***Pak. J. Bot.*, 56(2): 419-426, 2024.** **DOI: http://dx.doi.org/10.30848/PJB2024-2(31)**

# SEED PRIMING OF PEPPER (*CAPSICUM ANNUUM* L.) WITH Β-AMINOBUTYRIC ACID (BABA) ALLEVIATES DROUGHT STRESS

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## Abstract

The problem of drought in many major crops such as *Capsicum annuum* are onerous and numerous but not insurmountable. β- aminobutyric acid (BABA) seed priming was used in the present study to investigate its effectiveness as a panacea in relieving drought stress in *C. annuum*. The drought regimes included the withdrawal of irrigation for 0, 3, 5 and 7 days. *C. annuum* seeds were primed with 1.0 mM BABA. These four drought conditions were paired in relation to the BABA primed and un-primed seeds to make 8 treatments and each treatments had 3 replicates. Measurements to assess manifestations of drought with or without BABA were taken morphologically, physiologically and biochemically. The outcome of the findings revealed that the progressive drought stress was telling adversely on the performance of the plant. BABA, in no small measure, was able to sustain the stable growth of the plant accordingly with the severity of drought. Highlights of its efficacies include improving the plant height, leaf area, stomatal conductance, and transpiration, scavenging ROS production through boosting of antioxidants, preventing lipid peroxidation, maintaining chlorophyll content and promoting proline accumulation. The use of BABA at such concentrations as 1.0 mM is thus prescribed in battling water deficit challenges in *C. annuum*.

**Key words:** β-aminobutyric acid (BABA), Seed priming, Drought stress, *Capsicum annuum*.

## Introduction

*C. annuum*, commonly known as pepper, holds an important position in global agriculture, ranking as the second most significant vegetable crop worldwide, following only tomato (Anon., 2021). This dicotyledonous flowering plant, valued not only for its culinary applications but also for its medicinal, ornamental, and environmental significance, occupies approximately 4.5 million hectares of cultivated land across the globe (Rajametov *et al.*, 2021).

Despite its widespread cultivation, *C. annuum* faces a formidable adversary in the form of drought stress, a critical factor challenging the production of numerous major crops globally, including pepper. Drought stress poses a substantial obstacle to meeting the demands of an ever-expanding world population, with yield losses in pepper, according to Poobalan *et al.*, (2020), potentially reaching as high as 85-100%. This adversity manifests through a cascade of detrimental effects on pepper plants, including reductions in stem and leaf dry mass, decreased leaf relative water content, stomatal closure, diminished stomatal conductance, decreased chlorophyll and carotenoid levels, lowered transpiration, and compromised net CO2 assimilation. Ultimately, these impacts threaten yield and related productivity parameters (Khazaei *et al.*, 2020; Iqbal *et al.*, 2023).

Traditional approaches to mitigate drought stress in crops encompass a range of agronomic practices, such as irrigation, crop rotation, and fertilization, along with dedicated plant breeding programs (Ghalkhani *et al.*, 2023). However, these methods exhibit varying levels of success and often fall short in providing the resilience necessary to counteract the evolving challenges posed by climate change. In response, seed priming has emerged as a promising avenue to enhance drought tolerance in crops (Paul *et al.*, 2022). Among priming agents, BABA has gained attention for its potential to bolster plants' ability to withstand drought stress (Shehu *et al.*, 2019).

While previous research has explored the effects of seed priming on *C. annuum* under stressed conditions, there remains a conspicuous gap in knowledge concerning the specific responses elicited when BABA is employed as the priming agent against drought stress. This knowledge gap presents a significant opportunity for scientific inquiry and innovation in sustainable agriculture. The present research aims to address this gap, delving into the unique impacts of BABA as a priming agent for enhancing drought tolerance in *C. annuum*.

Through a comprehensive investigation encompassing plant growth, physiological responses, and biochemical parameters, this study seeks to elucidate the potential of BABA to serve as a potent tool in the ongoing battle against drought stress in pepper cultivation. By filling this critical knowledge void, this research takes a promising step towards more resilient and sustainable agricultural practices, ultimately contributing to the global effort to secure our food supply in the face of climate uncertainty.

## Materials and Methods

**Experimental design:** The seeds of *C. annuum* L. cv. *California Wonder* were procured from a local agricultural store in the city of Jeddah, Saudi Arabia. The experiment was arranged in a complete randomized design (CRD) with three replications. A total of 24 pots were used following a factorial design of 2 x 4 x 3 where 2 represents the priming conditions (primed and non-primed seeds), 4 represents the stress conditions (by withholding irrigation as similarly employed by (Showemimo & Olarewaju, 2006) and 3 represents the number of replicates. Thus, the treatments were as follows;

T1 (Control without BABA); receiving daily watering without primed seeds. T2; watering after every 3 days without primed seeds. T3; watering after every 5 days without primed seeds. T4; watering after every 7 days without primed seeds. T5 (Control with BABA); receiving daily watering with primed seeds. T6; watering after every 3 days with primed seeds. T7; watering after every 5 days with primed seeds. T8; watering after every 7 days with primed seeds. The stress condition commenced after the three-leaf stage when the plant could cope with drought incidence as it is well established able to manufacture its food independently.

**Seed treatment:** Seeds of *C. annuum* were washed thoroughly with water, surface-sterilized with 0.01% mercuric chloride and washed again with distilled water prior to soaking in the BABA solution. Following from Jisha & Puthur (2016), and Abid *et al*., (2020), seeds were treated with 1.0 mM for 12 hours. The treated seeds were then sown in 3 litre pots containing light peat moss soil. Six seeds were sown in each pot.

**Data collection:** Morphological parameters like plant height were measured at 2, 4 and 6 weeks after the application of the drought treatment. The leaf area was determined using the formula LA=0.57×L(length) W(width) as adopted by Padrón *et al.,* (2016). At 6 weeks after the treatment, readings on physiological features such as stomatal conductance (gs) were taken using the CIRAS-3 device. Fresh leaves from each replicate of the different treatments were collected at this stage for the biochemical assays.

The superoxide anion radical ($^{\cdot}O_2^{-}$) content was determined using the method described by Ajiboye *et al*., (2016). The method described by Velikova *et al*., (2000) was used in the estimation of Hydrogen peroxide ($H_2O_2$) concentration. The total superoxide dismustase (SOD) activity was measured by the method described by Afolabi *et al*., (2015). Catalase (CAT) activity was assessed by the method described by Hadwan & Abed (2016). Nakano & Asada (1981) method was used to assay the activity of Ascorbate peroxidase (APX). Following the procedure given by Jollow *et al*., (1974), reduced glutathione (GSH) was measured. Lipid peroxidation was measured by estimating the malondialdehyde content (MDA) using the method described by Reilly & Aust (1999) The methods of Bates *et al*., (1973), Coombs *et al*., (1985) and Bradford (1976) were used to assay proline, total chlorophyll and total soluble protein respectively.

## Statistical analysis

Data obtained were subjected to a one-way Analysis of Variance (ANOVA) using statistical package (SPSS 25.0) software to determine if the treatments have any significant effect on the parameters measured. The means were separated by Duncan Multiple Range Test (DMRT) and considered statistically significant at $p \leq 0.05$.

## Results and Discussion

**Morphological parameters:** In this experiment, progressive drought could not support the progressive height of *C. annuum* (Table 1). Remarkably, after 6 weeks of drought exposure; the control (treatment 1) had a height which is about 3 cm taller than the most drought-stressed plant (treatment 4). Drought significantly lowered the height of the plant at 2, 4 and 6 weeks after setting in. The reduction in height was proportional with drought severity and as such the lowest plant height was measured in the treatments 3 and 4 plants. In the same vein, According to Khazaei *et al*., (2020) and Iqbal *et al*., (2023), drought greatly declined the height/length of *C. annuum*. In all the drought treatments, the BABA primed plants outperformed the corresponding un-primed plants (Table 1). This outcome echoes Mahmud *et al*., (2020) findings, where BABA pre-treatment facilitated the restoration of plant height of *Brassica napus* plants under stress.

Just like the plant height, the effect of drought and the countering effect of BABA in the number of leaves and leaf area of *C. annuum* were alike (Table 1). The increasing water deficit translated to corresponding reduction in the number of leaves and leaf area. Hence the treatment 4 was the worst hit; recording the lowest number of leaves and leaf area at 2, 4 and 6 weeks after drought treatment (Table 1). Goto *et al*., (2021) and Widuri *et al*., (2020) also reported a decrease in leaf area in pepper due to drought. BABA helped significantly in improving the number of leaves of the plant when compared with the un-primed plant during drought. At 6 weeks after drought, leaves were found to fall from the un-primed plants. Equally, BABA did not only counter the diminishing leaf area caused by drought but it also ensured increase in it. Treatments 6, 7 and 8 for instance had 35 $cm^2$, 34.8 $cm^2$ and 32 $cm^2$ respectively for leaf area at 2 weeks after drought and at 6 weeks after drought their leaf area got increased to 45 $cm^2$, 45.3 $cm^2$, and 41.73 $cm^2$ respectively. Like BABA in the present research, seed priming with *Bacillus thuringiensis* + carrot extract catered for the increase in the number of leaves of Pea plant despite drought stress (Arafa *et al.,* 2021). The recorded reduced number of leaves and leaf area as evident in the moderate and severe drought treatments is reputed as an approach devised for water conservation (Tribulato *et al.,* 2019).

**Physiological parameters:** Without BABA, *C. annuum* performed poorly under drought for the estimates of stomatal conductance (gs) and transpiration (*E*) (Fig. 1). The reductions in the two parameters were proportional with the intensity of the drought. It is normal to find the transpiration crash following stomatal closure (i.e. from low stomatal conductance) as the openings for water escape is reduced. The reductions in stomatal conductance and transpiration reported in the present investigation due to drought tally with the findings of (Widuri *et al*., 2020). These modifications are from the water use strategies of plants under water shortage conditions. With BABA, a significant positive change to this narrative ensued as it helped maintain a stomatal conductance not far from the unstressed plant (Fig. 1). While Karimi *et al*., (2017) reported faster stomatal closure for BABA treated plants under drought stress, Abid *et al*., (2020) observed BABA promoted stomatal conductance and transpiration during drought. Owing to the relevance of stomatal limitation in photosynthesis, it is deduced from the foregoing that the rate of photosynthesis would be lowered. In addition, stomatal conductance in *C. annuum* is a quite useful factor during the selection for developing tolerant varieties (Malika *et al*., 2019).

**Table 1. Effect of drought stress and BABA on plant height, number of leaves and leaf area of *Capsicum annuum*.**

| Treatment | Plant height (cm) | | | Number of leaves | | | Leaf area (cm$^2$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2WAT | 4WAT | 6WAT | 2WAT | 4WAT | 6WAT | 2WAT | 4WAT | 6WAT |
| T1 | 5.80±0.12$^{ab}$ | 8.69±0.15$^{a}$ | 13.55±0.26$^{b}$ | 13.00±1.15$^{ab}$ | 27.00±0.5$^{b}$ | 40.00±0.5$^{b}$ | 35.30±6.4$^{a}$ | 42.50±1.26$^{a}$ | 44.60±0.4$^{b}$ |
| T5 | 6.11±0.08$^{a}$ | 9.00±0.36$^{a}$ | 14.89±0.17$^{a}$ | 15.00±0.58$^{a}$ | 29.00±0.5$^{a}$ | 42.00±0.5$^{a}$ | 38.00±3.0$^{a}$ | 45.00±1.78$^{a}$ | 54.00±0.7$^{a}$ |
| T2 | 5.49±0.11$^{b}$ | 7.64±0.23$^{b}$ | 12.60±0.25$^{c}$ | 12.00±0.00$^{b}$ | 22.00±0.5$^{d}$ | 21.00±0.5$^{d}$ | 30.00±1.6$^{a}$ | 22.00±1.95$^{b}$ | 20.00±1.5$^{c}$ |
| T6 | 5.90±0.17$^{ab}$ | 8.75±0.09$^{a}$ | 13.50±0.29$^{b}$ | 13.00±0.58$^{ab}$ | 25.00±0.5$^{c}$ | 28.00±0.5$^{c}$ | 35.00±4.2$^{a}$ | 43.00±1.53$^{a}$ | 45.00±0.5$^{b}$ |
| T3 | 4.26±0.19$^{c}$ | 6.33±0.38$^{c}$ | 11.12±0.51$^{d}$ | 12.00±0.00$^{b}$ | 22.00±0.5$^{d}$ | 20.00±0.5$^{d}$ | 29.30±1.7$^{a}$ | 21.20±0.23$^{b}$ | 18.90±0.3$^{c}$ |
| T7 | 5.71±0.15$^{ab}$ | 7.91±0.11$^{b}$ | 12.95±0.09$^{bc}$ | 12.00±0.00$^{b}$ | 24.00±0.5$^{c}$ | 28.00±0.0$^{c}$ | 34.80±2.7$^{a}$ | 42.70±1.10$^{a}$ | 45.30±0.5$^{b}$ |
| T4 | 4.19±0.19$^{c}$ | 6.00±0.23$^{c}$ | 10.88±0.27$^{d}$ | 8.00±0.28$^{c}$ | 11.00±0.5$^{e}$ | 9.00±0.58$^{e}$ | 29.00±2.0$^{a}$ | 19.00±1.79$^{b}$ | 17.00±1.7$^{c}$ |
| T8 | 5.65±0.16$^{ab}$ | 7.79±0.17$^{b}$ | 12.78±0.12$^{bc}$ | 11.00±1.71$^{b}$ | 21.00±0.5$^{d}$ | 21.00±0.5$^{d}$ | 32.00±1.5$^{a}$ | 40.20±1.59$^{a}$ | 41.73±1.8$^{b}$ |

cm: centimetres, cm$^2$: centimetres square, 2WAT: 2 weeks after treatment, 4WAT: 4 weeks after treatment, 6WAT: 6 weeks after treatment. T1 (Control without BABA); receiving daily watering without primed seeds, T2; watering after every 3 days without primed seeds. T3; watering after every 5 days without primed seeds. T4; watering after every 7 days without primed seeds. T5 (Control with BABA); receiving daily watering with primed seeds. T6; watering after every 3 days with primed seeds. T7; watering after every 5 days with primed seeds T8; watering after every 7 days with primed seeds. Values represented with the different superscripts are significantly different at $p \leq 0.05$

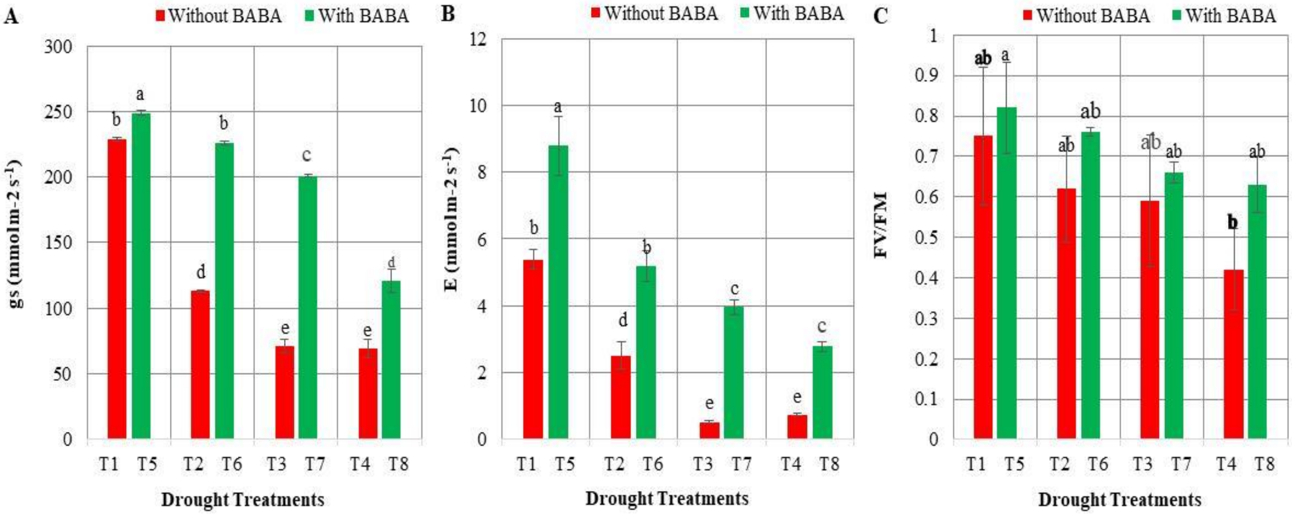

Fig 1. Effect of drought stress and BABA on (A) Stomatal conductance (gs), (B) Transpiration (*E*) and (C) Chlorophyll fluorescence (Fv/Fm) of *C. annuum*. T1-T4 (Un-primed seeds with daily, 3, 5 and 7 interval watering respectively). T5-T8 (Primed seeds with daily, 3, 5 and 7 interval watering respectively). Each bar represents the mean value and mean with different letters among the drought treatments indicate significant difference at $p \leq 0.05$.

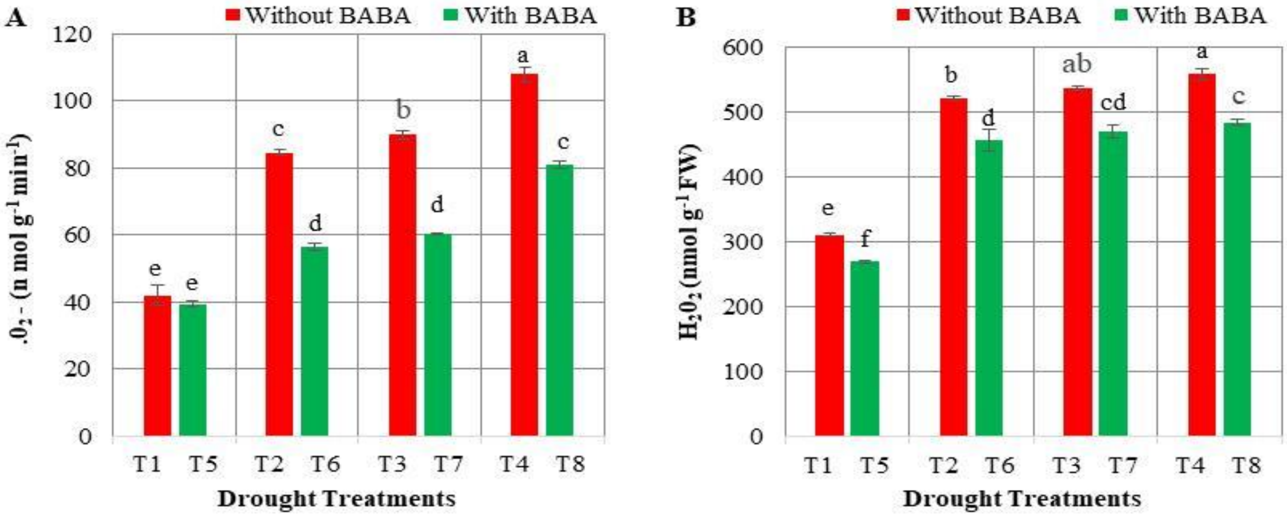

Fig. 2. Effect of drought stress and BABA on (a) Superoxide anion radical ($^{\cdot}0_2^-$) and (B) Hydrogen peroxide ($H_20_2$) of *C. annuum*. T1-T4 (Un-primed seeds with daily, 3, 5 and 7 interval watering respectively). T5-T8 (Primed seeds with daily, 3, 5 and 7 interval watering respectively). Each bar represents the mean value and mean with different letters among the drought treatments indicate significant difference at $p \leq 0.05$.

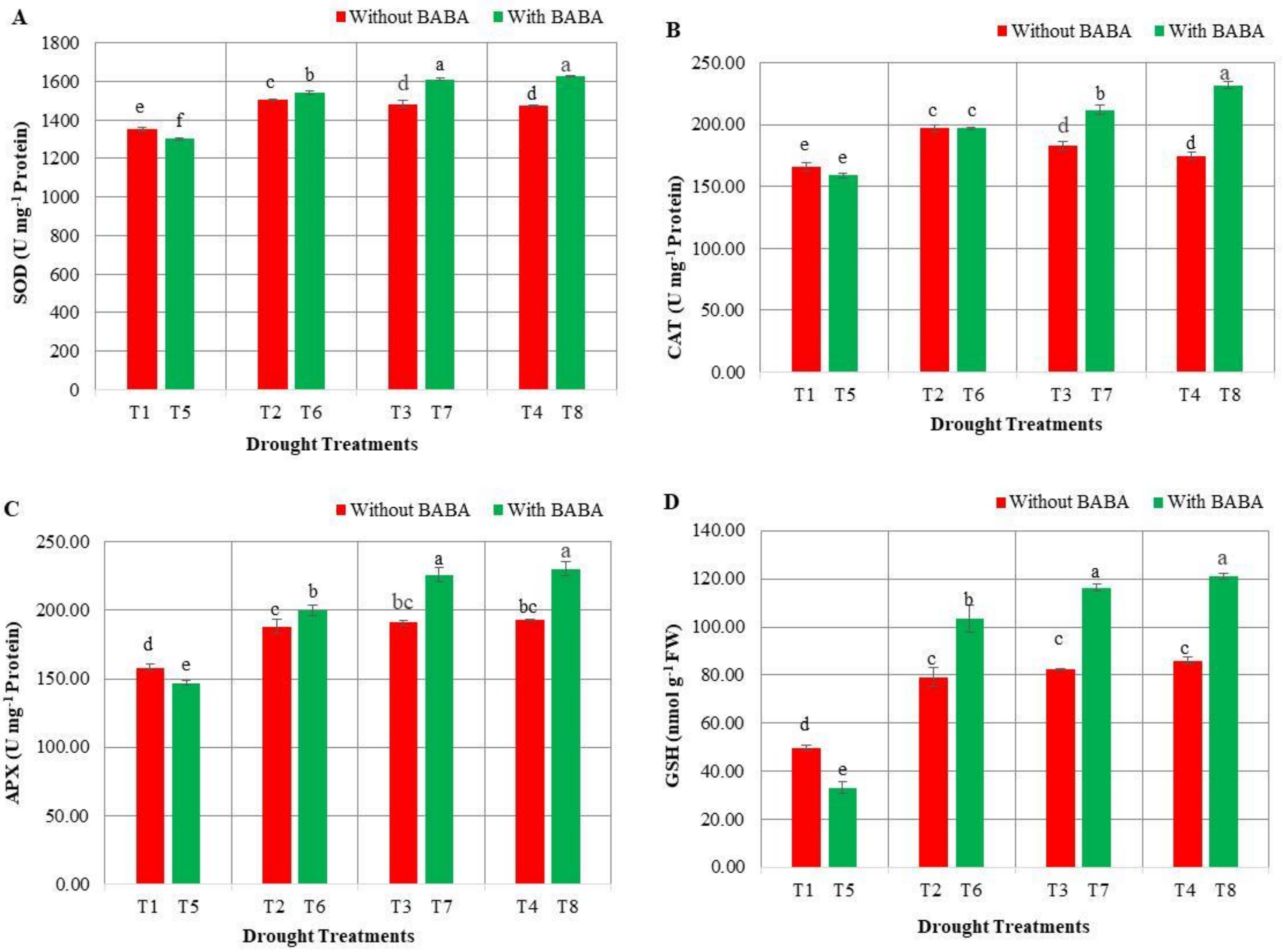

Fig. 3. Effect of drought stress and BABA on (A) Superoxide dismutase (SOD), (B) Catalase (CAT), (C) Ascorbate peroxidase (APX) and (D) Reduced glutathione (GSH) of *C. annuum*. T1-T4 (Un-primed seeds with daily, 3, 5 and 7 interval watering respectively)**.** T5-T8 (Primed seeds with daily, 3, 5 and 7 interval watering respectively). Each bar represents the mean value and mean with different letters among the drought treatments indicate significant difference at $p \leq 0.05$.

The chlorophyll fluorescence, $F_V/F_M$, unlike the two previous parameters, only differed greatly when the drought was highest. And it is only in such condition, in addition to the control, that BABA could be said to have caused a monumental change (Fig. 1). This confirms that the existing stress state mostly affected the photosystem II (PSII) in a dark state especially when it was higher as expressed by Agrawal *et al*., (2021). Pepper plants exposed to UV-B radiation by Rodríguez-Calzada *et al*., (2019) similarly did not show major effects in the leaf photosynthetic performance. With the corroborating evidences, the severity of stress is thus important before changes in $F_V/F_M$ can be statistically significant. The observed changes in the physiological parameters like the fall in transpiration during drought is not unexpected since drought had altered the leaf morphology. That is, decrease in leaf area translates to reduction in openings through which transpiration takes place.

## Biochemical parameters

**Reactive oxygen species (ROS):** With drought stress**,** many literatures report the over-production of $H_2O_2$ and other ROS in various plants including *C. annuum* (Khazaei & Estaji, 2020). The current inquiry did not shy away from that. Both $\cdot O_2^-$ and $H_2O_2$ were boosted in no small measure with the shortage of water (Fig. 2). The most values for both oxides were also recorded in the severe drought treatment. BABA treated plants recorded lower $O_2^-$ and $H_2O_2$ (Fig. 2) and therefore would do better in avoiding the challenges posed by ROS over-production. Such was the case with drought-stressed faba bean plant (Abid *et al.*, 2020) and salt-stressed *Brassica napus* (Mahmud *et al.*, 2020) following BABA treatment but was not the case with Cowpea plant where there was more ROS accumulation in the embryonic tissues following priming (Boucelha *et al.*, 2019).

**Antioxidant enzymes:** In the plants not pre-treated with BABA, there was an initial rise in SOD, CAT and APX production as drought sets in (i.e. from the treatments 2). However, unlike CAT and APX there was sharp fall in the value of SOD as drought intensifies as seen in treatments 3 and 4 (Fig. 3). SOD is considered as the first-line of defence against ROS which catalyses the partition of $O_2^-$ by transforming it to $H_2O_2$. The resulting $H_2O_2$ after further scavenging by peroxidases (like APX) and catalases then yields $H_2O$ and $O_2$ (Wang *et al.*, 2019; Olayinka *et al.*, 2021). These scenarios are deemed to have taken place as there was increase in the SOD, CAT and APX concentrations due to drought (Fig. 3). In addition, several authors have also mentioned the upward regulation in SOD (Beyaz, 2022), CAT (Wen *et al.*, 2022) and APX (Khazaei & Estaji, 2020) as a result of water deficit.

The effectiveness of BABA in this regard comes from the fact that it helped to improve the activity of these antioxidants especially where the plants devoid of BABA can no longer produce more of them to salvage their growth. For instance, despite the elevation in the production of SOD and APX in maize, it was still insufficient to counter the oxidative disorder caused by drought in the plant (Shaw *et al.*, 2016). It is Interesting to note that without drought, the levels of these antioxidants were relatively lower in BABA primed plants (Fig. 3). BABA is thus considered sensitive to the prevailing environmental conditions.

**Antioxidant compounds (GSH):** When the GSH content in the BABA primed plants under drought (treatments 6, 7 and 8) are compared with the un-primed plants (treatments 2, 3, and 4), it was very apparent that the BABA primed plants had superior amounts of GSH. Also, as the drought stress increases in the BABA plants, the GSH content was also increasing (Fig. 3). In the drought treatments of the un-primed plants (treatments 2, 3 and 4), the GSH contents could be described as stagnant because the difference between the treatments 2, 3 and 4 is negligible (Fig. 3). The seemingly non-improvement in value is quite peculiar to GSH contents different from the antioxidant enzymes. Thus, the role of BABA in enhancing the GSH content was more glaring than the antioxidants enzymes. The active role of GSH in BABA induced ROS scavenging can therefore be deduced. Roach *et al.*, (2023) described the upregulation in the activity of glutathione reductase (GR) as the major player for enhanced GSH content under stress.

**Lipid Peroxidation (MDA content):** MDA received a very much boost following drought prevalence in the un-primed plants (treatments 2, 3 and 4). MDA had its overall highest value recorded with the plants mostly deprived of water (i.e. treatment 4) (Fig. 4). Malondialdehyde (MDA) is a notable by product from lipid peroxidation (Senthilkumar *et al.*, 2021). Consequentially, from the recorded oxidative stress, lipid stability or membrane integrity is expected to be jeopardized and MDA is expected to be on the high (Fig. 4). The question of why there was significant increase in MDA following drought is thus answered sufficiently. In similar vein, the MDA level well managed in BABA plants since the oxidative stress in the BABA plants was lower as hence MDA content was relatively very low in all the drought treatments (treatments 6, 7 and 8) demonstrating little or no stress (Fig. 4). The reduction of MDA content associated with seed priming has also been documented in other plants like flax (Aghaee & Rahmani, 2019).

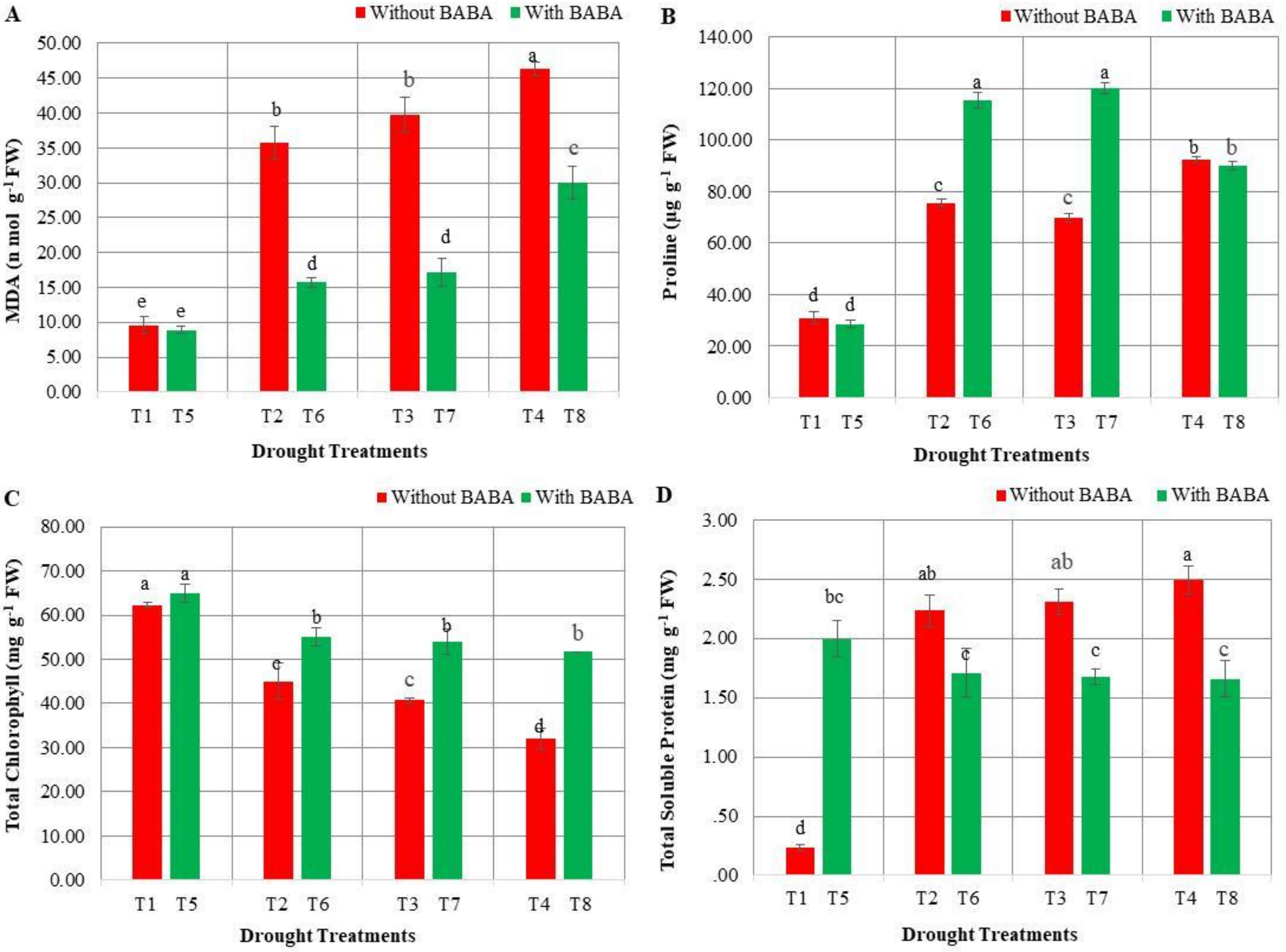

Fig. 4. Effect of drought stress and BABA on (A) Lipid peroxidation (MDA content), (B) Proline, (C) Total chlorophyll and (D) Total soluble protein of *C. annuum*. T1-T4 (Un-primed seeds with daily, 3, 5 and 7 interval watering respectively)**.** T5-T8 (Primed seeds with daily, 3, 5 and 7 interval watering respectively). Each bar represents the mean value and mean with different letters among the drought treatments indicate significant difference at p≤0.05.

**Proline concentration:** From the manifestations of stress in un-primed *C. annuum* is the gradual upsurge in the accumulation of proline which was noticed as drought intensifies. The cumulation of osmolytes like proline aids in preserving tissue water and in maintaining cell integrity against osmotic and oxidative stresses. The accumulation was found positive in *C. annuum* (Fig. 4) just like it was shown in similar experiments (Shafi *et al.*, 2019; Iftikhar *et al.*, 2022). Like Shaw *et al.*, (2016), despite this high accumulation of proline, some of the effects of stress was still felt in the *C. annuum* plant. However, unlike Shaw *et al.*, (2016), BABA was able to promote the accumulation of proline up to moderate drought in this experiment (Fig. 4). This shows that BABA is helpful in promoting proline accumulation needed to maintain cell integrity during stress conditions. In agreement with the current finding is the result of (Abid *et al.*, 2020).

**Total chlorophyll:** As water became more unreadily available, the chlorophyll level significantly declined in the un-primed plants (Fig. 4). ROS usually affect total chlorophyll negatively (Kim, 2020) and therefore the decline in total chlorophyll of leaves in *C. annuum* during drought stress in the present experiment could be from the notable manifestations of ROS disruptions. The fall in chlorophyll is from the non-stomatal limitations to photosynthesis. In fact, Helm *et al.*, (2020) regards it as a significant inactivator of photosynthesis during drought. Furthermore, as mentioned by Li *et al.*, (2019), the severity of drought was quite prominent in the declination as the treatment 4 was reckoned with the lowest total chlorophyll level in all of the treatments. The prevention of cell damage caused by the stress-induced upregulation of chlorophyllase is a possible reason for such (Lee *et al.*, 2022). BABA assisted in preventing much decline in chlorophyll level. As the drought thickens, the BABA plants were still able to maintain stable levels of chlorophyll and they were higher than the un-primed rivals (Fig. 4). Such improvement could be accredited to the ability of BABA to influence the biosynthesis of chlorophyll positively (Quan *et al.*, 2022).

**Total soluble protein (TSP):** Synthesis of new proteins in response to drought is a common feature of water stress (Guo *et al.*, 2023). Different from the BABA treatments, relatively high TSP was recorded with drought in the leaves of *C. annuum* (Fig. 4). Guo *et al.*, (2023) observed similarly in drought-stressed maize. Contrastingly, drought reduced ultimately the concentration of TSP in soybean (Iqbal *et al.*, 2019).

Quite consistent with BABA from the current result, seed priming application did not positively impact the TSP in drought-stressed *Vicia faba* plant (Kasim *et al.*, 2019). These findings raise the possibility that the elevated TSP levels in un-primed plants under drought conditions may be temporary, preceding an eventual decline. Alternatively, it could be suggested that the enhanced stability of BABA-treated plants against drought might obviate the need for excessive production of soluble proteins as a response to stress, such as dehydrins (Sharma & Dubey, 2016). Consequently, this could account for the lower levels of total soluble protein in BABA-treated plants compared to their un-primed counterparts, which exhibited higher TSP concentrations.

## Conclusion

Following from the results of the investigations made on the morphological, physiological and biochemical parameters, drought was definitely a clog in the wheel of stable growth of *C. annuum* especially as it increased in its severity. BABA, at 1.0mM used as seed priming agent proved generally remarkable in ameliorating the imbalanced consequences occasioned by drought. Therefore, the adoption of BABA in countering the water-deficit problems of *C. annuum* especially during the early stage of growth is highly encouraged. Studies on the transgenerational efficacy of BABA would be worthwhile in the future.

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(Received for publication 10 October 2022)