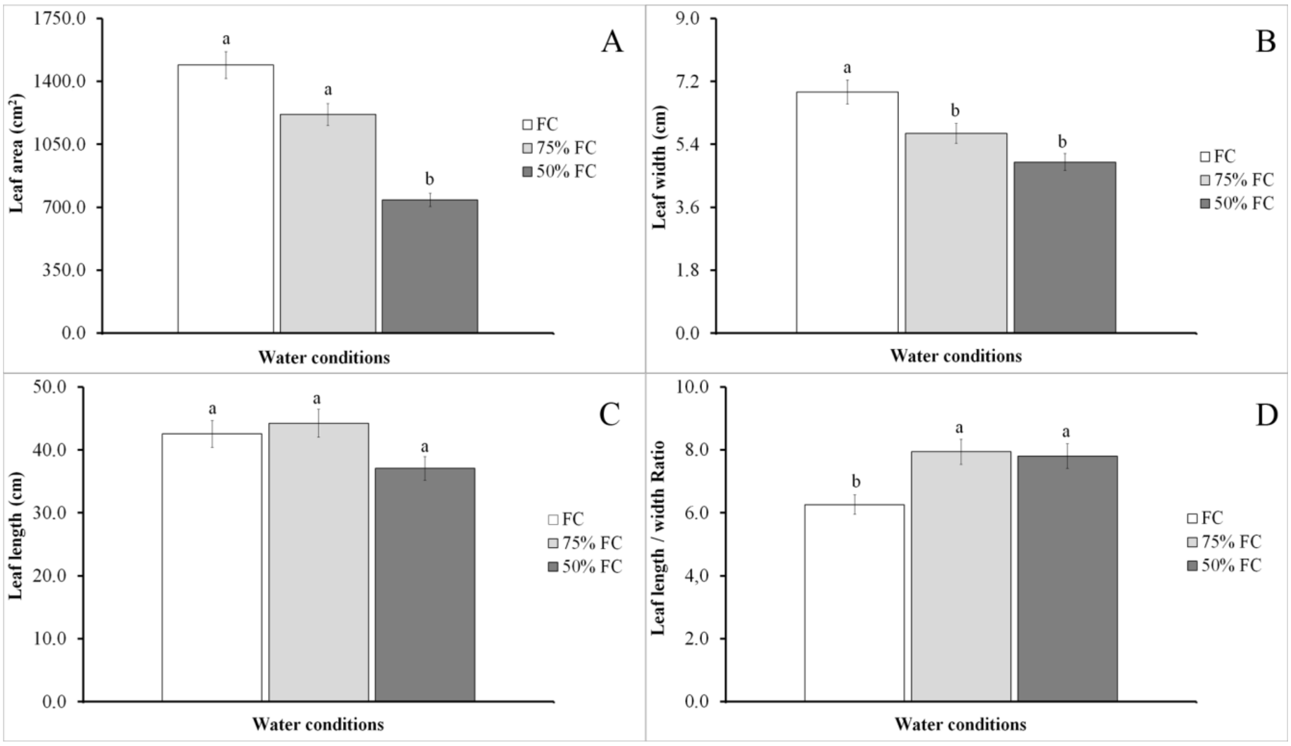
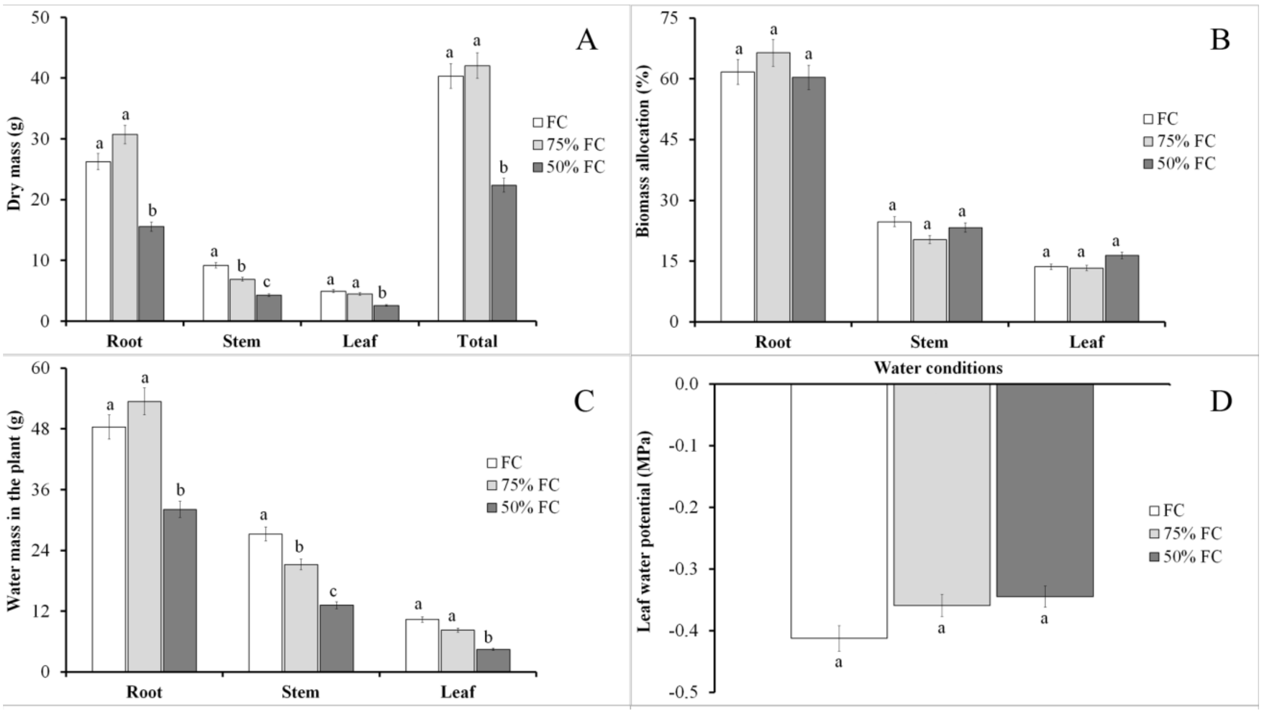


Figure 1 Leaf morphometrics in *Zea mays* under field capacity (FC), 75% FC, and 50% FC

Note: No significant difference according to the Scott-Knott test at p<0.05. The same letter in a panel denotes.

Bars = standard error.



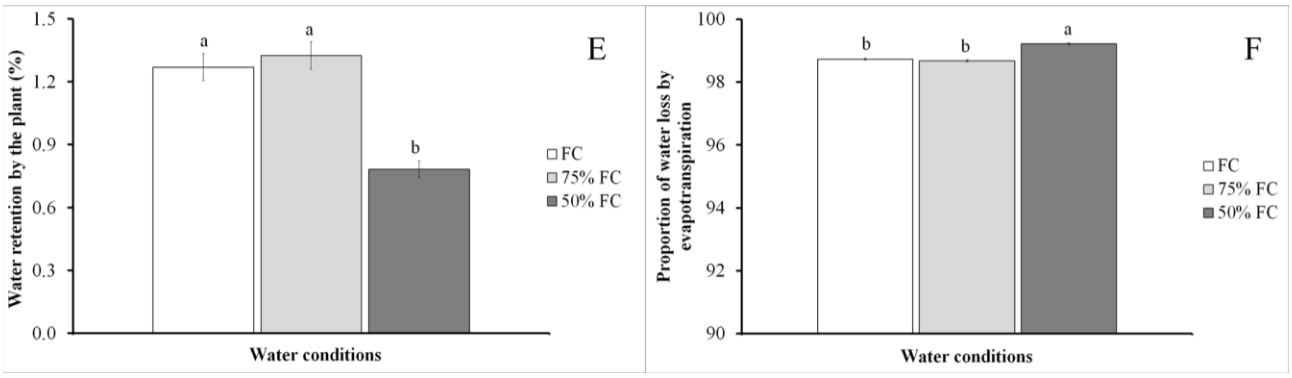


Figure 2 Growth parameters and water relations of *Zea mays* under field capacity (FC), 75% FC, and 50% FC

Note: The same letter in a panel denotes no significant difference according to the Scott-Knott test at $p<0.05$.

Bars = standard error.

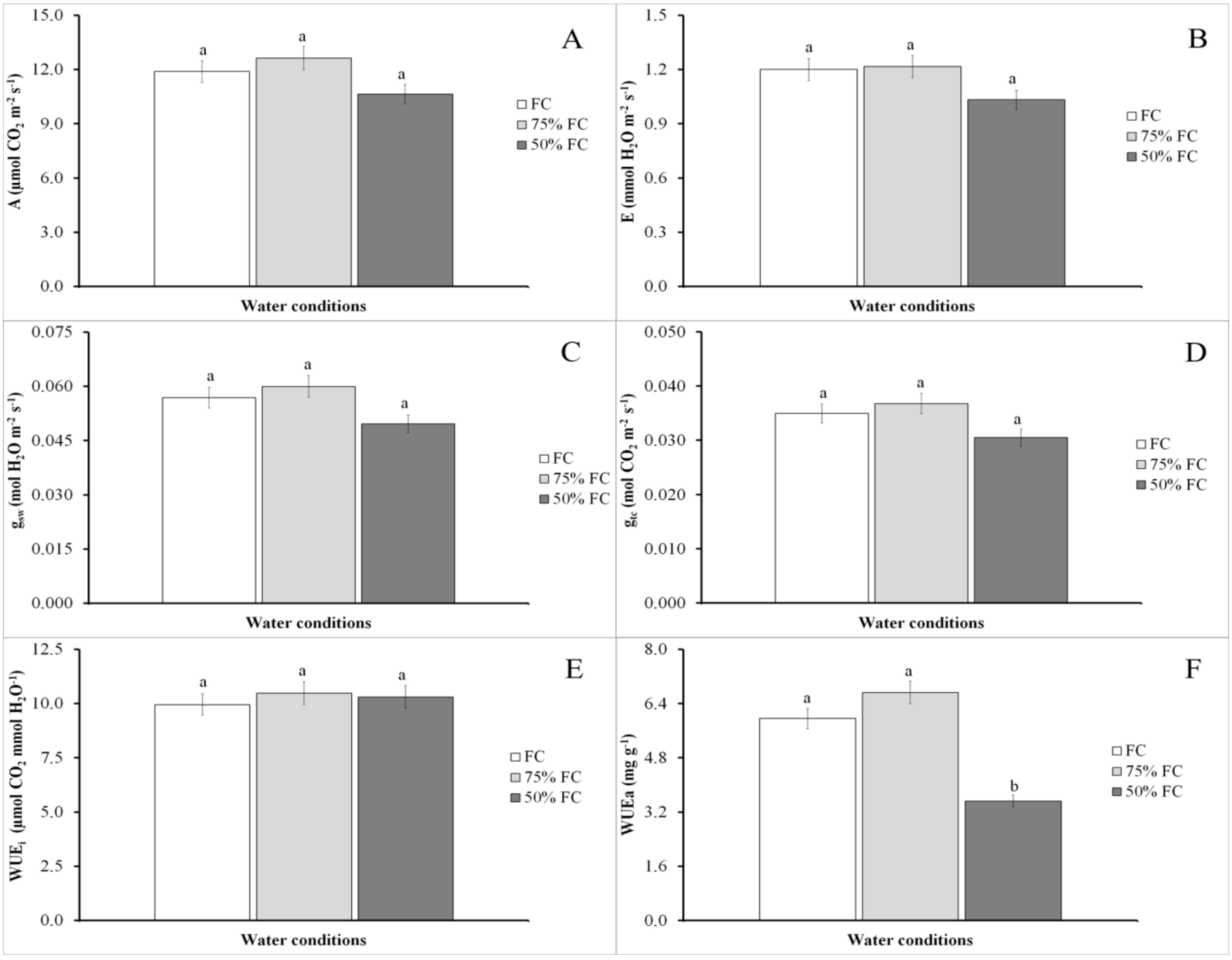


Figure 3 Gas exchange in leaves in *Zea mays* under field capacity (FC), 75% FC, and 50% FC

Note:A = net photosynthesis rate; E = transpiration rate; g_{sw}= stomatal conductance to water vapor; g_{tc} = total conductance to CO_2 in leaves; WUE_i = instantaneous water-use efficiency; WUE_a = accumulated water-use efficiency. The same letter in a panel denotes no significant difference according to the Scott-Knott test at $p<0.05$. Bars = standard error.

Drought did not promote significant changes in net photosynthesis (Figure 3A), transpiration (Figure 3B), stomatal conductance to water vapor (Figure 3C), or stomatal conductance to CO2 (Figure 3D). Zea mays showed no changes in the instantaneous water use efficiency (Figure 3E). Nevertheless, there was a significant difference in the accumulated water use efficiency under drought conditions (Figure 3F).However, when gas exchange was calculated for the whole plant, significant differences between treatments were observed in photosynthesis of the whole plant (Figure 4A), transpiration (Figure 4B), and stomatal conductance to water vapor (Figure 4C). Drought did not promote a significant effect on the chlorophyll content of Zea mays (Figure 4D).

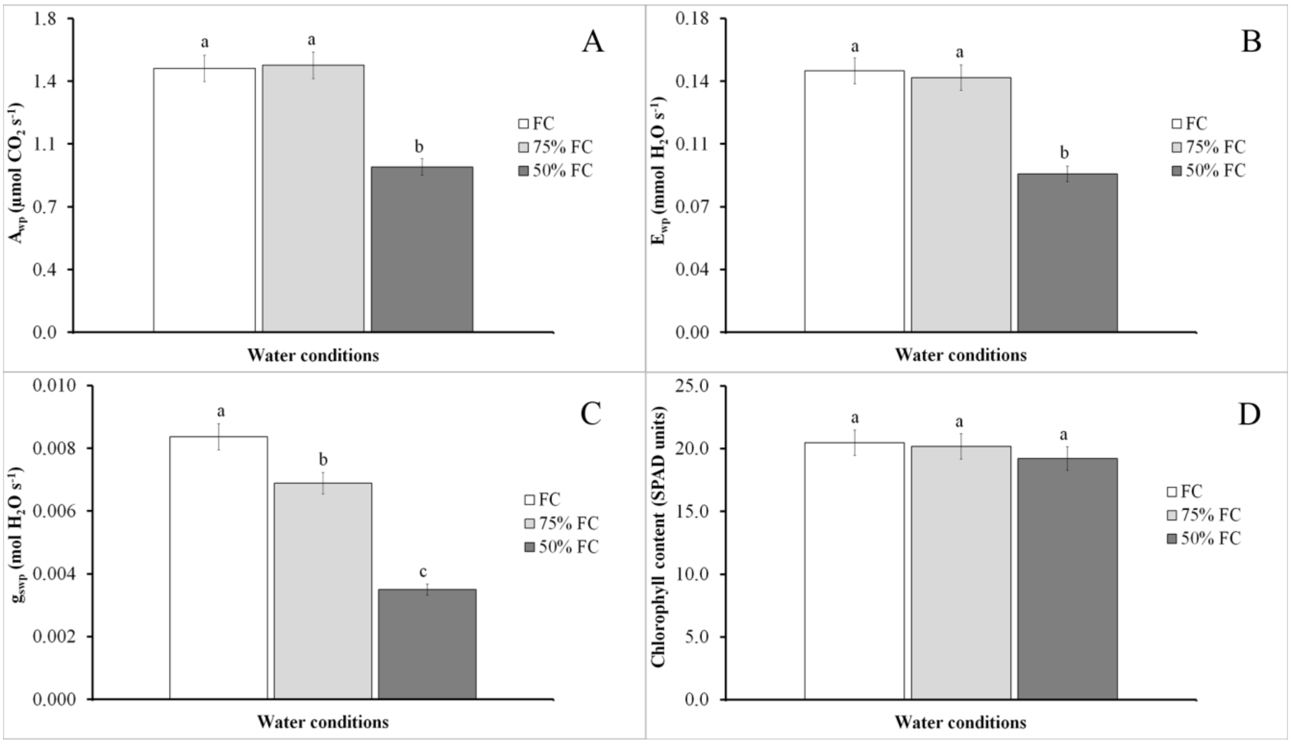


Figure 4 Gas exchange and chlorophyll content of *Zea mays* under field capacity (FC), 75% FC, and 50% FC

Note: The same letter in a panel denotes no significant difference according to the Scott-Knott test at $p<0.05$. (A_{wp}) = whole-plant photosynthesis; (E_{wp}) = whole-plant transpiration; (g_{swp}) = whole-plant stomal water conductance.

Bars = standard error.

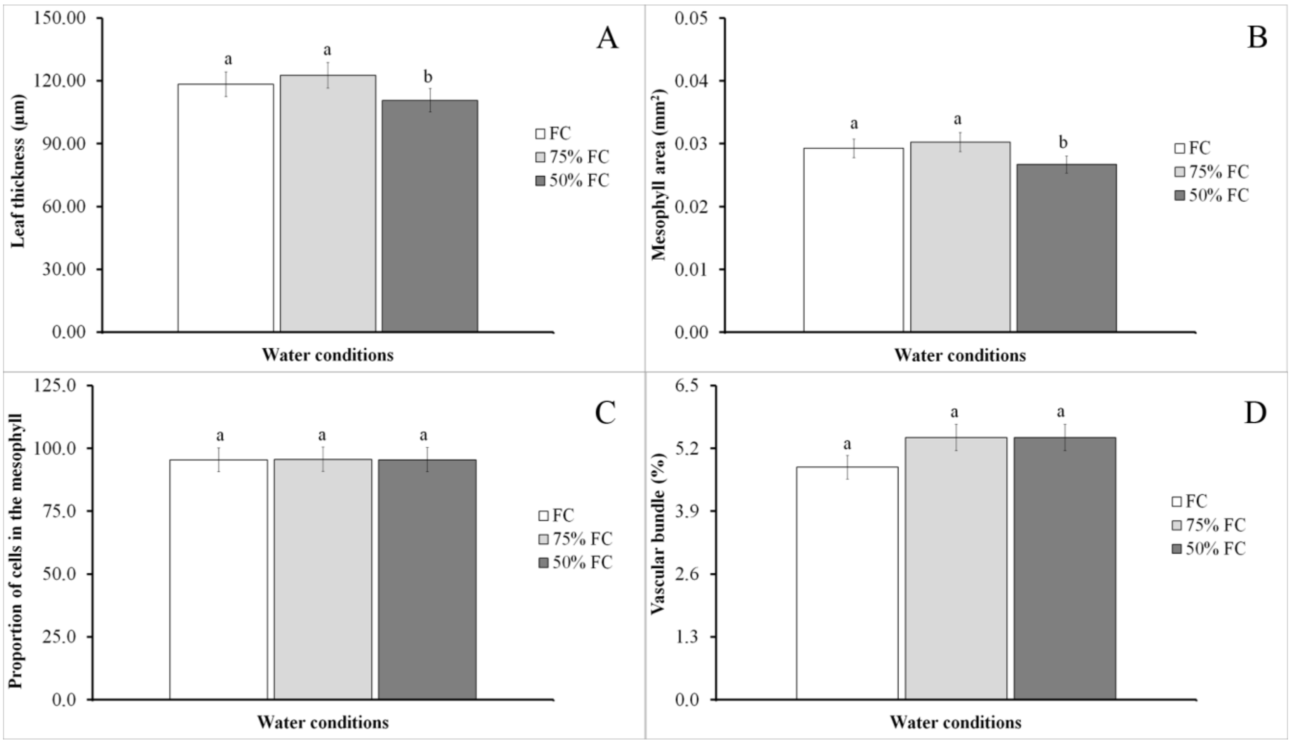


Figure 5 Leaf tissue characteristics of *Zea mays* grown under field capacity (FC), 75% FC, and 50% FC.

Note: The same letter in a panel denotes no significant difference according to the Scott-Knott test at $p<0.05$.

Bars = standard error.

Drought significantly reduced the leaf thickness of the maize plants (Figure 5A), but no leaf tissues or structures showed severe deformation. The lower water levels decreased the mesophyll area of *Zea* *mays* leaves (Figure 5B). The proportion of cells and vascular bundles of leaves was not altered (Figure 5, C and D).Drought did not have a significant effect on the stomatal density of maize (Figure 6A). However, the stomatal index on the adaxial side was lower under water deficit conditions, but not on the abaxial side (Figure 6B). The lack of water promoted a significant reduction in the total number of stomata per plant on the abaxial and adaxial sides (Figure 6C). No significant changes were detected in the number of open or closed stomata on the adaxial or abaxial side of the leaf (Figure 6, D and E). The stomatal pore area and the stomatal pore area per plant were not significantly affected by drought on the adaxial side (Figure 6, F and G), but the 50% FC treatment reduced the stomatal pore area on the abaxial side (Figure 6F and G).

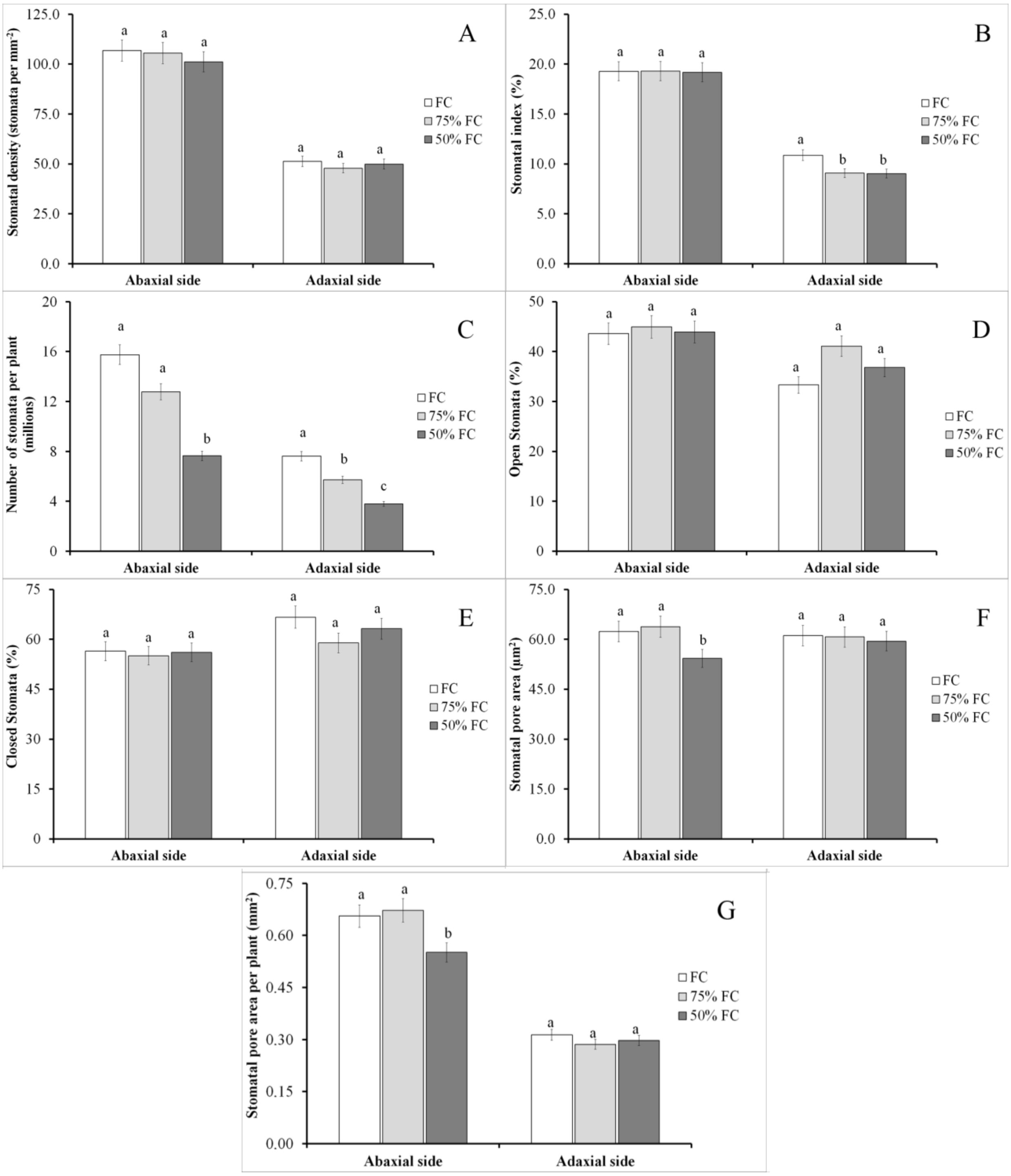


Figure 6 Leaf anatomical characteristics in paradermal sections of *Zea mays* under field capacity (CF), 75% FC, and 50% FC.
Note: The same letter in a panel denotes no significant difference according to the Scott-Knott test at p<0.05.
Bars = standard error.

4 Discussion

All maize plants survived under drought conditions. Water deficit can lead to premature death of plants sensitive to severe drought conditions (Cruz et al., 2019). Although the plants survived, the results showed that water availability negatively affected the physiological and structural characteristics of the maize plants, decreasing growth throughout the experimental period. Similar results were found in other studies where water deficit reduced the growth of maize plants (Song et al., 2018; Zhou et al., 2020). In addition, the lack of water reduced the number of leaves from the 40th day onwards. Reducing the number of leaves is considered a common response to drought (Widuri et al., 2020). In addition, the low turgor pressure caused by drought reduces leaf expansion, influencing gas exchange (Wyka et al., 2019). Thus, leaf expansion is directly related to leaf area. Early leaf fall reduces leaf area, decreasing photosynthetic area and plant growth, but this response is related to less water loss by transpiration.

Drought conditions reduced leaf expansion, significantly reducing the leaf area. This change promoted a smaller photosynthetic and transpiratory area, leading to a decrease in these parameters, which limited maize growth under drought conditions. In fact, leaf area is more sensitive to drought (Leuschner, 2020), and its reduction is considered one of the most common responses to water deficit, allowing plants to reduce transpiration and water loss (Clauw et al., 2015; Hsie et al., 2015). Therefore, drought promotes a reduction in leaf area, which also decreases gas exchange, reducing water loss and CO_2 uptake, and these reductions result in lower biomass production in these plants.

The reduction we saw in the leaf area of maize under drought conditions was related to the decrease in the width of these leaves under such conditions because there was no change in leaf length. This change caused an increase in the leaf length-to-width ratio. A common response to drought is to reduce both leaf length and width (Wyka et al., 2019). Maintaining the length and reducing only the leaf width is an interesting response since maize leaves have different photosynthetic rates and a different density of stomata along the length of the leaf (lower in the apical part of the leaf where the width is smaller). Thus, the reduction in leaf area promoted by drought in maize can be defined by the decrease in width and not only by the decrease in the number of leaves. In fact, the reduction in leaf area has a direct relationship with the inhibition of leaf expansion and leaf abscission under drought conditions (Zhou et al., 2020). Therefore, drought reduces the number and width of maize leaves, reducing the total leaf area while the length of the leaves remains unchanged.

This reduction in leaf width may also be related to a reduction in the activity of the marginal meristem because the marginal meristem is directly related to leaf width (Pereira et al., 2017). In fact, water deficit can promote the interruption of the water supply to meristematic cells, severely decreasing cell expansion and even influencing the process of cell division by reducing cell proliferation (Traas and Bohn-Courseau, 2005). The smaller number of cells produced by meristems reduces leaf area and, consequently, plant growth. Therefore, the reduced leaf area promoted by drought in maize leaves reduces CO_2 uptake and growth.

Under drought conditions, maize plants had smaller leaf area, which decreased the production of total biomass and of the biomass of each organ. In fact, the reduction in the root biomass, stem biomass, leaf dry mass, and total dry mass is a common response under water deficit (Cai et al., 2020) affecting plant productivity and growth because dry mass is considered the basis of vegetative organ formation (Zhou et al., 2020). Even so, drought did not promote changes in the allocation of biomass to roots, stems, or leaves, demonstrating the absence of deformation in the vegetative organs and thus allowing plant survival. However, the lack of greater investment in biomass to the roots impaired maize growth. In fact, the common response of drought-tolerant plants is to invest in the biomass of the root system, as this increases the probability of obtaining water (Zhao et al., 2018), facilitating plant growth (Gleason et al., 2019). The maize plants here could not acquire sufficient biomass to invest in roots under drought conditions, which may have limited the photosynthetic rate by reducing the absorption of water and nutrients.

The lack of investment in the root system may have limited the water uptake in maize plants under drought conditions. In fact, increasing the number of roots increases water uptake, promoting plant growth (Zhao et al., 2018; Gleason et al., 2019). In this context, the low amount of water may have hindered the process of stomatal opening and closing, affecting the photosynthesis, transpiration, and stomatal conductance of the plant. In fact, the water deficit promotes changes in stomatal morphology and stomatal movements (opening and closing), affecting the exchange of water vapor, carbon dioxide, and stomatal conductance between the leaf and the atmosphere (Gerardin et al., 2018). In addition, lower water uptake by maize plants increased the water lost by evapotranspiration under drought conditions. According to Gorthi et al. (2019), the increase in evapotranspiration is related to soil evaporation. In fact, there was a decrease in the transpiration rate by these maize plants, which reduced their water loss. However, most of the water saved remained in the substrate and evaporated, which increased evapotranspiration since there was no efficient water retention in the maize during the drought period. Therefore, drought severely hinders the retention and flow of water from the substrate to the plant, and much of the water ends up being lost by evapotranspiration. This behavior may be related to the lack of investment in the root system due to the decrease in leaf area, as this impaired the biomass gain in the plant.

Despite the reduction in water mass, the water potential remained unchanged in maize plants under drought conditions. This indicates that the maize plants maintained the water flow in the xylem. The constancy of this parameter also demonstrates that the reduction in water mass in the plant has a direct relationship with the lack of investment in the root system because the water flow was maintained; therefore, there was lower uptake, and a smaller amount of water was retained in the plant. Water potential is also related to the water stress in the xylem and therefore indicates that adjustments occur in the vessels to maintain hydraulic conductivity under drought conditions. In fact, low tension in xylem vessels can lead to embolism, which affects hydraulic conductivity (Gleason et al., 2019). This allowed the vascular tissues to remain unchanged and even maintain the same number of vascular bundles, thus contributing to the stability of water transport to the leaves. Maintaining a water supply through the xylem is essential because the leaves need to constantly replenish the water lost through transpiration (Carminati and Javaux, 2020). The maintenance of water transport also allowed the stomatal opening process to not be significantly affected by drought. Soil hydraulic conductivity is the main trigger of stomatal closure (Carminati and Javaux, 2020). Therefore, the lack of changes in water potential allowed the water transport in the plant to remain unchanged. However, it is important to note that total transpiration in the plant decreased, which may be related to the reduction in leaf area.

Another important aspect of the present study was the localized analysis of gas exchange, which showed that photosynthesis, transpiration, and stomatal conductance were maintained for *Zea mays* under drought conditions. However, the calculation of these parameters for the whole plant showed significant reductions under drought conditions. We took the localized measurements directly in the infrared gas analyzer chamber, which had an area of 6.0 cm^2, and these parameters for the whole plant were calculated for each square meter. When photosynthesis, transpiration, and stomatal conductance are calculated for the actual leaf area of the plant, the results are more reasonable because the conditions are closer to the actual photosynthetic capacity of the plants. Therefore, drought reduced the gas exchange parameters of maize. This is related to the smaller leaf area, which reduced CO_2 uptake, biomass gain, and plant growth.

Another finding obtained directly in the localized analysis with the infrared gas analyzer was the maintenance of the instantaneous water use efficiency under drought conditions. This is related to transpiration and photosynthesis, which did not significantly change in *Zea mays* plants. However, these plants cannot avoid water loss under drought conditions. In this context, *Zea mays* do not have optimized water use efficiency. This corroborates the result of the accumulated water use efficiency, which decreased under drought conditions. This parameter is directly related to the actual production of biomass by plants and the water consumed for growth. Therefore, the accumulated water use efficiency showed reasonable results and was supported by the extreme growth reduction observed in maize plants under drought.

Drought reduced leaf thickness and mesophyll area but did not affect the proportion of cells or chlorophyll content. Reductions in leaf thickness and mesophyll area, as well as the maintenance of chlorophyll content, are a common response of plants to drought (Liu et al., 2019), and they lead to reduced photosynthetic capacity. This reduction in leaf tissues reduced the photosynthetic capacity, which affected plant growth under drought conditions.

There were also changes in stomatal characteristics due to drought. The stomatal density showed no significant change in the adaxial or abaxial side. The stomatal index decreased under drought conditions on the leaf adaxial side but did not change on the leaf abaxial side. According to Gerardin et al. (2018), plants are known to adjust their stomatal density and index during leaf development as a common strategy to fight drought. Nevertheless, more information is needed to understand the stomatal adjustments made by maize plants under drought conditions, since plants under such conditions can decrease (Mansoor et al., 2019) or increase their stomatal density (Hsie et al., 2015). Thus, there is a contradiction in the literature about these parameters. In this context, the calculation of the total number of stomata per plant was extremely important. This parameter showed a significant reduction on both the abaxial and adaxial sides. The decrease in the number of stomata is a common response to drought that increases the probability of plant survival by reducing water loss. A smaller number of stomata can reduce transpiration and water loss (Gerardin et al., 2018). However, it also promotes a reduction in the photosynthetic rate, affecting biomass gain and plant growth. Plants control their gas exchange through stomata, and the reduction in these structures may restrict CO_2 flow (Cai et al., 2020). Under water stress, the most common response of plants is to close the stomata to prevent water loss (Liu et al., 2019), which in turn reduces gas exchange and is considered an adaptive mechanism (escape) to reduce water loss (Zia et al., 2021). The maintaining the opening and closing of the stomata was not responsible for the decrease in gas exchange. This decrease was caused by the lower number of stomata in the whole plant. Therefore, the reduction in the total number of stomata allowed the survival of the plants but reduced the gas exchange in the plant as a whole.

In this context, the water deficit significantly reduced the stomatal pore area on the abaxial side. This indicates that the abaxial side had a smaller pore area for gas exchange, which reduced transpiration and photosynthesis. In fact, dynamic adjustments in the opening of stomatal pores are responsible for the regulation of stomatal conductance, allowing plants to quickly reduce transpiration and water loss under drought conditions (Hasanagić et al., 2020). The reduction in stomatal pore area is an important response by maize to conserve water under drought conditions.

5 Conclusions

Drought promoted significant changes in *Zea mays*, as evidenced by the reduction in growth and number of leaves of these plants. In this context, the leaf area played a direct role in the sensitivity of these plants because its reduction influenced the whole plant gas exchange, and the biomass gain of the plants and even the total number of stomata; it is important to evaluate the whole shoot response since punctual evaluations can not totally explain the growth reductions. In addition, this study shows the relevance of calculating the total number of stomata for a more useful interpretation of the data due to the contradicting results on stomatal density and index.

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References

Avramova, V., H. AbdElgawad, Z. Zhang, B. Fotschki, R. Casadevall, L. Vergauwen, D. Knapen, E. Taleisnik, Y. Guisez, H. Asard, and G. T. S. Beemster. 2015. Drought induces distinct growth response, protection, and recovery mechanisms in the maize leaf growth zone. *Plant Physiology*, 169(2): 1382-1396.

Cai, F., Y. Zhang, N. Mi, H. Ming, S. Zhang, H. Zhang, and X. Zhao. 2020. Maize (*Zea mays* L.) physiological responses to drought and rewatering, and the associations with water stress degree. *Agricultural Water Management*, 241: 106379.

Carminati, A., and M. Javaux. 2020. Soil rather than xylem vulnerability controls stomatal response to drought. *Trends in Plant Science*, 25(9): 868-880.

Clauw, P., F. Coppens, K. De Beuf, S. Dhondt, T. Van Daele, K. Maleux, V. Storme, L. Clement, N. Gonzalez, and D. Inzé. 2015. Leaf responses to mild drought stress in natural variants of Arabidopsis. *Plant Physiology*, 167(3): 800-816.

Cowan, M. F., C. K. Blomstedt, S. L. Norton, R. J. Henry, B. L. Møller, and R. Gleadow. 2020. Crop wild relatives as a genetic resource for generating low-cyanide, drought-tolerant *Sorghum*. *Environmental and Experimental Botany*, 169: 103884.

Cruz, Y. C., A. L. M. Scarpa, M. P. Pereira, E. M. de Castro, and F. J. Pereira. 2019. Growth of *Typha domingensis* as related to leaf physiological and anatomical modifications under drought conditions. *Acta Physiologiae Plantarum*, 41: 64.

Díaz, A. S., G. M. Aguiar, M. P. Pereira, E. M. Castro, P. C. Magalhães, and F. J. Pereira. 2018. Aerenchyma development in different root zones of maize genotypes under water limitation and different phosphorus nutrition. *Biologia Plantarum*, 62: 561-568.

Ferreira, D. F. 2011. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, 35: 1039-1042.

Gerardin, T., C. Douthe, J. Flexas, and O. Brendel. 2018. Shade and drought growth conditions strongly impact dynamic responses of stomata to variations in irradiance in *Nicotiana tabacum*. *Environmental and Experimental Botany*, 153: 188-197.

Gleason, S. M., M. Cooper, D. R. Wiggans, C. A. Bliss, M. C. Romay, M. A. Gore, M. V. Mickelbart, C. N. Topp, H. Zhang, K. C. DeJonge, and L. H. Comas. 2019. Stomatal conductance, xylem water transport, and root traits underpin improved performance under drought and well-watered conditions across a diverse panel of maize inbred lines. *Field Crops Research*, 234: 119-128.

Gorthi, A., J. J. Volenec, and L. R. Welp. 2019. Stomatal response in soybean during drought improves leaf-scale and field-scale water use efficiencies. *Agricultural and Forest Meteorology*, 276: 107629.

Hasanagić, D., I. Koleška, D. Kojić, S. Vlaisavljević, N. Janjić, and B. Kukavica. 2020. Long term drought effects on tomato leaves: anatomical, gas exchange and antioxidant modifications. *Acta Physiologiae Plantarum*, 42: 121.

Hoagland, D. R., and D. I. Arnon. 1950. The water-culture method for growing plants without soil. *California Agricultural Experimental Station*, 347: 1-34.

Hsie, B. S., K. R. Mendes, W. C. Antunes, L. Endres, M. L. O. Campos, F. C. Souza, N. D. Santos, B. Singh, E. C. P. Arruda, and M. F. Pompelli. 2015. *Jatropha curcas* L.

(Euphorbiaceae) modulates stomatal traits in response to leaf-to-air vapor pressure deficit. *Biomass and Bioenergy*, 81: 273-281.

Johansen, D. A. 1940. *Plant Microtechnique*. New York, USA: McGraw-Hill.

Leuschner, C. 2020. Drought response of European beech (*Fagus sylvatica* L.)-A review. *Perspectives in Plant Ecology, Evolution and Systematics*, 47: 125576.

Liu, B., J. Liang, G. Tang, X. Wang, F. Liu, and D. Zhao. 2019. Drought stress affects on growth, water use efficiency, gas exchange and chlorophyll fluorescence of *Juglans* rootstocks. *Scientia Horticulturae*, 250: 230-235.

Mansoor, U., S. Fatima, M. Hameed, M. Naseer, M. S. A. Ahmad, M. Ashraf, and M. Waseem. 2019. Structural modifications for drought tolerance in stem and leaves of *Cenchrus ciliaris* L. ecotypes from the Cholistan Desert. *Flora*, 261: 151485.

Pereira, M. P., F. F. Corrêa, E. M. Castro, J. P. V. Oliveira, and F. J. Pereira. 2017. Leaf ontogeny of *Schinus molle* L. plants under cadmium contamination: the meristematic origin of leaf structural changes. *Protoplasma*, 254: 2117-2126.

Santos, K. R., M. P. Pereira, A. C. G. Ferreira, L. C. De Almeida Rodrigues, E. M. de Castro, F. F. Corrêa, and F. J. Pereira. 2015. *Typha domingensis* Pers. Growth responses to leaf anatomy and photosynthesis as influenced by phosphorus. *Aquatic Botany*, 122: 47-53.

Sapeta, H., J. M. Costa, T. Lourenco, J. Maroco, P. Van der Linde, and M. M. Oliveira. 2013. Drought stress response in *Jatropha curcas*: growth and physiology. *Environmental and Experimental Botany*, 85: 76-84.

Song, H., Y. Li, L. Zhou, Z. Xu, and G. Zhou. 2018. Maize leaf functional responses to drought episode and rewatering. *Agricultural and Forest Meteorology*, 249: 57-70.

Traas, J., and I. Bohn-Courseau. 2005. Cell proliferation patterns at the shoot apical meristem. *Current Opinion in Plant Biology*, 8(6): 587-592.

Widuri, L. I., B. Lakitan, J. Sakagami, S. Yabuta, K. Kartika, and E. Siaga. 2020. Short-term drought exposure decelerated growth and photosynthetic activities in chili pepper (*Capsicum annuum* L.). *Annals of Agricultural Sciences*, 65(2): 149-158.

Wyka, T. P., A. Bagniewska-Zadworna, A. Kuczyńska, K. Mikołajczak, P. Ogrodowicz, M. Żytkowiak, M. Surma, and T. Adamski. 2019. Drought-induced anatomical modifications of barley (*Hordeum vulgare* L.) leaves: An allometric perspective. *Environmental and Experimental Botany*, 166: 103798.

Zhao, J., Q. Xue, K. E. Jessup, X. Hou, B. Hao, T. H. Marek, W. Xu, S. R. Evett, S. A. O’Shaughnessy, and D. K. Brauer. 2018. Shoot and root traits in drought tolerant maize (*Zea mays* L.) hybrids. *Journal of Integrative Agriculture*, 17(5): 1093-1105.

Zhou, H., G. Zhou, Q. He, L. Zhou, Y. Ji, and M. Zhou. 2020. Environmental explanation of maize specific leaf area under varying water stress regimes. *Environmental and Experimental Botany*, 171: 103932.

Zia, R., M. S. Nawaz, M. J. Siddique, S. Hakim, and A. Imran. 2021. Plant survival under drought stress: implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. *Microbiological Research*, 242: 126626.