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## Research Article

# Elevated CO<sub>2</sub> Improves Vegetative Growth of Water-stressed *Zea mays* L. Under Low Photosynthetic Photon Flux Density

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## Abstract

**Background and Objective:** Elevated carbon dioxide (CO<sub>2</sub>) has the ability to mitigate plant drought stress. Not many studies were done to find the combined effects of drought stress and elevated CO<sub>2</sub> on C4 plant growth under low photosynthetic photon flux density (PPFD). In this study, drought mitigating effects of elevated CO<sub>2</sub> were assessed on the vegetative growth of maize (*Zea mays* L.) plants under low PPFD. **Materials and Methods:** Maize plants were grown in two growth chambers, one maintained at ambient CO<sub>2</sub> (435 μmol mol<sup>-1</sup>) and the other at elevated CO<sub>2</sub> (1000 μmol mol<sup>-1</sup>), conditions under low PPFD. Plants were subjected to three water stress treatments by reducing irrigation: Once every 7 days (SD, control), every 14 days (FD) and every 21 days (TD). Data were collected on chlorophyll content, stomatal and epidermal characteristics and plant vegetative growth (height and girth). Data were analyzed using SPSS and MANOVA. **Results:** The elevated CO<sub>2</sub> in combination with TD water stress treatment, induced higher chlorophyll 'a' (6.95 vs 10.93 μg cm<sup>-2</sup>), chlorophyll 'b' (2.63 vs 3.80 μg cm<sup>-2</sup>) and total chlorophyll content (14.73 vs 9.38 μg cm<sup>-2</sup>) compared to TD water stress treatment in ambient CO<sub>2</sub>. The longest and the widest stomata were recorded under elevated CO<sub>2</sub> conditions in the FD (39.40 ± 1.56 cm) and TD (26.63 ± 2.29 cm), respectively. Further, the highest stomatal density was observed in TD under elevated CO<sub>2</sub> (151 stomata/mm<sup>2</sup>). The combination of elevated CO<sub>2</sub> and FD water stress treatment registered the tallest (mean = 112.80 cm) and thickest (mean = 2.97 cm) maize plants in the experiment. **Conclusion:** Elevated CO<sub>2</sub> increased vegetative growth of maize plants especially under FD water stress treatment. The low PPFD was not a constraint for elevated CO<sub>2</sub> in mitigating the drought stress.

**Key words:** Elevated CO<sub>2</sub>, drought stress, low PPFD, maize (*Zea mays* L.), chlorophyll content, stomatal density, vegetative growth

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Maize (*Zea mays* L.) is a C4 plant and one of the most important food crops in the world in terms of production<sup>1</sup>. Maize production has been increasing in the last 70 years, due to improved plant breeding methods and cultivation techniques, in major maize producing countries like USA and China<sup>2,3</sup>. However, recent data show that global climate change has negatively impacted maize production in the world<sup>4,5,6</sup>. Water stress and increasing atmospheric CO<sub>2</sub> have been reported as the major results of the climate change<sup>7</sup>. Water stress reduces maize yield up to 30% by negatively impacting photosynthesis<sup>8</sup>. This is mainly due to the reduced water availability at reproductive stages and restricted leaf growth under water stress<sup>2</sup>. Non-expanding leaves intercept less photosynthetically active radiation (PAR) and result in reduced net CO<sub>2</sub> assimilation rate and photosynthesis<sup>5,9</sup>. In addition, water stress forces stomata to close, which leads to a fall in CO<sub>2</sub> absorption by leaves, which in turn reduces photosynthesis<sup>10</sup>. Further, water stress reduces plant chlorophyll content either by forcing chlorophyll break down<sup>11</sup>, hindering chlorophyll synthesis<sup>12</sup> or by altering activities of enzymes involved in chlorophyll synthesis<sup>13</sup>.

Even though water stress reduces CO<sub>2</sub> intake by closing stomata, C4 plants already have high bundle sheath CO<sub>2</sub> content which is almost 10 times that of atmospheric CO<sub>2</sub> content<sup>14</sup>. Thus, CO<sub>2</sub> enrichment is thought not to increase the photosynthesis in C4 plants<sup>7</sup>. However, exposing C4 plants to double atmospheric CO<sub>2</sub> concentration, promoted plant growth and increased CO<sub>2</sub> assimilation rate<sup>15,16</sup>. In contrast to the above observation, some C4 plants were reported not to exhibit any positive growth responses to elevated CO<sub>2</sub> conditions<sup>17</sup>. Leakey *et al.*<sup>7</sup>, demonstrated that water stress is an inevitable element for positive response in maize growth under CO<sub>2</sub> enrichment.

Like the drought-driven stomatal closure, increased atmospheric CO<sub>2</sub> concentration also forces stomatal closure due to localized increase in temperature at the leaf level. Artificial CO<sub>2</sub> enrichment acts in the same way by altering leaf microclimate and reduces photosynthesis due to reduction in CO<sub>2</sub> uptake<sup>10</sup>. Apart from the effect on stomata, enriched CO<sub>2</sub> environment also affects chlorophyll content in plants. CO<sub>2</sub> enrichment can increase<sup>18,19</sup>, decrease<sup>20</sup> or have no effect<sup>21</sup> on chlorophyll content, in comparison to ambient CO<sub>2</sub> conditions. Previous researches, on the effects of CO<sub>2</sub> enrichment and water stress in C4 plants, have reported contrasting results on the positive effect of CO<sub>2</sub> enrichment<sup>22,23</sup>. Earlier studies concentrated on the effects of water stress and CO<sub>2</sub> enrichment at reproductive stage. Therefore, it is acceptable

to conclude that if vegetative stages are affected by water stress, the crop yield will be reduced.

Based on these previous research findings, the effects of CO<sub>2</sub> enrichment during the vegetative growth stages were investigated, starting from two-leaves-stage up to the full-fledged development of 10th leaf. The main objective of this study was to assess the vegetative growth from plant growth traits such as height and stem girth. In addition, total chlorophyll content, length, width and density of stomata, length and width of epidermal cells surrounding stomata, were determined. Overall, this study was aimed to test the feasibility of using CO<sub>2</sub> enrichment as tool to mitigate the negative impact of water stress on the vegetative growth of maize plants.

## MATERIALS AND METHODS

**Plant growth conditions:** Hybrid maize seeds ('Merit', Seminis) were water soaked for 24 h and sown into bog peat media (Desert group, UAE) filled in 72 well plastic trays and were placed inside growth chambers (Binder KBW-720). There were five 'Narva cool daylight colourex plus (LT 30W/865) electric tubes' inside each growth chamber, with an overall light intensity of  $63.15 \pm 10 \mu\text{mol m}^{-2} \text{sec}^{-1}$ . The irradiance was measured using an LI-189 quantum/Radiometer/Photometer (Li-Cor, Inc., Lincoln, NE, USA). The measurements were done at 2 cm below the tube (on the full fledged 10th leaf) and at 50 cm below the lamp (halfway down, on the 6th leaf), to have a look at the effect of light penetration in between the leaves. The irradiance recorded were  $28.02 \text{ Wm}^{-2}$  at 2 cm and  $9.56 \text{ Wm}^{-2}$  at 50 cm.

Two growth chambers were used, one for ambient CO<sub>2</sub> ( $435 \mu\text{mol mol}^{-1}$ ) and the other for enriched CO<sub>2</sub> ( $1000 \mu\text{mol mol}^{-1}$ ) treatments. One week old maize seedlings were transplanted into the bog peat media filled in black plastic pots (19.3 cm diameter and 16.4 cm height). The seedlings were transplanted inside each chamber at a spacing of 20×20 cm (between rows and columns). There were 12 such seedlings allotted to each chamber totaling 24 seedlings in the whole experiment. The plants were arranged in a 2 factor completely randomized design (CRD) with multiple levels. The first factor, CO<sub>2</sub>, had 2 levels (two growth chambers) and the second factor, the water stress treatment, had 3 levels. Each treatment combination (CO<sub>2</sub>-waterstress) was repeated four times. A photoperiod of 15 h of light and 9 h of darkness was used. The temperature was set at 25°C during the light period and 19°C during the dark period. One week after transplanting maize seedlings, one of the growth chambers was enriched with a CO<sub>2</sub> concentration of  $1000 \mu\text{mol mol}^{-1}$

using a CO<sub>2</sub> tank (23 kg). The CO<sub>2</sub> flow through a 0.4 cm (inner diameter) plastic tube was regulated using a CO<sub>2</sub> system (Hydrofarm, CA, USA) and an autopilot digital environmental controller (Hydrofarm, CA, USA). The ambient CO<sub>2</sub> concentration (435 μmol mol<sup>-1</sup>) inside the second chamber was attained automatically through a hole on the side of the growth chamber, which equilibrated the CO<sub>2</sub> concentration inside the chamber with that of outside air. Both growth chambers were kept at these settings until the completion of the experiment on the 70th day after transplanting. The whole experiment was repeated two times to confirm the results.

#### **Determination of irrigation volume for the experiment:**

Water stress levels were determined using a pre-experimental set up. For this purpose, 400, 500, 600 and 700 mL of water were added to bog peat potting mix in different plastic pots. Three replications were used for each water treatment. The soil was kept on lab benches at 22°C temperature and 60% relative humidity for 2-3 days. The samples were then oven dried at 105°C for 24 h and the field capacity was calculated. The results from the pre-trial showed that, 600 mL of water was needed for the potting soil mix to reach its field capacity. Based on this value, three different water stress treatments were applied in the pots under ambient and enriched CO<sub>2</sub> treatments, which were:

- Irrigation to field capacity every 7 days, abbreviated as SD
- Irrigation to field capacity every 14 days, abbreviated as FD
- Irrigation to field capacity every 21 days, abbreviated as TD

After seed germination, fertilizers were given with the irrigation water to all the plants for the first 14 days. The TUROFORT 20+20+20+2MgO+TE (Adfert, AD, UAE) fertilizer containing micro nutrients was used for the fertigation of maize plants (pH = 6 and Electrical Conductivity = 2.5). After first 14 days, fertilizer application was regulated to once in every 21 days. Non-regulation of fertilizer application could have resulted in faulty measurements of plant growth traits due to the interaction of fertilizer and water stress treatments. Consequently, plants in SD could have been the biggest under both CO<sub>2</sub> treatments.

The water stress treatments were applied starting from one week after transplanting the maize seedlings. The SD treatment was used as the control treatment for the water stress treatments, since the plants were irrigated to field capacity in the SD. Maize leaves were detached and

immediately processed to determine chlorophyll content, stomatal density, stomatal length, stomatal width, epidermal cell length and width. For the chlorophyll content determination, samples were collected from the 3rd leaf of each maize plant from the bottom due to the long period of exposure to light and CO<sub>2</sub> treatments. An additional reason to use the 3rd leaf was that the lower most leaves (leaves 1 and 2) were dried-up when the leaves were collected on the 70th day ('in-treatment') for chlorophyll analysis.

#### **Chlorophyll extraction and quantity determination:**

Chlorophyll content was determined by a method described by Richardson *et al.*<sup>24</sup>, with slight modifications. Instead of leaf discs, rectangular pieces (1 cm<sup>2</sup> each, 100 mg total fresh weight) were cut from the third leaf counted from the bottom of the maize plant. The cut leaf pieces were immersed into 7 mL of pre-heated DMSO (65°C) contained in a glass vial. After, the glass vials were heated inside a water-bath for 30 min. The volume of DMSO inside the glass vials were then topped up to 10 mL using a Pasteur pipette. The absorbance of each DMSO-extracted chlorophyll sample was determined by a Lambda 25 UV/VIS spectrometer (PerkinElmer, USA). The spectrometer was calibrated to zero absorbance using DMSO in a quartz cuvette as a blank. We used 663 nm, 645 nm wavelengths for chlorophyll determination based on chlorophyll absorption spectra of 600-680 nm reported by Richardson *et al.*<sup>24</sup>. Five replications were done for each leaf sample from ambient and enriched drought treatments for both 663 and 645 nm wavelengths. The equations reported by Schlemmer *et al.*<sup>25</sup>, were used to convert the absorbance values to chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents. The chlorophyll contents calculated from these equations (g L<sup>-1</sup>) were then converted to leaf chlorophyll content per unit leaf area (μg cm<sup>-2</sup>).

**Stomatal length, width and density:** Stomatal length, width and density were determined from 70 days old maize leaves. Clear nail polish was applied onto the maize leaves and epidermal peels of 6×4 mm were removed from the leaves once the nail polish had dried. The peels were put on a glass slide and pressed with a cover slip. These samples were immediately observed under Olympus BX41 laboratory microscope fitted with a DP 71 microscopic camera (Olympus Corporation, Tokyo, Japan). Micrographs were taken for stomatal density from 200×200 μm<sup>2</sup> focal area (set using scale option) of the DP 71 camera field set under 40X objective and 10X eyepiece (400X magnification). Micrographs were taken to measure stomatal length, stomatal width and stomatal density. All measurements were done in 3 replications.

**Epidermal cell length and width:** The epidermal peels observed for stomatal characteristics were also used to determine epidermal cell length and width. Micrographs were taken using Olympus BX41 laboratory microscope fitted with a DP 71 microscopic camera (Olympus Corporation, Tokyo, Japan) from  $200 \times 200 \mu\text{m}^2$  focal area of the DP 71 camera at 400X magnification to measure the lengths and widths of the epidermal cells surrounding stomata. All measurements were done in 3 replications.

**Plant growth measurements:** Maize plant height and stem girth were measured during four different time points to get an idea on the combined effects of water stress and  $\text{CO}_2$  treatments. The height and girth of maize plants on 15th, 24th, 31st, 47th and 70th days were measured while they were in treatments, referred from here onwards as DIT (days in treatment). The aim was to assess the gradual differences in growth of maize caused by enriched  $\text{CO}_2$  environment and water stress treatments.

**Statistical analysis:** Data were analyzed using IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY). All the dependent variables except stomatal density followed a normal distribution and showed homogeneity of variances. Multivariate analysis of variance (MANOVA) GLM procedure was done on all the other dependent variables except for stomatal density ( $p < 0.05$ ). The stomatal density followed Poisson distribution and thus was analyzed using generalized linear model of SPSS. Bonferroni adjustment was used to report the significant pairwise comparisons of the water stress treatments and  $\text{CO}_2$  levels.

## RESULTS

**Chlorophyll content:** Overall, maize chlorophyll content ( $\mu\text{g cm}^{-2}$ ) was significantly higher in  $1000 \mu\text{mol mol}^{-1} \text{CO}_2$  (enriched  $\text{CO}_2$ ) compared to ambient  $\text{CO}_2$  ( $p > 0.001$ ). Under enriched  $\text{CO}_2$ , chlorophyll 'a' was significantly higher in both TD and FD treatments, compared to the ambient  $\text{CO}_2$  (Fig. 1). The SD (control) also showed higher chlorophyll 'a' under  $\text{CO}_2$  enrichment but it was not high enough to have a significant statistical difference in comparison to ambient  $\text{CO}_2$  ( $p > 0.05$ ). Similarly, under enriched  $\text{CO}_2$ , Chlorophyll 'b' content was higher in the FD and TD treatments compared to ambient  $\text{CO}_2$ , however the difference was not statistically significant ( $p > 0.05$ ). Total chlorophyll content (TCC) followed the similar trend of that of chlorophyll 'b' but was significantly higher in both FD and TD under enriched  $\text{CO}_2$  compared to ambient

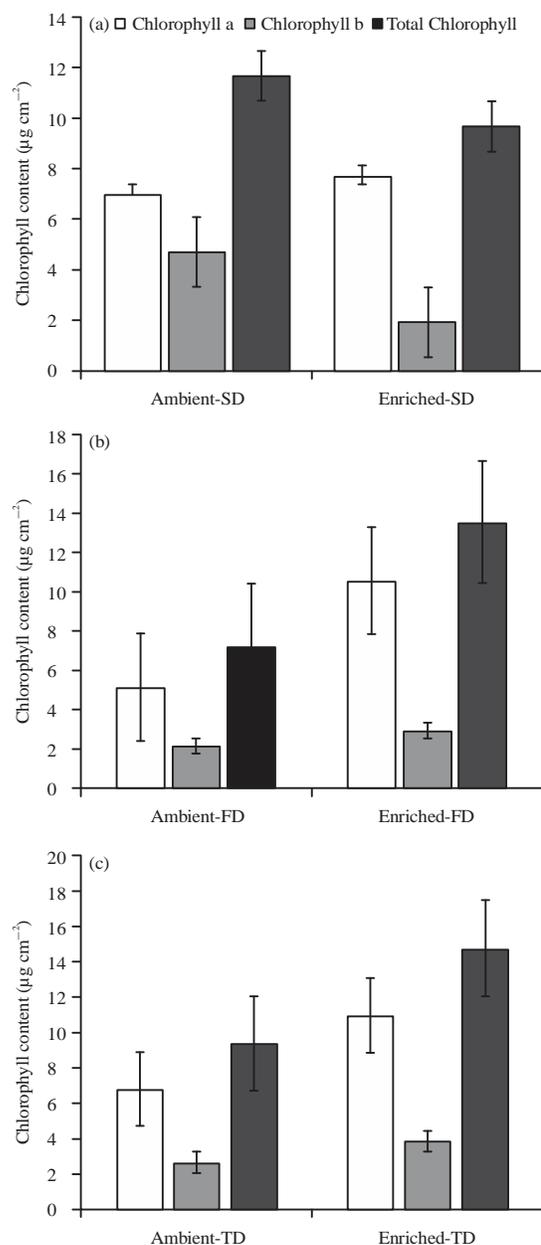


Fig. 1(a-c): Chlorophyll 'a', chlorophyll 'b' and the total chlorophyll content (Mean  $\pm$  SEM) of maize leaves in three water stress treatments (a) SD: 7 days, (b) FD: 14 days and (c) TD: 21 days under ambient ( $435 \mu\text{mol mol}^{-1}$ ) and enriched ( $1000 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$

$\text{CO}_2$ . Enriched  $\text{CO}_2$  actually reduced the TCC in maize plants under the control (SD) treatment.

**Stomatal and epidermal characteristics:** The highest stomatal length was measured in the FD treatment under

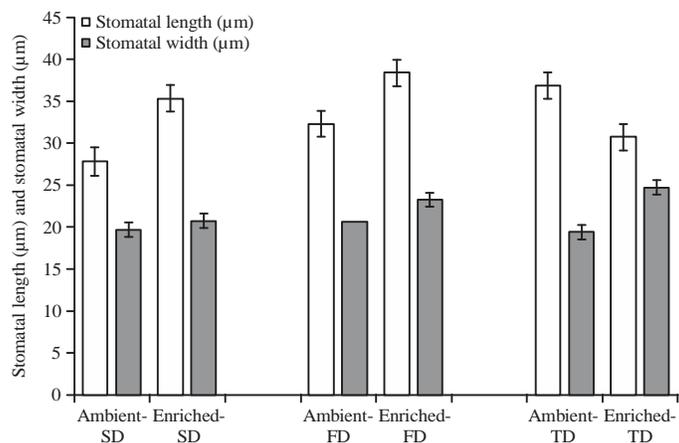


Fig. 2: Stomatal length and width (Mean  $\pm$  SEM) of maize leaves in three water stress treatments, SD: 7 days, FD: 14 days and TD: 21 days) under ambient ( $435 \mu\text{mol mol}^{-1}$ ) and enriched ( $1000 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$

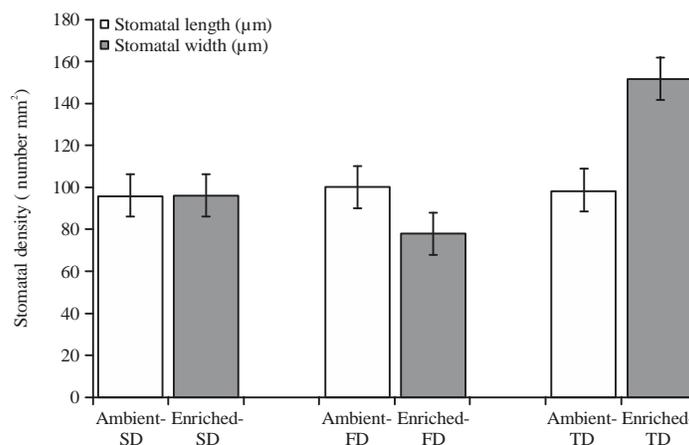


Fig. 3: Stomatal density (Mean  $\pm$  SEM) of maize leaves in three water stress treatments (SD: 7 days, FD: 14 days and TD: 21 days) under ambient ( $435 \mu\text{mol mol}^{-1}$ ) and enriched ( $1000 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$

enriched  $\text{CO}_2$  and the lowest in SD, under ambient environment conditions. The order of increasing stomatal length under ambient environment was  $\text{SD} < \text{FD} < \text{TD}$  (Fig. 2). Enriched  $\text{CO}_2$  significantly increased ( $p < 0.05$ ) the stomatal length in SD and FD treatments but decreased in TD compared to that of ambient  $\text{CO}_2$ . In the enriched  $\text{CO}_2$ , stomatal length increased in the order  $\text{TD} < \text{SD} < \text{FD}$  for the water stress treatments.

Stomatal widths were almost similar ( $p > 0.05$ ) among all water stress treatments (SD, FD and TD) under ambient  $\text{CO}_2$  conditions. A similar trend was also noticed under enriched  $\text{CO}_2$  environments (Fig. 2). However, stomatal width was significantly higher in TD under enriched  $\text{CO}_2$  compared to ambient  $\text{CO}_2$  ( $p < 0.05$ ). Overall, even though not significant, enriched  $\text{CO}_2$  increased the stomatal width compared to ambient  $\text{CO}_2$ . The widest stomata were found in TD water

stress treatment under enriched  $\text{CO}_2$  and the narrowest stomata was found in TD under ambient conditions.

Stomatal densities (Fig. 3) were not significantly different between the ambient-FD vs enriched-FD and ambient-SD vs enriched-SD treatments ( $p > 0.05$ ). However, for TD water stress treatment, stomatal density was significantly higher ( $p < 0.05$ ) under enriched  $\text{CO}_2$  environment compared to ambient  $\text{CO}_2$  environment. In addition, under ambient  $\text{CO}_2$ , stomatal densities were similar under all water stress treatments. However, under enriched  $\text{CO}_2$ , stomatal density in the water stress treatments increased in the order  $\text{FD} < \text{SD} < \text{TD}$ . The TD treatment showed significantly higher stomatal density ( $p < 0.05$ ) compared to both SD and FD, as mentioned earlier.

Epidermal cell lengths ( $p > 0.05$ ) and cell widths ( $p > 0.05$ ) were not significantly affected by the water stress and  $\text{CO}_2$  interaction (Fig. 4). However, epidermal cell width was

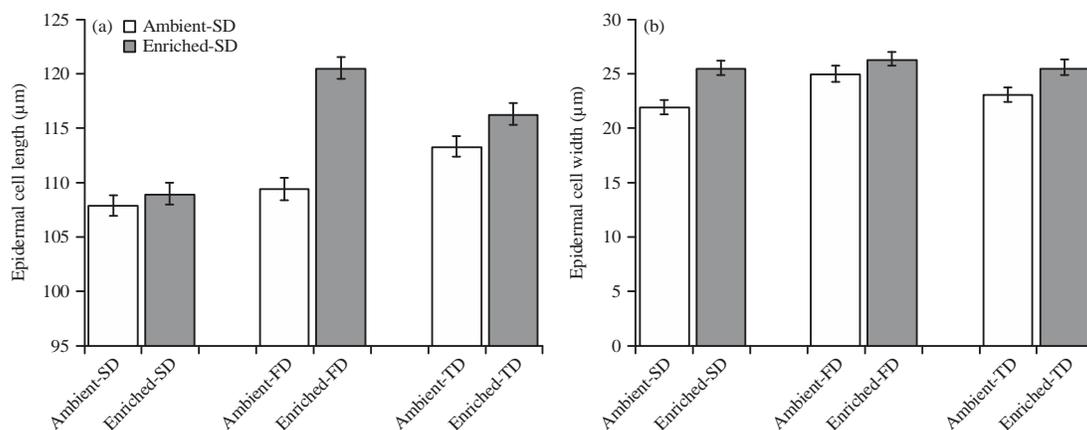


Fig. 4(a-b): Length and width of epidermal cells (Mean  $\pm$  SEM) of maize leaves in three water stress treatments SD: 7 days, FD: 14 days and TD: 21 days) under ambient ( $435 \mu\text{mol mol}^{-1}$ ) and enriched ( $1000 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$

significantly different among the  $\text{CO}_2$  levels ( $p < 0.05$ ). A slight increase in epidermal cell lengths and widths were observed under FD and TD in both of the  $\text{CO}_2$  environments.

**Plant growth measurements:** Plant height was significantly higher under the combined effect of the water stress treatments and the  $\text{CO}_2$  enrichment treatments ( $p < 0.05$ ). The maize plants under the ambient  $\text{CO}_2$  environment were growing slow until 15 DIT in the SD, FD and TD treatments, compared to enriched  $\text{CO}_2$  environment. Gradually they grew taller and caught up with the plants in the same treatments under the enriched  $\text{CO}_2$ , within 31 DIT (Fig. 5). From then until the end of the experiment, the water stressed plants under enriched  $\text{CO}_2$  treatments started to grow faster compared to their counter parts in the ambient  $\text{CO}_2$  treatments. In the comparison between the  $\text{CO}_2$  environments, largest height difference was observed in TD followed by SD and FD.

Plant girth in SD, FD and TD water stress treatments under the ambient  $\text{CO}_2$  were not significantly different from the enriched  $\text{CO}_2$  ( $p > 0.05$ ). The girth showed the opposite trend in growth compared to plant height. Until 31 DIT, girths of maize plants were lower in enriched treatments in comparison with ambient treatment; afterwards it surpassed the ambient treatment (Fig. 6). By 70th DIT, girths of water stressed plants under enriched  $\text{CO}_2$  treatments were higher than their ambient counterparts in ambient  $\text{CO}_2$ . Plants under FD treatment showed the highest girth difference in between the  $\text{CO}_2$  environments. Plant girth values under enriched-TD and enriched-SD treatments were also higher compared to ambient-TD and ambient-SD, respectively.

## DISCUSSION

Enriched  $\text{CO}_2$  environment significantly increased total chlorophyll content (TCC) in the drought treatments compared to the ambient  $\text{CO}_2$  environments. In enriched drought environments, 21 day-drought treatment (TD) showed highest total chlorophyll content, followed by 14 day-drought treatment (FD) and 7 day-drought treatment (SD). In the ambient drought environment, the SD treatment showed the highest TCC followed by TD and FD. However, the highest TCC recorded in ambient drought environment ( $11.67 \mu\text{g cm}^{-2}$  in SD) was still less than that of second highest ( $13.50 \mu\text{g cm}^{-2}$  in FD) in enriched drought environment. These results demonstrate that the  $\text{CO}_2$  enrichment effectively mitigated the negative effect of water stress on chlorophyll production.

Previously, it has been reported that total chlorophyll content was lower in the middle leaves of maize under  $\text{CO}_2$  enrichment, where the  $\text{CO}_2$  concentration was double of the ambient atmospheric  $\text{CO}_2$ <sup>20</sup>. Moreover, higher TCC was reported in that research compared to this study, both in ambient (around  $27 \mu\text{g cm}^{-2}$  vs  $12.5 \mu\text{g cm}^{-2}$ ) and enriched ( $15 \mu\text{g cm}^{-2}$  vs  $14 \mu\text{g cm}^{-2}$ ) conditions. Driscoll *et al.*<sup>20</sup>, did not apply any water stress treatments and that might be the reason for the higher TCC in their study compared to this study. In addition, Driscoll *et al.*<sup>20</sup>, used higher levels of light intensity in their study ( $800 \mu\text{mol m}^{-2} \text{sec}^{-1}$ ) compared to this study ( $163.15 \pm 10 \mu\text{mol m}^{-2} \text{sec}^{-1}$ ), probably another reason for higher reported chlorophyll content. In this study, topmost fully fledged maize leaf (3rd leaf) received a PAR of  $22.5 \pm 5 \mu\text{mol m}^{-2} \text{sec}^{-1}$ , when the plants were two weeks old and about  $163.15 \pm 10 \mu\text{mol m}^{-2} \text{sec}^{-1}$ , when they were 70 DIT

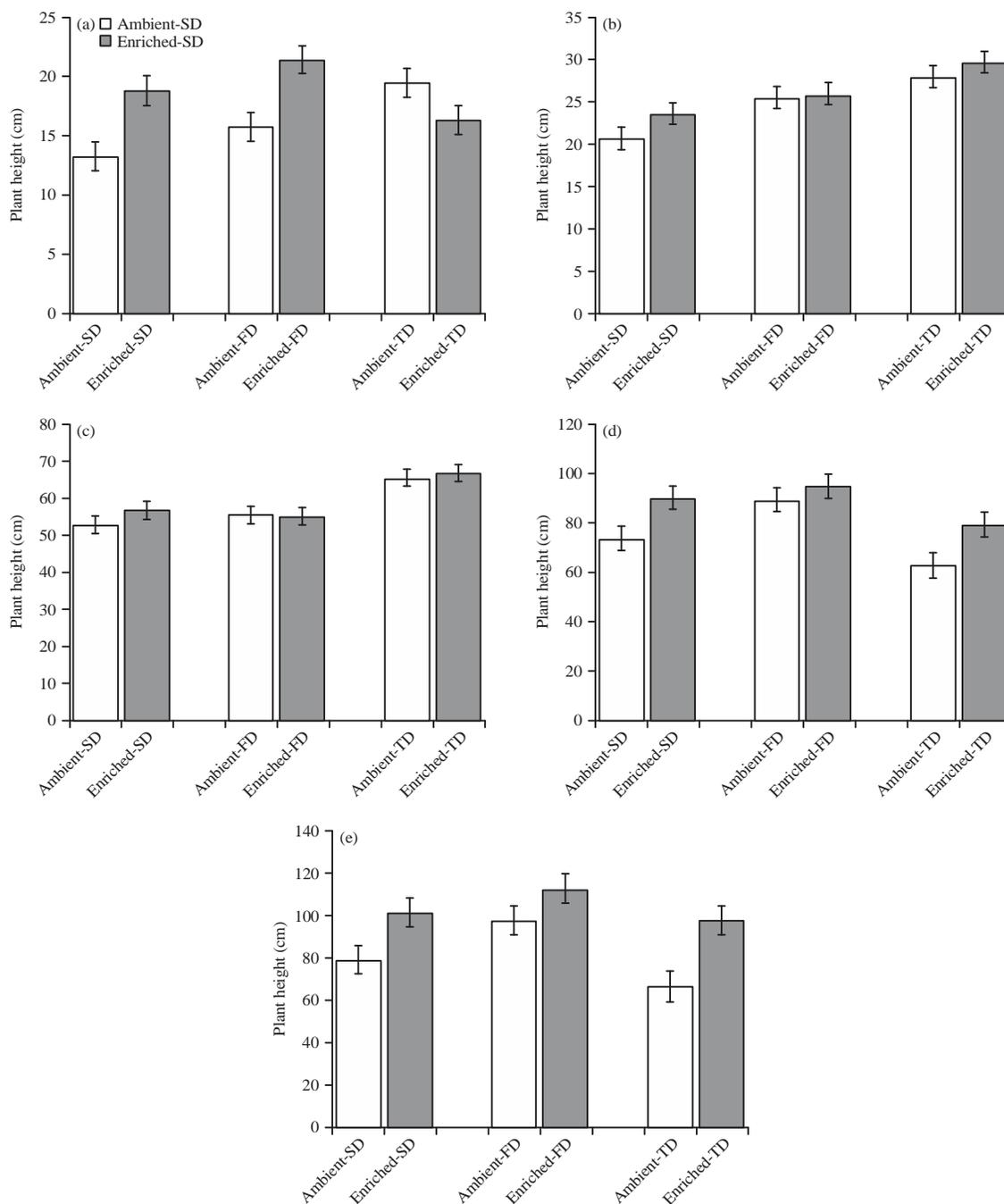


Fig. 5(a-e): Height (Mean  $\pm$  SEM) of the maize plants in three water stress treatments SD: 7 days, FD: 14 days and TD: 21 days under ambient ( $435 \mu\text{mol mol}^{-1}$ ) and enriched ( $1000 \mu\text{mol mol}^{-1}$ ) CO<sub>2</sub>

(fully fledged 10th leaf). Along with this, exposure to longer photoperiod (16 h of light Vs 15 h of light) for longer period of time (until 13th leaf stage Vs until 10th leaf stage) might also have contributed to the higher TCC in the above-mentioned study. Longer photoperiod has been reported to influence radiation use efficiency of maize leaves<sup>9</sup>. In addition, as per a previous study, TCC gradually

increased up to VT stage of maize growth (13th leaf stage)<sup>26</sup>. In general chlorophyll 'a' was quantified higher than chlorophyll 'b', similar to those reported in non-water-stressed maize plants under ambient CO<sub>2</sub><sup>27</sup>. Zhu *et al.*<sup>28</sup> reported that quantities of chlorophyll 'a' and chlorophyll 'b' in maize were not significantly different under drought stress.

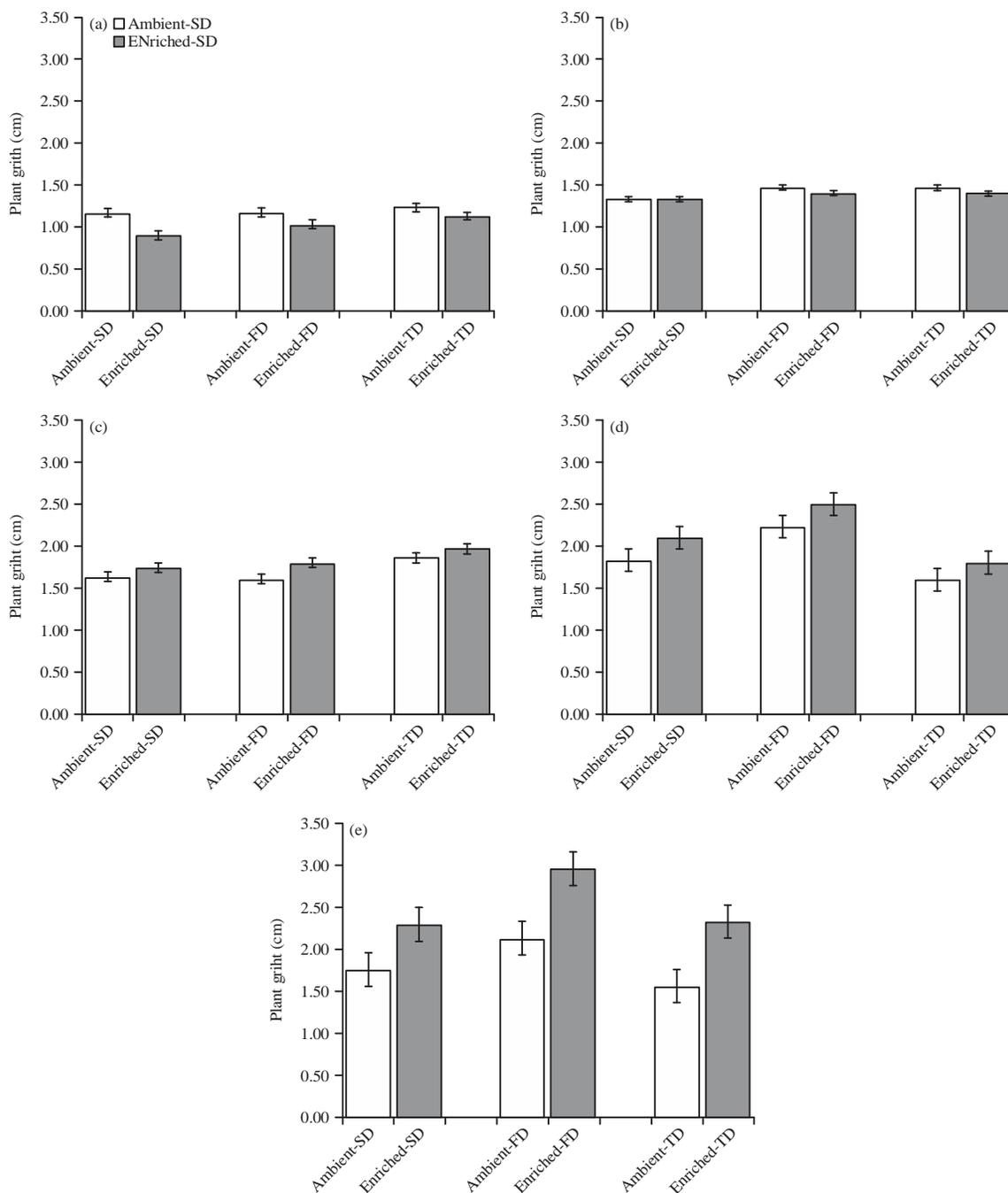


Fig. 6(a-e): Stem girth (Mean  $\pm$  SEM) of the maize plants in three water stress treatments SD:7 days, FD: 14 days and TD: 21 days under ambient ( $435\mu\text{mol mol}^{-1}$ ) and enriched ( $1000\mu\text{mol mol}^{-1}$ ) CO<sub>2</sub>

Stomatal length was significantly higher ( $p < 0.05$ ) in enriched-SD and enriched-FD treatments compared to ambient-SD and ambient-SD treatments. In addition, stomatal length decreased ( $30.71\mu\text{m}$ ) in enriched-TD when compared to enriched-FD ( $38.35\mu\text{m}$ ). This indicates, that, as the water stress increases, further stomatal length reduction can be observed under enriched CO<sub>2</sub> environment. However, Sarker

and Hara<sup>29</sup>, reported that eggplant (C3 plant) grown under enriched CO<sub>2</sub>, stomatal length increased in 21-day water stress treatment. The difference in C3 and C4 stomatal arrangement and structure might have caused this difference. In this research, stomatal widths were not significantly different among the water stress treatments between ambient and enriched CO<sub>2</sub> environments. The main effects, which were CO<sub>2</sub>

environments and water stress treatments, also did not show any significant impact on stomatal widths of maize leaves. In contrast to these results on stomatal widths, Sarker and Hara<sup>29</sup>, reported comparatively narrower stomata in 21-day water stress treatments under enriched CO<sub>2</sub> environment compared to ambient CO<sub>2</sub> environment. The contradictory results on stomatal length and width of this study, comparing to Sarker and Hara<sup>29</sup>, could be attributed again to the differences in monocot and dicot plant stomatal morphology and arrangement. In this study CO<sub>2</sub> enrichment resulted in larger epidermal cells compared to ambient CO<sub>2</sub>. However, in other crops (e.g., rice) no significant change in the epidermal cell width under elevated CO<sub>2</sub> levels has been reported<sup>30</sup>.

After 31 DIT of growth till the end of experiment (70 DIT), plants grown under enriched conditions grew taller than those under ambient conditions. The maximum plant growth is recorded in FD, while the maximum growth in between ambient and enriched conditions was recorded in TD. A previous study reported that plant height increased 14% only after 3 years of exposure to CO<sub>2</sub> enrichment<sup>31</sup>. Further, two other studies reported that there is no difference in plant growth between ambient and enriched conditions<sup>32,33</sup>. Furthermore, Leakey *et al.*<sup>7</sup>, reported that in absence of water stress maize growth is not affected by elevated CO<sub>2</sub>. In these experiments, maize plants did grow bigger in presence of CO<sub>2</sub> enrichment, contradicting the reports of Leakey *et al.*<sup>7</sup> and Kim *et al.*<sup>32,33</sup>.

### CONCLUSION

In the controlled environment growth chambers, enriched CO<sub>2</sub> (1000 μmol mol<sup>-1</sup>) increased vegetative growth of maize plants compared to ambient CO<sub>2</sub>, under low light condition. Although low PPFD affected the chlorophyll content, it did not hinder the overall drought mitigating effects of elevated CO<sub>2</sub> on maize plants. The growth enhancement was much pronounced in 14-days water stress treatments. Under 1000 μmol mol<sup>-1</sup> CO<sub>2</sub> and 14-days water stress, chlorophyll 'a' and chlorophyll 'b' and stomatal characteristics improved in such a way that they indirectly contributed to the overall plant growth. More levels of water stress treatments and CO<sub>2</sub> enrichments should be studied in both growth chambers and outside growth chamber conditions (e.g., greenhouse or field), to find out how the maize plants react to the combined treatments of enriched CO<sub>2</sub> and water stress.

### SIGNIFICANCE STATEMENTS

The current research used elevated CO<sub>2</sub> to mitigate the effect of drought stress in maize growing under low PPFD. The

results showed that negative effect of drought stress, especially withholding irrigation for 2 weeks, was not pronounced in maize vegetative growth due to the positive effects of elevated CO<sub>2</sub> conditions.

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