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Influence of Exogenous Ascorbic Acid Levels on Growth and Physiological Responses of Wheat (*Triticum aestivum*) Exposed to Drought Stress

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ABSTRACT

Drought stress is a major environmental constraint that significantly threatens wheat (Triticum aestivum L.) production, particularly in regions like Pakistan, where wheat yields are relatively low. Water scarcity adversely affects plant growth and physiology, leading to decreased productivity. To mitigate these effects, various strategies have been explored, including the application of exogenous plant growth stimulants such as ascorbic acid (AsA). AsA is known to enhance plant tolerance against abiotic stresses by improving biochemical and physiological attributes. Despite its potential benefits, limited research has been conducted on the role of AsA in enhancing drought tolerance in wheat. There is a need for further investigation to determine the most effective concentration of AsA for improving wheat growth under water-limited conditions. In this study, a completely randomized design (CRD) experiment with three replications was conducted to assess the impact of AsA on wheat under drought stress (50% field capacity). Wheat plants were sprayed with three different concentrations of AsA (200 µM, 400 µM, and 600 µM), and their physiological and growth responses were analyzed using various methodologies, including spectrometry, the digestion method, flame photometry, microscopy, moisture content measurement, and field capacity assessment. The data were statistically evaluated using the Statistix-8.1 software. Results demonstrated that drought stress significantly reduced growth parameters and ion concentrations while increasing hydrogen peroxide levels compared to the control. However, AsA application improved plant resilience, enhancing growth metrics, soluble sugar, flavonoids, anthocyanin, ascorbic acid content, chlorophyll, and ion concentrations under drought conditions. The 600 µM AsA concentration had the most pronounced positive effects on all tested variables. These findings suggest that AsA can serve as a potential growth promoter, alleviating drought-induced damage and optimizing the morpho-physiological and biochemical attributes of wheat.

INTRODUCTION

Wheat a significant grain crop belongs to Poaceae family, a vital constituent of calories and protein, and the main source with almost 30% of worldwide grain productivity and 50% of global trade (Khalid *et al.*, 2023). It is predicted that human wheat utilization will grow up to 60% in 2050. FAO calculated that the world would need approximately 198 million tons of wheat grain by 2050 to meet future demand, which would necessitate a 77% increase in wheat production in developing nations. Wheat takes up more area than any other crop on a global scale (Farooq *et al.*, 2023).

Wheat is a widely consumed grain cereal since more than half of the world's population depends on it as a main meal. (Mahmood *et al.*, 2020). Wheat accounts for over half of the calorie consumption in North Africa, West, and Central Asia. To fulfil future demands, the estimated yearly rise in worldwide wheat production must increase to at least 1.6% out of its present value of less than 1% (Eskola *et al.*, 2020).

One of the main risks to wheat production is drought. Drought stress is thought to influence plant intake, transport, and accumulation of important inorganic nutrients (Hossain et al., 2021). The primary obstacles to increasing productivity are abiotic and biotic stresses. Drought is a major abiotic stress that has an impact on the environment to a large extent, wheat production (Kopecká *et al.*, 2023).

Drought is often regarded as a water shortage sufficient to affect the plant growth significantly. A sufficient amount of moisture is required for normal growth. One of the most important abiotic stresses, it affects approximately 32% of 99 million hectares of wheat output in low-income countries and at least 60% of wheat production in high-income countries (Mishra *et al.*, 2023). A lack of water could reduce wheat production by 17 to 70% (Chowdhury *et al.*, 2021). Because of the bad effect on the number of inflorescences and the double ridge to flower initiation stage is the most susceptible growth period in terms of wheat production to water deficiency. Water scarcity reduces grain production by interfering at anthesis and grain filling stages (Daryanto *et al.*, 2016).

Ascorbic acid (AsA) is widely considered as one of the most potent antioxidants and growth regulators in combating various types of stress (Wu *et al.*, 2024). In plants, it promotes cell division; its growth, differentiation, metabolism, and resistance against various oxidative stress (Nandy *et al.*, 2023). In addition to acting as an antioxidant, AsA levels in the body are linked to the activation of complicated biological defense mechanisms (Demirci-Çekiç *et al.*, 2022). It assists plants in managing and overcoming the harmful effects of salt stress as well as drought (Horchani *et al.*, 2024). It has hypothesized functions in plant metabolism as a whole (Castro *et al.*, 2023). Though AsA is an effective natural antioxidant, its concentration in plants is insufficient. As a result, AsA exogenous methods is now common practice in many crops, including wheat to alleviate oxidative stress and promote plant growth (Farooq et al., 2020).

Drought can cause oxidative damage in plants due to ROS production, which damage the wheat productivity (Seleiman *et al.*, 2021). Thus to overcome this damage plants use several antioxidant enzymes such as catalase, SOD and certain antioxidants such as AsA (Hasanuzzaman *et al.*, 2020). Therefore, in plants exogenously applied AsA spray greatly enhances the plant's capacity to withstand various stresses, including drought, ozone (O_3) stress, and salinity (El Sabagh *et al.*, 2021).

The plant's response to drought conditions is determined by the genotype and species of the plant, the degree and intensity of the drought and the plant's growth and developmental phase (Kapoor *et al.*, 2020). Plants' tolerance to abiotic factors has been investigated using metabolomics studies, which may reveal the unique responses of natural systems to genetic and environmental modifications (Reza. A. 2022). One of the main objectives of Pakistan's national wheat breeding programs is, in fact, to develop wheat genotypes that are resistant to drought. Even while wheat breeders have increased yield on a national level, they have not made much progress in the agro-ecological region, where environmental conditions are variable and many problems, including heat, salinity, and drought, are prevalent.

My objective was to examine how wheat responds to drought stress when treated with ascorbic acid (AsA) applied as a foliar spray. It sought to understand the mechanisms of drought tolerance at different AsA levels by comparing control and stressed plants. The research assessed how wheat plants react to varying drought conditions, examining physiological and biochemical changes that contribute to their survival. Additionally, the study evaluated the duration for which the plants can endure drought stress and how AsA influences their resilience, ultimately providing insights into improving wheat's drought tolerance.

MATERIALS AND METHODS

Soil Quality Analysis

Moisture Contents

A 100g soil sample was oven-dried overnight, cooled, weighed, and its moisture content calculated using a specific equation.

$$\%\ moisture = \frac{Wet\ Soil\ (g) - Dry\ soil\ (g)}{Dry\ Soil\ (g)} \times 100$$

Saturation %

A 100g soil sample was mixed with water to form a paste, allowed to stand for 60 minutes, and saturation% was calculated using Gavlak *et al.* (2003) method.

$$Saturation\ percentage = \frac{Amount\ of\ water\ added\ in\ mL \times 10}{Mass\ of\ air\ dried\ soil\ (g) \times [(100 - Pw) \div 100]}$$

Field Capacity

The following equation was used to compute the field capacity of soil:

Field Capacity = Saturation percentage/2

Growth Parameters Analysis

Shoot length and root length (cm)

Plant shoot and root lengths were measured using a measuring tape, and mean values were calculated immediately after harvesting.

Shoot and Root Fresh Weight (g)

Fresh weight of shoot and root was computed immediately after harvesting the plants by utilizing weighing balance and after that computed their mean values.

Shoot and Root Dry Weight (g)

Plant shoots and roots were placed in an oven at 65°C for 72 hours and after withdrawing the samples from the oven dry weight was assessed by a weighing machine.

Shoot Diameter (cm)
Shoot diameter of each plant was calculated by vernier caliper and after that determined their means.

Number of Leaves Per Plant
The total number of leaves on each plant was counted, in three replications was counted and then the mean ratios were determined.

No. of Roots
Total number of roots on each plant was counted and then the means values were computed.

Leaf Area (cm^{-2})
To determine leaf area, the length and width of each plant leaf were determined by utilizing a measuring tape, and the leaf area was calculated by multiplying leaf length and width.

Physiological Contents Analysis
Chlorophyll Content
Chlorophyll content was calculated with this formula

Chl. a (mg mL-1) = [12.7 (OD 663) - 2.69 (OD645)] × V/1000 × W

Chl. b (mg mL-1) = [22.9 (OD 645) - 4.68 (OD663)] × V/1000 W

Where V= Volume of the extract (mL)

W = Weight of the fresh leaf tissue (g)

Carotenoids = [(OD 480) + 0.114 (OD 663)-0.638 (OD645)]

E 100% Cm = 2500

Carotenoids (g mL^{-1}) = Acar / Em100% × 100

Anthocyanin, flavonoids, ascorbic acids, hydrogen peroxides, soluble sugars and different ions like sodium, potassium and calcium were calculated by following their respective procedures.

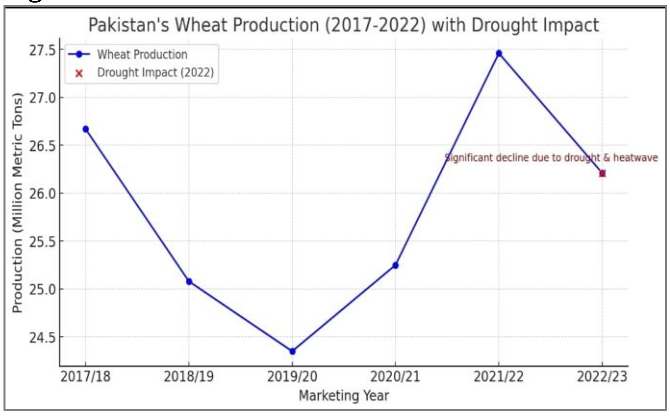
RESULTS AND DISCUSSION
Pakistan wheat has gone through its ups and downs during past years. Here are the figures illustrating the Pakistan wheat production from 2017 to 2022, highlighting the years affected by drought (table 1).

Table 1

Year	Production (Million metric tons)
2017/2018	26.67
2018/2019	25.08
2019/2020	24.35
2020/2021	25.25
2021/2022	27.46

Here is another graphical representation of wheat effected by drought during previous years in Pakistan (fig.3.2):

Figure 3.2



Morphological Analysis
Drought stress significantly hampers wheat growth, leading to reduced biomass, stunted root and shoot development, and overall diminished plant vigor. However, the application of ascorbic acid (AsA) has been shown to counteract these adverse effects, promoting resilience and growth in drought-stressed wheat as shown in (fig.3.3).

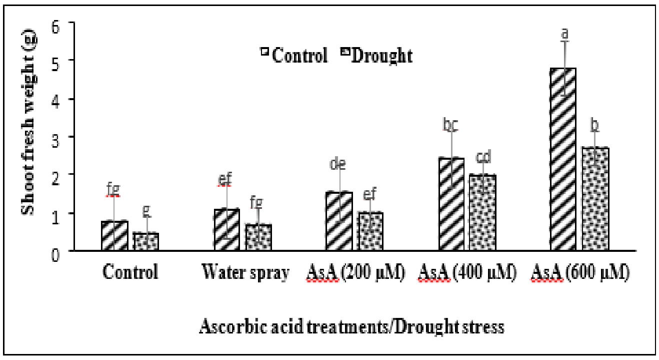
Impact on Biomass and Growth Parameters
Drought stress leads to a notable decline in shoot and root fresh and dry weights, impairing plant development (fig.3.3). In contrast, wheat treated with ascorbic acid exhibited substantial improvements in these metrics. The application of AsA enhanced water uptake and osmotic adjustment, leading to increased biomass accumulation. Treated plants demonstrated longer shoots and deeper root systems, which facilitated improved water absorption and overall structural integrity. Notably, the optimal enhancement was observed at a 600 µM AsA dosage, aligning with previous findings by Khadr *et al.*, (2021).

Effect on Vegetative Traits
The impact of drought stress extends beyond biomass reduction, affecting key vegetative traits such as leaf count, root proliferation, shoot diameter, and leaf area. Drought-stricken wheat plants showed a considerable decline in these parameters, whereas ascorbic acid treatment led to significant improvements. AsA-treated plants exhibited an increased number of leaves, suggesting enhanced photosynthetic capacity and greater vigor. Root development was also more pronounced, contributing to better water and nutrient uptake. Additionally, shoot diameter increased, reinforcing structural support and facilitating efficient nutrient transport. Leaf area expansion in treated plants further underscored the protective role of ascorbic acid, improving overall growth and drought tolerance. These results align with previous studies, such as those reported by Ahmad et al. (2021), which confirm AsA's effectiveness in mitigating drought stress. The comparative analysis highlights ascorbic acid as a powerful protective agent against drought-induced stress

in wheat. While drought stress severely impairs plant growth, AsA application—particularly at 600 µM—significantly enhances shoot and root biomass, vegetative traits, and overall plant health (fig.3.3). These findings underscore the potential of ascorbic acid as a practical solution to improve wheat resilience in drought-prone environments, offering a promising strategy for sustainable agricultural practices.

Figure 3.3.
Effect of ascorbic acid on (A) shoot fresh weight (g), (B) root fresh weight (g), (C) shoot fresh weight (g) and (D) root dry weight (g) of wheat plant under drought stress. Error bars above indicate the ± of three replicates. Means the same letter for a parameter do not differ significantly at $p \leq 0.05$. AsA= ascorbic acid; µM =Micro-molar; control (x-axis) = No exogenous AsA application; water spray= normal water application; (200/400/600µM) = levels of ascorbic acid application; Bras with lines=no drought only AsA application; Bars with dots= drought with AsA application.

A



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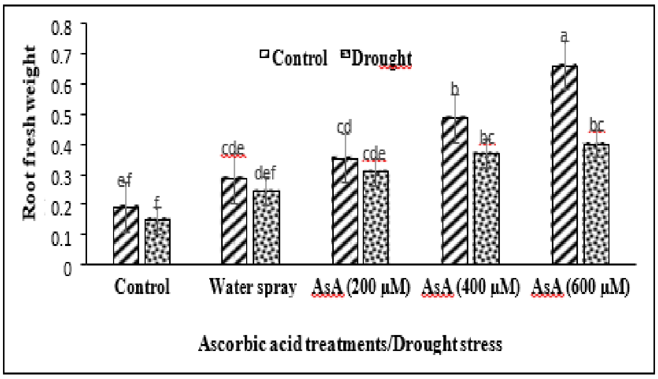

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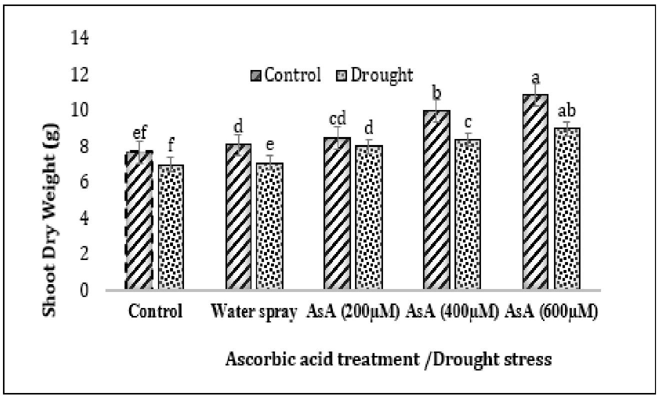

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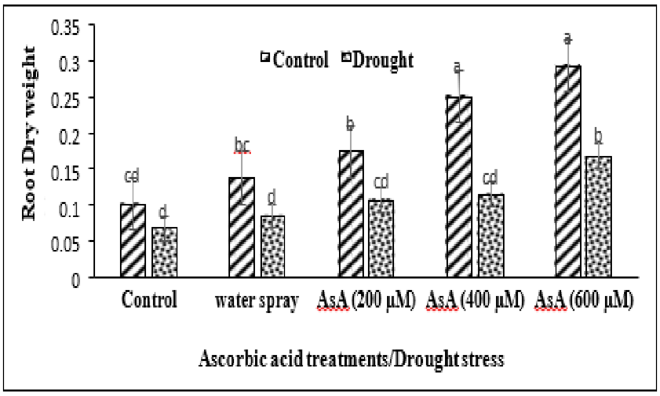

Impact of Drought on Shoot and Root Ramification
The research proved that wheat exhibits variation in root length and diameter under drought, with same genotypes developing longer, thinner roots (reduced diameter) to enhance water uptake, same results were discussed by Awad *et al.* (2018) in Great Plains winter wheat. The result showed that increasing the root-to-shoot ratio improves root water influxes, suggesting that drought-stressed plants allocate more resources to root growth at the expense of shoot development, these same findings align with Bacher *et al.* (2022). The length of root is slightly resistant to drought than the shoot, and root showed more growth under drought stress due to the exogenous application of ascorbic acid than shoot under the same stress and mitigation. Mahpara *et al.* (2022) reported that drought stress reduces shoot length while increasing the root-to-shoot ratio. In control conditions, plants tend to have balanced shoot and root growth, whereas under drought stress, root systems often expand to access deeper water while shoot growth is stunted. This adaptation helps plants survive by improving water acquisition but can limit biomass accumulation and yield.

Drought stress reduced shoot diameter due to limited water availability as shown in (fig.3.4) as Mahpara (2022) and Awad *et al.* (2018) observed that root number varies among wheat genotypes, with some increasing root proliferation under stress. suggested that Modification of root-to-shoot ratios can enhance root water influxes, potentially increasing root number to compensate for reduced shoot growth. These finding align with Bacher *et al.* (2022)

Figure 3.4
Effect of ascorbic acid on (A) shoot length (cm), (B) root length (cm), (C) shoot diameter (mm) and (D) number of roots/plant of wheat plant under drought stress. The error bars represent the ± standard deviation of three replicates. Means sharing the same letter for a given parameter are not significantly different at $p \leq 0.05$. AsA= ascorbic acid; µM =Micro-molar; control (x-axis) = No exogenous AsA application; water spray= normal water application; (200/400/600µM) = levels of ascorbic

acid application; Bras with lines=no drought only AsA application; Bars with dots= drought with AsA application.

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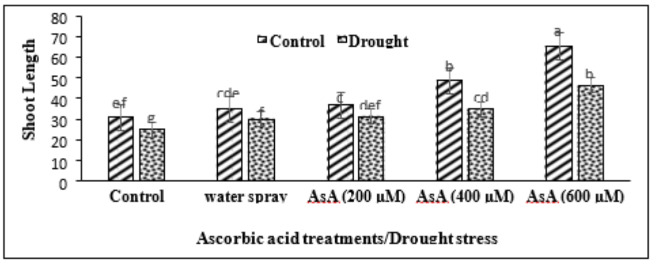

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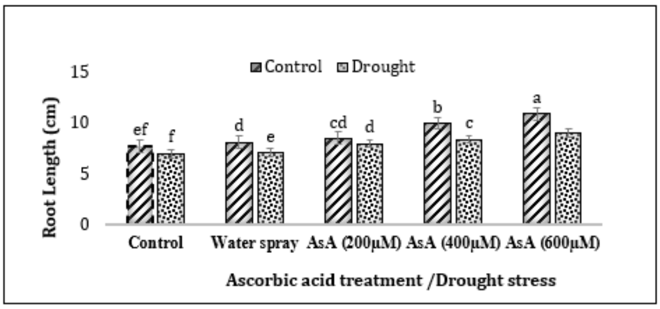

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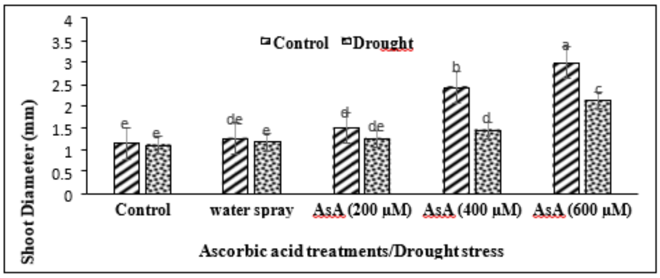

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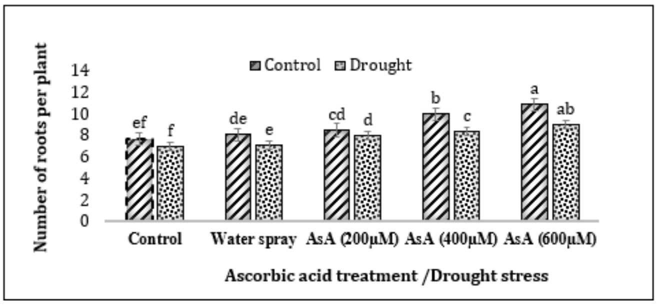

Fallout in Leaf Growth

Drought stress reduced the number of leaves and leaf area in wheat, negatively impacting growth. Control plants (unstressed) maintain normal leaf development, while drought-stressed plants show fewer leaves and reduced leaf expansion due to water limitation. Results showed that ascorbic acid application improved leaf morphology under stress. Treated plants had a higher leaf count and greater leaf area than untreated stressed plants (fig.3.5), these same finding matches with Malik *et al.* (2015) and Hafez & Gharib (2016). Ascorbic acid enhanced cell expansion, mitigated oxidative stress, and maintained turgor pressure. Overall As.a preserved leaf morphology and improved drought tolerance in wheat.

Figure 3.5

Effect of ascorbic acid on (A) no. of leaves per plant and (B) Leaf area of wheat plant under drought stress. The error bars represent the ± standard deviation of three replicates. Means sharing the same letter for a given parameter are not significantly different at $p \leq 0.05$. AsA= ascorbic acid; µM =Micro-molar; control (x-axis) = No exogenous AsA application; water spray= normal water application; (200/400/600µM) = levels of ascorbic acid application; Bras with lines=no drought only AsA application; Bars with dots= drought with AsA application.

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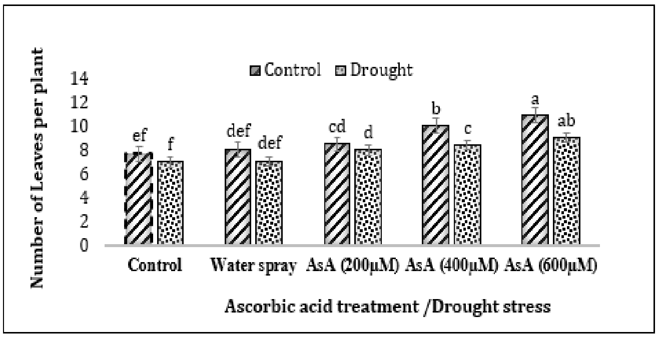

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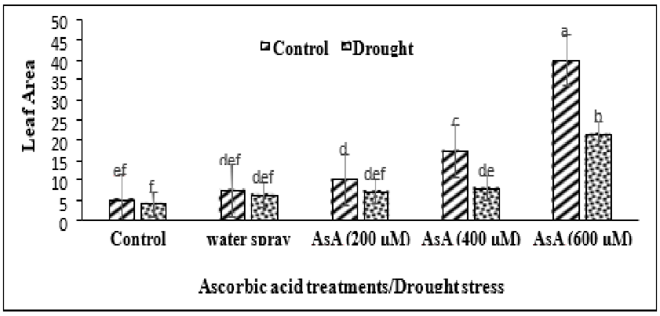

Biochemical Analysis

The application of exogenous ascorbic acid significantly improved physiological and biochemical traits in wheat (*Triticum aestivum* L.) under drought stress. Drought conditions led to a marked reduction in chlorophyll a, chlorophyll b, soluble sugars, ascorbic acid and carotenoid contents, impairing photosynthesis. However, ascorbic acid treatment enhanced these pigments, improving photosynthetic efficiency, consistent with findings by (Niroula *et al.*, 2021). Similarly, anthocyanin and flavonoid levels, which declined under drought stress, were significantly restored with ascorbic acid, reinforcing the plant's antioxidant defense system, as also reported by Alayafi (2020). Hydrogen peroxide (H_2O_2) accumulation increased in drought-stressed plants, contributing to oxidative stress, but ascorbic acid mitigated this effect by reducing ROS levels. Endogenous ascorbic acid naturally increased in response to drought, and its exogenous application further strengthened stress tolerance as shown in the graph (fig. 3.6).

Moreover, drought stress negatively impacted germination and growth by inhibiting growth enzyme activity and disrupting cell division. In contrast, ascorbic acid improved these parameters, aligning with previous studies (Hamid *et al.*, 2024; Azeem *et al.*, 2023). Leaf area reduction was evident under drought conditions, but ascorbic acid promoted leaf expansion, further supporting its protective role. Additionally, soluble sugar content, which declined under drought stress, was significantly restored with ascorbic acid, aiding in osmotic regulation and plant resilience. These findings align with Aziz *et al.* (2018), further emphasizing the role of ascorbic acid in mitigating drought-induced biochemical disruptions.

Figure 3.6

Effect of ascorbic acid on (A) shoot ascorbic acid and (B) shoot soluble sugar of wheat plant under drought stress. The error bars represent the ± standard deviation of three replicates. Means sharing the same letter for a given parameter are not significantly different at $p \leq 0.05$. AsA= ascorbic acid; µM =Micro-molar; control (x-axis) = No exogenous AsA application; water spray= normal water application; (200/400/600µM) = levels of ascorbic acid application; Bras with lines=no drought only AsA application; Bars with dots= drought with AsA application.

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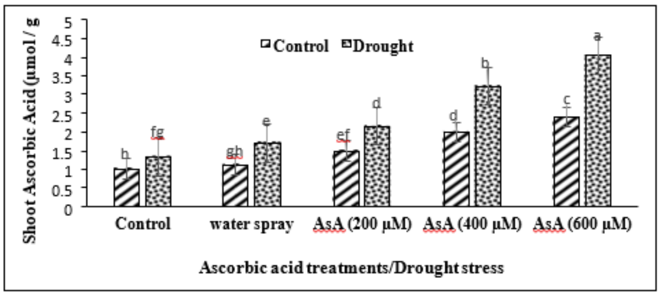

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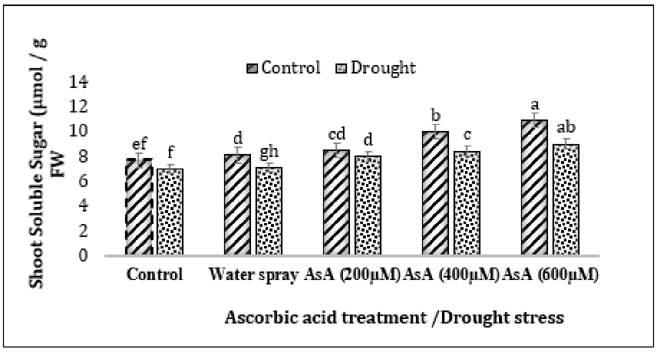

Changes in Chlorophyll and Photosynthetic Machinery

The study examined the effects of externally applied ascorbic acid on wheat subjected to water stress and revealed that it notably improved chlorophyll content and photosynthetic activity. Drought stress caused a significant reduction in chlorophyll a, chlorophyll b, and total chlorophyll levels, thereby impairing the plant's photosynthetic efficiency. Nevertheless, the application of ascorbic acid counteracted this reduction by preserving chlorophyll pigments and safeguarding the photosynthetic machinery against oxidative damage (fig.3.7). Ascorbic acid-treated plants exhibited higher chlorophyll retention, which contributed to improved photosynthetic efficiency and overall growth under drought conditions, same findings align with Hafez and Gharib (2016)

Experiment also highlighted the role of organic osmolytes, including ascorbic acid, in improving drought tolerance in plants. They emphasized that ascorbic acid functions as an antioxidant, reducing oxidative stress-induced chlorophyll degradation. This antioxidant activity protects chloroplast membranes and photosynthetic machinery, ensuring better light absorption and carbon fixation under drought stress. The study also noted that ascorbic acid helps maintain stomatal regulation, which enhances photosynthetic rates by optimizing CO_2 uptake while minimizing water loss. Water deficiency hindered the production of soluble sugars and effected the functions of accessory pigments such as carotenoids and anthocyanin (fig.3.7). The research concluded that ascorbic acid application improves chlorophyll stability, safeguards photosynthesis, and enhances wheat resilience to drought stress, same results were reported in research by Celi *et al.* (2023).

Figure 3.7

Effect of ascorbic acid on (A) Chlorophyll A (mg), (B) Chlorophyll B (mg), (C) Carotenoids (mg) and (D) shoot Anthocyanin of wheat plant under drought stress. The error bars represent the ± standard deviation of three replicates. Means sharing the same letter for a given parameter are not significantly different at $p \leq 0.05$. AsA= ascorbic acid; µM =Micro-molar; control (x-axis) = No exogenous AsA application; water spray= normal water application; (200/400/600µM) = levels of ascorbic acid application; Bras with lines=no drought only AsA application; Bars with dots= drought with AsA application.

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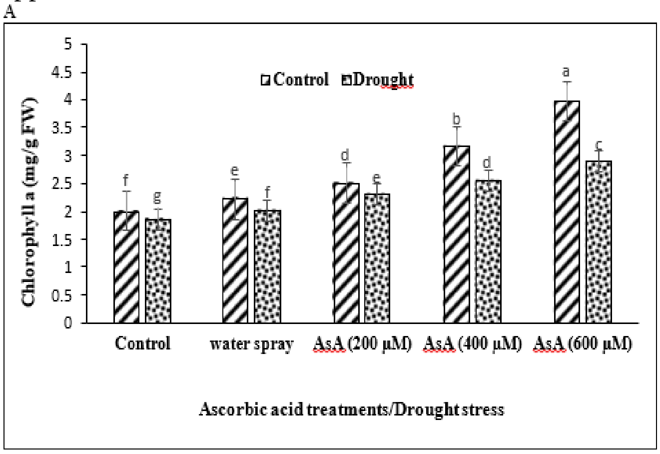

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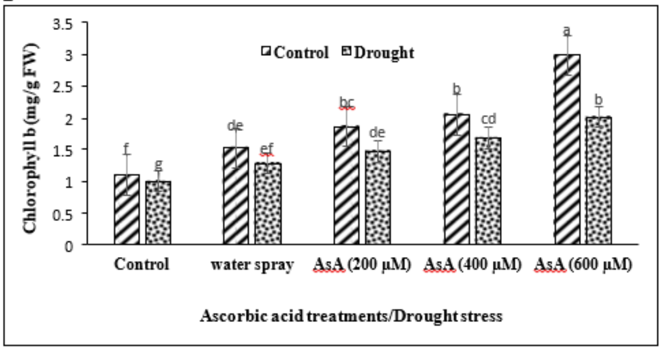

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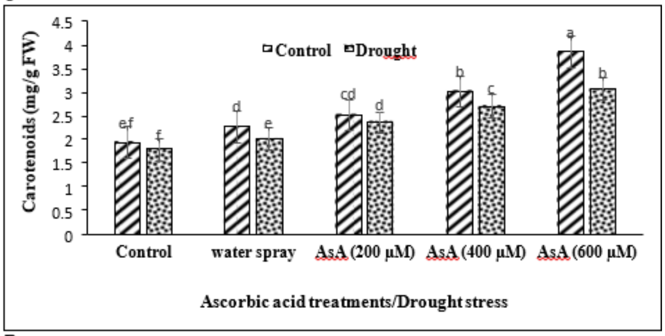

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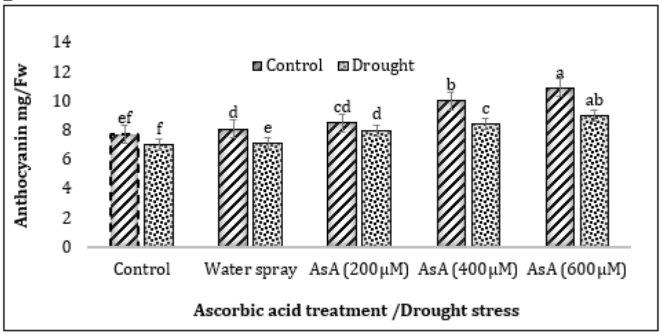

Oxidative damage by ROS

The present results indicated that oxidative stress, indicated by increased reactive oxygen species (ROS) production, was significantly higher under stress conditions compared to control plants. Elevated ROS levels led to membrane damage and disrupted metabolic activities. The study highlighted that antioxidant compounds, such as ascorbic acid, could help mitigate oxidative stress by enhancing membrane stability and reducing ROS accumulation. Primarily the studied align with the findings of (Vijayaraghavareddy *et al.*, 2022; Kotb *et al.*, 2021) about gas exchange, membrane permeability, and ion uptake in salt-stressed Indian jujube species.

Xiong *et al*. (2018) examined the effects of fullerol on Brassica napus under water stress, noting that drought-stressed plants exhibited a significant rise in ROS levels compared to control plants. This study found that ROS overproduction under drought led to oxidative damage, impairing photosynthesis and growth. However, antioxidant compounds, including ascorbic acid, played a crucial role in scavenging ROS, thereby reducing oxidative stress and improving plant resilience. The study emphasized that ascorbic acid contributed to maintaining cellular redox balance, protecting chloroplast integrity, and supporting overall plant health under drought conditions.

The research focused on ROS production in drought-stressed plants. It leads to the idea that drought stress leads to excessive ROS accumulation, while antioxidant treatments, including ascorbic acid, effectively mitigate oxidative damage and improve plant tolerance (fig.3.8).

Figure 3.8
Effect of ascorbic acid on (A) shoot Hydrogen peroxide and (B) Root hydrogen peroxide of wheat plant under drought stress. The error bars represent the ± standard deviation of three replicates. Means sharing the same letter for a given parameter are not significantly different at $p \leq 0.05$. AsA= ascorbic acid; µM =Micro-molar; control (x-axis) = No exogenous AsA application; water spray= normal water application; (200/400/600µM) = levels of ascorbic acid application; Bras with lines=no drought only AsA application; Bars with dots= drought with AsA application.

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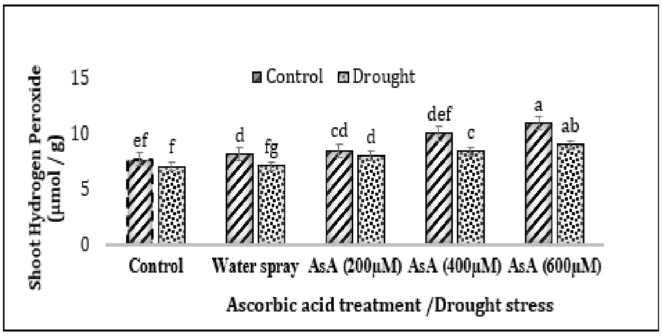

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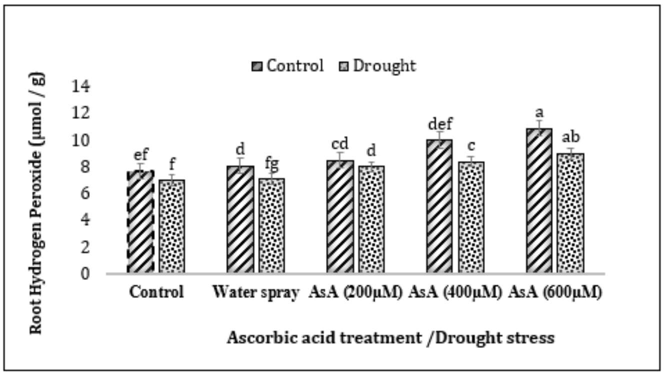


Ion Analysis

These graphs observed an increase in H_2O_2 levels in plants subjected to drought stress. However, ascorbic acid application assuaged the adverse effects of water stress and protected the crop from stress-induced damage by significantly reducing ROS accumulation under drought conditions. These results are consistent with those reported by (Vijayaraghavareddy *et al.*, 2022; Kotb *et al.*, 2021).

In this study, it was examined that the impact of ascorbic acid on the levels of non-enzymatic antioxidants, particularly ascorbic acid, under water deficit conditions. Ascorbic acid is a crucial and potent intracellular antioxidant that plays a key role in ROS scavenging (Gupta et al., 2016). The findings indicated that drought stress increased ascorbic acid levels, while exogenously applied ascorbic acid further enhanced its content, thereby mitigating the harmful effects of drought. These results align with previous studies conducted by various researchers (Xiong *et al.*, 2018; Alam *et al.*, 2014).

Additionally, this study demonstrated that water stress led to a decline in the ionic content of crops. However, increasing the dosage of ascorbic acid resulted in higher concentrations of Na^+, K^+, and Ca^{2+} ions. Similar findings were reported by (Hasanuzzaman *et al.*, 2023; Hussein and Khursheed 2014).

In conclusion, drought stress negatively impacted all growth parameters, ionic balance, and several physiological and biochemical attributes. However, various doses of ascorbic acid effectively reduced the detrimental effects of water stress by promoting growth, improving functional and biochemical characteristics, and enhancing ionic content while inhibiting ROS production, including H_2O_2. Regular application of ascorbic acid may benefit other plants and cereal crops, contributing to increased agricultural productivity.

Fluctuations in Plant's Ionic Balance

Horchani *et al.*, (2023) studied the effects of exogenous ascorbic acid on barley under salt stress, and while their focus was on salt-induced oxidative stress, their findings are relevant to drought stress as well. The graphs indicated that under stress conditions, there was an imbalance in essential ions, with increased sodium (Na^+) accumulation and a reduction in potassium (K^+), calcium (Ca^{2+}), and other essential nutrients (fig.3.9). The application of ascorbic acid helped restore ion homeostasis by reducing Na^+ uptake and augmenting the absorption of K^+ and Ca^{2+}, which are crucial for maintaining cellular functions and osmotic balance.

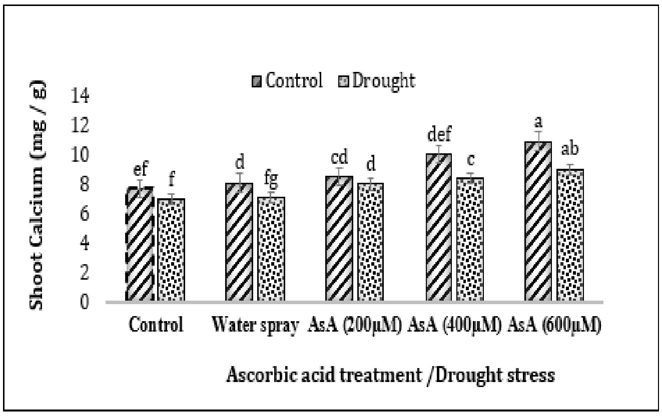
The graph represented that Sodium (Na^+) levels increased under drought conditions, while potassium (K^+), calcium (Ca^{2+}), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), and nitrogen (N) levels declined (fig.3.9). These changes negatively affected plant metabolism and growth. However, ascorbic acid application improved ionic balance by decreasing Na^+ levels and increasing the uptake of K^+, Ca^{2+}, NO_3^-, PO_4^{3-}, SO_4^{2-}, and N. This enhancement contributed to better nutrient availability, improved photosynthetic efficiency, and increased drought tolerance in wheat plants. The same findings align with Shafiq *et al.*, (2020) as the foliar use of fulleroll on wheat altered ion concentrations significantly under salt stress.

Overall experiment highlight that drought stress disrupts ion homeostasis, leading to nutrient deficiencies, but ascorbic acid treatment helps restore balance by regulating ion uptake and improving overall plant health.

Figure 3.9

Influence of ascorbic acid on (A) shoot Calcium, (B) shoot potassium, (C) shoot sodium, (D) shoot nitrate (D) shoot phosphate and (E) shoot sulphate of wheat plant under drought stress. The error bars represent the ± standard deviation of three replicates. Means sharing the same letter for a given parameter are not significantly different at $p \leq 0.05$. AsA= ascorbic acid; µM =Micro-molar; control (x-axis) = No exogenous AsA application; water spray= normal water application; (200/400/600µM) = levels of ascorbic acid application; Bras with lines=no drought only AsA application; Bars with dots= drought with AsA application.

A



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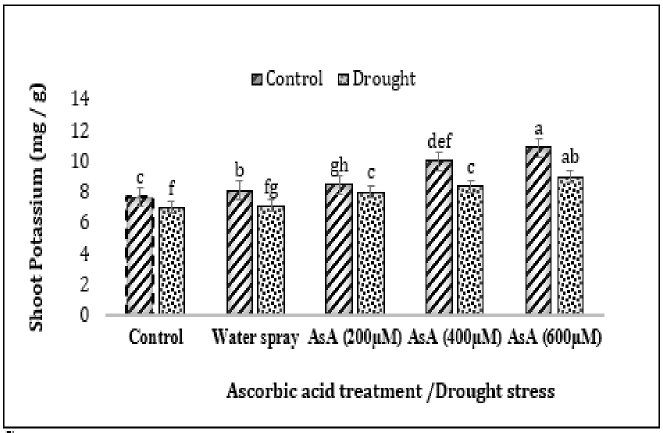

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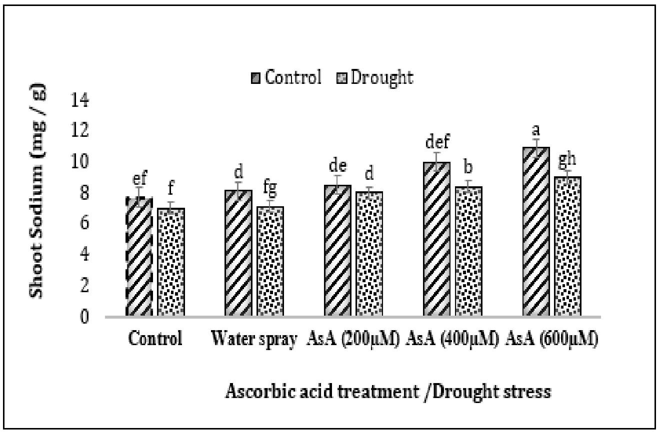

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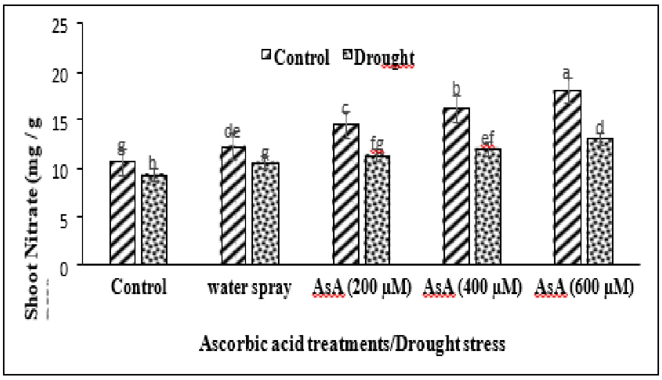

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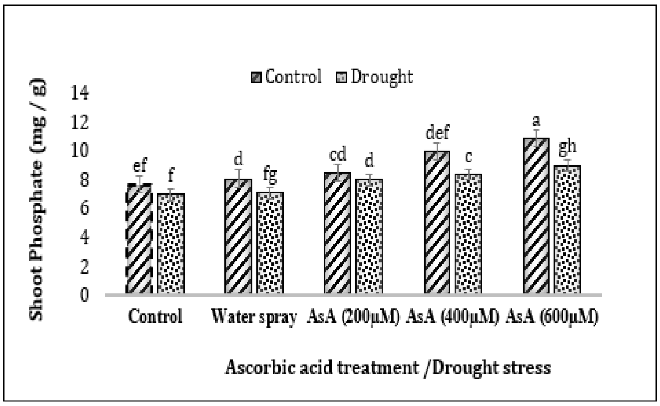

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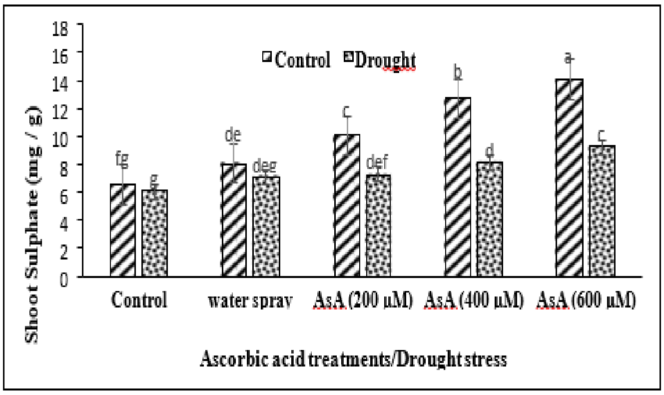


DISCUSSION

Wheat (*Triticum aestivum* L.) is an almanac grass that belongs to the family Poaceae. It is the maximum planted crop worldwide due to its nutritional value (Moshawih *et al.*, 2022). It is consumed as a food throughout the world. It contains basic nutrients like carbs and proteins which are essential for the human diet (Khalid *et al.*, 2023). Now a day's wheat crop is facing various biotic and abiotic stresses that reduce the annual yield across the planet. Abiotic stressors include drought stress, which poses a serious threat to agricultural productivity and lowers physiological and growth characteristics in plants (Al-Khayri *et al.*, 2023).

The current research was conducted in the winter of 2021–2022 in the Old Botanical Garden University of Agriculture Faisalabad. The goal of this research was to determine how foliar ascorbic acid influenced wheat under water-scarce conditions. The experiment was completely randomized sequence with three replications. Seeds were sown in pots uniformly. After 30 days of sprouting, the plants were exposed to water deficit followed by ascorbic acid spray. After 30 days of growth under water deficit conditions, the crop was harvested and evaluated different growth metrics. Various biochemical and ion investigations were carried out utilizing various appropriate protocols.

The current research demonstrated that under drought stress, plant growth is severely abridged as illustrated by vegetative parameters such as root and shoot stretch, fresh and dry weight of root and shoot and plant size. In contrast, ascorbic acid application enhanced all plant growth metrics when compared to control group. Considering the interaction effects, it is evident that ascorbic acid markedly improved every aspect of wheat germination. The optimum enhance in 600 µM ascorbic acid dosage. These results are aligned with those that were obtained by (Khadr *et al.*, 2021).

Optimizing Ascorbic Acid Dosage for Wheat Growth

A comparative analysis of different AsA dosages (200 µM, 400 µM, and 600 µM) revealed that higher concentrations had a more pronounced influence on plant progress and biochemical attributes. The ascending order of effectiveness followed the trend: 600 µM > 400 µM > 200 µM > water spray > control. Among these, the 600 µM concentration proved to be the most effective in enhancing growth metrics, improving stress tolerance, and promoting overall plant vigor. This finding aligns with prior research suggesting that optimal AsA concentrations can maximize plant resilience against abiotic stress (Hameed *et al.*, 2021).

In the current trial, water stress results in a considerable fall in shoot diameter, and the number of leaves and roots, whereas an increase is shown in these metrics when ascorbic acid is sprayed particularly at 600 µM dosage. These findings are in line with those reported by (Khazaei and Estaji 2020; El-Beltagi *et al.*, 2022).

Bestowing to recent studies, the leaf area of plants that were exposed to water deficit showed a considerable decline, whereas the plants treated with ascorbic acid exhibited improved leaf area. Similar findings were obtained by preliminary research studies on numerous crops (Aziz *et al.*, 2018; Desoky *et al.*, 2020). The recent research revealed that drought treatment diminished wheat germination and development by inhibiting the activities of growth enzymes and cell division procedure. In contrast, ascorbic acid promoted these metrics. These results are aligned with those that were found by (Chieb and Gachomo 2023).

Present studies described that chlorophyll contents drastically lowered during drought conditions. While externally applied ascorbic acid stimulated the chlorophyll components i-e. chlorophyll a, chlorophyll b, and carotenoid contents. Hafez and Gharib (2016) also established similar findings. Anthocynain contents declined during water stress whereas ascorbic acid sprays increased these contents in current study. Similar findings were determined by early study carried out by (Alayafi, 2020). However, the mechanism is not much studied.

The current research explained that there is a decline in falvonoids and soluble sugar contents. I noticed that optimal doses of ascorbic acid had optimistic influence on flavonoids, and soluble sugar contents. These findings are in agreement with the ones that were published by (Amira and Qados, 2014; Aziz *et al.*, 2018).

In this research, the enhancement in the ratios of H_2O_2 noted in drought-treated plants. The spray of ascorbic acid mitigated the negative effects of water stress and prevented the crop from the destruction caused by stress, which manifested in a significant decrease in the deposition of ROS under the drought-stressed conditions. These results are consistent with the findings reported by (Singh *et al.*, 2016; Parveen *et al.*, 2024).

In this trial, I monitored the influence of ascorbic acid on the ratio of non-enzymatic antioxidants such as ascorbic acid under water deficit. It is the vital and potent non-enzymatic intracellular antioxidants that effectively involved in the exclusion of ROS (Gupta *et al.*, 2016). In the current trial, the findings indicated that water deficiency caused a spike in the quantities of ascorbic acid. While ascorbic acid also boosted the ascorbic acid contents, which assisted to mitigate the adverse impacts of drought. These findings align closely with the findings that were found and published by a number of other investigators (Xiong *et al.*, 2018).

Current study explained that water stress declines the ionic contents of crops. While there is an increase in ions of Na+, K+ and Ca+2 by enhancing the ascorbic acid dosages. Similar findings were reported by other researchers (Hasanuzzaman *et al.*, 2023)

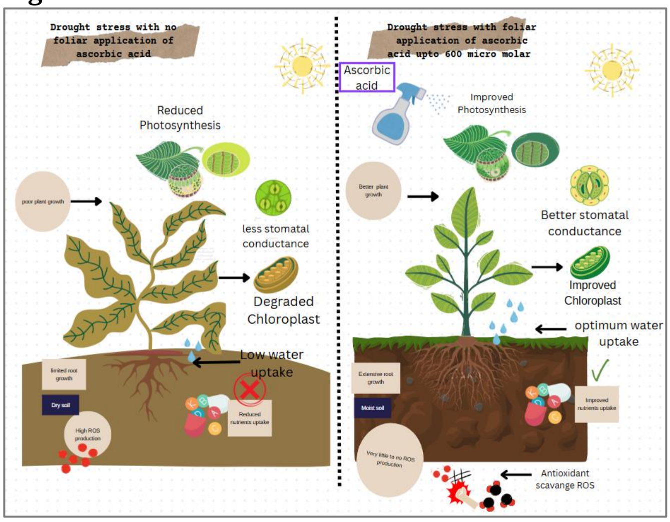
Hence in conclusion, all growth metrics, ionic contents, and several physio-chemical attributes were inhibited by drought stress. Whereas ascorbic acid various dosages were found significant in minimizing the deleterious impacts of water stress conditions by promoting all growth and numerous physiochemical attributes and ionic contents and inhibiting the ROS production such as H2O2. Other plants and cereal crops may benefit from regular ascorbic acid dosages, and the increasing demand for food can be met by applying ascorbic acid on large scale to mitigate harmful drought affects.

Gaps in Research and Future Perspectives

While the effectiveness of ascorbic acid in enhancing drought tolerance is well-documented, several gaps remain in our understanding. The prolong effects of AsA exposure on soil health, plant microbiome, and yield stability remain unexplored. Future studies should focus on integrating AsA application with other agronomic practices to develop sustainable strategies for wheat production under water-deficit conditions.

The visible sensitivity to stress by the plant can be seen via the physical parameters, As the difference in the growth of both the plants could be analyzed in the fig. (3.10). Plant started its response to stress very early from the exposure, that cause its phenotypical factors quite noticeable at the very beginning of drought. These phenotypical changes occurred due to the degradation of essential organelles fig. (3.10) and disruption of plant mechanism such as photosynthesis, ions imbalance and production of reactive oxygenated species due to prolonged drought stress. While on the other hand plants with the mitigation of foliar spray of ascorbic acid coped with drought stress and maintained its growth as a health plant, can be seen in fig. (3.10) .

Figure 3.10.



A conceptual model illustrates two distinct responses of wheat plants to drought stress, with and without the foliar application of ascorbic acid. Under drought conditions, wheat experienced negative impacts on its morphological, physiological, and biochemical traits. These effects include reduced uptake of essential nutrients, disruption of water balance, increased oxidative stress, and damage to cellular organelles such as mitochondria and chloroplasts. These disruptions ultimately lead to decreased photosynthetic efficiency and stunted plant growth. Additionally, the plant's inherent stress-response mechanisms are insufficient to fully protect cells from the damaging effects of reactive oxygen species (ROS). In contrast, wheat plants treated with foliar sprays of ascorbic acid, up to 600 micro-molar concentrations, exhibited a markedly different response. Ascorbic acid promoted stomatal closure to minimize water loss through evapotranspiration and activates stress-response pathways that helped mitigate cellular damage. This treatment supported the maintenance of chlorophyll content and enhanced osmolyte accumulation, resulting in improved photosynthesis, reduced oxidative damage, and ultimately higher plant productivity.

CONCLUSION

Wheat (Triticum aestivum L.) is a major cereal crop globally, meeting nearly 20% of people's nutritional needs. It is the primary food source in Pakistan and widely cultivated due to its high nutritional value. However, wheat production is severely impacted by drought stress. Various approaches, including the application of growth stimulators, are used to mitigate drought effects. Among them, ascorbic acid (AsA) is a widely recognized growth promoter.

This study aimed to evaluate the influence of AsA on wheat under drought conditions. A pot trial was conducted at the University of Agriculture Faisalabad using the Dhazi-11 variety. Ten seeds per pot were sown and initially watered adequately. Half of the pots were subjected to drought at 50% field capacity and later sprayed with AsA at concentrations of 200, 400, and 600 µM. The experiment followed a completely randomized design (CRD), and plants were harvested after 30 days.

Drought stress reduced growth metrics and ion contents, while hydrogen peroxide levels increased. AsA-treated plants exhibited enhanced growth attributes, increased soluble sugar, flavonoids, anthocyanin, ascorbic acid, and chlorophyll content. The highest improvements were observed at 600 µM AsA. These findings suggest that AsA foliar application effectively mitigates drought stress, enhancing wheat growth and stress tolerance.

REFERENCES

Awad, W., Byrne, P. F., Reid, S. D., Comas, L. H., & Haley, S. D. (2018). Great Plains winter wheat varies for root length and diameter under drought stress. *Agronomy Journal*, *110*(1), 226-235. https://doi.org/10.2134/agronj2017.07.0377

Alayafi, A. A. (2019). Exogenous ascorbic acid induces systemic heat stress tolerance in tomato seedlings: Transcriptional regulation mechanism. *Environmental Science and Pollution Research*, *27*(16), 19186-19199. https://doi.org/10.1007/s11356-019-06195-7

Al-Khayri, J. M., Rashmi, R., Surya Ulhas, R., Sudheer, W. N., Banadka, A., Nagella, P., Aldaej, M. I., Rezk, A. A., Shehata, W. F., & Almaghasla, M. I. (2023). The role of nanoparticles in response of plants to abiotic stress at physiological, biochemical, and molecular levels. *Plants*, *12*(2), 292. https://doi.org/10.3390/plants12020292

Azeem, M., Sultana, R., Mahmood, A., Qasim, M., Siddiqui, Z. S., Mumtaz, S., Javed, T., Umar, M., Adnan, M. Y., & Siddiqui, M. H. (2023). Ascorbic and salicylic acids vitalized growth, biochemical responses, antioxidant enzymes, photosynthetic efficiency, and Ionic regulation to alleviate salinity stress in sorghum bicolor. *Journal of Plant Growth Regulation*, *42*(8), 5266-5279. https://doi.org/10.1007/s00344-023-10907-2

Aziz, A., Akram, N. A., & Ashraf, M. (2018). Influence of natural and synthetic vitamin C (ascorbic acid) on primary and secondary metabolites and associated metabolism in quinoa (chenopodium quinoa Willd.) plants under water deficit regimes. *Plant Physiology and Biochemistry*, *123*, 192-203. https://doi.org/10.1016/j.plaphy.2017.12.004

Ahmad, A., Aslam, Z., Naz, M., Hussain, S., Javed, T., Aslam, S., Raza, A., Ali, H. M., Siddiqui, M. H., Salem, M. Z., Hano, C., Shabbir, R., Ahmar, S., Saeed, T., & Jamal, M. A. (2021). Exogenous salicylic acid-induced drought stress tolerance in wheat (Triticum aestivum L.) grown under hydroponic culture. *PLOS ONE*, *16*(12), e0260556. https://doi.org/10.1371/journal.pone.0260556

Bacher, H., Sharaby, Y., Walia, H., & Peleg, Z. (2021). Modifying root-to-shoot ratio improves root water influxes in wheat under drought stress. *Journal of Experimental Botany*, *73*(5), 1643-1654. https://doi.org/10.1093/jxb/erab500

Chowdhury, M. K., Hasan, M. A., Bahadur, M. M., Islam, M. R., Hakim, M. A., Iqbal, M. A., Javed, T., Raza, A., Shabbir, R., Sorour, S., Elsanafawy, N. E., Anwar, S., Alamri, S., Sabagh, A. E., & Islam, M. S. (2021). Evaluation of drought tolerance of some wheat (Triticum aestivum L.) genotypes through phenology, growth, and physiological indices. *Agronomy*, *11*(9), 1792. https://doi.org/10.3390/agronomy11091792

Celi, G. E., Gratão, P. L., Lanza, M. G., & Reis, A. R. (2023). Physiological and biochemical roles of ascorbic acid on mitigation of abiotic stresses in plants. *Plant Physiology and Biochemistry*, *202*, 107970. https://doi.org/10.1016/j.plaphy.2023.107970

Castro, J. C., Castro, C. G., & Cobos, M. (2023). Genetic and biochemical strategies for regulation of L-ascorbic acid biosynthesis in plants through the L-galactose pathway. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1099829

Chieb, M., & Gachomo, E. W. (2023). The role of plant growth promoting rhizobacteria in plant drought stress responses. *BMC Plant Biology*, *23*(1). https://doi.org/10.1186/s12870-023-04403-8

Demirci-Çekiç, S., Özkan, G., Avan, A. N., Uzunboy, S., Çapanoğlu, E., & Apak, R. (2022). Biomarkers of oxidative stress and antioxidant defense. *Journal of Pharmaceutical and Biomedical Analysis*, *209*, 114477. https://doi.org/10.1016/j.jpba.2021.114477

Desoky, E. M., Mansour, E., Yasin, M. A., El-Sobky, E. E., & Rady, M. M. (2020). Improvement of drought tolerance in five different cultivars of Vicia faba with foliar application of ascorbic acid or silicon. *Spanish Journal of Agricultural Research*, *18*(2), e0802. https://doi.org/10.5424/sjar/2020182-16122

Daryanto, S., Wang, L., & Jacinthe, P. (2016). Global synthesis of drought effects on maize and wheat production. *PLOS ONE*, *11*(5), e0156362. https://doi.org/10.1371/journal.pone.0156362

EL Sabagh, A., Islam, M. S., Skalicky, M., Ali Raza, M., Singh, K., Anwar Hossain, M., Hossain, A., Mahboob, W., Iqbal, M. A., Ratnasekera, D., Singhal, R. K., Ahmed, S., Kumari, A., Wasaya, A., Sytar, O., Brestic, M., ÇIG, F., Erman, M., Habib Ur Rahman, M., … Arshad, A. (2021). Salinity stress in wheat (Triticum aestivum L.) in the changing climate: Adaptation and management strategies. *Frontiers in Agronomy*, *3*. https://doi.org/10.3389/fagro.2021.661932

El-Beltagi, H. S., Sulaiman, Mohamed, M. E., Ullah, S., & Shah, S. (2022). Effects of ascorbic acid and/or α-tocopherol on agronomic and physio-biochemical traits of oat (Avena sativa L.) under drought condition. *Agronomy*, *12*(10), 2296. https://doi.org/10.3390/agronomy12102296

Eskola, M., Kos, G., Elliott, C. T., Hajšlová, J., Mayar, S., & Krska, R. (2019). Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%. *Critical Reviews in Food Science and Nutrition*, *60*(16), 2773-2789. https://doi.org/10.1080/10408398.2019.1658570

Farooq, A., Farooq, N., Akbar, H., Hassan, Z. U., & Gheewala, S. H. (2023). A critical review of climate change impact at a global scale on cereal crop production. *Agronomy*, *13*(1), 162. https://doi.org/10.3390/agronomy13010162

Gavlak, R., Horneck, D., Miller, R. O., & Kotuby-Amacher, J. (2003). Soil, plant and water reference methods for the western region. *WCC-103 Publication, Fort Collins, CO*, 1-207. https://www.unm.edu/~unmvclib/cascade/handouts/westernstatesmethodmanual2005.pdf

Gupta, D. K., Palma, J. M., & Corpas, F. J. (Eds.). (2016). *Redox state as a central regulator of plant-cell stress responses* (pp. 1-386). Berlin/Heidelberg, Germany: Springer. https://link.springer.com/book/10.1007/978-3-319-44081-1

Hamid, K., Mustafa, A., Salem, H., Ismaiel, S., & Amin, M. (2024). Effect of salinity and ascorbic acid treatments on growth, yield and antioxidant enzymes activity of Barley plants. *Al-Azhar Journal of Agricultural Research*, *49*(1), 117-129. https://doi.org/10.21608/ajar.2024.260498.1321

Hasanuzzaman, M., Raihan, M. R., Alharby, H. F., Al-Zahrani, H. S., Alsamadany, H., Alghamdi, K. M., Ahmed, N., & Nahar, K. (2023). Foliar application of ascorbic acid and tocopherol in conferring salt tolerance in rapeseed by enhancing K+/Na+ homeostasis, osmoregulation, antioxidant defense, and Glyoxalase system. *Agronomy*, *13*(2), 361. https://doi.org/10.3390/agronomy13020361

Hafez, E. M., & Gharib, H. S. (2016). Effect of exogenous application of ascorbic acid on physiological and biochemical characteristics of wheat under water stress. *International Journal of plant production*, *10*(4), 579-596.

Hameed, A., Farooq, T., Hameed, A., & Sheikh, M. A. (2021). Silicon-mediated priming induces acclimation to mild water-deficit stress by altering physio-biochemical attributes in wheat plants. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.625541

Hossain, A., Skalicky, M., Brestic, M., Maitra, S., Ashraful Alam, M., Syed, M. A., Hossain, J., Sarkar, S., Saha, S., Bhadra, P., Shankar, T., Bhatt, R., Kumar Chaki, A., EL Sabagh, A., & Islam, T. (2021). Consequences and mitigation strategies of abiotic stresses in wheat (Triticum aestivum L.) under the changing climate. *Agronomy*, *11*(2), 241. https://doi.org/10.3390/agronomy11020241

Hasanuzzaman, M., Bhuyan, M., Zulfiqar, F., Raza, A., Mohsin, S., Mahmud, J., Fujita, M., & Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants*, *9*(8), 681. https://doi.org/10.3390/antiox9080681

Horchani, F., Bouallegue, A., Namsi, A., & Abbes, Z. (2023). Exogenous application of ascorbic acid mitigates the adverse effects of salt stress in two contrasting Barley cultivars through modulation of physio-biochemical attributes, K+/Na+ homeostasis, osmoregulation and antioxidant defense system. *Russian Journal of Plant Physiology*, *70*(9). https://doi.org/10.1134/s1021443723602598

Horchani, F., Bouallegue, A., Namsi, A., & Abbes, Z. (2024). Simultaneous application of ascorbic acid and proline as a smart approach to mitigate the adverse effects of salt stress in wheat (Triticum aestivum). *Biology Bulletin*, *51*(5), 1346-1363. https://doi.org/10.1134/s1062359024607171

Hussein, Z. K., & Khursheed, M. Q. (2014). Effect of foliar application of ascorbic acid on growth , yield components and some chemical constituents of wheat under water stress conditions = تأثير الرش الورقي بحامض الأسكوربيك في النمو و مكونات الحاصل و في بعض المكونات الكيمياوية للحنطة تحت ظروف الإجهاد المائي. *Jordan Journal of Agricultural Sciences*, *10*(1), 1-15. https://doi.org/10.12816/0029871

Li, G., Wang, Y., Liu, J., Liu, H., Liu, H., & Kang, G. (2022). Exogenous melatonin mitigates cadmium toxicity through ascorbic acid and glutathione pathway in wheat. *Ecotoxicology and Environmental Safety*, *237*, 113533. https://doi.org/10.1016/j.ecoenv.2022.113533

Kopecká, R., Kameniarová, M., Černý, M., Brzobohatý, B., & Novák, J. (2023). Abiotic stress in crop production. *International Journal of Molecular Sciences*, *24*(7), 6603. https://doi.org/10.3390/ijms24076603

Khadr, S.A., El-Hamamsy, S.M., El-khamissi, H.A. & Saad, Z. H., (2021). The effect of ascorbic acid treatment on wheat (Triticum aestivum L.) seedlings under drought stress. (2021). *Egyptian Journal of Applied Science*, *36*(1), 30-42. https://doi.org/10.21608/ejas.2021.152334

Kapoor, D., Bhardwaj, S., Landi, M., Sharma, A., Ramakrishnan, M., & Sharma, A. (2020). The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. *Applied Sciences*, *10*(16), 5692. https://doi.org/10.3390/app10165692

Khazaei, Z., & Estaji, A. (2020). Effect of foliar application of ascorbic acid on sweet pepper (Capsicum annuum) plants under drought stress. *Acta Physiologiae Plantarum*, *42*(7). https://doi.org/10.1007/s11738-020-03106-z

Khalid, A., Hameed, A., & Tahir, M. F. (2023). Wheat quality: A review on chemical composition, nutritional attributes, grain anatomy, types, classification, and function of seed storage proteins in bread making quality. *Frontiers in Nutrition*, *10*. https://doi.org/10.3389/fnut.2023.1053196

Kotb, M., Yakout, G., Ali, F., & Abas, M. (2021). The interactive effect between water stress and foliar spraying with ascorbic acid or hydrogen peroxide on wheat productivity. *Zagazig Journal of Agricultural Research*, *48*(5), 1181-1195. https://doi.org/10.21608/zjar.2021.224028

Malik, S., Ashraf, M., Arshad, M., & Malik, T. A. (2015). EFFECT OF ASCORBIC ACID APPLICATION ON PHYSIOLOGY OF WHEAT UNDER DROUGHT STRESS. *Pakistan Journal of Agricultural Sciences*, *52*(1).

Moshawih, S., Abdullah Juperi, R. N., Paneerselvam, G. S., Ming, L. C., Liew, K. B., Goh, B. H., Al-Worafi, Y. M., Choo, C., Thuraisingam, S., Goh, H. P., & Kifli, N. (2022). General health benefits and pharmacological activities of triticum aestivum L. *Molecules*, *27*(6), 1948. https://doi.org/10.3390/molecules27061948

Mahmood, N., Arshad, M., Kächele, H., Ullah, A., & Müller, K. (2020). Economic efficiency of rainfed wheat farmers under changing climate: Evidence from Pakistan. *Environmental Science and Pollution Research*, *27*(27), 34453-34467. https://doi.org/10.1007/s11356-020-09673-5

Mishra, S., Kumar, R., & Kumar, M. (2023). Use of treated sewage or wastewater as an irrigation water for agricultural purposes- Environmental, health, and economic impacts. *Total Environment Research Themes*, *6*, 100051. https://doi.org/10.1016/j.totert.2023.100051

Mahpara, S., Zainab, A., Ullah, R., Kausar, S., Bilal, M., Latif, M. I., Arif, M., Akhtar, I., Al-Hashimi, A., Elshikh, M. S., Zivcak, M., & Zuan, A. T. (2022). The impact of PEG-induced drought stress on seed germination and seedling growth of different bread wheat (Triticum aestivum L.) genotypes. *PLOS ONE*, *17*(2), e0262937. https://doi.org/10.1371/journal.pone.0262937

Nejad, T. S. (2011). Effect of drought stress on shoot/root ratio. *World Academy of Science, Engineering and Technology*, *5*, 539-541.

Nandy, S., Mandal, S., Gupta, S. K., Anand, U., Ghorai, M., Mundhra, A., Rahman, M. H., Ray, P., Mitra, S., Ray, D., Lal, M. K., Tiwari, R. K., Nongdam, P., Pandey, D. K., Shekhawat, M. S., Jha, N. K., Jha, S. K., Kumar, M., Radha, … Dey, A. (2022). Role of Polyamines in molecular regulation and cross-talks against drought tolerance in plants. *Journal of Plant Growth Regulation*, *42*(8), 4901-4917. https://doi.org/10.1007/s00344-022-10802-2

Niroula, A., Amgain, N., KC, R., Adhikari, S., & Acharya, J. (2021). Pigments, ascorbic acid, total polyphenols and antioxidant capacities in deetiolated Barley (Hordeum vulgare) and wheat (Triticum aestivum) microgreens. *Food Chemistry*, *354*, 129491. https://doi.org/10.1016/j.foodchem.2021.129491

Parveen, S., Arfan, M., & Wahid, A. (2024). Exogenous applications of ascorbic acid improve wheat growth, physiology and yield under salinity stress via a balance in antioxidant production and ROS scavenging. *New Zealand Journal of Crop and Horticultural Science*, 1-24. https://doi.org/10.1080/01140671.2024.2347534

Raza, A. (2020). Metabolomics: A systems biology approach for enhancing heat stress tolerance in plants. *Plant Cell Reports*, *41*(3), 741-763. https://doi.org/10.1007/s00299-020-02635-8

Shafiq, F., Iqbal, M., Ashraf, M. A., & Ali, M. (2020). Foliar applied fullerol differentially improves salt tolerance in wheat through ion compartmentalization, osmotic adjustments and regulation of enzymatic antioxidants. *Physiology and Molecular Biology of Plants*, *26*(3), 475-487. https://doi.org/10.1007/s12298-020-00761-x

Seleiman, M. F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Abdul-Wajid, H. H., & Battaglia, M. L. (2021). Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants*, *10*(2), 259. https://doi.org/10.3390/plants10020259

Singh, N., & Bhardwaj, R. D. (2016). Ascorbic acid alleviates water deficit induced growth inhibition in wheat seedlings by modulating levels of endogenous antioxidants. *Biologia*, *71*(4), 402-413. https://doi.org/10.1515/biolog-2016-0050

Vijayaraghavareddy, P., Lekshmy, S. V., Struik, P. C., Makarla, U., Yin, X., & Sreeman, S. (2022). Production and scavenging of reactive oxygen species confer to differential sensitivity of rice and wheat to drought stress. *Crop and Environment*, *1*(1), 15-23. https://doi.org/10.1016/j.crope.2022.03.010

Wu, P., Li, B., Liu, Y., Bian, Z., Xiong, J., Wang, Y., & Zhu, B. (2024). Multiple physiological and biochemical functions of ascorbic acid in plant growth, development, and abiotic stress response. *International Journal of Molecular Sciences*, *25*(3), 1832. https://doi.org/10.3390/ijms25031832

Xiong, J., Li, J., Wang, H., Zhang, C., & Naeem, M. S. (2018). Fullerol improves seed germination, biomass accumulation, photosynthesis and antioxidant system in brassica napus L. under water stress. *Plant Physiology and Biochemistry*, *129*, 130-140. https://doi.org/10.1016/j.plaphy.2018.05.026