

Efficacy of Naphthyl Acetic Acid Foliar Spray in Moderating Drought Effects on the Morphological and Physiological Traits of Maize Plants (*Zea mays* L.)

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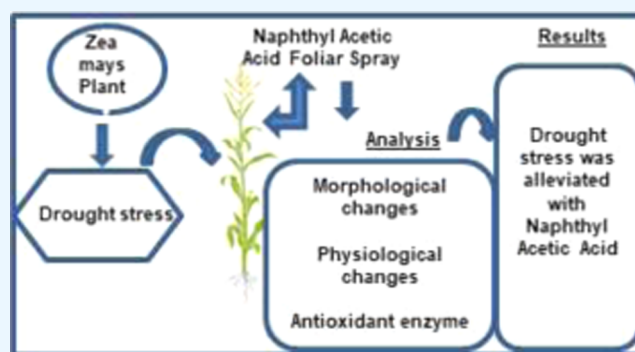
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ABSTRACT: The threat of varying global climates has greatly driven the attention of scientists, as climate change increases the odds of worsening drought in many parts of Pakistan and the world in the decades ahead. Keeping in view the forthcoming climate change, the present study aimed to evaluate the influence of varying levels of induced drought stress on the physiological mechanism of drought resistance in selected maize cultivars. The sandy loam rhizospheric soil with moisture content 0.43–0.5 g g⁻¹, organic matter (OM) 0.43–0.55 g/kg, N 0.022–0.027 g/kg, P 0.028–0.058 g/kg, and K 0.017–0.042 g/kg was used in the present experiment. The findings showed that a significant drop in the leaf water status, chlorophyll content, and carotenoid content was linked to an increase in sugar, proline, and antioxidant enzyme accumulation at $p < 0.05$ under induced drought stress, along with an increase in protein content as a dominant response for both cultivars. SVI-I & II, RSR, LAI, LAR, TB, CA, CB, CC, peroxidase (POD), and superoxide dismutase (SOD) content under drought stress were studied for variance analysis in terms of interactions between drought and NAA treatment and were found significant at $p < 0.05$ after 15 days. It has been found that the exogenous application of NAA alleviated the inhibitory effect of only short-term water stress, but yield loss due to long-term osmotic stress will not be faced employing growth regulators. Climate-smart agriculture is the only approach to reduce the detrimental impact of global fluctuations, such as drought stress, on crop adaptability before they have a significant influence on world crop production.



1. INTRODUCTION

Temperature fluctuation owing to climatic variations in the precipitation regime, and high-level increases in natural adversities due to climatological changes every year have an adverse effect on the dynamics of human beings including inevitable loss of crop production.¹ An increase in severe drought events will expectedly enhance loss of atmospheric evaporation via soil surfaces and water bodies due to climate change and concomitant climatic disasters instigating severe crop losses in crop production.² The plant's reaction to drought tolerance is determined by a variety of parameters, including timing, length, intensity, and frequency of drought stress, as well as inadequate soil–plant–environment interactions.³ Although climate change is now confined as a global singularity,^{4–6} however, its sternness is more extensively sensed in emerging countries, owing to their more liabilities and the low level of capability to alleviate its effects. The economies of most of the developing countries including

Pakistan are based on agriculture; their farming region is the utmost affected sector due to exposure to environmental constraints such as salinity,^{7,8} drought, heavy metals,^{9,10} nutrient deficiencies,^{11,12} and temperature. The broad spectrum changes in droughts,¹³ rain patterns, floods, and temperature are considered consequences of variations in climate, which will decline agricultural production in developing countries.^{14,15} The current investigation indicated that local and worldwide water needs for agricultural practices can be changed due to variations in climatic factors which can prompt water shortage for agricultural purposes and could

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cause potential food uncertainty for 9 billion people by 2050.¹⁶ As per the Global Climate Risk Index (GCRI), Pakistan was ranked 21st for the period from 1993 to 2012 based on exposure to severe climate conditions, e.g., drought and water shortage, floods, and temperature fluctuation, and thought to be an extremely vulnerable country to climate change.^{17–21}

Impacts of climate change include increasing water scarcity, termed as drought, which takes place when less water is available in the soil, creating a sort of complexity revealing the involvement of macro and micro molecules in the physiology of plant bodies, for example, proteins, minerals, enzymes, hormones, lipids, carbohydrates, nucleic acid, free radicals, and ions.²² Arid and semi-arid regions cover around 60% of the globe.²³ Water stress has had a significant impact on crop yield in Asian countries where the economy is mostly based on irrigated agriculture.^{24,25} In terms of the effect of drought stress on plant species and varieties, there are significant differences in physiological and metabolic changes, even in their organs.^{26,27} The degree of drought resistance induced by different genotypes is determined by the metabolic changes that the genotype undergoes when stressed, i.e., the physiological and biochemical reactions.²⁸

Maize (*Zea mays* L.) is effectively grown in Pakistan from below sea level (from 58 °N to 40 °S) to higher altitudes (3300 m) in a subtropical and temperature-controlled environment.²⁹ After rice and wheat, maize has been dubbed the king of grain crops.^{30–33} Maize is grown on an area of around 896 thousand hectares in Pakistan, with a yearly mass production of 4.9 million tons of grains.^{34,35} It is widely cultivated in Khyber Pakhtunkhwa (KPK) and Punjab, accounting for around 98% of Pakistan's total production.^{30,36–38} It is grown on around 1.11 million hectares of land and has an average yield of 3.62 t ha⁻¹. Maize is planted in rain-fed areas for the remaining 34%, with around 66% of the crop being grown in irrigated areas.³⁹ Compared to rice and wheat, maize has a lower protein level but a higher calorie density, with 365 kcal/100 g. Maize also comprises roughly 72% starch, 10% protein, and 4% fat. Many of the B vitamins, crucial minerals, and fiber are all present in maize.^{31,36,40,41} Maize is a crucial crop that offers a way to produce a variety of foods. Additionally, it acts as a raw material for numerous industries and has some therapeutic benefits. The most crucial component of a maize harvest is grain.^{37,40,42}

Drought is the abiotic factor that severely restricts maize yield. According to a rigorous study, only drought causes a 50% or greater decrease in global average yields.⁴³ The effects of drought stress on maize's morphological, physiological, anatomical, and biochemical processes resulted in poor growth and a small yield, including a decrease in the plant's length, diameter, dry weight, and fresh weight due to water shortage conditions, as well as the fresh weight of the seedlings.^{44,45} The growth responses of maize crops investigated via photosynthetic pigment efficiency revealed that chlorophyll "a" and "b" content were adversely affected by induced drought conditions provided to the experiment,²⁵ while carotenoids as an accessory light-harvesting pigment help the plant to withstand drought stress.⁴⁶ Chlorophyll and carotenoid concentrations have been reduced in tissues due to water stress^{47,48} as a result of the deterioration of their precursors. Osmolytes are low-molecular-weight organic molecules.⁴⁹ When under stress or in any pathological situation, the amount that is typically built up in tissues is fairly high.⁵⁰ These molecules aid in stabilizing aggregated proteins and

prevent them from folding improperly.⁵¹ Osmolytes have been demonstrated to aid in protein stabilization and to possess additional cytoprotective qualities in situations of salinity, temperature, and pressure stress.⁵² Reactive oxygen species (ROS) were also produced in the thylakoid membrane,^{53,54} and for their detoxification, a complex enzymatic and nonenzymatic antioxidant system has been evolved to minimize the affections of oxidative stress. The ROS-scavenging enzymes, e.g., ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD), and low-molecular-mass antioxidants, e.g., glutathione, carotenoids, and ascorbate, are produced during abiotic stresses.^{55,56} Drought stress causes the accumulation of soluble sugar, proline, and free amino acids, which are nontoxic at high cellular concentrations, have a low molecular weight, and are highly soluble compounds that protect cellular components from dehydration injury, known as compatible solutes and osmoprotectants.⁵⁷

As the population of the world is increasing exponentially and we need more food to survive. So we need to develop approaches to increase crop growth and yield. This study will investigate the effects of climatic variation in terms of providing water-deficit conditions as induced drought stress to the maize crop in order to investigate their growth responses, osmoprotection capabilities, and antioxidant enzyme system when the NAA growth regulator is present. This study is important since Pakistan is susceptible to water scarcity as a result of global climate change, and the country's overall food security condition is not very favorable because it is believed that as temperatures rise (0.5–2 °C), food production would decrease and agricultural productivity in Pakistan will decrease by 8–10% by 2040. To stabilize our agricultural development rate, Pakistan must immediately implement the adaptation measures indicated by the current research and the connected results of other researchers.

2. MATERIALS AND METHODS

2.1. Plant Material and Growth Conditions. During the maize (*Z. mays* L.) growing season, the experiment was carried out in the natural environment of the Department of Botany, Bacha Khan University, Charsadda having a 34°18'17N 71°46'24E, 359 m altitude. Maize cultivars, Azam and Iqbal, obtained from the Cereal Crop Research Institute (CCRI) Pirsabaq, Nowshera, after surface sterilization with 5% chlorox and 95% ethanol, were grown in earthen pots (15.6 cm height, 0.5 cm thick, 14.5 cm lower inside diameter, 19 cm upper inner diameter) composed of sandy loam soil containing OM (0.43–0.55), N (0.022–0.027), P (0.028–0.058), and K (0.017–0). The seeds were uniform in size and color, and they were wrinkle-free.⁵⁸ Plants were watered daily to keep the moisture content of the soil up to 0.55 gg⁻¹. The experiment used a 2 × 5 factorial design (two cultivars and five levels of soil water status). Five levels of the soil water potential (ψ_{soil}) were exposed from the early vegetative stage through maturity, including 5, 7, 10, 12, and 15 days of drought, while the second group (control) was watered normally. The water potential of soil was measured at a depth of 15–20 cm using a tension meter with a 5-cm-long sensor (Soil Science Research Institute, The University of Agriculture, Peshawar), which was used to monitor soil moisture in each pot. Readings of the tension meter were recorded every 6 to 10 h. The pots were protected from rain and pests by being placed in the greenhouse. Foliar materials were frozen in a refrigerator at 4 °C for physiological

and biochemical analysis including photosynthetic efficiency, osmoprotection, and antioxidant enzymes, whereas some of the materials were processed for agronomic studies.

2.2. Physiochemical Characteristics of Rhizospheric Soil. The pH and EC of rhizospheric soil were evaluated by preparing a 1:1 (soil:water) suspension via the method of ref 59. The % FC (field capacity) and % MC (moisture content) were determined following the method of Ullah et al.⁶⁰

$$\%FC = \frac{\text{Weight of wet soil (g)} - \text{Weight of dry soil (g)}}{\text{Weight of dry soil (g)}} \times 100$$

$$\%MC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Calculated 0.5 g of an air-ground, sieved, and air-dried soil sample was taken in a flask, and 20 mL of extracting solution (0.05 N HCl + 0.025 N H₂SO₄) was added to it. The mixture was shaken for 15 min in a mechanical shaker before being filtered through Whatman #42 filter paper. Filtrated samples were diluted to 50 mL in a conical flask. Potassium (K), nitrogen (N), phosphorous (P), and carbon (C) were determined using the atomic absorption method.⁶¹

2.3. Agronomic Characteristics. **2.3.1. Leaf Area Ratio (LAR) and the Leaf Area Index (LAI).** The LAR and LAI (leaf area ratio and leaf area index) were determined by the following formulas suggested by Shah et al.⁶² and Bibi et al.⁶³

$$LAR = \text{Final plant dry weight/Leaf area}$$

$$LAI = \text{Land area/(Leaf area)}$$

2.3.2. Root–Shoot Ratio (RSR). The formula proposed by Chuyong and Acidri⁶⁴ was used to calculate the root–shoot ratio as follows

$$\text{Root shoot Ratio} = \text{Shoot dry weight/Root dry weight}$$

2.3.3. Seed Vigor Index-I (SVI-I). The SVI-I regarding the seedling's length was determined through the standard method established by Bina and Bostani.⁶⁵

$$SVI - I = \text{Seedling length (cm)} \times \text{Seed germination \%}$$

2.3.4. Seed Vigor Index-II (SVI-II). The SVI-II regarding the seedling's length was determined through the standard method adopted by Sharma.⁶⁶

$$SVI - II = \text{Seedling dry weight (mg)} \times \text{Seed germination \%}$$

2.3.5. Germination Rate Index (GRI) and Total Biomass (TB). The germination rate index represents the proportion of germination that occurs each day throughout the germination period and was calculated using the standard procedure of Kader⁶⁷ along with the total biomass.

$$GRI = 12X/G_1 + G_2 + \dots + G_x$$

where G_1 represents the percentage of germinated seeds on the first day after sowing and G_2 represents the percentage of germinated seeds on the second day after sowing.

2.4. Plant Physiological and Biochemical Features. **2.4.1. Analysis of Total Chlorophyll Content (TCC).** The Zou et al.⁶⁸ methods were used to assess the chlorophyll content of leaves. Fresh leaves weighing 0.2 g were pulverized in 80%

acetone using a mortar and pestle and incubated for 24 h in the dark before being centrifuged. By using a spectrophotometer, absorbance values at 649 nm (chl "a"), 663 nm (chl "b"), and 470 nm (carotenoid) were measured against a blank of 80% acetone. The chlorophyll *a* and *b* content were assessed with the standard procedure as adopted by Arnon⁶⁹ by using the following formulas

$$\text{Chl 'a' (mg/100 mL)} = 0.999 A_{663} - 0.0989 A_{645}$$

$$\text{Chl'b' (mg/100 mL)} = -0.328 A_{663} + 1.77 A_{645}$$

2.4.2. Assessment of Soluble Sugar Content (SSC). With a few minor adjustments, the standard methodology of Marcińska et al.⁷⁰ was used to measure the amount of soluble sugar. 1 mL of 30% phenol was added to 0.1 mL of supernatant after 0.5 g of fresh leaves were pulverized and homogenized with 10 mL of distilled water. The mixture was then incubated for 20 min. After incubation, 5.0 mL of conc. H₂SO₄ was added, and readings were observed at 420 nm.

2.4.3. Analysis of Total Proline Content (TPC). Proline content was approximated using the Płazek et al.⁷¹ methods by homogenizing 0.5 g of leaves in 10 mL of 3% (w/v) sulfosalicylic acid, followed by filtration. 2.0 mL of filtrate was combined with 2.0 mL of glacial acetic acid and 2.0 mL of acid ninhydrin (1.87 g of ninhydrin + 40 mL of glacial acetic acid + 30 mL of 6 M phosphoric acid) in a test tube, which was then cooked for 1 h at 100 °C. The entire reaction came to an end in an ice bath. 4 mL of toluene was used for the extraction, and 520 nm of absorbance was used to measure it.

2.4.4. Assessment of Soluble Protein Content (SPC). The soluble protein content of the foliar material was determined using the accepted method of Lowery et al.⁷² 0.5 g of leaves were homogenized for 10 min after being pulverized in 1 mL of phosphate buffer solution (pH 7.0). 0.1 mL of the extract was extracted, and 1 mL of the reagent [0.75 g of Na₂CO₃, 0.1 n of NaOH, and 0.37 g of Na-K tartrate in 40 mL of distilled water] was added to make a total volume of 1 mL. A test tube was filled with the solution, shaken for 10 min, and then 0.1 mL of the foline phenol reagent was added. The sample absorbance was measured using a spectrophotometer at 650 nm.

2.4.5. Analysis of Peroxidase (POD) Activity. For the POD estimate, the Asthir et al.⁷³ methodology was employed. Fresh leaves (0.5 g) were homogenized for 20 min in a centrifuge after being mixed with 2 mL of a solution containing 12.5 g of poly(vinyl pyrrolidone), 0.2 mL of phosphate buffer solution (pH 7.0), and 4.6 g of ethylenediamine tetraacetic acid (EDTA). 0.1 mL of supernatant, 1.3 mL of MES buffer (970 mg of MES in 50 mL of distilled water), 0.1 mL of phenyl diamine (36 mg in 4 mL of distilled water), and a drop of 0.3% H₂O₂ were all included in the reaction mixture (3 mL). Using a spectrophotometer, the absorbance change was monitored for 3 min at 485 nm.

2.4.6. Analysis of Superoxide Dismutase (SOD) Activity. The Yuan et al.⁷⁴ standard method was used to determine the SOD content in the foliar material. The reaction mixture (3 mL) contained 0.1 mL of supernatant, 0.72 mL of methionine (58 mg of methionine in 30 mL of distilled water), 0.72 mL of NBT (1.89 mg of NBT in 30 mL of distilled water), 0.72 mL of EDTA (1.1 mg of EDTA in 30 mL of distilled water), and 0.72 mL of riboflavin (0.02 mg of riboflavin in 30 mL of distilled water), followed by 30 min in the dark and then 30 min in the light, with spectrophotometer readings at 560 nm.

Table 1. Impact of Naphthyl Acetic Acid Foliar Application Agronomic Features of Maize under Induced Drought Stress^a

varieties	treatments	GRI	SVI-I	SVI-II	LAI	LAR	RSR	TB
Azam	5 days	4.667 ± 0.577	555.0 ± 1.00	1.82 ± 0.25	0.786 ± 0.120	22.80 ± 1.94	0.336 ± 0.01	0.62 ± 0.09
	5 days + NAA	4.667 ± 0.577	920.6 ± 0.57	0.94 ± 0.27	0.976 ± 0.123	60.39 ± 17.4	0.702 ± 0.03	0.30 ± 0.06
	7 days	4.333 ± 1.155	629.6 ± 1.15	2.77 ± 0.30	0.872 ± 0.145	19.70 ± 4.12	0.079 ± 0.03	0.80 ± 0.06
	7 days + NAA	4.667 ± 0.577	401.6 ± 1.52	2.46 ± 0.32	1.189 ± 0.188	29.91 ± 6.88	0.211 ± 0.05	0.72 ± 0.04
	10 days	4.333 ± 1.155	587.3 ± 2.08	0.46 ± 0.10	0.557 ± 0.091	48.71 ± 13.4	0.370 ± 0.08	0.21 ± 0.04
	10 days + NAA	4.333 ± 0.577	345.0 ± 1.00	0.58 ± 0.21	0.468 ± 0.114	46.57 ± 13.2	0.131 ± 0.00	0.18 ± 0.01
	12 days	4.667 ± 0.577	423.0 ± 1.00	1.34 ± 0.74	0.766 ± 0.136	20.79 ± 2.92	0.163 ± 0.03	0.66 ± 0.06
	12 days + NAA	4.333 ± 1.155	373.6 ± 1.52	0.83 ± 0.44	0.778 ± 0.120	62.52 ± 6.09	0.251 ± 0.07	0.22 ± 0.01
	15 days	4.667 ± 0.577	485.6 ± 1.52	0.62 ± 0.06	0.504 ± 0.092	45.14 ± 5.77	0.151 ± 0.02	0.21 ± 0.01
	15 days + NAA	4.667 ± 0.577	374.6 ± 0.57	0.58 ± 0.22	0.545 ± 0.029	60.24 ± 11.3	0.347 ± 0.02	0.17 ± 0.03
	control	4.667 ± 0.577	433.6 ± 1.52	0.88 ± 0.60	0.602 ± 0.064	26.21 ± 6.74	0.175 ± 0.00	0.44 ± 0.11
	control + NAA	5.000 ± 0.000	1500.6 ± 1.1	4.23 ± 1.14	1.302 ± 0.099	24.69 ± 3.19	0.186 ± 0.04	0.95 ± 0.06
Iqbal	5 days	4.667 ± 0.577	881.0 ± 1.00	0.77 ± 0.38	0.893 ± 0.090	71.26 ± 4.90	0.477 ± 0.17	0.22 ± 0.02
	5 days + NAA	4.333 ± 0.577	2161.3 ± 1.5	1.73 ± 0.78	1.225 ± 0.044	33.70 ± 4.95	0.207 ± 0.03	0.66 ± 0.08
	7 days	4.667 ± 0.577	2384.3 ± 0.5	1.50 ± 0.55	1.120 ± 0.119	23.97 ± 2.07	0.230 ± 0.08	0.84 ± 0.02
	7 days + NAA	4.333 ± 0.577	1268.0 ± 1.0	1.13 ± 0.33	1.148 ± 0.655	39.43 ± 4.60	0.154 ± 0.03	0.53 ± 0.08
	10 days	4.667 ± 0.577	1620.6 ± 0.5	1.42 ± 0.35	0.159 ± 0.138	27.70 ± 6.89	0.259 ± 0.02	0.43 ± 0.01
	10 days + NAA	4.333 ± 1.155	880.6 ± 0.57	0.43 ± 0.10	0.949 ± 0.175	89.39 ± 26.1	0.599 ± 0.07	0.19 ± 0.02
	12 days	4.333 ± 0.577	673.3 ± 1.52	0.34 ± 0.01	0.563 ± 0.193	55.40 ± 14.1	0.291 ± 0.03	0.18 ± 0.02
	12 days + NAA	4.333 ± 0.577	801.3 ± 1.52	0.32 ± 0.06	0.836 ± 0.044	114.3 ± 19.0	0.295 ± 0.02	0.13 ± 0.03
	15 days	4.667 ± 0.577	1192.3 ± 2.0	1.08 ± 0.42	0.688 ± 0.118	32.11 ± 14.3	0.156 ± 0.02	0.43 ± 0.10
	15 days + NAA	4.333 ± 1.155	920.3 ± 0.57	0.75 ± 0.06	0.879 ± 0.227	21.74 ± 5.01	0.114 ± 0.02	0.72 ± 0.02
	control	4.667 ± 0.577	1002.0 ± 2.0	1.33 ± 0.08	1.198 ± 0.161	38.60 ± 3.08	0.595 ± 0.06	0.56 ± 0.11
	control + NAA	4.667 ± 0.577	2790.3 ± 0.5	2.62 ± 0.18	1.489 ± 0.111	25.44 ± 3.96	0.720 ± 0.06	1.06 ± 0.08

^aGRI—germination rate index; SVI-I—seed vigor index-I; SVI-II—seed vigor index-II; LAI—leaf area index; LAR—leaf area ratio; RSR—root—shoot ratio; TB—total biomass.

2.4.7. Assessment of Catalase (CAT) Activity. With some modifications, the Tyburski et al.⁷⁵ method for determining catalase (CAT) was used. 0.2 mL of enzyme extract, 0.4 mL of 30% H₂O₂, and 0.4 mL of 100 mM potassium phosphate buffer (pH 7.0) made up the reaction mixture (1 mL). The decrease in absorbance at 240 nm was used to calculate how much H₂O₂ was decomposed over a 3-minute period.

2.4.8. Quantification of Ascorbate Peroxidase (APOX) Activity. Salimi et al.⁷⁶ proposed a protocol for quantifying APOX content. 0.5 g of fresh leaves were homogenized with 5 mL of buffer solution and centrifuged for 10 min to form the reaction mixture (1 mL). 0.1 mL of enzyme extract was mixed with 1.8 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mL of 0.5 mM ascorbic acid, and 1 mL of 30% H₂O₂. The absorbance was determined as a reduction at 290 nm for 3 min when compared to a blank 30% H₂O₂ solution.

2.5. Statistical Analysis. The statistical analysis was done using a factorial design with different levels of induced drought stress. Statistix 10 and SPSS Statistics 22 were used to calculate data for several germination parameters and physiological and biochemical components. The significant difference at $p \leq 0.05$, mean separation, and standard deviation were calculated.⁷⁷

3. RESULTS

3.1. Assessment of Agronomic Characters. Plant characteristics associated with crop production, such as height, maturity, tiller number, panicle size, yield, and quality variables, are typically observed during plant growth. In maize, drought reduces the leaf area and ultimately lowers the grain yield. Results of agronomic characteristics including GRI, LAI, LAR, RSR, TB, SVI-I, and SVI-II mentioned in Table 1 estimated that maximum GRI, SVI-I, SVI-II, and RSR under control

conditions with naphthyl acetic acid (NAA) treatment in both cultivars indicating the adverse effect of induced drought stress forecasting the future yield loss of the crop as a consequence of the global variation ahead in the region. The maximum LAI and LAR were recorded during 5 days drought with NAA treatment in variety Azam and 10 days drought in variety Iqbal, respectively, which are the direct indication of the growth responses in the selected cultivar under induced drought stress, reflecting the second domain of the present findings suggesting the idea of using growth regulators to cope with the future scenario of climate change in Pakistan as per the Global Climate Risk Index (GCRI). Nonetheless, the maximum range for all agronomic characters in both cultivars has been reported after the foliar application of NAA as the growth regulator is considered to be an initiative toward the improvement of climate-resilient crops, and this climate-smart agriculture will be considered the mere option to reduce the adverse effects of global disparities in the region considered to be most vulnerable.

3.2. Evaluation of Physio-Biochemical Characteristics. Drought stress causes numerous physiological and biochemical changes in various agriculture crops.⁷⁸ Changes in protein expression, accumulation, and synthesis have been seen in many plant species as a result of drought stress during growth. Physical and biochemical analyses of the foliar material, including photosynthetic pigments, soluble protein content, osmolytes, and antioxidant enzyme activity, were performed throughout the vegetative stage of the proposed experiment under induced drought stress. Chlorophyll concentration is an important indicator for determining plant health and photosynthetic efficiency under drought stress.⁷⁹ Proteins synthesized in response to drought stress are known to be involved in the plant water stress response. Results

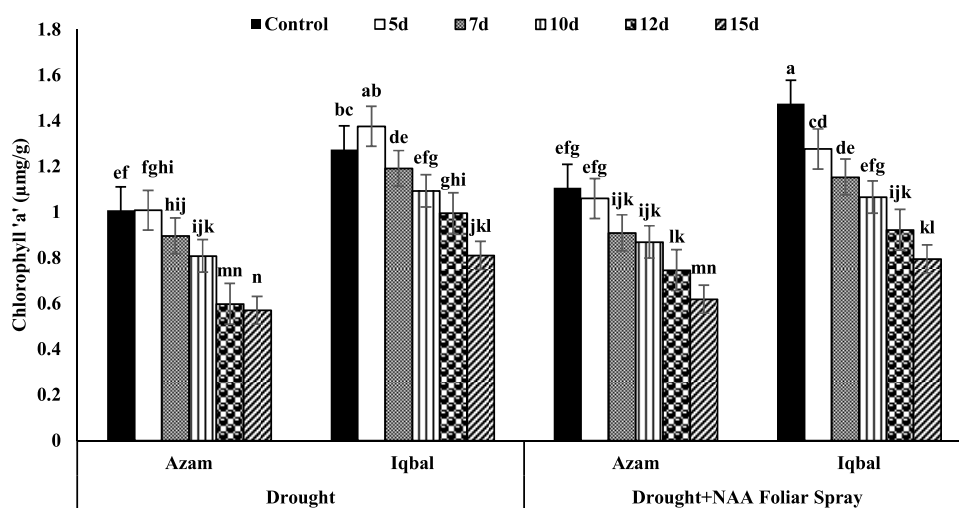


Figure 1. Impact of NAA foliar application on chlorophyll “a” content under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows, signifying significance at $p \leq 0.05$.

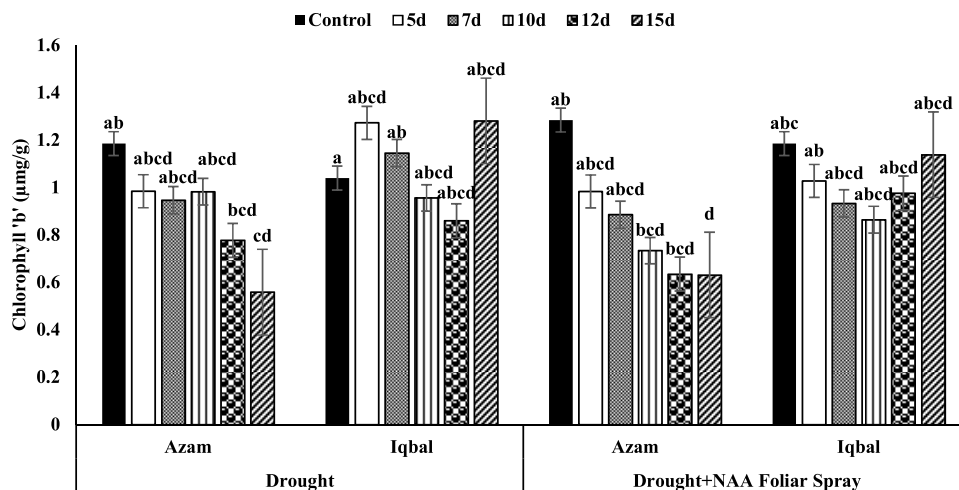


Figure 2. Impact of NAA foliar application on chlorophyll “b” content under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows, signifying significance at $p \leq 0.05$.

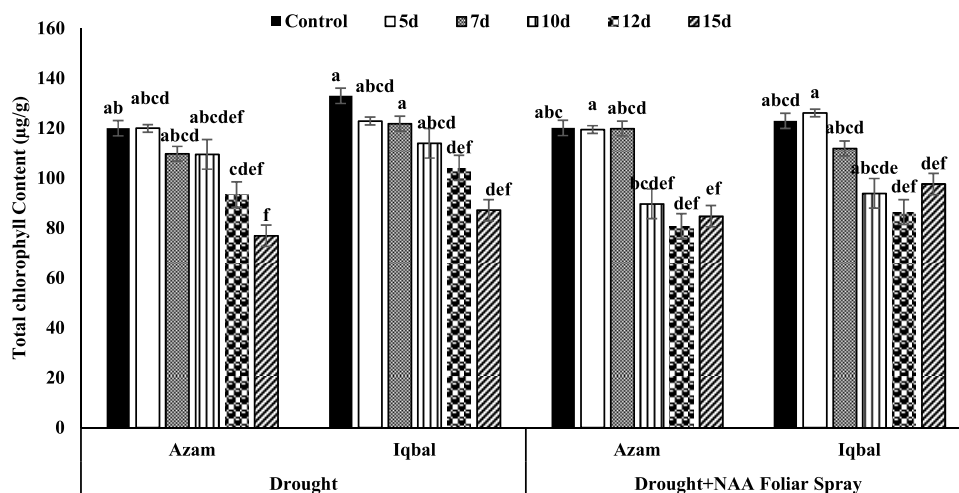


Figure 3. Impact of NAA foliar application on total chlorophyll content under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows signifying significance at $p \leq 0.05$.

shown in Figures 1–5 reveal that maximum chl “a,” chl “b,” chl a/b ratio, and total chl and carotenoid concentration have been

reported in variety Iqbal as well as Azam under induced stress of 5-day drought and 5-day drought with NAA as growth

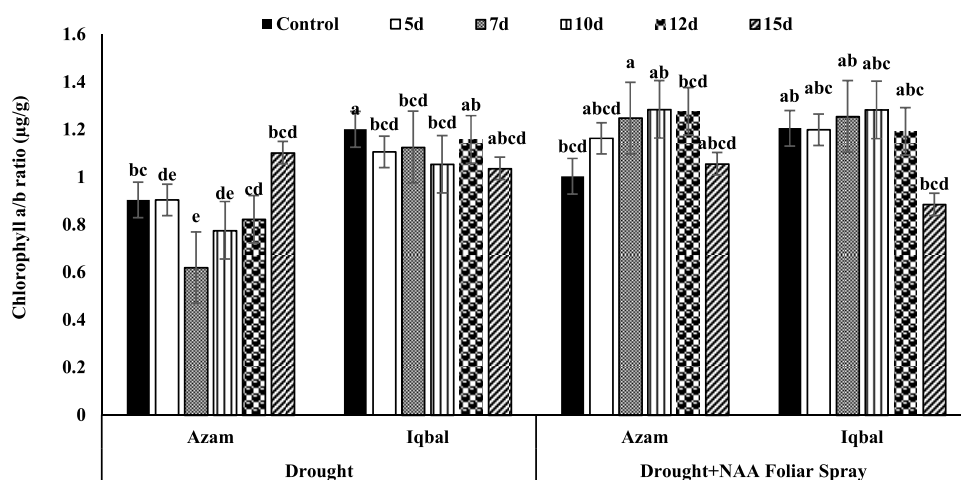


Figure 4. Impact of NAA foliar application on chlorophyll *a/b* under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows, signifying significance at $p \leq 0.05$.

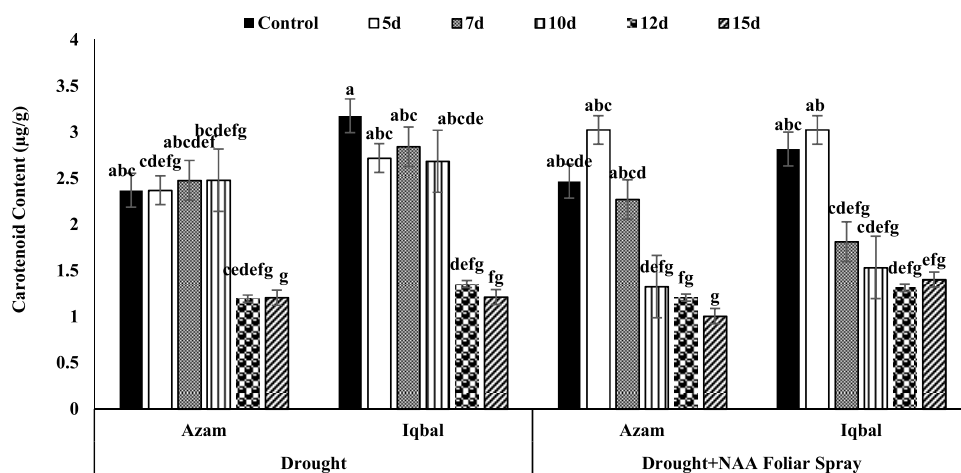


Figure 5. Impact of NAA foliar application on carotenoid content under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows, signifying significance at $p \leq 0.05$.

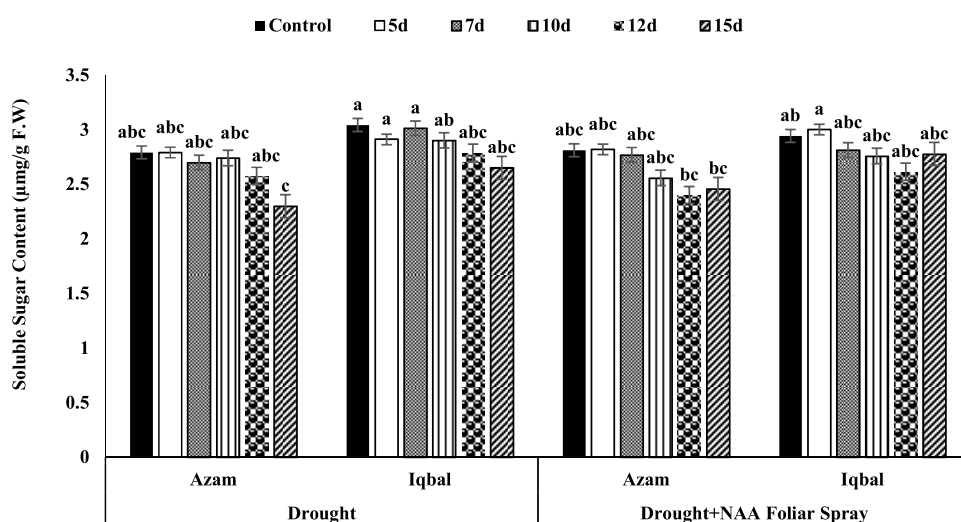


Figure 6. Impact of NAA foliar application on soluble sugar content under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows, signifying significance at $p \leq 0.05$.

regulators, respectively, that was considerably comparable with the untreated control at $p < 0.05$, reflecting the tolerance level of variety Iqbal under natural conditions, and can be

considered a suitable cultivar at considerable level of drought stress to cope with forthcoming global variation in the region. The minimum of all of the mentioned attributes in both

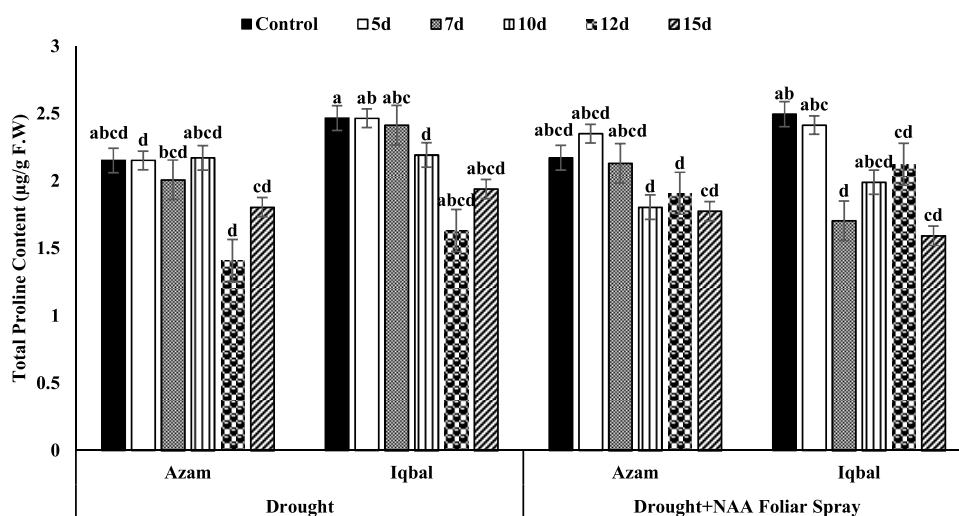


Figure 7. Impact of NAA foliar application on total proline content under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows, signifying significance at $p \leq 0.05$.

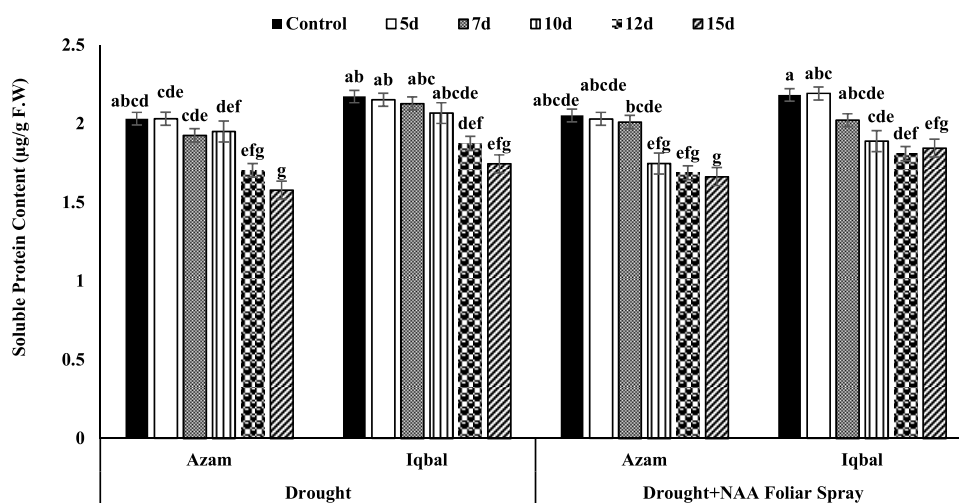


Figure 8. Impact of NAA foliar application on soluble protein content under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows, signifying significance at $p \leq 0.05$.

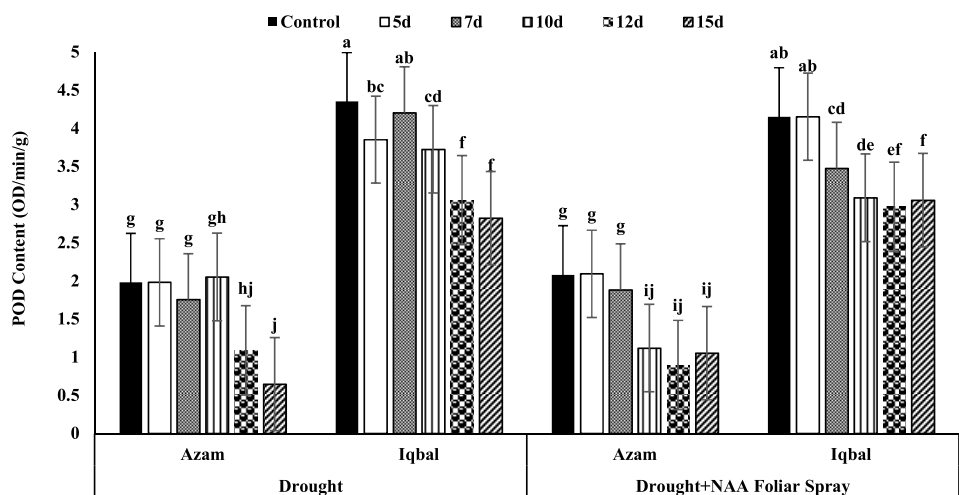


Figure 9. Impact of NAA foliar application on peroxidase content under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows, signifying significance at $p \leq 0.05$.

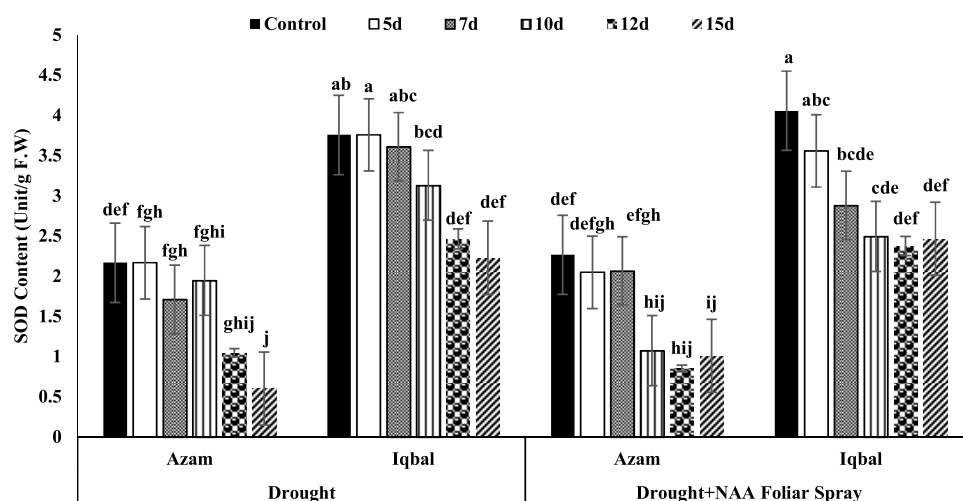


Figure 10. Impact of NAA foliar application on super dismutase content under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows, signifying significance at $p \leq 0.05$.

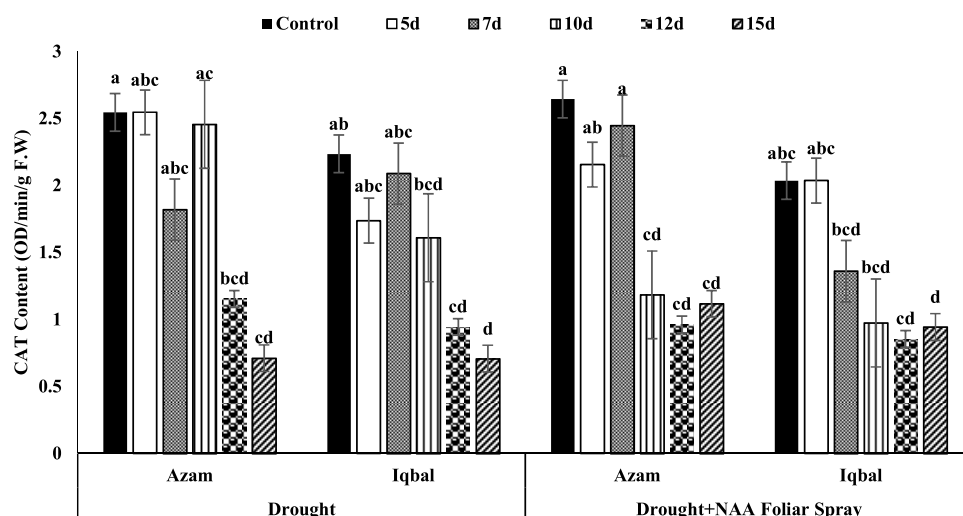


Figure 11. Impact of NAA foliar application on catalase content under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows, signifying significance at $p \leq 0.05$.

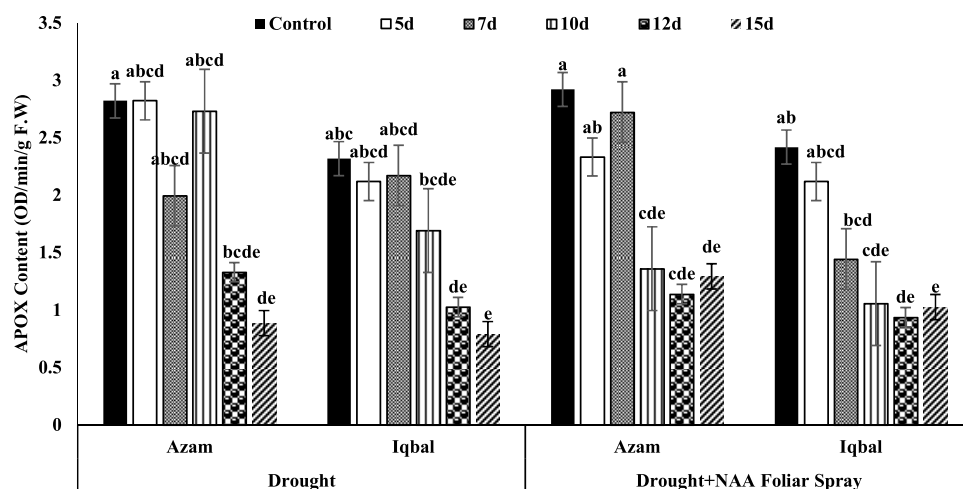


Figure 12. Impact of NAA foliar application on ascorbate peroxidase content under drought stress. Vertical bars represent SE. The treatments show disparate letters in rows, signifying significance at $p \leq 0.05$.

cultivars has been described in the treatment (15 days drought) and (15 days drought + NAA) at $p < 0.05$, indicating

the minimum growth responses showed in both varieties reflecting their susceptibility at the highest level of induced

Table 2. Traits of Maize Plants under Induced Drought Stress via Variance Analysis^a

trait	source of variation	SS	df	MS	F	p
GRI	genotype	0.09	1	0.09	0.49	0.48
	treatment	0.25	5	0.05	0.28	0.92
	genotype $\times$ treatment	0.07	5	0.01	0.08	0.99
	error	4.40	24	0.18		
SVI-I	genotype	0.40	1	0.40	4.13	0.05*
	treatment	2.39	5	0.47	10.95	<0.001***
	genotype $\times$ treatment	0.26	5	0.05	1.98	0.11
	error	0.64	24	0.02		
SVI-II	genotype	108	1	108	29.73	<0.001***
	treatment	646	5	129	2.30	0.07
	genotype $\times$ treatment	583	5	116	203.42	<0.001***
	error	137	24	573.9		
LAI	genotype	0.26	1	0.26	0.60	0.44
	treatment	12.10	5	2.42	22.69	<0.001***
	genotype $\times$ treatment	1.88	5	0.37	8.59	<0.001***
	error	1.05	24	0.04		
LAR	genotype	0.40	1	0.40	4.13	0.05*
	treatment	2.39	5	0.47	10.95	<0.001***
	genotype $\times$ treatment	0.26	5	0.05	1.98	0.11
	error	0.64	24	0.02		
RSR	genotype	0.14	1	0.14	1.34	0.25
	treatment	2.87	5	0.57	20.28	<0.001***
	genotype $\times$ treatment	0.59	5	0.12	26.3	<0.001***
	error	0.10	24	0.00		
TB	genotype	0.00	1	0.00	0.28	0.59
	treatment	0.04	5	0.00	1.70	0.16
	genotype $\times$ treatment	0.13	5	0.02	23.84	<0.001***
	error	0.02	24	0.00		
CA	genotype	0.14	1	0.14	1.34	0.25
	treatment	2.87	5	0.57	20.28	<0.001***
	genotype $\times$ treatment	0.59	5	0.12	26.33	<0.001***
	error	0.10	24	0.00		
CB	genotype	0.49	1	0.49	11.82	0.01**
	treatment	1.31	5	0.26	13.57	<0.001***
	genotype $\times$ treatment	0.06	5	0.01	9.23	<0.001***
	error	0.03	24	0.00		
TCC	genotype	0.22	1	0.22	6.29	0.01**
	treatment	0.93	5	0.18	11.08	<0.001***
	genotype $\times$ treatment	0.13	5	0.02	4.57	<0.01**
	error	0.14	24	0.00		
CABR	genotype	45.21	1	45.21	0.167	0.68
	treatment	798	5	159	38.02	<0.001***
	genotype $\times$ treatment	148.17	5	29.63	0.66	0.65
	error	106	24	44.4		
CC	genotype	0.00	1	0.00	0.15	0.69
	treatment	0.26	5	0.05	3.41	0.01**
	genotype $\times$ treatment	0.13	5	0.02	2.04	0.10
	Error	0.32	24	0.01		
SSC	Genotype	0.28	1	0.28	0.54	0.46
	treatment	14.97	5	2.99	30.37	<0.001***
	genotype $\times$ treatment	1.64	5	0.32	7.64	<0.001***
	error	1.03	24	0.04		
SPC	genotype	826	1	826	0.91	0.34
	treatment	174	5	349	7.40	<0.001***
	genotype $\times$ treatment	718	5	143	5.61	0.01**
	error	614	24	255		
TPC	genotype	304	1	304	12.2	0.001***
	treatment	649	5	129	7.75	<0.001***
	genotype $\times$ treatment	312	5	62.55	0.90	0.49
	error	166	24	69.35		
POD	genotype	158	1	158	5.32	0.02*

Table 2. continued

trait	source of variation	SS	df	MS	F	p
SOD	treatment	930	5	186	22.9	<0.001***
	genotype × treatment	297	5	59.45	2.60	0.05*
	error	547.846	24	22.827		
	genotype	34.07	1	34.07	107.83	<0.001***
	treatment	9.04	5	1.80	1.51	0.21
	genotype × treatment	0.40	5	0.08	1.519	0.22
CAT	error	1.29	24	0.05		
	genotype	19.07	1	19.07	42.15	<0.001***
	treatment	13.30	5	2.66	3.77	0.01**
	genotype × treatment	0.80	5	0.16	2.99	0.03*
APOX	error	1.28	24	0.05		
	genotype	1.37	1	1.37	24.06	<0.001***
	treatment	12.94	5	2.58	45.26	<0.001***
	genotype × treatment	0.99	5	0.19	3.46	0.01**
	error	1.37	24	0.05		

^aKeys: * Significant at $p \leq 0.05$, ** significant at $p \leq 0.01$, *** significant at $p \leq 0.001$.

Table 3. F-Ratio and the Significance Level of Agronomical Characteristics and Physio–Biochemical Parameters in Maize

parameters	treatment	amongst genotype	F	p	amongst drought factors	F	p
GRI	drought	0.003	0.014	0.90	0.027	0.02	1.0
	NAA	0.091	0.652	0.425	0.257	0.33	0.88
SVI-I	drought	597***	29.7	<0.001	402*	2.74	0.03
	NAA	0.401*	4.135	0.05	2.391***	10.95	<0.001
SVI-II	drought	107	3.71	0.06	210	1.43	0.24
	NAA	108***	29.73	<0.001	646	2.30	0.07
LAI	drought	0.01***	0.01	<0.001	0.01	0.01	0.36
	NAA	0.267	0.60	0.44	12.10***	22.6	<0.001
LAR	drought	107	3.71	0.06	210	1.43	0.24
	NAA	0.401*	4.135	0.05	2.391***	10.9	<0.001
RSR	drought	0.01*	5.43	0.02	0.04**	3.69	0.01
	NAA	0.142	1.346	0.25	2.876***	20.28	<0.001
TB	drought	107	3.71	0.06	210	1.43	0.24
	NAA	0.002	0.286	0.59	0.046	1.706	0.164
CA	drought	0.73***	19.6	<0.001	1.20***	8.93	<0.001
	NAA	0.142	1.346	0.25	2.876***	20.28	<0.001
CB	drought	0.31***	8.12	<0.01	1.02***	10.30	<0.001
	NAA	0.490***	11.82	0.002	1.317***	13.57	<0.001
CABR	drought	0.65***	43.45	<0.001	0.29	1.74	0.15
	NAA	45.21	0.167	0.68	798***	38.02	<0.001
TC	drought	279	0.93	0.34	796***	19.75	<0.001
	NAA	0.22**	6.29	0.017	0.93***	11.08	<0.001
CC	drought	1.65	2.95	0.09	13.08***	10.41	<0.001
	NAA	0.003	0.155	0.697	0.267**	3.416	0.015
SSC	drought	423***	14.47	0.001	7.41***	6.57	<0.001
	NAA	0.283	0.545	0.465	14.97***	30.37	<0.001
SPC	drought	2.73	8.72	0.06	8.81***	11.52	<0.001
	NAA	826	0.912	0.346	174***	7.40	<0.001
TPC	drought	108***	14.97	<0.001	983	2.30	0.06
	NAA	304***	12.22	0.001	649***	7.75	<0.001
POD	drought	38.35***	107	<0.001	9.39	1.37	0.26
	NAA	158*	5.32	0.02	930***	22.93	<0.001
SOD	drought	22.75***	52.66	<0.001	11.57*	2.68	0.04
	NAA	34.07***	107	<0.001	9.04	1.51	0.21
CAT	drought	0.41	0.92	0.74	10.9***	14.0	<0.001
	NAA	19.07***	42.15	<0.001	13.3***	3.77	<0.001
APOX	drought	0.78	1.57	0.21	12.0***	12.3	<0.001
	NAA	1.37*	3.05	0.08	12.94***	20.7	<0.001

drought stress under natural conditions as well as with the application of growth regulators. Growth responses of both

varieties can be enhanced using growth regulators conditionally if the water-deficit condition provided is not too high.

Table 4. Key Outcome of Naphthyl Acetic Acid Foliar Spray Application through Variance Analysis on Physiological Contents, Agronomical Parameters, and Biochemical Attributes of Maize over Induced Drought Stress^a

treatment	parameters	factor levels											
		5 days		7 days		10 days		12 days		15 days		control	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
naphthyl acetic acid (NAA)	GRI	0.16	0.70	0.70	0.85	0.0	1.0	0.0	1.0	0.63	0.47	1.25	0.32
	SVI-I	117	<0.001***	302	<0.001***	249	<0.001***	171	<0.001***	282	<0.001***	652	<0.001***
	SVI-II	4.32	0.10	2.64	0.17	4.25	0.10	19.9	0.02*	91.2	0.01**	0.04	0.84
	LAI	5.93	0.07	39.3	0.003**	6.09	0.06	3.02	0.15	94.2	0.001***	720	<0.001***
	LAR	4.32	0.10	2.64	0.17	4.25	0.10	12.9	0.02*	19.2	0.01**	0.04	0.84
	RSR	39	0.003**	0.75	0.43	35	0.004**	0.16	0.70	11	0.02*	33	0.004**
	TB	4.3	0.10	2.6	0.17	4.2	0.10	12.9	0.02*	19.2	0.01**	0.04	0.84
	CA	40	0.003**	70	0.001***	81	0.001***	13	0.02*	53	0.002**	106	<0.001***
	CB	102	0.001***	0.5	0.49	13	0.02*	6.6	0.06	13	0.02*	0.7	0.42
	TCC	0.07	0.79	0.08	0.78	1.2	0.31	0.8	0.40	0.4	0.54	0.6	0.46
	CABR	3.6	0.12	1.9	0.23	0.1	0.69	0.1	0.70	1.6	0.26	38	0.003**
	CC	1.5	0.28	45	0.002**	4.3	0.10	6.5	0.06	8.3	0.04*	25	0.007**
	SSC	19	0.01**	35	0.004**	10	0.03*	5.3	0.08	3.9	0.11	14	0.01*
	SPC	22	0.009**	2.8	0.16	14	0.01**	17	0.01**	2.3	0.19	71	0.001***
	TPC	1.7	0.26	581	<0.001***	6.7	0.06	0.3	0.61	0.07	0.80	148	<0.001***
	POD	153	<0.001***	148	<0.001***	558	0.001***	46	0.002***	85	0.001***	337	<0.001***
	SOD	841	<0.001***	81	0.001***	39	0.003**	23	0.008**	44	0.003**	96	<0.001***
	CAT	5.4	0.08	93	0.001***	0.07	0.80	0.83	0.41	2.31	0.20	76	0.001***
	APOX	5.6	0.07	132	<0.001***	0.00	0.94	1.54	0.28	3.8	0.12	97	0.001***

^aKeys: * Significant at $p \leq 0.05$, ** significant at $p \leq 0.01$, *** significant at $p \leq 0.001$.

However, similar pattern results have been shown by the same treatment of 5 days drought + NAA in the case of osmolites presented in Figures 6 and 7. But results in the declared tolerant variety Iqbal at the highest level of induced drought along with NAA in the treatment (15 days drought + NAA) similar to the control treatment, indicating the role of sugar and proline as an osmoprotectant in true sense.

Antioxidants can signal a plant's resistance to certain stressors.³⁸ Drought stress reduces the activity of numerous enzymes and causes a net increase in free amino acids, which slows protein synthesis and/or activation and accelerates protein breakdown in plants. The lowest protein content in both cultivars reported at the highest induced drought of 15 days at $p < 0.05$ demonstrated how the crop plant response is affected by the drought intensity and duration, plant species, developmental stage, and growing season (Figure 8). However, in the current investigation, the lowest drought of 5 days, along with the growth regulator NAA, did not result in a decrease in total soluble protein content, indicating that proteins associated with antioxidant enzymes may have increased.

ROS detoxification mechanisms exist in all plants and can be classified as enzymatic or nonenzymatic.^{80,81} The extent to which antioxidant enzyme activities increase during drought stress varies greatly between plant species and even between cultivars of the same species. Results in Figures 9 and 10 revealed that (5-day drought + NAA) in Azam and Iqbal has high peroxidase and superoxide dismutase activity, whereas the lowermost of both activities has been reported in treatment with 15-day drought along with NAA at $p < 0.05$. This means that under maximal amplitude-caused drought stress, these activities cannot be enhanced with naphthyl acetic acid (NAA) spray as a growth regulator. Figures 11 and 12 indicated that the lowermost catalase activity in Azam and Iqbal has been reported in treatment (10-day drought + NAA), (12-day drought + NAA), and (15-day drought + NAA) at $p \leq 0.05$

along with ascorbate peroxidase reflecting less severity of the global variation in the start but will surely affect the crop productivity crossing the borderline even if the use of growth regulator will not protect the declination of crop productivity in the region.

3.3. Analysis of Variance of the Measured Characters.

The largest effect of NAA therapy was observed on SVI-I, LAI, LAR, RSR, CA, CB, TCC, CABR, SSC, SPC, TPC, POD, and APOX at $p \leq 0.001$, whereas on CC and CAT at $p \leq 0.01$, according to the variance analysis results in Table 2. However, the relations concerning the genotype and treatment were estimated on SVI-II, LAI, RSR, TB, CA, CB, and SSC up to a significant level at $p \leq 0.01$, TCC, SPC, and APOX at $p \leq 0.01$, and POD and CAT at $p \leq 0.05$. The observable results are shown in Table 3 for all parameters excluding GRI, TB, CC, and SPC; the ANOVA interactions among the genotype and naphthyl acetic acid treatment and between the genotype and drought stress were accounted significant at $p \leq 0.05$, while the interactions between elements and naphthyl acetic acid were established to be significant at $p \leq 0.05$ for GRI, SVI-II, and SOD, individually. It was determined that the genotype differences in the interactions between drought factors under the NAA treatment had a significant disparity. At $p \leq 0.05$, the effect of NAA therapy on SVI-I, SVI-II, RSR, LAI, LAR, TB, CA, CB, CC, POD, and SOD was considered to be significant (Table 4). Low levels of drought stress and NAA treatment were shown to be significantly correlated with SVI-I, CB, POD, and SOD, with $p \leq 0.001$; RSR, CB, and SPC, with $p \leq 0.01$; and SSC, with $p \leq 0.01$.

3.4. Biological Constituents via Principal Component Analysis. The principal component analysis results were based on 19 characters and explained 70.99% of the total variance, with the first PC1 accounting for 57.18% and being significantly correlated with GRI, SVI-I, LAI, LAR, TB, CA, CB, TCC, CC, SSC, SPC, TPC, APOX growth response,

osmolytes, and antioxidant enzyme activity. The second PC2, on the other hand, explained 13.81% of the total variation and was associated with the plant growth regulator and the responsorial component (SVI-II, RSR, and CABR) (Table 5 and Figure 13).

Table 5. Eigen Values (EV), Variation Explained (VE) in %, Cumulative Variance (CV) in %, and Coefficients of Determination (CD) of the First Three Principal Components Based on the Correlation Matrix (CM) of Biological Components (BC) under Induced Drought Stress

traits	eigen values	variance (%)		components		
		individual	cumulative	PC1	PC2	PC3
GRI	10.865	57.185	57.185	0.759	0.188	0.384
SVI-I	2.624	13.810	70.996	0.628	0.288	0.488
SVI-II	1.860	9.788	80.784	0.542	0.690	0.154
LAI	1.040	5.475	86.259	0.759	0.188	0.384
LAR	0.802	4.220	90.479	0.636	0.630	0.357
RSR	0.619	3.258	93.737	0.392	0.425	0.125
TB	0.331	1.743	95.480	0.636	0.630	0.357
CA	0.248	1.308	96.788	0.884	0.294	0.038
CB	0.205	1.081	97.868	0.916	0.063	0.125
TCC	0.148	0.780	98.648	0.884	0.072	0.368
CABR	0.119	0.625	99.273	0.187	0.436	0.305
CC	0.078	0.412	99.685	0.909	0.042	0.302
SSC	0.031	0.161	99.847	0.914	0.220	0.090
SPC	0.015	0.078	99.924	0.743	0.266	0.130
TPC	0.009	0.045	99.970	0.963	0.141	0.150
POD	0.005	0.027	99.996	0.778	0.472	0.271
SOD	0.001	0.004	100.000	0.873	0.385	0.162
CAT	2.21	1.164	100.000	0.762	0.320	0.519
APOX	8.37	4.407	100.000	0.732	0.345	0.528

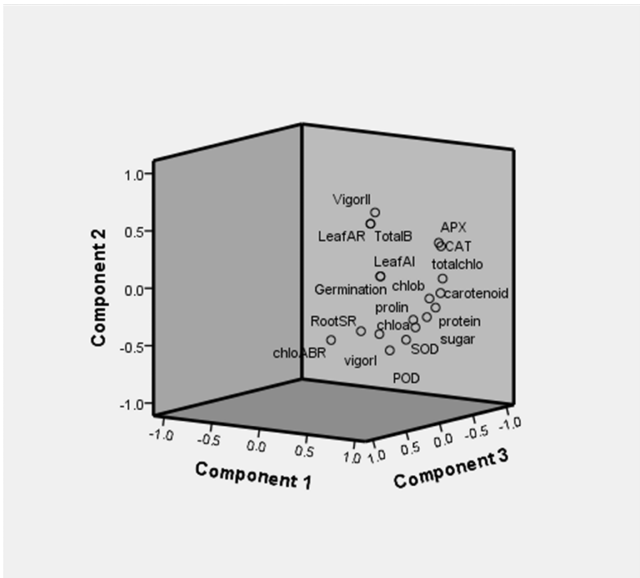


Figure 13. Loading plot of PC1, PC2, and PC3 in rotated space.

4. DISCUSSION

With extreme temperatures and rainfall, decreased water availability, and changes in soil quality,^{82,83} climate change is the most pressing issue confronting humanity today.^{84–86}

These effects will have an impact on agricultural productivity and will make it more difficult for plants to grow and thrive globally.^{87,88} Human actions are the primary cause of climatic fluctuations that are distinct throughout decades to millennia, according to ref 89. Since plants are subjected to a variety of abiotic pressures, reactions are more complicated and many stress pathways overlap, making plant responses to abiotic stresses dynamic, complex, and both elastic and plastic.⁹⁰ According to "Adapting Crops to Climate Change," drought is the main abiotic stress that is anticipated to worsen as a result of climate change. In genetic engineering and breeding efforts around the world today, drought resistance and water use efficiency are given the most consideration. By the end of the 20th century, predictions state that extreme drought will affect 30% of the area.⁹¹ Because agriculture uses over 70% of water utilized globally, there will be a significant increase in the demand for irrigation in the future.

The current study looked specifically at how the physiological state and productivity of *Z. mays* L. were affected by induced drought stress. It may be an indication of the impending regional climate change and its impact on crop yield declines, which will have an impact on Pakistan's economic stability as an agricultural nation. Water stress in the current study had an impact on every aspect of maize's vegetative character, including its germination rate index, vigor index, weight, leaf surface area index, root/shoot ratio, root density, fresh and dry weight, total biomass, and more. With the foliar application of NAA as a growth regulator and a lower level of induced drought stress of 5 days, a reduction in the amplitude of all of the agronomic qualities described above was easily regulated and improved. However, high drought stress of 15 days negatively impacted plant growth both with and without NAA, showing that water scarcity of high amplitude caused by or due to the impending climate variation will not be encountered by the use of a growth regulator and will undoubtedly reduce biomass and yield of the crop plants. Similarly, our findings demonstrated that under stress conditions, plant mass decreased due to inappropriate cell division and elongation, resulting in tuberization of the roots, and root size decreased due to the low osmotic potential and cellular expansion.⁴⁸ The plants' heights also showed significant variation in growth.^{92,93}

Our findings are consistent with prior research revealing that under drought stress, tissue concentrations of chlorophyll and carotenoid content are reduced, among other alterations caused by ROS production in the thylakoids. Carotenoid reduces triplet chlorophyll in photosynthetic tissues, resulting in the generation of singlet oxygen, which protects against oxidative damage.²⁵ Our work demonstrated that both maize varieties undergo considerable physiological and biochemical alterations. At the vegetative stage, drought stress of a lesser amplitude of 5 days along with NAA foliar spray maintained the normal range of growth responses in terms of chlorophyll "a," chlorophyll "b," total chlorophyll, and chlorophyll a/b ratio, along with carotenoid content, whereas the long-term exposure to 15-day drought adversely affects the mentioned growth responses, which are in accordance with Parekh et al.⁹⁴ who reported that decreased chlorophyll content in plants under environmental stress, plant illness, and photosynthetic productivity also changed pigment content. Water scarcity affects the actions of photosynthetic enzymes, reduces metabolic competence, and eventually destroys photosynthetic machinery.⁹⁵ Because of water stress conditions, stomata

remain closed, which in turn disturbs CO₂ fixation and their translation into a complex energy-rich compound and retards the process of photosynthesis.⁹⁶ However, the second assumption is that ROS containing OH, H₂O₂, and singlet oxygen are derivatives of imbalanced metabolism consequent upon the environmental stress triggered, which can deteriorate the photosynthetic membrane system.⁹⁷

A significant increase in the sugar and proline content was reported under induced drought stress conditions in both cultivars in the designed experiment representing similarity with the work of Hamada⁹² and Yadav et al.,⁹⁸ who explained that water stress increased proline and sugar accumulation in *Gossypium hirsutum* as both act as osmoprotectants of cytosolic enzymes and cellular structures. These osmolytes gathering can help the crop survive short periods of drought (5 days in the current study) and recover from such stress, but long-term exposure to water-deficit conditions, as predicted by future climate change, will require not only the use of growth regulators but also biotechnological and breeding efforts. In the present work, both an increase as well as a decrease in the content of protein reported in the designed experiment reflect the different nature of proteins, though some are upregulating during stress conditions, and the level of that particular protein will be enhanced, whereas some type of protein undergoes downregulation, and their level will be decreased when the plant undergoes stress conditions. The increase and/or decrease also depend upon the nature and duration of oxidative stress induced. This perception comes from the designed experiment supported by the reduction in soluble protein content in moth beans reported by ref 99 and the increase in maize reported by ref 100.

Under water-deficit conditions, numerous mechanisms have been adopted by plants including tolerance, escape, and evasion, whereas the best mechanism is antioxidant enzymes production, where an excessive amount of ROS was scavenged via activation of strong antioxidant enzymes comprising catalase, peroxidase, ascorbate peroxidase, and superoxide dismutase.¹⁰¹ The present findings revealed that short-term drought treatment at the vegetative stage increased antioxidant enzymes including SOD, POD, CAT, and APOX activities in both cultivars when NAA was used as growth regulators, whereas long-term exposure decreased these activities even with the availability of NAA. Our results are similar to ref¹⁰⁰, revealing that plants have developed various antioxidant enzyme systems to survive with oxidative stress of lower levels caused by unfavorable environmental conditions.

5. CONCLUSIONS

The current study evaluated the effect of various levels of induced drought stress on the physiological mechanism of drought resistance in chosen maize cultivars. It is concluded that under induced drought stress, a significant decrease in leaf water status and chlorophyll and carotenoid contents was associated with an increase in the accumulation of sugar, proline, and antioxidant enzymes at $p < 0.05$, along with an increase in protein content as a dominant response for both cultivars. Under high drought stress, variance analysis in terms of interactions between drought and NAA treatment was explored and found significant at $p < 0.05$ for physiological and biochemical features after 15 days. The inhibitory effect of short-term water stress was alleviated by exogenous NAA application; however, yield loss due to long-term osmotic stress

will not be overcome by employing growth regulators. According to the results of the planned experiment, it is expected that a changing climate will cause drought stress, affecting the production of Pakistan's most valuable crops, notably maize, as well as negatively impacting ecological fitness. As a result, the groundwork for developing exceptional crop types that are ready for climate change has been laid. To address the food and fuel security issues of a growing population, biotechnology and breeding efforts to harness these mechanisms must be increased during the next decade. Regardless, crop development professionals must exercise caution and ensure that potentially exploited approaches are practical in the context of Pakistan's ever-changing environment. A drought-mitigation strategy is critical for Pakistan's drought-prone regions. This should also include the effects of climate change on the availability of water resources, as well as the development of coping mechanisms to deal with the effects of drought. This plan must be integrated into the overall prospective development plan so that all sectorial development plans consider drought mitigation in their routine development activities.

■ ASSOCIATED CONTENT

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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ABBREVIATIONS

NAA, naphthyl acetic acid; GRI, growth rate index; SVI-I, seed vigor index (plant height); SVI-II, seed vigor index (plant weight); LAI, leaf area index; LAR, leaf area ratio; RSR, root–shoot ratio; TB, total biomass; CA, chlorophyll “a”; CB, chlorophyll “b”; TCC, total chlorophyll content; CABR, chlorophyll *a/b* ratio; CC, carotenoid content; SSC, soluble sugar content; SPC, soluble protein content; TPC, total proline content; POD, peroxidase; SOD, superoxide dismutase; CAT, catalase; APOX, ascorbate peroxidase; GCRI, Global Climate Risk Index; ROS, reactive oxygen species; EC, electrical conductivity; pH, power of hydrogen ion concentration; K, potassium; C, carbon; N, nitrogen; P, phosphorus

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