RESEARCH ARTICLE

CO₂ enrichment affects eco-physiological growth of maize and alfalfa under different water stress regimes in the UAE

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Abstract Water stress has been reported to alter morphology and physiology of plants affecting chlorophyll content, stomatal size and density. In this study, drought stress mitigating effects of CO₂ enrichment was assessed in greenhouse conditions in the hot climate of UAE. Commercially purchased maize (*Zea mays* L.) and alfalfa (*Medicago sativa* L.) were seeded in three different custom-built cage structures, inside a greenhouse. One cage was kept at 1000 ppm CO₂, the second at 700 ppm CO₂, and the third at ambient greenhouse CO₂ environment (i.e. 435 ppm). Three water stress treatments HWS (200 ml per week), MWS (400 ml per week), and CWS (600 ml per week) were given to each cage so that five maize pots and five alfalfa pots in each cage received same water stress treatments. In maize, total chlorophyll content was similar or higher in water stress treatments compared to control for all CO₂ concentrations. Stomatal lengths were higher in enriched CO₂ environments under water stress. At 700 ppm CO₂, stomatal widths decreased as water stress increased from MWS to HWS. At both enriched CO₂ environments, stomatal densities decreased compared to ambient CO₂ environment. In alfalfa, there was no significant increase in total chlorophyll content under enriched CO₂ environments, even though a slight increase was noticed.

Keywords CO₂ fertilization · Drought stress · Maize · Alfalfa · Total chlorophyll content · Stomatal density

Introduction

Plant growth and crop production are under threat due to an alarming increase in drought affected areas along with increasing temperatures (Asseng et al. 2015) and global climate change (Zhao and Running 2010). Availability of fresh water for irrigation is becoming a major problem, especially in the middle-eastern countries with large desert area and prevailing high temperature. The United Arab Emirates (UAE) shares first rank with seven other countries in the list published by the World Resources Institute, which are going to be severely drought-affected by the year 2040 (Luo et al. 2015). In addition, large amounts of fresh water have been used for farming activities, at different parts of UAE (Hirich and Choukr-Allah 2017; Shihab 2001). Currently, more greenhouses, hydroponics farms and nurseries are being set up, making the fresh water availability for irrigation an even bigger concern in the coming years.

Water stress may vary from small fluctuations in atmospheric humidity to extreme soil water deficits and low humidity (Thriveni et al. 2017). The ability of plants to tolerate water stress is determined by various biochemical pathways that help in retention and/or acquisition of water and that protect chloroplast functions (Gill and Tuteja 2010). Drought results in decrease in soil moisture content, lowers the plant water potential and reduces stomatal transpiration, causing cell turgor reduction and decreased relative water content, bringing about a series of damages (Anjum et al. 2011a).

Maize (C4) is one of the important food crops in the world (Dahmardeh 2011) and alfalfa (C3) is an important fodder crop (Kim 2015). Water stress has been known to influence radiation use efficiency and photosynthesis leading to reduction in overall plant growth and economic

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yield of maize plant (Dahmardeh 2011). Similarly, Hamidi et al. (2010) reported that water stress severely affected alfalfa plant growth. Water stress decreases chlorophyll content (Din et al. 2011; Keyvan et al. 2010) leading to reduced photosynthesis (Peng et al. 2011). Both chlorophyll 'a' and 'b' were reported to be lost from mesophyll cells under water stress (Anjum et al. 2011b). Gitelson and collaborators (Gitelson et al. 2014) found that photosynthetic capacity of maize is high during vegetative- and low during reproductive stages and observed that lack of nitrogen uptake at reproductive stages would have resulted in reduced chlorophyll content. This suggests that water stress during reproductive stages might reduce photosynthesis due to reduced nitrogen uptake, ultimately resulting in yield reduction, as was reported by Pandey et al. (2000). In alfalfa severe decrease in chlorophyll 'a', and chlorophyll 'b' contents were reported in water-stressed condition (at 25% field capacity) (Abid et al. 2016). Water stress results in leaf rolling, which in turn reduces the leaf area used for PAR interception leading to poor plant growth (Aslam et al. 2013; Saglam et al. 2014). Water stress also reduces leaf area resulting in reduced stomatal density and transpiration (Zhao et al. 2015). Additionally, water stress negatively affects stomatal density and size leading to plant growth reduction. Stomatal density and size were reported to decrease under water stress (Zhao et al. 2015). To cope with these damages inflicted by water stress, techniques need to be implemented for the wise use of irrigation water without compromising the yield potential of the plant. A few earlier studies suggest that carbon dioxide (CO₂) enrichment can ameliorate water stress tolerance in C3 and C4 plants along with its positive effect on yield (Kauwe et al. 2013; Wang et al. 2012).

CO₂ enrichment stimulates plant growth by increasing photosynthesis and improves biomass accumulation resulting in higher economic yield. The profitable use of supplemental CO₂ over years of greenhouse practice points to the importance of CO₂ in plant production (Li et al. 2017; Jin et al. 2009). Maize plants grew taller under enriched CO₂ conditions (Driscoll et al. 2006). However, the total chlorophyll content in maize plants was reported to decrease under elevated CO₂ conditions, but no changes in stomatal density were observed (Driscoll et al. 2006). To the contrary, Casson and Gray (Casson and Gray 2008) reported that stomatal density decreased in many species under CO₂ enriched conditions and amphistomatous species showed pronounced differences in stomatal density compared to hypostomatous species. In alfalfa, elevated CO₂ increased total growth and yield along with an increase in mineral nutrient absorption (Sanz-Saez et al. 2012). Chlorophyll contents in alfalfa were reported not different under elevated CO₂ condition in comparison with ambient ones (Al-Rawahy et al. 2013).

There are only a handful of published investigations on the effect of water stress on maize (Leakey et al. 2006; Yang et al. 2014; Erbs et al. 2015) and alfalfa (Ahmadani 2014; Erice et al. 2007; Sgherri et al. 1998; De Luis et al. 1999), under enriched CO₂ conditions. Some of these studies were done in either field or growth chamber conditions (Leakey et al. 2006; Yang et al. 2014; Ksiksi et al. 2017). Under these circumstances it would be worthwhile to do a research on the combined effect of CO₂ and water stress under greenhouse conditions, as was suggested by other researchers as well (Ksiksi 2016). The greenhouse used here is in UAE desert, where it is very challenging to keep constant temperature and humidity. In this study, maize and alfalfa were grown in different pots under the combined effects of elevated CO₂ and water stress. It was therefore hypothesized that, elevated CO₂ may compensate for the reduction in chlorophyll content and the stomatal architecture as a whole leading to better survival and growth of both maize and alfalfa.

Materials and methods

Determination of irrigation volume for the experiment

The volumes of water, used as drought stress treatments, were determined based on a pre-experiment set up using 3-l pots (19.3 cm diameter and 16.4 cm height) and potting mix (Gardeners mix 1, Desert Group, Dubai, UAE), inside the greenhouse. For this purpose, field capacity of the potting mix was calculated first by adding different volumes of water (400, 500, 600, and 700 ml) to the potting mix, keeping three replications. From our initial experiment, we found that 600 ml of water was needed for the potting soil mix to reach its field capacity (control treatment in the experiment). Based on this value, other two values were determined which were 70% of field capacity (420 ml) and 30% of field capacity (180 ml). For the ease of application, we changed these values to 400 ml (moderate stress) and 200 ml (high stress). Here after, the control (field capacity), 70%-field-capacity, and 30%-field-capacity treatments will be referred to as 'CWS', 'MWS', and 'HWS', respectively.

Growing maize and alfalfa in the greenhouse

Commercially purchased maize (*Zea mays* L.) and alfalfa (*Medicago sativa* L.) were seeded in three different custom-built cage structures, inside a greenhouse in UAE University farm in Alfoah, U.A.E (N24°–E55°). Each cage structure was made up of aluminium frames and transparent plastic material (98% sunlight transmittance) of 2 mm thickness.

Further, each cage had three sections, 2 of those section had rectangular structure and made in such a way that they could be stacked up on each other. Each of them was $1.5\text{ m} \times 1.5\text{ m} \times 0.6\text{ m}$ (Length $\times$ Height $\times$ Width). The third section was a trapezoid shaped one, with same length and width and could be stacked up on top of the other two sections. The bottom section has holes for incoming and outgoing electrical and other instrument wires and was fitted with a small kitchen exhaust fan ($12\text{ cm} \times 12\text{ cm} \times 4\text{ cm}$) to remove extra moisture due to humidity, in the morning. The topmost trapezium section had an outward opening door on the topside for the same purpose.

Maize and alfalfa were seeded in a RCBD design (cages as blocks) in an inter-cropping style into different plastic pots (19.3 cm diameter and 16.4 cm height.) containing tightly packed potting mix (gardeners mix 1, Desert Group, Dubai, UAE). We maintained 90 such pots with 45 of them for maize and 45 for alfalfa. As per treatment design, each cage had 30 pots and 15 of them were maize and 15 were alfalfa. Maize and alfalfa seeds were irrigated with only water and once they germinated manual fertigation started for first 14 days as in a usual cropping environment. TUROFORT 20 + 20 + 20 + 2MgO + TE NPK fertilizer with trace elements (TE) (Adfert, AD, UAE) for the vegetative stages and ALASKAFORT 12 – 12 – 36 + TE (Adfert, AD, UAE) was used after flowering for fertigation (EC:2.5 and pH: 6.2). After 14 days, CO₂ enrichment and water stress treatments were applied. Atlas 4 control (Titan controls, ON, CAN) system with multi-room CO₂ sensors were used for the CO₂ enrichment treatments. In one cage, the sensor was set at 1000 ppm CO₂, the second at 700 ppm CO₂, and the third at ambient greenhouse CO₂ environment (435 ppm). Three water stress treatments were allotted to each cage so that 5 maize (out of 15) and 5 alfalfa (out of 15) plants received each water stress treatment per cage. The remaining five plants were used as control. The treatments were high water stress (HWS), moderate water stress (MWS), and control (CWS), as mentioned before. Nine GS3 soil moisture sensors (Decagon Devices Inc., WA, USA) were inserted into the soil mixes in the pots. Each of the cage structure got 3 sensors for the three different water stress treatments. The other ends of the GS3 sensors were connected to two EM50 dataloggers (Decagon Devices Inc., WA, USA) from which periodic data on soil moisture were downloaded (data is not presented here) and used to schedule irrigation time.

Chlorophyll extraction and quantification

Chlorophyll was extracted from the leaves of maize and alfalfa collected on the last day (day 105). Chlorophyll was quantified following a procedure described by Schlemmer et al. (2013) with slight modification on the plant material

added. Nine leaf squares ($1\text{ cm} \times 1\text{ cm}$) were cut from both alfalfa and maize leaves and were separately added to DMSO in glass tubes, which was pre-heated to 65°C, and were kept in DMSO for 30 min. The samples were immediately read for absorbance spectra using a Lambda 25 UV/VIS spectrometer (PerkinElmer, USA). Two samples were read for absorbance from each maize and alfalfa leaf taken from ambient and two enriched treatments. The spectrometer was calibrated to zero absorbance using DMSO as blank in a quartz cuvette. The absorbance spectra were read at 665, 649 nm wavelengths for chlorophyll ‘a’ and Chlorophyll ‘b’, respectively. The calculations were made using equations described by Schlemmer et al. (2013). Additionally, absorbance readings were taken at 480 nm in the DMSO extracted plant sample of maize to determine the carotenoid concentrations and were quantified using an equation described by Pompelli et al. (2013). Chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll contents and carotenoids concentrations were calculated from the equations ($\mu\text{g/ml}$) which were then converted to chlorophyll content per unit area ($\mu\text{g/cm}^2$).

Stomatal length, width and density of maize

Stomatal length, width and density were determined from the epidermal peels prepared from the adaxial surface of 105 days old maize leaves using a method described by Zhang and Meinzer (2013) with slight modifications. Clear nail polish was applied onto the maize. Using the tip of surgical knife, $6 \times 4\text{ mm}^2$ sized epidermal peels were removed and were put on a glass slide and covered with a cover slip. An Olympus BX41 laboratory microscope fitted with a DP71 microscopic camera (Olympus Corporation, Tokyo, Japan) was used to observe these epidermal peels. Stomata from three different locations on the epidermal peels were observed individually for length and width and in group for stomatal density. Stomatal densities (per $200 \times 200\text{ }\mu\text{m}^2$ peel area) were quantified at $400\times$ magnification and were converted into stomatal density per square millimeter. Stomatal length and width were recorded at $1000\times$ magnification of the BX41 microscope DP 71 camera combination. Alfalfa leaves were too small to apply this technique of stomatal imprinting and was practically difficult to separate the peels without breaking the nail polish impressions. Thus, we decided not to measure the stomatal dimensions of alfalfa leaves.

Data Analysis

Data were analyzed using IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY). Two-way Analysis of Variance (ANOVA) procedure was used to analyze the data with CO₂ and water stress as two independent variables. Pairwise

comparisons (at $P \leq 0.05$) were also performed with bonferroni corrections to identify the mean differences.

Results

Chlorophyll content

In maize, a significant interaction effect is observed between CO₂ levels and water stress treatments ($P \leq 0.05$). Maize plants in the HWS and MWS stress treatments of the 700 ppm CO₂, showed total chlorophyll content (TCC) on par with the CWS (control) (Fig. 1). In contrast, HWS and MWS treatments of ambient showed higher TCC in comparison to CWS in ambient and 1000 ppm CO₂ environments showed higher total chlorophyll content compared to CWS, for unknown reasons. In addition, in both of these CO₂ environments (700 and 1000 ppm), highly water-stressed plants (HWS) showed comparatively higher TCC compared to MWS and CWS. In this context, the maize plants in 700 ppm CO₂ showed more consistent and reliable results. We believe that this is a sign of the positive effect of CO₂ enrichment. Chlorophyll 'a' content was showing the same trend as TCC. A more reliable data, in this case as well, is observed at 700 ppm CO₂, where the HWS and MWS treatments could not affect the chlorophyll 'a' content, severely, compared to CWS (Fig. 2). Further, at 700 ppm CO₂, both HWS and MWS did affect chlorophyll 'b' content in the maize plants compared to CWS. This was again evident from the comparison of chlorophyll

Fig. 1 Total chlorophyll content (µg/cm²) of maize leaves (mean ± SEM, N = 5) under water stress treatments HWS (200 ml/week), MWS(400 ml/week) and CWS (600 ml/week) in ambient and two CO₂ enrichment levels: 700 and 1000 ppm

Fig. 2 Chlorophyll 'a' content (µg/cm²) of maize leaves (mean ± SEM, N = 5) under water stress treatments HWS (200 ml/week), MWS (400 ml/week) and CWS (600 ml/week) in ambient and two CO₂ enrichment levels: 700 and 1000 ppm

'b' contents of ambient and 700 ppm CO₂ environments (Fig. 3). However, the 1000 ppm CO₂ showed higher chlorophyll 'b' contents in the HWS and MWS compared to control, a drastic deviation from the chlorophyll 'b' decreasing trends with the increasing CO₂ concentration. Since, the interaction was statistically significant, the main effects of CO₂ enrichment and water stress can be ignored. However, it would give an insight to what actually

Fig. 3 Chlorophyll 'b' content (µg/cm²) of maize leaves (mean ± SEM, N = 5) under water stress treatments HWS (200 ml/week), MWS (400 ml/week) and CWS (600 ml/week) in ambient and two CO₂ enrichment levels: 700 and 1000 ppm

contributed more to the chlorophyll content. Maize plants in 700 ppm CO₂ showed highest average total chlorophyll content (20.87 µg/cm²) compared to ambient (18.34 µg/cm²) and 1000 ppm (19.33 µg/cm²) CO₂ treatments. This shows that water stress actually increased the TCC in the maize plants. In case of carotenoids, all the water stress treatments in 1000 ppm showed higher content compared to 700 ppm ($P \leq 0.05$) and ambient ($P \leq 0.05$) (Fig. 4).

Chlorophyll 'a', 'b', and total chlorophyll content in alfalfa were not significantly different among CO₂ and water treatments. A closer look will reveal that CO₂ enrichment actually reduced the chlorophyll content compared to ambient condition. The total chlorophyll content (Fig. 5) was highest in ambient condition (19.45 µg/cm²) followed by 1000 ppm (19.35 µg/cm²) and by 700 ppm (18.86 µg/cm²) CO₂ environments. Chlorophyll 'a' (Fig. 6) was highest (11.16 µg/cm²) in 1000 ppm CO₂ and lowest (10.07 µg/cm²) in ambient condition, while chlorophyll 'b' (Fig. 7) was highest (9.38 µg/cm²) in ambient condition and lowest (8.18 µg/cm²) in 1000 ppm CO₂ environment.

Stomatal length, width and density

Stomatal length and width

Stomatal length was lowest (45.22 µm) in ambient and highest (49.12 µm) at 700 ppm CO₂ level (Fig. 8). The most important observations were, CO₂ treatments 700 ppm and 1000 ppm increased stomatal length of maize plants in the high and moderate water stress treatments compared to that of control ($P \leq 0.05$). Especially, in the

Fig. 4 Carotenoid content (µg/cm²) of maize leaves (mean ± SEM, N = 5) under water stress treatments HWS (200 ml/week), MWS (400 ml/week) and CWS (600 ml/week) in ambient and two CO₂ enrichment levels: 700 and 1000 ppm

Fig. 5 Total chlorophyll content (µg/cm²) of alfalfa leaves (Mean ± SEM, N = 5) under water stress treatments HWS (200 ml/week), MWS (400 ml/week) and CWS (600 ml/week) in ambient and two CO₂ enrichment levels: 700 and 1000 ppm

Fig. 6 Chlorophyll 'a' content (µg/cm²) of alfalfa leaves (Mean ± SEM, N = 5) under water stress treatments HWS (200 ml/week), MWS (400 ml/week) and CWS (600 ml/week) in ambient and two CO₂ enrichment levels: 700 and 1000 ppm

700 ppm CO₂ treatment, stomata in the HWS and MWS were longer than that of CWS.

Stomatal width was highest in 1000 ppm CO₂ (15.86 µm) and lowest in 700 ppm CO₂ (14.90 µm). We observed that stomatal width of maize plants decreased in high and moderate water stress treatments under CO₂ enriched environments (700 ppm and 1000 ppm) compared to ambient environment (Fig. 9). As the plants received more water

Fig. 7 Chlorophyll 'b' content (µg/cm²) of alfalfa leaves (Mean ± SEM, N = 5) under water stress treatments HWS (200 ml/week), MWS (400 ml/week) and CWS (600 ml/week) in ambient and two CO₂ enrichment levels: 700 and 1000 ppm

Fig. 8 Stomatal length (µm) of maize leaves (mean ± SEM, N = 5) under water stress treatments HWS (200 ml/week), MWS (400 ml/week) and CWS (600 ml/week) in ambient and two CO₂ enrichment levels: 700 and 1000 ppm

(control), stomatal width increased with increase in CO₂ enrichment. It was noteworthy that wider stomata were observed in water stress treatments (HWS and MWS) compared to CWS under ambient condition.

Stomatal density

Stomatal density (per mm²) of maize was not affected by CO₂ enrichment and in fact showed irregular response to CO₂ enrichment. In 1000 ppm and ambient CO₂ environments, the HWS and MWS treatments increased stomatal

Fig. 9 Stomatal width (µm) of maize leaves (mean ± SEM, N = 5) under water stress treatments HWS (200 ml/week), MWS (400 ml/week) and CWS (600 ml/week) in ambient and two CO₂ enrichment levels: 700 and 1000 ppm

Fig. 10 Stomatal density (per mm²) of maize leaves (mean ± SEM, N = 5) under water stress treatments HWS (200 ml/week), MWS (400 ml/week) and CWS (600 ml/week) in ambient and two CO₂ enrichment levels: 700 and 1000 ppm

density compared to control (Fig. 10). However, in 700 ppm CO₂, water stress treatments (HWS and MWS) decreased stomatal density compared to CWS. The highest stomatal density (110/mm²) was recorded in ambient and lowest stomatal density (93/mm²) was recorded in 700 ppm CO₂ environments.

Discussion

In maize, chlorophyll 'a' was the major contributor in quantity to the total chlorophyll content compared to chlorophyll 'b'. Also, a close analysis revealed that

chlorophyll ‘b’ did not significantly influence the total chlorophyll content as compared to chlorophyll ‘a’. At 700 ppm CO₂, chlorophyll production in the HWS and MWS water stress treatments was on par with the control treatment. High (1000 ppm) and ambient CO₂ concentrations led to an increased total chlorophyll content in the HWS and MWS treatments, when compared to the control. It is important to note that there are no recently published works directly assessing the combined effect of water stress and CO₂ changing chlorophyll content. But if we consider results from only one CO₂ environment (for e.g.: 700 ppm), this result was contrary to the earlier study reported by Sanchez and Colleagues (Sanchez et al. 1983) who observed significantly lower chlorophyll content in water stressed maize plants. The only variant in our study compared to the above listed study (Sanchez et al. 1983) is the CO₂. So, it is clear that CO₂ actually mitigated the negative effect of water stress on chlorophyll production in maize plants. Plants in 1000 ppm CO₂ showed the highest carotenoid content followed by 700 ppm and then ambient condition. Carotenoid content was calculated based on chlorophyll content, so whichever plants had high chlorophyll contents (700 ppm), they were reported to be lower in carotenoid contents.

Alfalfa plants in the enriched CO₂ environments showed similar total chlorophyll contents compared to ambient CO₂ environment. In alfalfa also, recent works were not published showing the assessment of the combined effects of water stress and CO₂ enrichment and thus an earlier work is cited for the discussion in this paper. Sgherri et al. (1998), reported that water stress reduced chlorophyll content in alfalfa and the rate of reduction was higher in the ambient (300 ppm) environment compared to enriched (600 ppm) environment. In contrast, our experiment showed that the HWS plants in the ambient environment showed higher chlorophyll content compared to their counterparts in the enriched CO₂ environments. This indicates the inability of CO₂ treatments to show any positive effects on alfalfa plants under high water stress. However, CO₂ enrichment did increase chlorophyll contents in moderately water stressed alfalfa plants, and which is in agreement with the findings of Sgherri et al. (1998). In addition, chlorophyll ‘a’ and chlorophyll ‘b’ contents in the ambient condition (only water stress, no CO₂ enrichment), showed similar results compared to a previously published study (Abid et al. 2016). In that study, lower chlorophyll ‘a’ content was reported in drought stressed alfalfa plants compared to control. Therefore, it is clear that 1000 ppm CO₂ enrichment increased the chlorophyll ‘a’ and chlorophyll ‘b’ production in the water stressed alfalfa plants.

In the maize plants, higher levels of CO₂ increased stomatal lengths even in water stressed treatments. In both of the CO₂ enriched conditions, stomatal lengths were

similar or higher than the control treatments. It is noteworthy to highlight that the CO₂ enrichment in general increased stomatal lengths in the HWS and MWS treatments compared to their counter parts in the ambient environment. For example, stomatal lengths were 49.90, and 49.93 µm, respectively at the HWS and MWS treatments in the 700 ppm and 41.72 µm and 45.01 µm, respectively at the HWS and MWS environments in the ambient condition. Zhao et al. (2015) also reported an increase in stomatal length as the water stress increased, however, without CO₂ enrichment. In addition, the ambient treatment (without CO₂ enrichment) in our experiment showed the exact reverse observation on stomatal length in comparison with the findings of Zhao and Collaborators (Zhao et al. 2015). Therefore, in our study it can be summarized as the CO₂ enrichment actually increased stomatal length in water stressed maize plants and 700 ppm was the most effective CO₂ treatment.

Stomatal width in the moderately water stressed treatment (MWS) showed irregular trends, as the CO₂ concentration increased, in comparison with ambient environment, in maize plants. For the HWS treatments, a clearly visible reduction in stomatal widths was recorded at enriched CO₂ concentrations, when compared to ambient (Fig. 9). A previous study reported that stomatal width was not different in the water stress treatments compared to control (Zhao et al. 2015). However in our results, stomatal width increased under water stress for the ambient and 1000 ppm (from MWS to HWS) and decreased for 700 ppm (from MWS to HWS) treatments. In the maize plants, stomatal density decreased, in both MWS and CWS water stress treatments, as the CO₂ concentration increased (Fig. 10). The highest decrease under CO₂ conditions, was observed in 700 ppm (96.11/mm²) followed by 1000 ppm (100.97 mm²) compared to ambient (103.33 mm²). The results reported were contrary to two previous studies, where CO₂ enrichment (Driscoll et al. 2006), and water stress (Zhao et al. 2015) were reported to individually increase stomatal densities. So, actually decrease in stomatal density is good for the plants under water stress to reduce the water loss. By enriching water stressed plants with CO₂, that condition could be achieved. In short, CO₂ enrichment did decrease stomatal density, which in turn positively influenced the maize plant growth by reducing transpiration loss.

Conclusions

CO₂ enrichments, especially at the 700 ppm level, increased the total chlorophyll content in maize plants. This is a good sign as the increase in TCC could be positively correlated with plant growth. However, in alfalfa,

CO₂ enrichments did not show a desired impact under the experimental conditions and slightly reduced the total chlorophyll content. In addition, the slightly higher stomatal lengths and widths under enriched CO₂ environments points to the fact that the leaves still have higher relative water content. This further indicates that the overall water content inside the plants are higher due to enriched CO₂ environments. Moreover, reduction in stomatal density also indicates to the same direction of more water storage under drought stress in presence of CO₂ enrichment. The results in our study provide a big positive boost to move forward with further research on reducing irrigation waters coupled with CO₂ entrenchments, without compromising crop and forage yield. More future studies are required to confirm the above results.

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Authors contributions TSK and SBTP have planned the experiments. SBTP executed the experimental work with help from ARP. ARP reviewed the drafts.

Compliance with ethical standards

Conflict of interest The authors declares that they have no conflict of interest.

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