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ENHANCING DROUGHT STRESS RESILIENCE IN MAIZE PLANTS BY EXOGENOUS APPLICATION OF GIBBERELLIC ACID (GA_3)

UMBREEN BIBI AND SARA ZAFAR*

Department of Botany, Government College University, Faisalabad, Pakistan
**Corresponding author's email: sarazafar@gcuf.edu.pk*

Abstract

Gibberellic acid (GA_3) foliar application is an effective approach for maintaining plant integrity under various environmental stresses. The study was conducted to determine the influence of varying concentrations of GA_3 (control, 50, 100, 150 and 200 ppm) on key physico-biochemical traits of two maize cultivars (CIMMYT PAK and Gohar-19) grown under 100 and 60%, field capacity (FC). A significant reduction in shoot and root fresh biomass (3.66g, 2.29g), as well as dry biomass (2.09g, 1.3g), was recorded in Gohar-19 at 60% FC compared to 100% FC (4.33g, 2.68g), (2.43, 1.60g). However, under water-deficit conditions, Gohar-19 performed better than CIMMYT PAK in terms of biomass accumulation. Foliar spray of GA3 at 150 ppm effectively lessened the influence of drought stress by modulating the growth and increasing the biosynthesis of essential metabolites. In conclusion, topical application of GA3 improved drought tolerance by promoting growth, pigment levels, and non-enzymatic antioxidant activity. Foliar application of GA_3 is a great latent potential in the diminution of the negative impacts of water scarcity on crop production. Principal component analysis (PCA) revealed distinct variations in plant traits in response to GA3 foliar treatment under water shortage conditions. The novelty of this research is the identification of the optimal GA3 concentration as a viable approach for reducing the harmful impact of water scarcity on maize plants.

Key words: Water shortage, GA_3, Maize, Plant production.

Introduction

Maize (*Zea mays* L.), a member of the Poaceae family, is one of the most widely cultivated crops in tropical and subtropical regions around the world (Khan *et al.*, 2014). It ranks as the third-largest grain crop in Pakistan after rice and wheat (Harris *et al.*, 2007). To meet the growing demands of the world's rapidly increasing population, maize requires an adequate supply of essential nutrients to ensure high productivity. According to the Pakistan Economic Survey (Anon., 2022-2023), maize contributes 0.7% to the national GDP and accounts for 3.4% of the value added in agriculture.

A critical challenge to global food security, in addition to malnutrition, is the limited availability of freshwater for irrigation. Water scarcity, driven by the imbalance between food supply demand, is a major constraint to meet the agricultural needs (Wanders & Wada, 2015; Ali *et al.*, 2022). Accurate prediction of drought periods remains difficult due to the interaction of various factors such as evapotranspiration, soil absorption capacity, rainfall variability, and moisture retention (Barik *et al.*, 2019). Under drought conditions, plant growth is impaired due to disrupted gas exchange, nutrient imbalances, and altered plant-water relationships, ultimately leading to ionic disturbances and reduced yield (Liu *et al.*, 2018).

Low and irregular precipitation in arid and semi-arid areas leads to moisture stress, which restricts crop growth. Of all the environmental stresses, drought is particularly detrimental to yield of maize (Nuccio *et al.*, 2015). It negatively affects post-pollination processes such as embryo development and seedling survival, resulting in decreased productivity (Mao *et al.*, 2015). While some crop varieties are more resistant than others, all plants experience reductions in growth and yield under water deficit conditions. Nevertheless, various adaptive strategies are used by the plants to cope with drought stress, including morphological and physiological modifications (Mohammadi & Asadi-Gharneh, 2018). Plants respond to drought stress by initiating a range of adaptive mechanisms. These include morphological changes such as leaf rolling; the accumulation of osmoprotectants like proline; activation of defense systems; and the modulation of plant hormones. Drought also triggers the expression of stress-responsive genes, initiates complex signaling pathways, adjusts stomatal conductance to minimize water loss, and influences root exudates that affect rhizosphere microbial communities and promote beneficial symbiotic interactions (Kong *et al.*, 2017). Water scarcity disrupts anatomical, morphological, physiological, and biochemical processes, thus severely limiting maize development. The extent of drought damage depends on the crop's growth stage and the duration of stress (Setter *et al.*, 2001). When the moisture level around a plant roots drops too low for adequate water uptake, drought stress occurs (Benjamin, 2007). Drought also shares physiological similarities with other abiotic stresses, particularly due to similar osmotic imbalances and metabolic responses (Djibril *et al.*, 2005; Ahmed *et al.*, 2024).

Among drought mitigation strategies, agronomic approaches such as the exogenous application of growth regulators have shown promise. In contrast, plant feeding for drought resistance remains a long and complex process (Tinker, 2002). Plant growth regulators (PGRs), however, play a critical role in modulating physiological processes under drought stress. They improve water use efficiency by promoting stomatal closure and stimulating antioxidant accumulation to protect plants from oxidative stress. Additionally, PGRs influence root and shoot growth, even at low concentrations, by functioning as signaling molecules that facilitate intercellular communication (Bakhsh *et al.*, 2011; Baranova *et al.*, 2014). Among these regulators, gibberellic acid (GA3) plays a key role in seed germination, dormancy breaking, stem elongation, and flower induction (Ayyub *et al.*, 2013; Gupta & Chakrabarty, 2013). Gibberellins (GA3) are naturally released from the leaves and aid in promoting both root and shoot growth. When applied exogenously, GA3 supports plant cell elongation, promotes parthenocarpic fruit development, counters genetic dwarfism, and enhances branching, flowering, and fruiting. It also contributes to photosynthesis, stimulates cell division, and increases overall plant height, leaf area, and root size (Kondhare *et al.*, 2014).

The present study is planned to explore whether exogenous treatment of GA3, particularly at optimal concentrations, enhances drought stress resilience in maize plants through enhancing the physiological and biochemical characteristics and hence reducing the effects of water scarcity on plant growth and metabolism.

Material and Methods

The experiment was conducted at the Botanic Garden, Government College University, Faisalabad. Gohar-19 and CIMMYT PAK, maize cultivars were taken from the Maize & Millet Research Institute (MMRI), Yusafwala, Sahiwal. Seeds were sown in plastic pots in a completely randomized design (CRD), with three replications per treatment. There were 120 experimental units, including 60 pots at well-watered levels (100% FC) and 60 pots as drought stress (60% FC), each pot containing five seeds.

Gibberellic acid (GA3) was used at five rates (0, 50, 100, 150, and 200 ppm) to control and stressed plants of each maize cultivar. After germination, two seedlings were removed from each pot to ensure equal growth. On the seventh day after germination, plants were subjected to two drought stress levels to evaluate their responses under water-limited conditions. Twenty days after germination, one plant pot was removed from each pot to determine the important growth traits, including shoot and root length, and biomass.

Estimation of plant shoot length, root length and biomass: Shoot and root length was measured in centimeters with the help of a meter rod. Fresh and dry biomass of plant samples was measured in grams. In case of dry weight, plant samples were dried in an oven at 65˚C until constant weight was obtained.

Quantification of pigments: Chlorophyll a and Chlorophyll b contents were approximated according to Arnon's method (1949), and calculated by (Davies, 1977). GA3 treated and control plant leaves were harvested, and 0.5g of fresh tissue was homogenized in 5 mL of 80% acetone. The suspension was centrifuged at 1000 x g for 5 minutes. Supernatant absorbance was measured at 645, 663 and 480 nm.

Measurement of phenolic contents (TPC): The TPC was determined according to the method of Sakanaka *et al.*, (2005). Leaves were homogenized in 80% acetone, and the extract was centrifuged for 12 minutes at 15,000 rpm. To 0.1 mL supernatant, 250 µL of 20% Na_2CO_3, and 500 µL of Folin-Ciocalteu reagent were added. The mixture was incubated, and absorbance was read at 750 nm.

Total soluble proteins (TSPs): TSP content was measured by using the Bradford assay (1976). Fresh maize leaves equivalent to approximately 250 mg were homogenized in 5 mL of phosphate buffer with a pH of 7.0. The mixture was centrifuged at 12000 rpm for 12 minutes. Absorbance was measured at 595 nm.

Total soluble sugar contents (TSS): TSS content was measured by the Liu *et al.*, (2004) method. Fresh maize leaves (250 mg) were extracted in 5 ml of 1% acidic methanol. The reaction solution was centrifuged for 12 minutes at 12,000 rpm. In another tube, added 3 ml of anthrone reagent was mixed with 200 µL of the supernatant. The test tubes were placed at room temperature and then heated at 95°C for almost 10 minutes. Absorbance was read at 625 nm.

Total free amino acid contents (TFA): TFA was determined according to the procedure of Crampton *et al.*, (1957). Fresh maize leaves (0.25g) were blended with 1 ml of 10% pyridine and one ml of 2% ninhydrin. The reaction mixture was placed at room temperature; and heated for almost 30 minutes. The absorbance was recorded at 570 nm.

Total flavonoid content (TFC): The estimation of TFC was done according to the procedure of Marinova *et al.*, (2005). Maize plant leaves 0.5 g were homogenized in five ml of 80% acetone and filtered. In another tube, 0.5 mL of the filtrate was combined with 2 mL of distilled water and 0.6 ml of 5% $NaNO_2$. The liquid was cooled at ambient temperature, and 0.5 mL of 10% aluminum chloride was added. To the reaction mixture 2ml of 1 M NaOH was added. Absorbance was measured at 510 nm using a UV-visible spectrophotometer.

Statistical analysis: Data was analyzed statistically with the help of Statistix 8.1 software. Mean and standard error were computed on Microsoft Excel 2007. Least significant difference (LSD) test was used to make treatment means comparison at 5% probability level.

Results

Morphological and biochemical traits: Drought stress resulted in a decrease in shoot length in both maize cultivars. Foliar application of gibberellic acid (GA_3) at 150 ppm, however, greatly increased the shoot length, especially in the Gohar-19 cultivar, to 38.6 cm (100% FC) and 34.7 cm (60% FC). CIMMYT PAK, in comparison, had shoot length of 35.3 cm and 33.1 cm under the same conditions. Similarly, an upsurge in root length was observed at 150 ppm GA_3 (20.5 cm) under 60% FC in Gohar-19 cultivar as compared to CIMMYT PAK (20 cm) (Fig. 1).

Foliar spray with GA3 remarkably increased the shoot fresh weight under both irrigated and water scarcity conditions. At 150 ppm GA3, Gohar-19 had the maximum shoot fresh weight of 4.8 g (100% FC) and 4.0 g (60% FC). CIMMYT PAK had slightly lower values of 4.1g and 3.8 g under the same conditions. Among all the treatments, 150 ppm GA3 always produced the maximum shoot fresh weight in both the cultivars.

The GA3 treatment also enhanced shoot dry weight under water stress. In Gohar-19, 150 ppm GA3 application showed 2.8g and 2.4g shoot dry weight at 100% and 60% FC. However, at 150 ppm CIMMYT PAK cultivar exhibited 2.6g and 2.4g shoot dry weight. Furthermore, at 150 ppm of GA3, an elevated shoot dry weight was recorded in comparison to other levels. Foliar application of 150 ppm of GA3 recorded higher root fresh weight under drought stress. CIMMYT PAK cultivar, compared to the Gohar-19 had an elevated root fresh wt. at 150 ppm of GA3. Regardless of varietal differences, drought stress greatly reduced the fresh wt. of the roots.

Similarly, at 100% and 60% FC, the root dry weight was greater in the GA3 treatment at 150 ppm. Compared to CIMMYT PAK, Gohar-19 had greater root dry weight at 150 ppm of GA3 than the other levels.

Chlorophyll a and b levels showed considerable improvement in Gohar-19 compared to CIMMYT PAK cultivar. Foliar spray of GA3 at 150 ppm had higher chl a, b, chlt and carotenoid contents under 100% and 60% FC in Gohar-19 compared to CIMMYT PAK (Fig. 2). This characteristic was drastically reduced under drought stress, regardless of cultivar differences.

Foliar application of GA3 showed improved phenolics and flavonoid contents under 100% and 60% FC (Figs. 3, 4). In Gohar-19 at 50,100,150 and 200 ppm GA3 showed 6.0, 6.5,6.9 and 6.6 mg g^{-1} f. increased weight in phenolic contents under 60% FC respectively. Similarly, 5.6, 5.7, 6.9 and 6.3 mg g^{-1} f.wt. increase in phenolic contents was noticed in CIMMYT PAK under 60% FC. Again, the maximum values were recorded at 150 ppm.

Foliar application of GA3 at 150 ppm significantly increased TSS (1.4 and 1.6 mg g^{-1} f.wt.) in Gohar-19 plants under 100% and 60% FC as compared to plants without any application. In CIMMYT PAK, 1.3 and 1.4 mg g^{-1} f.wt. was observed in this trait with the treatment of 150 ppm under normal and stressed conditions, respectively.

Similarly, GA3 application at 150 ppm notably improved TSP under normal as well as water scarcity condition. In CIMMYT PAK cultivar, 3.9 and 3.7 mg g^{-1} f. wt in this trait was detected with the treatment of 150 ppm of GA3 under normal and stressed conditions, respectively. Regardless of cultivar differences water shortage notably decreased the TFAA. However, an increase was seen in CIMMYT PAK compared to Gohar-19 with the treatment of GA3 at 150 ppm relative to other treatments.

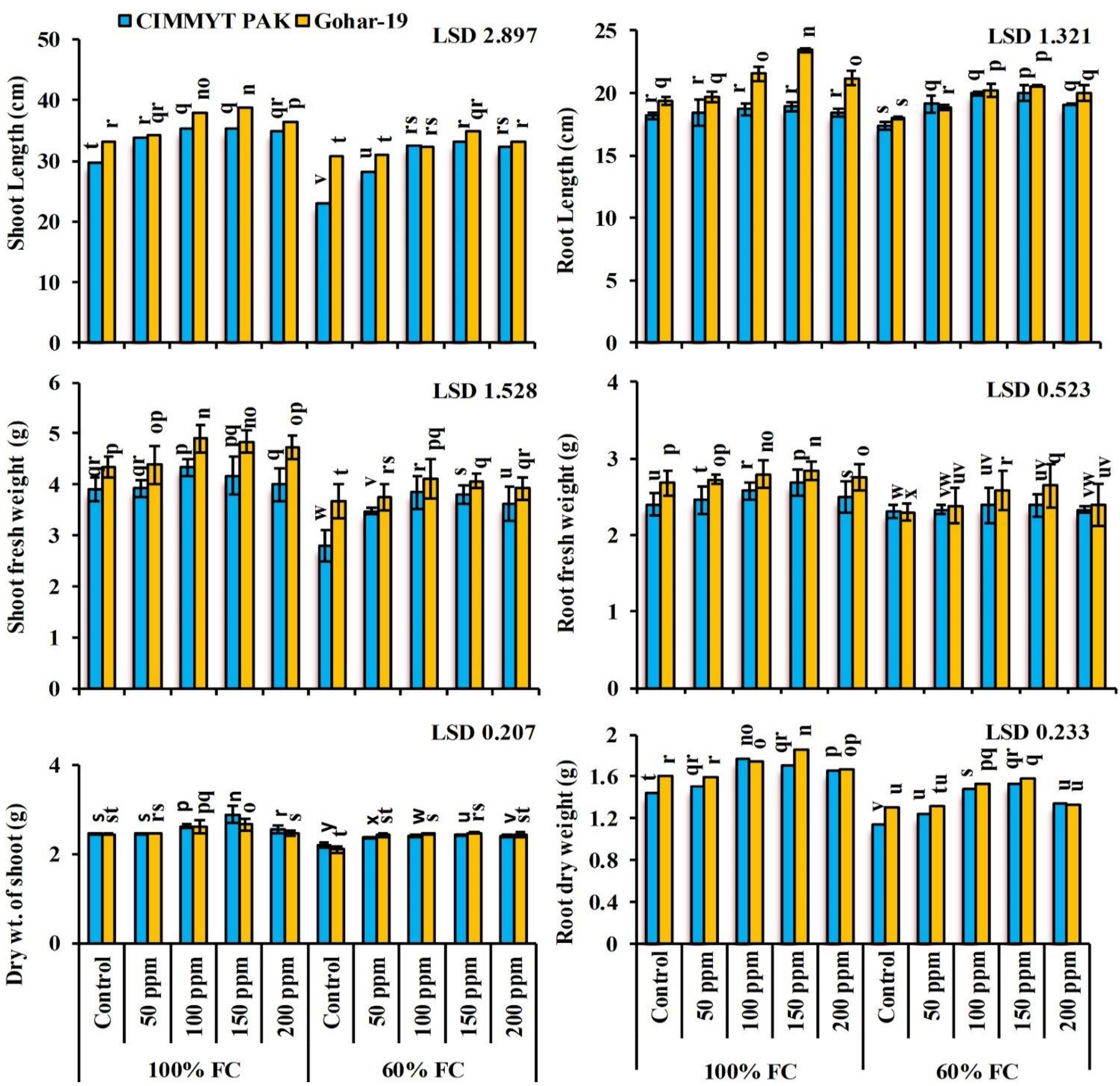

Fig. 1. Effect of foliarly applied GA3 on growth attributes of maize genotypes under 100% and 60% field capacity.

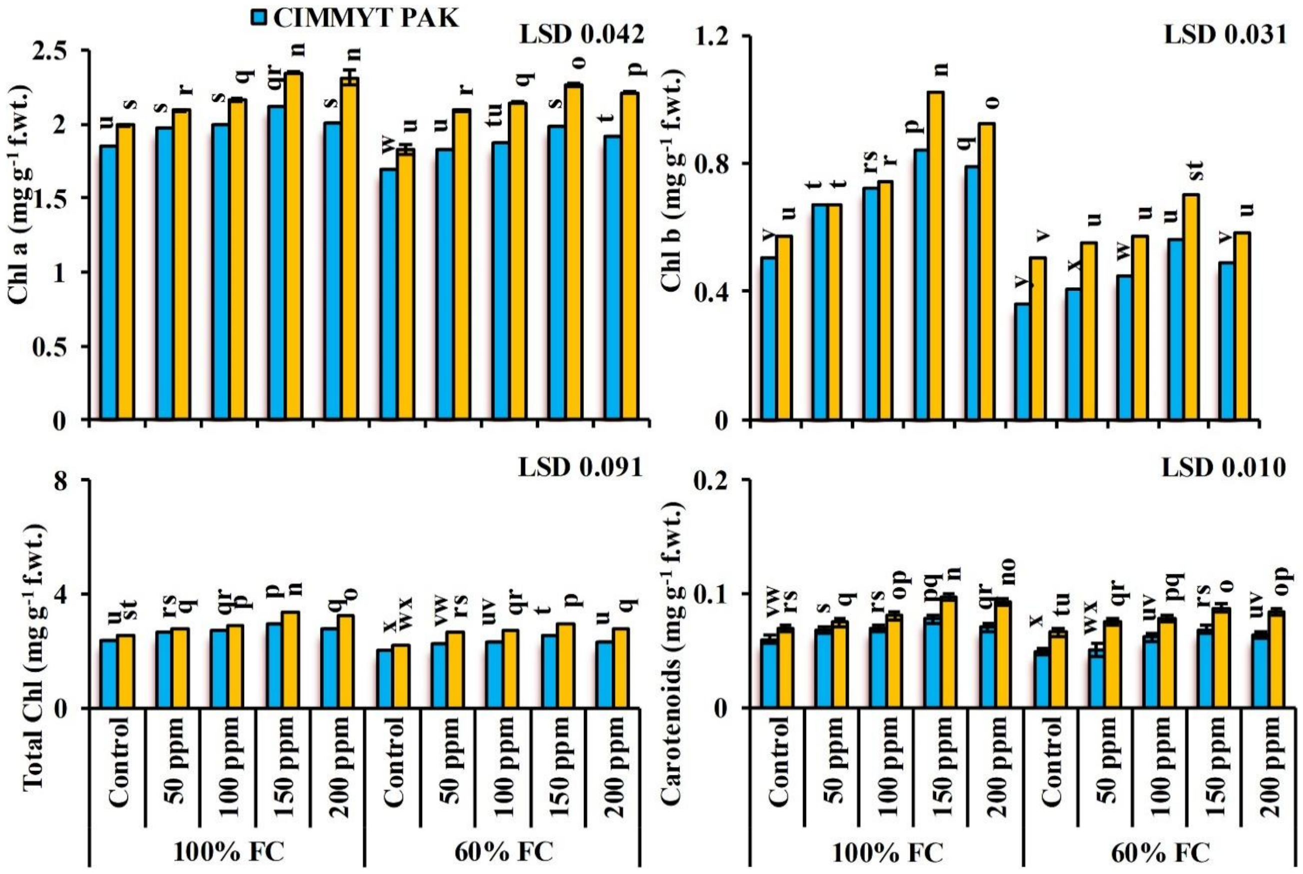

Fig. 2. Effect of foliarly applied GA3 on biochemical attributes of maize genotypes under 100% and 60% field capacity.

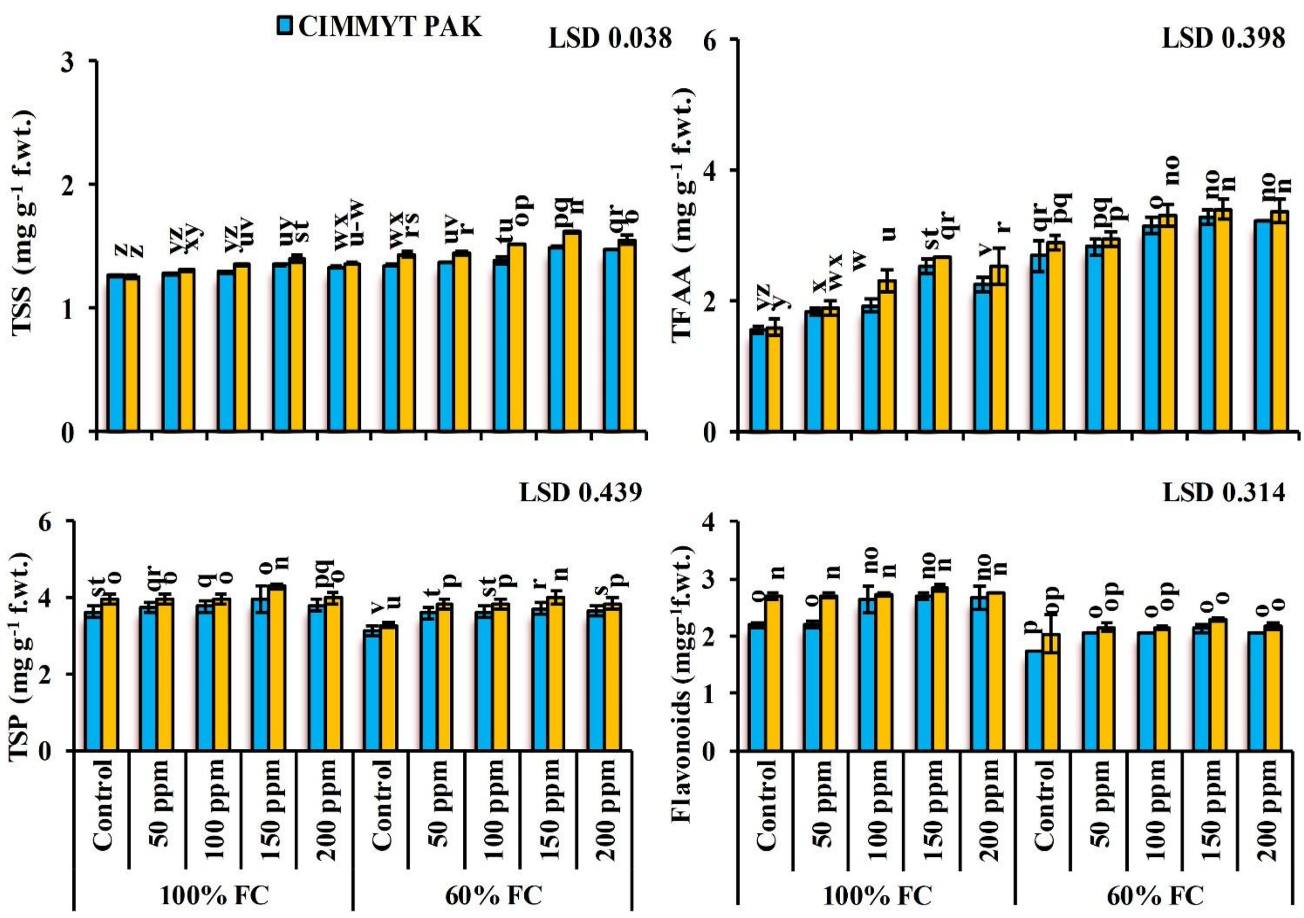

Fig. 3. Effect of foliarly applied GA3 on biochemical attributes of maize genotypes under 100% and 60% field capacity.

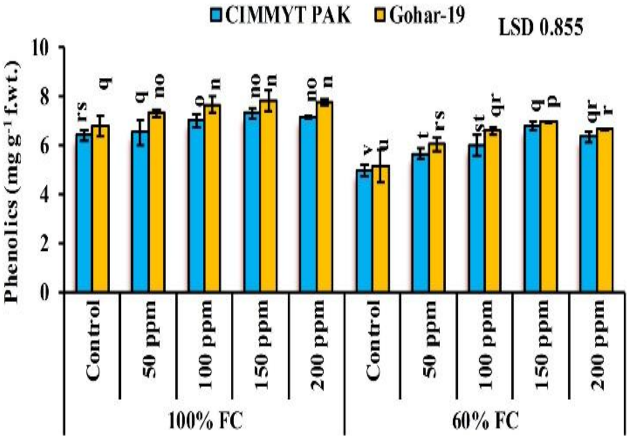

Fig. 4. Effect of foliarly applied GA3 on phenolic contents of maize genotypes under 100% and 60% field capacity.

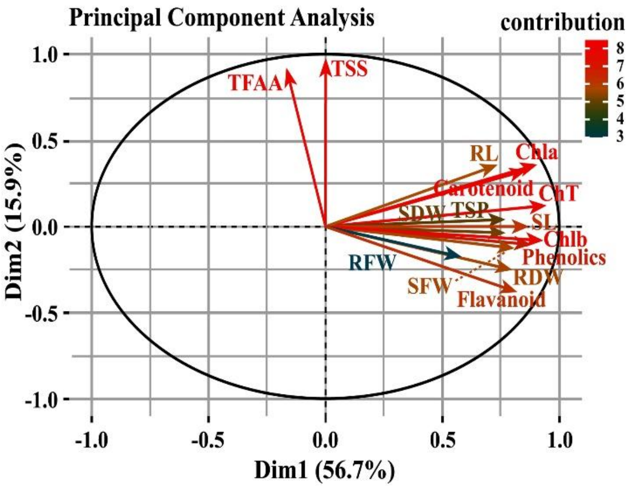

Fig. 5. Loading plots of principal component analysis showing interaction between various traits of maize plants under 100% and 60% field capacity.

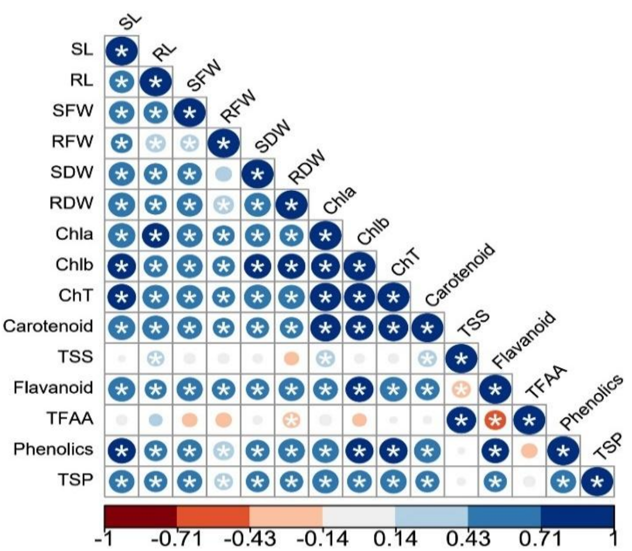

Fig. 6. Correlation among various traits of maize genotypes under 100% and 60% field capacity. Abbreviations: SL: shoot length; RL: root length; SFW: shoot fresh weight; RFW: root fresh weight; SDW: shoot dry weight; RDW: root dry weight; Chl a: chlorophyll a; Chl b: chlorophyll b; Chl t: chlorophyll t; TFA: total free amino acids; TSS: total soluble sugars; TSP: total soluble proteins.

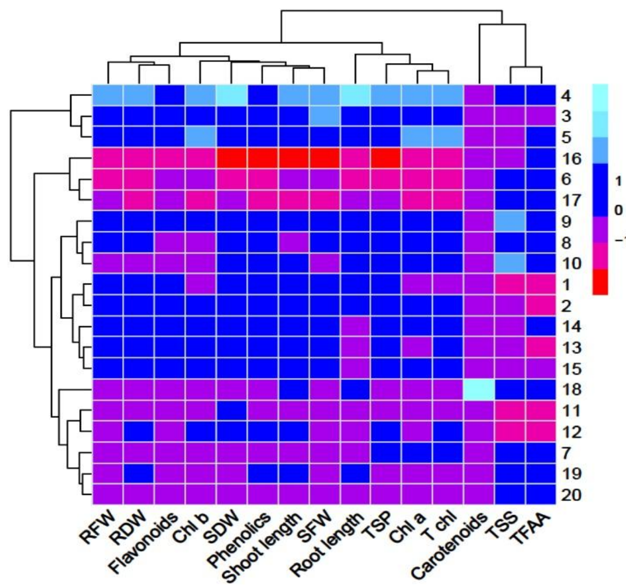

Fig. 7. Correlation of heat map histograms between several characteristics of maize plants under 100% and 60% field capacity (FC) in CIMMYT PAK GA3 0 ppm [1], GA3 50 ppm [2], GA3 100 ppm [3]), GA3 150 ppm [4], GA3 200 ppm [5], GA3 0 ppm (60% FC) [6]), GA3 50 ppm (60% FC) [7], GA3 100 ppm (60% FC) [8], GA3 150 ppm (60% FC) [9]), and GA3 200 ppm (60% FC) [10], in Gohar-19 GA3 0 ppm [11], GA3 50 ppm [12], GA3 100 ppm [13]), GA3 150 ppm [14], GA3 200 ppm [15], GA3 0 ppm (60% FC) [16]), GA3 50 ppm (60% FC) [17], GA3 100 ppm (60% FC) [18], GA3 150 ppm (60% FC) [19]), and GA3 200 ppm (60% FC) [20]. Abbreviations: TSP stands for total soluble protein; TFAA for total free amino acid; TSS for total soluble sugars; RFW for fresh weight of roots; RDW for dry weight of roots; SFW for fresh weight of shoots; Chl. a for chlorophyll a; Chl. b for chlorophyll b; Chl T for total chlorophyll.

Principal component analysis (PCA): It summarizes various plant traits to different GA3 treatments under drought and control conditions. Dim1 (56.7%) and Dim2 (15.9%) are the two principal components. Together, they explain about 72.6% of the total variation in data. It shows most of the differences between treatments and traits can be seen in this two-dimensional plot. The PCA confirms that GA3 foliar application, especially at 150 ppm, significantly alters the expression of several key traits under drought stress. Traits related to osmoprotection and antioxidants contributed most to the variation, supporting that GA3 enhanced drought tolerance by modulating metabolic and growth responses (Fig. 5).

Correlation matrix plot: Traits like shoot length, root length and biomass related traits are strongly positively correlated (dark blue with stars) (Fig. 6). GA3 treatment enhanced photosynthetic pigment production aiding stress resilience. Non enzymatic antioxidant defenses and osmolyte accumulation exhibited a coordinated metabolic response to drought and GA3. Soluble protein accumulation responded to GA3 and drought but is tightly linked to growth or other metabolic traits.

Heat map histogram: The heatmap graphically summarizes GA3 application modulation of plant water stress response (Fig. 7). Pigment contents and defense responses exhibited significant improvement with GA3, whereas biomass was suppressed under drought but showed improvement with GA3 application. This corroborates the conclusion of the abstract that GA3 foliar spraying, especially at 150 ppm, is an effective approach for alleviating water stress in maize by promoting growth and biochemical tolerance.

Discussion

Global crop productivity is adversely affected by water scarcity. The study aimed to explore the role of GA3 as a growth-promoting agent while assessing the drought response of two maize cultivars, CIMMYT PAK and Gohar-19. Water scarcity drastically affected shoot and root lengths, as well as biomass. This is in agreement with previous research in other crops like beans (Hussain *et al.*, 2020), rice (Wang *et al.*, 2019; Tahir *et al.*, 2023; Ali *et al.*, 2025), and soybeans (Chimungu *et al.*, 2014). Ali *et al.*, (2020) explained this decrease in maize due to interruptions in metabolic transport and damages in physiological and biochemical processes under water- deficit conditions. Among the different drought mitigation techniques, foliar spraying with growth regulators such as GA3 has been shown to be effective in promoting plant stress tolerance (Zafar *et al.*, 2018). Root and shoot growth enhancement through biostimulant treatment has been noted in mung beans and grams by Mahajan *et al.*, (2011). In the present research, 150 ppm GA3 greatly enhanced shoot and root length under drought stress, with a more positive response of Gohar-19 compared to CIMMYT PAK. The findings of Zafar *et al.*, (2021) were in line with our results, who showed that foliar spray of Mel increased total free amino acid and total soluble sugars levels thus increasing the growth of wheat plants under abiotic stress. The current study noticed that under water shortage conditions, morphological or growth factors like the length and biomass of the root and shoot was decreased. Application of GA3, however, lessened the negative effects of the drought stress. The decrease in growth attributes under drought stress was caused by changes in the plant-water relationship, disruptions to the photosynthetic pigments, and oxidative damage to macromolecules resulted in membrane degradation (Arif *et al.*, 2021).

Drought stress was also reported to be detrimental to photosynthetic pigments like chlorophyll a, b and carotenoids. This reduction is usually attributed to stomatal closure and downregulation of photosynthetic genes, particularly in the Calvin cycle. Foliar application of GA3 enhanced chlorophyll content under stress and non-stress conditions indicating its protective effect on the photosynthetic machinery. These results were akin to those found in spinach by Munné-Bosch & Alegre (2000). According to Sharma *et al.*, (2020), injuries to the photosynthetic system under stress result in a reduced amount of chlorophyll content, disrupted chloroplasts, and elevated levels of the enzyme chlorophyllase in plants. Gibberellic acid protective effects are linked to decreased breakdown of chlorophyll and increased photosynthetic activity (Khanna, 2012). In this investigation, exogenously given GA3 (150 ppm) increased chlorophyll concentration and decreased symptoms associated with stress, it may be due to the fact that some genotypes are more susceptible to drought stress (Zafar *et al.*, 2018).

In addition to growth and pigments, biochemical responses were also influenced by drought and GA3 treatment (Ma *et al.*, 2016). Our results showed that contents of total free amino acids, total soluble sugars and phenolic compounds were higher under drought stress. These compounds are reported to act as osmoprotectants and antioxidants enabling plants to preserve cellular integrity and turgor pressure during stress (Elahi *et al.*, 2022). Phenolic compounds in plants provide a range of secondary metabolites with antioxidant qualities that help prevent oxidative damage caused by stress (Krol *et al.*, 2014; Zhang *et al.*, 2020). The total phenolic contents vary in stressed conditions (Hassan *et al.*, 2015). These results were in agreement with those of Rivas-Ubach *et al.*, (2012), who observed higher concentration of total phenolic compounds in grapevine roots under water shortage conditions. The flavonoid accumulation also enhanced under water stress, in line with findings from research on Adonis species (Gao *et al.*, 2020) and *Oudeneya africana* (Talbi *et al.*, 2020). The plants of both maize cultivars exhibited some variation in the accumulation of total soluble protein during drought stress. Total soluble protein level in the leafy areas of *Zea mays* genotypes under drought condition initially increase and then decline, according to Riccardi *et al.*, (1998).

When exposed to drought stress, the total soluble sugar content of both cultivars of maize was increased. These outcomes resemble those of Sperdouli & Moustakas (2012), indicating a stress-protecting role of sugars in signaling and membrane stabilization (Watanabe, 2000). Soluble sugars, which are generated as hydrolytic activity byproducts, substrates in biosynthetic processes, energy sources, and parts of systems that sense and interact with sugar, are recognized to play a complicated and crucial role in plant metabolism. According to recent research, even sugar flow may serve as a signal for metabolic control in drought-stressed environments (Kishor *et al.*, 2005). Moreover, soluble sugars function as an osmo-protectant, continuously maintains turgor pressure and stabilizes cell membranes. Gibberellic acid, a plant growth regulator, improves morphological and biochemical markers; however, the optimum outcome was noted at 150 ppm. As GA3 concentration is increased, growth, yield, and quality metrics of plants showed significant improvement. Growth and biochemical markers improved most when GA3 was applied topically at a dose of 150 mg L^{-1} (Elahi *et al.*, 2022). The exogenous use of GA3 creates novel pathways to enhance the mechanisms of abiotic resistance, particularly under water scarcity, which could alleviate the financial problem of low output and aid in the treatment and rehabilitation of chronically malnourished individuals under climate variability. The PCA component (PC) captured most of the activity of the data with Dim 1 showing 56.7% of total variance and strongly related Chlb, SDW, phenolics, RFW, SFW, RDW, Flavonoids attributes. While Dim 2 explained 15.9% of the data with TFAA and TSS content.

To explore the association between growth and biochemical attributes, Pearson's correlation graph was constructed (Fig. 3). All the growth parameters are correlated with each other. However total free aminoacids and total soluble sugars were increased under stress conditions compared to other growth parameters indicating the adaptability of plants to tolerate harsh conditions.

In order to illustrate the correlations between the morpho-physio-biochemical characteristics of maize plants by exogenous GA3 administration, histogram correlation analysis was carried out. In a histogram analysis, cyan and red colors represented the differences in the treatments, while blue depicted similar behavior.

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