Physiological Plasticity of Green Gram Stomata to Photosynthesis Traits under Interactive Effects of Elevated CO$_2$, Drought and Heat Stress

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Authors’ contributions

The authors collaboratively did this work. All the authors have reviewed and approved the content of the submitted manuscript.

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ABSTRACT

Heat waves and droughts are projected to become more widespread as a result of climate change. At the same time, CO$_2$ levels are predicted to have doubled by 2100. The stomatal regulation and gas exchange characteristics were assessed in 25 days old plants of green gram (var Co 8) by exposing them to six different treatments namely, T$_1$: a [CO$_2$] + a T+ irrigation (100%), T$_2$: a [CO$_2$] + a T+ irrigation (50%), T$_3$: a [CO$_2$] + e T (40ºC) + irrigation (100%), T$_4$: e [CO$_2$] − 800 ppm + a T+ irrigation (100%), T$_5$: a [CO$_2$] + combined stress [e T (40ºC) + irrigation (50%) T$_6$: e [CO$_2$] − 800 ppm + combined stress [e T (40ºC) + irrigation (50%)]. The experiment was carried out using Completely Randomized Design (CRD) with three replications. All gas exchange parameters viz., (photosynthesis rate, stomatal conductance, and transpiration rate) were determined before imposing stress and two weeks after imposing stress. Stomatal characters was examined two weeks after imposing stress. Elevated CO$_2$ stress caused a reduction in stomatal frequency accompanied by larger stomatal size. The study revealed the positive effect of higher CO$_2$ concentration on gas exchange traits of the C3 crops viz., green gram.
Keywords: Green gram; elevated CO₂; drought; heat; photosynthetic rate; stomatal frequency; stomatal area.

1. INTRODUCTION

Green gram is an important pulse crop with short duration, and an excellent source of high-quality protein. Drought, waterlogging, and high temperatures, are extremely harmful to it. Along with these major stresses, scientists are more concentrated on today’s global warming with increasing levels of CO₂ concentration in the atmosphere. CO₂ levels in the atmosphere have been steadily rising, and are now around 410 parts per million (Scripps Institution of Oceanography 2020; Schmidt, 2020). When atmospheric CO₂ levels rise, plant carbon balance is expected to improve due to CO₂-fertilization and less transpirative water loss [1]. Plant stomata serve as an essential link between the plant and the environment, and they play a key part in plant responses to changing conditions [2]. For investigating plant stress responses in shifting climates, effective stomatal control is essential, especially in fast-growing crop species like green gram, which has the highest rates of stomatal conductance [3]. Decrease in stomatal conductance leads to an improvement in water use efficiency [1].

The degree and length of abiotic stress exposure and the stage of crop growth, determine the number of stomatal variables limiting gas exchange parameters. Due to variation in stomatal frequency and stomatal area under different abiotic stress, green gram may have different gas exchange capabilities. Through morphological variations in the number of stomata and physiological alteration in stomatal aperture size, stomatal control allows for the best balance of CO₂ uptake and water loss [4]. Physiological control of stomatal conductance allows plants to maintain water use efficiency while balancing CO₂ intake for photosynthesis and water loss.

It has been reported that elevated CO₂ alone increased net photosynthetic rate and water use efficiency while decreasing green gram’s stomatal conductance and transpiration rate [5]. In green gram, the deficit in soil moisture reduces the net photosynthetic rate, stomatal conductance and transpiration rate, whereas heat stress decrease the net photosynthetic rate and stomatal conductance while increased the transpiration rate [6]. Plant responses to elevated CO₂, heat, and drought stress have been the focus of the majority of investigations so far. According to Jiang et al., [7] the e [CO₂] reduced the damage caused by moderate and severe drought stress. During drought stress, the e [CO₂] could improve water usage efficiency and plant water relations by lowering leaf stomatal conductance [8].

So far, there have been no reports on the combined effects of e [CO₂], drought, and heat so far. Hence the study was undertaken with the following objectives a) to study the interactive effects of elevated CO₂, drought and heat stress on different stomatal traits in green gram. b) to understand the stomatal anatomical plasticity in regulating the gas exchange traits and WUE. c) to ascertain the effects of elevated CO₂ on photosynthetic efficiency and WUEs in the changing climate scenario.

2. MATERIALS AND METHODS

2.1 Plant Materials and Stress Treatments

This work was carried out from February 2021 to May 2021 in the popular green gram variety (Co 8) of TNAU at the Open Top Chamber (OTC) at the Department of Crop Physiology, TNAU, Coimbatore. OTC of 4 m x 4 m x 4 m fabricated with polycarbonate sheet was used. The chambers were equipped with SCADA integration technology with CO₂, temperature and humidity monitoring control and wireless signal transmission. Four OTCs were used for this study. The first chamber was designated as the ambient chamber. In the second chamber, the temperature was maintained at 40°C. In the third chamber, CO₂ was maintained at 800 ppm, and in the fourth chamber, the temperature at 40°C and e CO₂ at 800 ppm was maintained. Drought stress was imposed by the dry down method [9]. The seeds of green gram (variety – Co 8) were sown in a pot size of 37x 35 cm (20 kg pot). The pots were maintained under normal conditions and watered daily upto 25 days. Crop management and protection measures were taken as per TNAU crop production guidelines.

After 25 days, the pots were kept inside OTC for inducing stress. Pots of each treatment were divided and arranged in Completely Randomized Design (CRD) with three replications. Plants were subjected to six different treatments viz.,

- T1: a [CO₂] + a T+ irrigation (100%) (absolute control)
• T2: a [CO₂] + a T+ irrigation (50%) (drought stress only)
• T3: a [CO₂] + e T (40ºC) + irrigation (100%) (heat stress only)
• T4: e [CO₂] – 800 ppm + a T+ irrigation (100%) (elevated CO₂ stress only)
• T5: a [CO₂] + combined stress [e T (40ºC) + irrigation (50%)]
• T6: e [CO₂] – 800 ppm + combined stress [e T (40ºC) + irrigation (50%)].

Elevated CO₂, high temperature and drought stress were given to the plants for 21 days. The temperature during this period ranged from 34.66 ºC to 40.92 ºC. The RH in the ambient chamber ranged from 55.8 % to 59.93 %, and in the elevated chamber from 39.27 % to 42.55 % (Fig. 1). Stomatal parameters (SL, SW, SP, SA, SSI and SF) and gas exchange parameters were measured two weeks after imposing stress.

2.2 Characterization of Stomata

2.2.1 Sample preparation

The leaf impression approach assessed the stomatal characters such as length, width, perimeter, area, and stomatal shape index (SSI). The replica-based method developed by Meidner and Mansfield (1968) was used to observe the stomatal characters. Original imprints were obtained by evenly smearing the quick fix on each treatment’s freshly collected green gram leaves. After 15 minutes, the thin film layer (approximately 5 mm x 5 mm) was peeled from the leaf surface, and then the thin film was mounted on a glass slide and covered with cover slip.

2.2.2 Observation of stomatal parameters

The stomatal length, stomatal width, stomatal perimeter, stomatal area, stomatal shape index (SSI) and stomatal frequency for each film strip were measured and counted immediately under the light microscope (Olympus – bright field) with computer attachment. Three replicates were prepared from the same leaf at different positions. For each replica, three photographs were taken at different locations in the slide. Stomata number was counted for each picture, excluding those cut by the picture border and stomatal frequency was calculated per unit leaf area and expressed as stomatal frequency/ mm² (Baruah et al.,[10]).

Fig. 1. Air temperature and relative humidity during stress period at OTC

AC – Ambient chamber, EC – Elevated chamber
Bars represent the standard errors of mean values
Stomatal length, stomatal width, and stomatal perimeter were measured and expressed as μm, whereas stomatal area was expressed in μm$^2$. The stomatal shape index (SSI) was calculated by the function that shape index $= \sqrt{\frac{SA}{SP}} \times 100$ where SA is the stomatal area and SP is the stomatal perimeter and expressed in %.

2.3 Gas Exchange Parameters

The Portable Photosynthesis System (PPS) (Model LI-6400 of LICOR inc., Lincoln, Nebraska, USA) was used to collect data on leaf gas exchange characters, two weeks after imposing stress. Totally, three measurements were taken in the same leaf. The readings were taken between 10.00 and 12.30 h, using PPS system, the gas exchange parameters viz., Photosynthetic rate ($Pn$: μmol CO$_2$ m$^{-2}$ s$^{-1}$), Stomatal conductance ($Gs$: mmol H$_2$O m$^{-2}$ s$^{-1}$) and Transpiration rate ($E$: mmol H$_2$O m$^{-2}$ s$^{-1}$) were recorded.

2.4 Intrinsic Water Use Efficiency (WUE$_i$) (mmol CO$_2$ mol$^{-1}$ H$_2$O)

The intrinsic water use efficiency (WUE$_i$) was calculated as $Pn/E$ (Guha et al. 2010). Where $Pn$ is photosynthetic rate and $E$ is transpiration rate. The $Pn$ and $E$ was measured using Portable Photosynthesis System (PPS) (Model LI6400 of LICOR inc., Lincoln, Nebraska, USA).

2.5 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) as suggested by Gomez and Gomez (2010) [11]. ANOVA was performed for each variable and subsequently used to determine whether there were statistical differences between the treatments. The significance level was at 5 %. Treatments were arranged in a completely randomized design with three replications. Each replicant consists of five sampled means. Pearson correlation was used to investigate the relationship between the stomatal characters and gas exchange parameters.

3. RESULTS AND DISCUSSION

In general CO$_2$ enrichment led to a significant increase in photosynthetic rate in green gram (Fig. 2A). Significant variation was recorded for photosynthetic rate among different treatments(T1-T6). Generally light-saturated photosynthesis for C3 plants increased by an average of 31% in Free Air CO$_2$ Enrichment (FACE) studies [12]. In the present experiment, the photosynthetic rate at elevated CO$_2$ (T4) was significantly higher when compared to ambient CO$_2$ at absolute control conditions (T1). The photosynthetic rate and stomatal conductance of green gram at drought stress (T2) and heat stress (T3) were significantly lower when compared to control conditions (Fig. 2A and 2B). It was also observed that, the photosynthetic rate and stomatal conductance of green gram leaves decreased greatly under combined heat and drought stress than individual stress. On the other hand, photosynthetic rate and stomatal conductance under elevated CO$_2$ and combined stress increased slightly when compared to combined stress at ambient CO$_2$ conditions. This is because e (CO$_2$) could alleviate the reduction in photosynthetic rate brought by combined heat and drought stresses. This alleviating effect on plants grown at combined heat and drought stress has been reported in Arabidopsis thaliana [13], Triticum aestivum [14] and C3 grassland [15]. Furthermore, under severe and combined stress, the stomatal conductance is not always associated with plant photosynthetic capability [16]. Under e (CO$_2$), despite the reduction of Gs, Tr was not much affected (Fig. 2D).

Elevated CO$_2$ led to a significant (P<0.01) increase in WUE$_i$ in green gram (Fig. 3) when compared to control, individual stresses and also under combined stress. This was supported by Gao et al., [5], who noticed an increase in water use efficiency in green gram under e (CO$_2$) conditions in FACE experiments. Transpiration rate under different treatments varied significantly in green gram (Fig. 2B). Under high-temperature stress (T3), transpiration gets increased, whereas it decreases under drought stress (T2), and combined stresses (T5) at a (CO$_2$) and e (CO$_2$) conditions (T6) (Fig. 2B). Plants cultivated at a (CO$_2$) were more susceptible to combined stress than those grown at e (CO$_2$). As a result, cumulative stress causes a larger decline in WUE$_i$ in plants cultivated at a (CO$_2$) than in those grown at e (CO$_2$). The study clearly explains that, green gram grown at e (CO$_2$) + combined stress had a more photosynthetic rate when compared to those grown at a (CO$_2$) + combined stress. The transpiration rate at combined stress was lesser when compared to individual stresses. Similar results were reported in maize by Hussain et al. [17], who noticed a decrease in photosynthetic rate, Gs and E under combined stress than individual stress.
Fig. 2. Effect of different abiotic stresses on A) photosynthetic rate, B) stomatal conductance and transpiration rate, C) percent increase or decrease in net photosynthetic rate, D) percent increase or decrease in stomatal conductance and transpiration rate
WUE is influenced by stomatal behaviour, patterning, and morphology (Lawson and Blatt [18]) but little is known about how changes in stomatal features affect plant physiological responses under various environmental situations. The findings of the current research showed that, when CO₂ concentrations were low, a reduction in stomatal area (sa) and an increase in stomatal frequency (sf) was observed (Plate 1). On the other hand, when atmospheric CO₂ levels have been high, the stomatal area has increased and frequency has decreased. When correlating the gas exchange parameters with stomatal frequency and stomatal area, it was found that the plants grown under elevated CO₂ with lower stomatal frequency and the larger stomatal area maintained a higher rate of photosynthesis. This decrease in stomatal frequency under e (CO₂) have been reported in soybean, when plants were grown under elevated CO₂, with a consequent decrease of gas conductance [19]. The experiment revealed that, photosynthetic rate increases with increased stomatal area.
The stomatal area, photosynthetic rate and stomatal conductance were positively correlated to WUE whereas stomatal frequency was negatively correlated (Table 1). The stomatal frequency and stomatal area may have a higher degree of plasticity in response to different abiotic stresses. Many studies have reported a reduction in stomatal frequency with increased CO₂. Contradictory to the above findings, there was a minimum reduction in stomatal frequency with an increase in stomatal area under e (CO₂) conditions in green gram (var. Co 8).

The increased stomatal frequency may increase transpiration potential, which is employed for evaporative cooling, at high temperatures. An increase in stomatal frequency and decrease in stomatal area under-drought stress may be due to partial closure of stomata which indicates the adaptive mechanism under drought conditions. As a result, several-fold decreases in the stomatal area were observed under both drought stress and combined stresses. The stomatal area under combined stress at a (CO₂) gets drastically reduced when compared to all other treatments (Fig. 4). When compared to combined stress at a (CO₂) conditions the stomatal area under combined stress at e (CO₂) gets slightly increased. This may be due to the effect of e (CO₂) on the stomatal area at combined stress conditions.

**Plate 1. Images on measurement of stomatal frequency in green gram variety (Co 8)**

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**Fig. 4. Percentage increment of stomatal length (SL), stomatal width (SW), stomatal perimeter (SP), stomatal area (SA), stomatal shape index (SSI) and stomatal frequency (SF) in green gram (var Co 8) grown under elevated CO2 compared over interactive effects of heat and drought stress**
Table 1. Correlating water use efficiency (WUE) with stomatal traits and gas exchange parameters

|                      | Photosynthetic rate | Gas conductance | Stomatal perimeter | Stomatal area | Stomatal frequency |
|----------------------|---------------------|-----------------|--------------------|---------------|-------------------|
| WUEi                 | 2                   | 0.4             | 0.6                | 0.8           | 1                 |
|                      | 2                   | 6               | 5                  | 4             | 3                 |
|                      | 6                   | 0.374           | 0.374              | 0.374         | 0.374             |
|                      | 2                   | 100             | 120                | 140           | 5                 |
|                      | 0.4                 | 6               | 5                  | 4             | 3                 |
|                      | 6                   | 0.728           | 0.782              | 0.7691        | 0.5048            |
|                      | 2                   | 600             | 800                | 1000          | 1200              |
|                      | 0.4                 | 140             | 150                | 160           | 170               |
|                      | 6                   | 140             | 150                | 160           | 170               |

R² = 0.8374, R² = 0.374, R² = 0.782, R² = 0.7691, R² = 0.5048
4. CONCLUSION

The study concludes that elevated CO$_2$ caused a reduction in stomatal frequency accompanied by increased stomatal area. Compared with a (CO$_2$), e (CO$_2$) increased the photosynthetic rate of green gram. In comparison to a (CO$_2$), e (CO$_2$) raised the plant’s WUEi. Because of its negative effects on plant water relations, the e (CO$_2$) cannot always be considered to impact positively. This study offers insight into understanding gas exchange parameters and stomatal traits to the interactive effects of future climate conditions, which is vital to identify resilient crops for future climate regimes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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