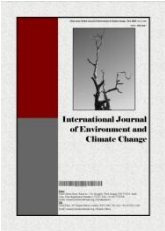

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Physiological Plasticity of Green Gram Stomata to Photosynthesis Traits under Interactive Effects of Elevated CO_2, Drought and Heat Stress

J. Ranjani Priya[1], D. Vijayalakshmi[1*], A. Vinitha[1], M. Raveendran[2] and V. Babu Rajendra Prasad[1]

[1]Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, India.
[2]Department of Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India.

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.

**Corresponding author: E-mail: vijiphysiology@gmail.com;*

Keywords: Green gram; elevated CO_2; 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 CO_2 concentration in the atmosphere. CO_2 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 CO_2 levels rise, plant carbon balance is expected to improve due to CO_2-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 CO_2 intake for photosynthesis and water loss.

It has been reported that elevated CO_2 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 e [CO_2], heat, and drought stress have been the focus of the majority of investigations so far. According to Jiang et al., [7] the e [CO_2] reduced the damage caused by moderate and severe drought stress. During drought stress, the e [CO_2] 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 [CO_2], drought, and heat so far. Hence the study was undertaken with the following objectives a) to study the interactive effects of elevated CO_2, 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_i. c) to ascertain the effects of elevated CO_2 on photosynthetic efficiency and WUE_i 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 CO_2, 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 40ºC. In the third chamber, CO_2 was maintained at 800 ppm, and in the fourth chamber, the temperature at 40ºC and e CO_2 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 [CO_2] + a T+ irrigation (100%) (absolute control)

- T2: a [CO_2] + a T+ irrigation (50%) (drought stress only)
- T3: a [CO_2] + e T (40ºC) + irrigation (100%) (heat stress only)
- T4: e [CO_2] – 800 ppm + a T+ irrigation (100%) (elevated CO_2 stress only)
- T5: a [CO_2] + combined stress [e T (40ºC) + irrigation (50%)]
- T6: e [CO_2] – 800 ppm + combined stress [e T (40ºC) + irrigation (50%)].

Elevated CO_2, 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/ mm^2 (Baruah *et al,*.[10]).

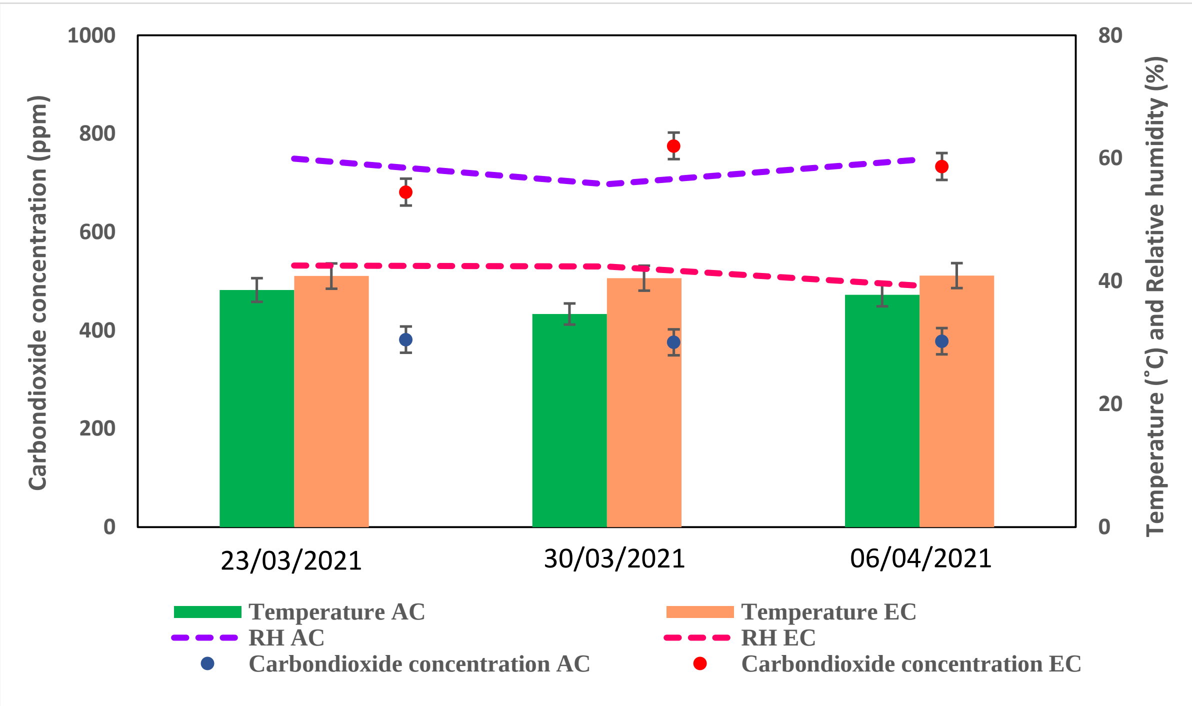


Fig. 1. Air temperature and relative humidity during stress period at OTC

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 µm². The stomatal shape index (SSI) was calculated by the function that shape index = $\frac{\sqrt{SA}}{SP} X\ 100$ 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: mol H_2Om^{-2} s^{-1}) and Transpiration rate (E: mmol H_2Om^{-2} s^{-1}) were recorded.

2.4 Intrinsic Water Use Efficiency (WUE_i) (mmol CO_2 mol^{-1} H_2O)

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).

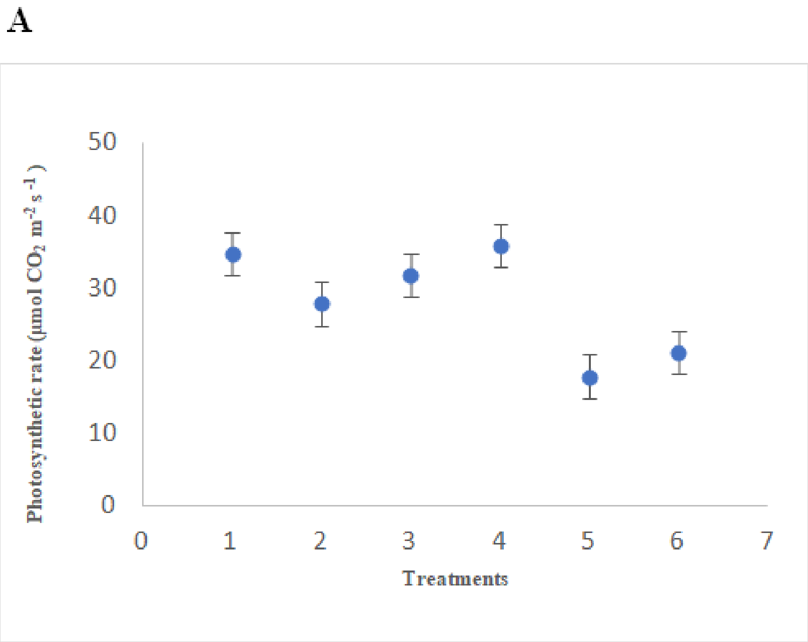
2.5 Statistical Analysis

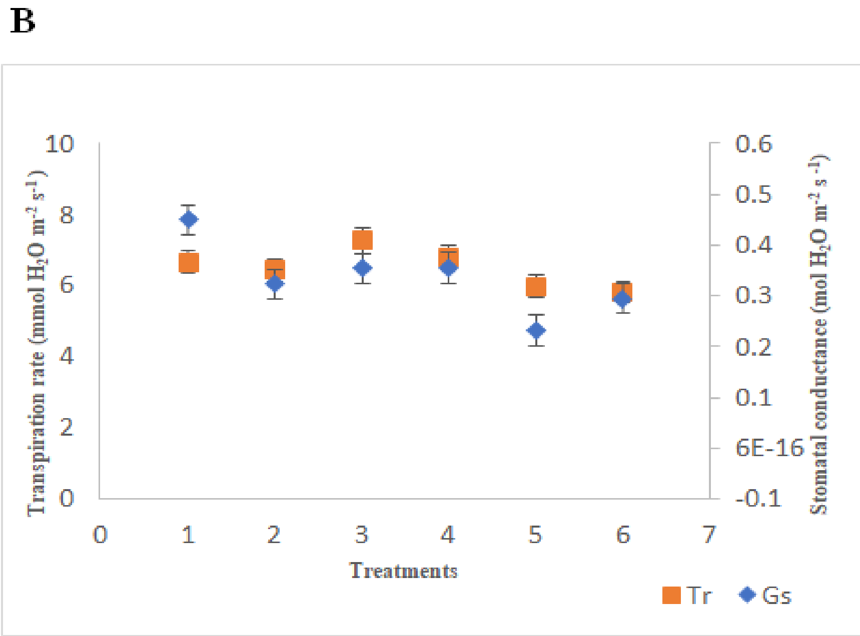
Data were subjected to analysis of variance (ANOVA) as suggested by Gomez and Gomez (2010) [11]. ANOVA was performed for each variable and subsequently used to determine whether there were statistical differences between the treatments. The significance level was at 5 %. Treatments were arranged in a 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 rateat 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 WUEi 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, GsandE under combined stress than individual stress.


A
50
40
30
20
10
0
0 1 2 3 4 5 6 7
Treatments
Photosynthetic rate (µmol CO2 m-2 s-1)


B
10
8
6
4
2
0
0 1 2 3 4 5 6 7
Treatments
Transpiration rate (mmol H2O m-2 s-1)
0.6
0.5
0.4
0.3
0.2
0.1
6E-16
-0.1
Stomatal conductance (mol H2O m-2 s-1)
Tr
Gs

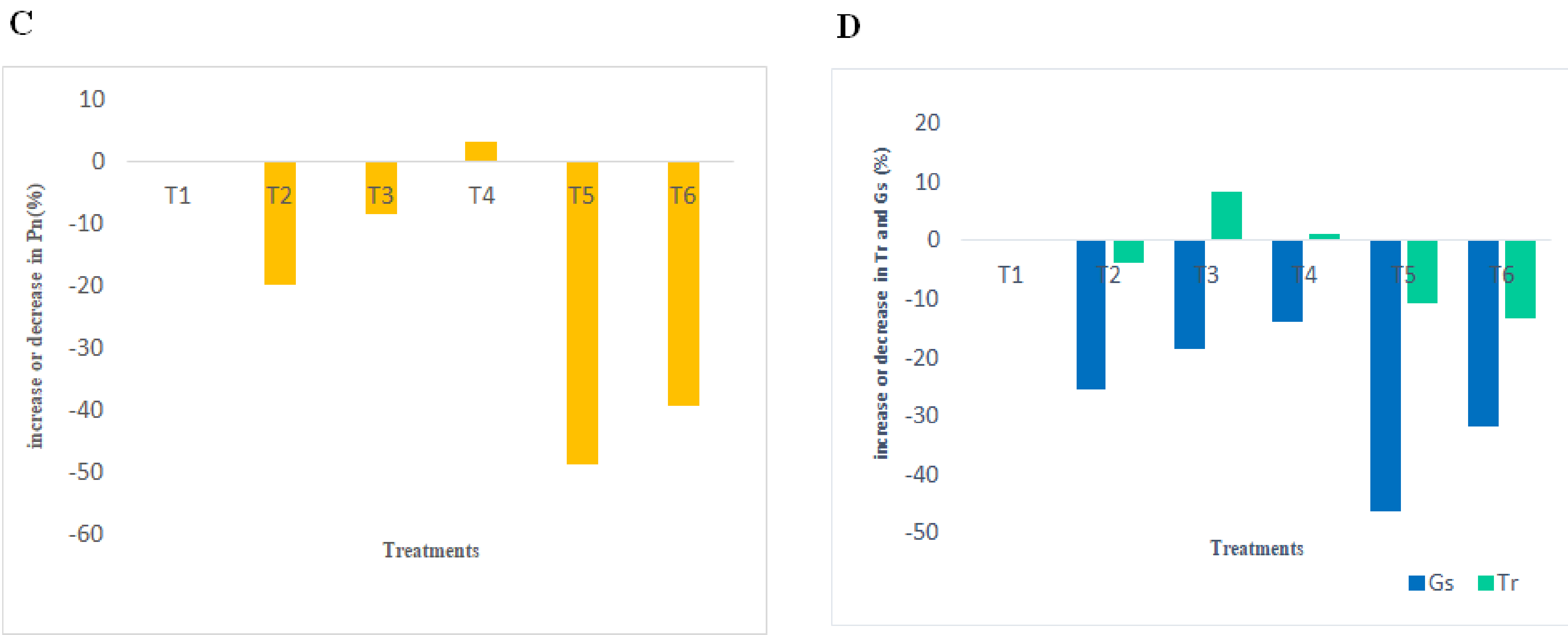


Fig. 2. Effect of different abiotic stresses on A) photosynthetic rate, B) stomatal conductance and transpiration rate, C) percent increase or decrease in net photosynthetic rate, D) percent increase or decrease in stomatal conductance and transpiration rate

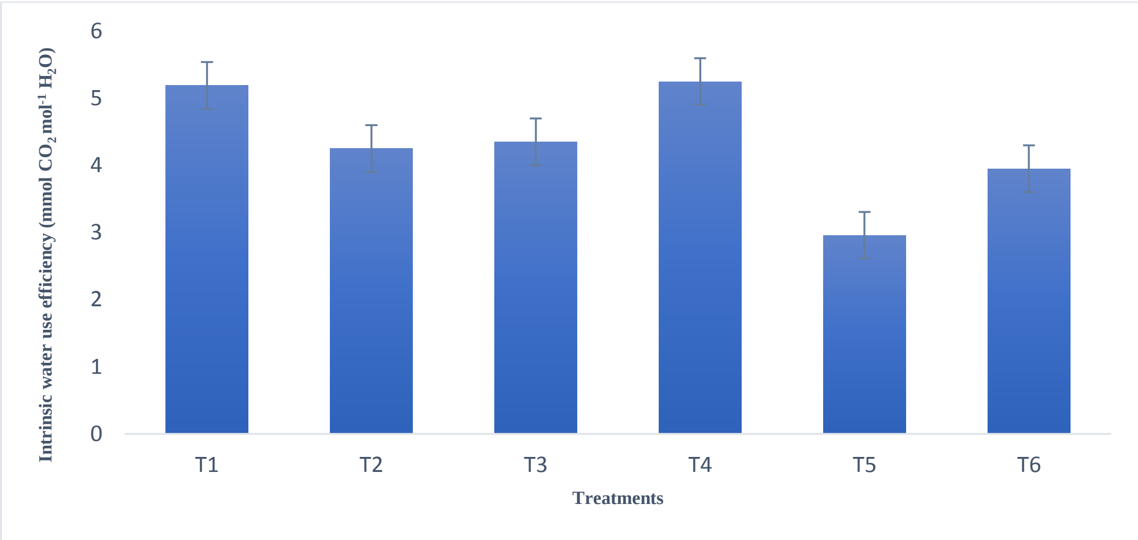
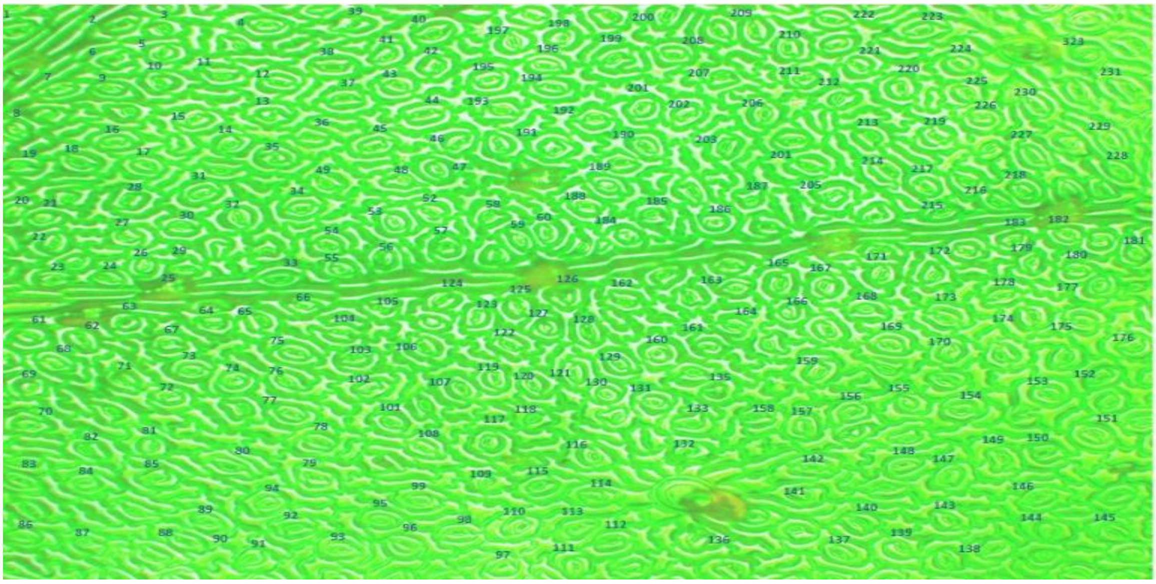


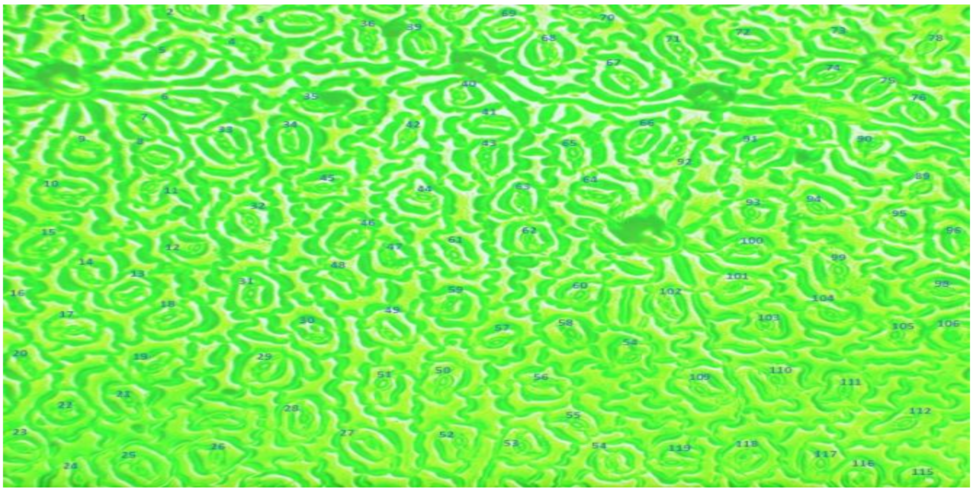
Fig. 3. Variation in intrinsic water use efficiency (WUE_i) of green gram exposed to various abiotic stress

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 CO_2 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 CO_2 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 CO_2 with lower stomatal frequency and the larger stomatal area maintained a higher rate of photosynthesis. This decrease in stomatal frequency under e (CO_2) have been reported in *soybean*, when plants were grown under elevated CO_2, with a consequent decrease of gas conductance [19]. The experiment revealed that, photosynthetic rate increases with increased stomatal area.



T5 – a (CO_2) + combined heat and drought stress



T4 – elevated CO_2 alone

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

The stomatal area, photosynthetic rate and stomatal conductance were positively correlated to WUE_i 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 CO_2. Contradictory to the above findings, there was a minimum reduction in stomatal frequency with an increase in stomatal area under e (CO_2) 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 (CO_2) gets drastically reduced when compared to all other treatments (Fig. 4). When compared to combined stress at a (CO_2) conditions the stomatal area under combined stress at e (CO_2) gets slightly increased. This may be due to the effect of e (CO_2) on the stomatal area at combined stress conditions.

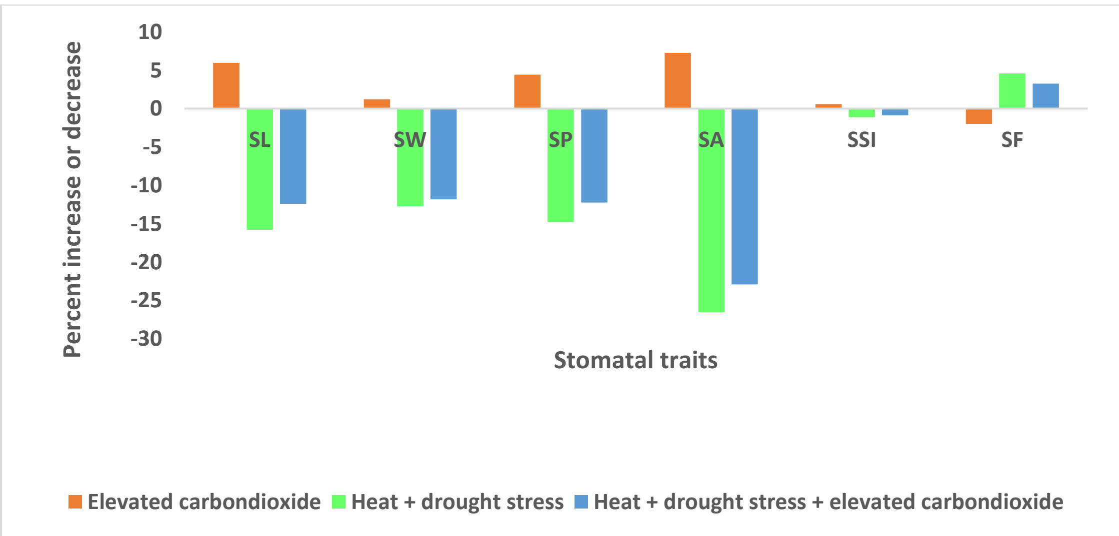
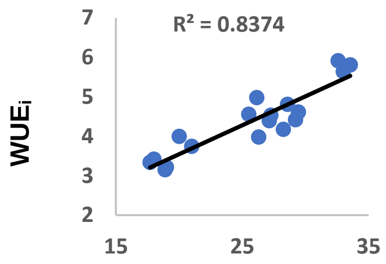
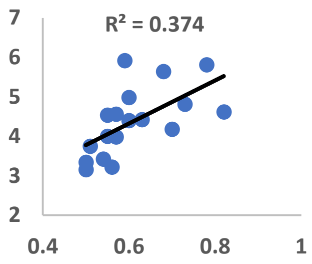
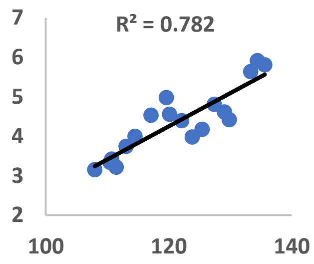
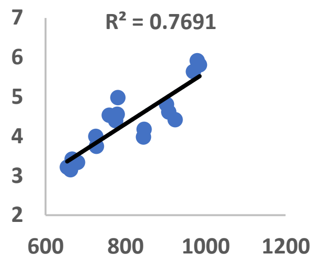
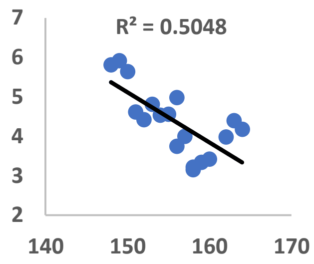


Fig. 4. Percentage increment of stomatal length (SL), stomatal width (SW), stomatal perimeter (SP), stomatal area (SA), stomatal shape index (SSI) and stomatal frequency (SF) in green gram (var Co 8) grown under elevated CO2 compared over interactive effects of heat and drought stress

Table 1. Correlating water use efficiency (WUE_i) with stomatal traits and gas exchange parameters

Photosynthetic rate	Gas conductance	Stomatal perimeter	Stomatal area	Stomatal frequency
				

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.

REFERENCES

1. Haworth M, Killi D, Materassi A, Raschi A and Centritto M. Impaired Stomatal Control Is Associated with Reduced Photosynthetic Physiology in Crop Species Grown at Elevated [CO_2]. Frontiers in Plant Science. 2016;7.
2. Xu Z and Zhou G. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. Journal of Experimental Botany. 2008;59(12):3317–3325.
3. Killi D, Bussotti F, Raschi A and Haworth M. Adaptation to high temperature mitigates the impact of water deficit during combined heat and drought stress in C3 sunflower and C4 maize varieties with contrasting drought tolerance. Physiologia Plantarum. 2016;159(2).
4. Haworth M, Killi D, Materassi A andRaschi A. Co-ordination of stomatal physiological behavior and morphology with carbon dioxide determines stomatal control. American Journal of Botany. 2015;102:677–688.
5. Gao Ji, Hao X, Seneweera S, Li P, Zong Y, Dong Q and Hao X. Leaf photosynthesis and yield components of mung bean under fully open-air elevated [CO_2]. Journal of Integrative Agriculture. 2015;14(5):977–983.
6. Basu PS, Pratap A, Gupta S, Sharma K, Tomar R and Singh NP. Physiological Traits for Shortening Crop Duration and Improving Productivity of Greengram (*Vigna radiata* L. *Wilczek*) Under High Temperature. Frontiers in Plant Science. 2019;10.
7. Jiang YL, Yao YG, Zhang QG, Yue W, Chen TF and Fan LL. Changes of photosynthetic pigment contents in soybean under elevated atmospheric CO_2 concentrations. Crop Research. 2006;2:144–146.
8. Pazzagli PT, Weiner J and Liu F. Effects of CO_2 elevation and irrigation regimes on leaf gas exchange, plant water relations, and water use efficiency of two tomato cultivars. Agricultural Water Management. 2016;169:26–33.
9. Durgadevi R and Vijayalakshmi D. Mulberry with increased stomatal frequency regulates gas exchange traits for improved drought tolerance. Plant Physiology Reports. 2020;25(1):24-32.
10. Baruah KK, Gogoi B, Borah L, Gogoi M and Boruah R. Plant morphophysiological and anatomical factors associated with nitrous oxide flux from wheat (*Triticum aestivum*). Journal of Plant Research. 2012; 125(4), 507–516.
11. Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research. 2nd edn. New York: John Wiley and Sons; 2010.
12. Ainsworth EA and Rogers A. The response of photosynthesis and stomatal conductance to rising [CO_2]. mechanisms and environmental interactions. Plant, Cell and Environment. 2007;30(3):258–270.
13. Zinta G, AbdElgawad H, Peshev D, Weedon JT, Van den Ende W and Nijs I. Dynamics of metabolic responses to periods of combined heat and drought in *Arabidopsis thaliana* under ambient and elevated atmospheric CO_2. Journal of Experimental Botany. 2018;20;69:2159–70.
14. Fitzgerald GJ, Tausz M, Leary G, Mollah, MR, Tausz-Posch S and Seneweera S. Elevated atmospheric [CO_2] can dramatically increase wheat yields in semi-arid environments and buffer against heat waves. Global Change Biology. 2016;22: 2269–84.

15. Roy J, Picon-Cochard C, Augusti A, Benot ML, Thiery L and Darsonville O. Elevated CO_2 maintains grassland net carbon uptake under a future heat and drought extreme. Proceedings of the National Academy of Sciences of the United States of America. 2016;113:6224–9.
16. Xu Z and Zhou GS. Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism and lipid peroxidation of a perennial grass *Leymus chinensis*. Planta. 2006; 224:1080–1090.
17. Hussain HA, Men S and Hussain S. Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. Scientific Reports. 2019;9:3890.
18. Lawson T and Blatt MR. Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. Plant Physiology. 2014;164: 1556–1570.
19. Teng N, Wang J, Chen T, Wu X, Wang Y and Lin J. Elevated CO_2 induces physiological, biochemical and structural changes in leaves of *Arabidopsis thaliana*. New Phytologist. 2006;172:92–103.

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