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Penicillium citrinum-infused Biochar and externally applied auxin enhances drought tolerance and growth of *Solanum lycopersicum* L. by modulating physiological, biochemical and antioxidant properties

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Abstract

Drought stress is a critical issue in agricultural crops as it can affect crop development, growth, physiological processes and yield. To meet nutritional needs amid rapidly dwindling water resources, it is crucial to adopt precise and efficient strategies through various amendments. Organic treatments such as biochar and the growth hormone auxin (IAA) can play a vital role in this regard. However, it is necessary to refine the existing amendment strategies to attain improved outcomes. Therefore, the present study used *Penicillium citrinum*-Loaded Biochar (PBC) with and without IAA as amendments under no drought stress in tomato plants. The effects of PBC and IAA on the tomato plants have been examined using several physiological, morphological and biochemical parameters. Results showed that plants sprayed with various doses (0, 10 ppm, 20 ppm, 40 ppm) of IAA and 3.0% (w/w) PBC increased root length (11.11 cm), shoot length (17.88 cm), plant height (38.12 cm), and leaf surface area (40.77 cm²). The highest increase in root fresh weight was 9.5 g, shoot fresh weight was 17.11 g, plant fresh weight was 25.44 g, while the root dry weight was 9.01 g, shoot dry weight was 15.99 g, and plant dry weight was 22.45 g was determined after the synergistic application of PBC and IAA. The proline contents (160.77 μmol g⁻¹ FW), total soluble sugar (45.33 μmol g⁻¹ FW), chl a (9.99 mg g⁻¹ FW), chl b (4.88 mg g⁻¹ FW), and carotenoids (8.88 mg g⁻¹ FW), SOD (3.88 mmol g⁻¹ FW), POD (7.66 mmol g⁻¹ FW), and CAT (3.91 mmol g⁻¹ FW), whereas relative water content and electrical conductivity were increased to 48.88% and 30.11% in T6. The H₂O₂ and MDA were decreased to (6.11 μmol/g FW) and (0.29 ± 0.20 μmol/g FW) respectively in T6 as compared to T1. In conclusion the combination of PBC and IAA is an effective, and eco-friendly way to meliorate the growth, biochemical, physiological, and antioxidant enzyme activities of tomato plants under drought stress.

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Keywords *Penicillium citrinum*-Loaded biochar, Osmotic stress, Physiological parameters, Biochemical attributes, Tomato

Introduction

By 2050, there is a world imperative to double agricultural products to fulfill the rising requirement for food. The Asia is having the second highest population globally and is vulnerable to the impact of climate change [1]. Drought, accountable for to decrease around 50% of crop yield and disturbing 40% of arable land, poses a risk to food security internationally [2]. Meanwhile, Pakistan's population is growing at a rate of 2.1%, significantly outpacing the global average of 1.1% [3]. Drought stress, a recurring and complex challenge for farmers, severely affects plant reproduction, molecular activity, and crop yield [4]. As a prolonged water shortage, drought impacts multiple dimensions, hydrological, meteorological, socio-economic, and agricultural [5]. This situation highlights the urgent need to develop strategies and technologies that can enhance soil water and nutrient retention, boost crop yields, and improve overall agricultural productivity. Recently, biochar has gained global attention as a promising solution due to its ability to improve soil physico-chemical properties, offering an effective approach to mitigate the crisis.

Addressing the drought's impact, biochar arises as a critical substance in protecting crops from abiotic stresses like drought and salinity [6]. Biochar (BC) is a high carbon content material prepared as a result of pyrolysis of the organic material expected to be applied as a soil amendment [7] enhance soil functions and properties [8]. BC can boost the soil water retention potential, in various soil conditions [9]. It also enhanced the microbial activity and nutrient retention and soil exchange capacity [10]. It has a large, porous surface area which is facilitative in decreasing the drought stress (DS) [11]. Biochar's amendment enhances soil porosity, alleviate water transmission and aeration, thus enhancing crop growth in a water stress [12]. Furthermore, BC positively enhance nutrient uptake, maintaining plant cell osmotic potential and decreasing rate of transpiration [7]. In addition, BC addition enhances antioxidant enzymes activity (CAT, SOD, and POD) contributing to increase antioxidant activity, water relationships, and seedling growth under drought stress [13].

Various studies have proved that microbial species associated with different parts of plants can enhance plant growth and health via Phyto-stimulation, microbe-originated compounds such as biocontrol and fertilizers [14]. Symbiotic associations with beneficial bacterial and fungal strain are an efficacious plant survival tactic that assist them adopt in stressed conditions [15]. Different bacterial and fungal strain are are found

within plant species considered endophytes [16]. Some fungal species obtained nutrients and shelter via symbiotic relation from plants tissue. Fungal endophytes produce different signaling molecules to bypass intrinsic plant defense system that benefit the host plant in fighting extreme environments [17]. Endophytic fungi are advantageous microorganisms due to their ability to produce bioactive metabolites [18], which perform a vital part in maintaining the host plants growth [19]. These fungi release volatile compounds, phytohormones like gibberellic acid and indole-3-acetic acid (IAA), along with mineral-solubilizing enzymes and antioxidants, all of which assist to maintain the physiological balance of the host plant. Additionally, fungal endophytes synthesize various stress-responsive compounds crucial for plant survival under stress conditions [20]. Plant growth promoters and protectants originate from the phyla Basidiomycota, Mucoromycota, Ascomycota, and Oomycota, functioning under both ordinary and abiotic stress [21].

Plant growth regulators (PGRs) are organic substance that are either naturally occurring or synthetic and alter the biochemical reactions of plants [22]. The normal biochemical processes of PGRs can be modify to enhance or reduce the development and growth of plant body [23]. These compounds are referred to as plant growth regulators (PGRs) when applied exogenously [24]. They are also recognized as key factors in plant responses to abiotic and biotic stresses, functioning as signaling molecules that help maintain developmental and growth plasticity [25]. PGRs perform a vital role in vindicating drought stress through inducing various developmental and physiological changes [26]. They improve the plant's capacity to utilize water and essential nutrients more efficiently while also helping to alleviate the toxic effects of salt [27]. Moreover, PGRs significantly influence the antioxidant enzymes activity in plants [28]. By boosting the function of reactive oxygen species (ROS) scavenging enzymes, PGRs help maintain ROS at non-damaging levels under stress conditions, thereby contributing to improved salt tolerance [29]. The effectiveness of PGRs in stress alleviation is influenced by the plant's physiological condition, the applied concentration of PGRs, and environmental factors affecting their uptake [30, 31].

Abiotic stress typically leads to a decline in plant development and yield, primarily by disrupting the function of PGRs. These regulators play a vibrant role in orchestrating plant responses to stress by modulating biochemical, physiological, and molecular mechanisms [32]. Among PGRs, auxins are particularly key regulator

for plant development, influencing a range of physiological activities such as cell elongation, phototropism, gravitropism, hydrotropism, leaf arrangement (phyllotaxy), apical dominance maintenance, and root growth regulation [33]. Auxins also interact with other phytohormones to regulate various aspects of cell growth and development [34]. Their application has been shown to enhance berry size, yield, and carbon allocation, while moderating sugar accumulation, synchronizing ripening, and delaying fruit maturation beneficial traits for extending harvest periods and optimizing fruit processing [35]. Furthermore, auxins influence the sensory and volatile profiles of berries, indicating their potential in controlling fruit quality [36]. Auxins also regulate the expression of stress-responsive genes, with numerous studies highlighting their roles in auxin metabolism, transport, and signaling under environmental stress conditions [33, 37].

Solanum lycopersicum L. (Tomato) is a widely cultivated annual or perennial herb grown primarily for its edible fruit. Tomatoes are produced on over five million hectares worldwide, with an annual global yield exceeding 186.8 million metric tons [38]. Among the top ten tomato-producing countries, China ranks first, followed by India and the United States [39]. Essential nutrients like N, K, and P are critical for optimal tomato growth. Guo et al. (2021) [40] reported that the application of biochar resulted in a non-linear rise in tomato yield, with the highest yield observed at a rate of 40 t ha⁻¹ which is 1.6 times better than the untreated control. The usage of 10 t ha⁻¹ sewage sludge-derived biochar give rise to an increase in dry matter, along with more uniform fruit shape and size in the treated plants [41]. Biotic, abiotic, and socio-economic constraints hinder the full potential of tomato production. To address these challenges, specific integrated management strategies are required, which are economically viable, socially acceptable, and environmentally sustainable.

Based on the significance of biochar and auxin in plant growth and the devastating effect of drought in agriculture the ongoing study aims to study the effectiveness of PBC, both alone and in combination with the IAA, in enhancing the growth and stress resistance of tomato under DS. Specifically, the study aimed to evaluate how these organic amendments influence various physiological, morphological, and biochemical traits of tomato plants, such as plant height, leaf area, biomass, chlorophyll content, and antioxidant enzyme activities. The objective was to determine whether the synergistic application of PBC and IAA could serve as a sustainable and efficient strategy to promote plant development and improve tolerance mechanisms against osmotic stress, offering a viable resolution to the challenges posed by declining water resources in agriculture.

Materials and methods

The experiment was executed in the Plant Sciences Department at Quaid-I-Azam University (QAU), Islamabad, Pakistan to evaluate the drought tolerance of the Tomato in mid-October, 2024 using Biochar, IAA and *P. citrinum* microbial inoculants.

Preparation of biochar

Grass feedstock was collected from the agricultural farmland at the Islamabad capital territory (ICT), Pakistan, for the biochar production. The grass feedstock was chopped into small pieces, properly cleaned to remove dust and contaminants, and washed 03 times with deionised water. It was then shade-dried for 48 h. After that, the dried material was kept at 70 °C in a oven for 8 h, according to the standard protocol described by Pan et al. (2021) [42] with slight amendments. Subsequently, 200 g of the oven-dried feedstock was subjected to pyrolysis in a muffle furnace (Model: NEYO M-525 Series) at 450 °C for 5 h in a fastened pots to create an anaerobic condition. The resulting BC was disseminated in 10 mL of double distil water, sonicated for 15 min, and then dried again using the earlier described method. After desiccation, the BC was strained through an 80-mesh screen and stowed for later practice. Its basic physicochemical characteristics were analyzed according to the methodology of Amalina et al. (2022) [43].

Synthesis of of *P. citrinum* charged biochar

The *P. citrinum* strain was obtained from a preserved culture maintained in the Laboratory of Plant Pathology. To prepare *P. citrinum*-loaded biochar (PBC), the *P. citrinum* was cultured on potato dextrose medium and incubated with autoclaved seeds of *Sorghum bicolor* (L.) for two weeks at 25 °C, allowing fungal growth on the seed surfaces. The *P. citrinum* was initially combined with sorghum seeds to supply essential nutrients to the fungus before its attachment to the biochar surface. Subsequently, the fungus-coated sorghum seeds were assorted with the prepared biochar in a 1:1 weight-to-weight. This mixture was then stored in a sealed container at 4 °C in the dark for 7 days. During this period, fungal hyphae spread and occupy the biochar surface. Following incubation, the *Sorghum* seeds were separated, and the resulting PBC was analyzed with the help of a scanning electron microscope (SEM) (JEOLJSM, 25910). For comparison, pure biochar (without fungal inoculation) was also observed under SEM.

Soil sampling and characterization of soil

Soil samples for this study were collected from the top 0–20 cm layer of an uncontaminated field in Islamabad, Pakistan, situated at an elevation of 1,663 feet from sea level, with a 25-year history of continuous cultivation.

Any unwanted materials, such as roots, were removed from the collected samples of soil. The soil was then dried in an air and sieved through a 2 mm mesh sieve. To ensure homogeneity, the soil was thoroughly mixed. Key soil properties, including texture, pH, electrical conductivity (EC), cation exchange capacity (CEC), organic matter, and total concentrations of carbon (C), potassium (K), phosphorus (P), and sodium (Na), were analyzed following the method of Meimaroglou et al. (2019) [44].

Experimental design

The seeds of tomato buy from a local seed supplier in Islamabad. A pot culture study was performed in a control condition using growth chamber at the Quaid-I-Azam University, Islamabad. The seeds of tomato weresterilized with 1% sodium hypochlorite for 2 min. For the current work, pots of 16 cm height and 22 cm width were filled with 650 g of soil. For treatments, PBC (3% w/w) was assorted in individual pots [45] and the soil was incubated under a under desiccated conditions for one week prior to the seed were cultivated. Six seedlings were grown in each pot and placed under controlled conditions. The stock solution of IAA was synthesized for various treatments. IAA was exogenously applied to the plants at different concentrations such as 0, 10 ppm, 20 ppm, and 40 ppm over a period of three consecutive weeks. To induce drought stress, it was applied at the heading stage by supplying a small amount of water and field capacity was maintained at 40%, while temperature was 30 °C in the growth maintained consistently throughout the experiment. Control plants were watered with tap water. The details of various treatments are given in Table 1. All the plants were harvested after three weeks, and their physicochemical properties, and antioxidant activities were subsequently analyzed.

Growth attributes

Various growth attributes were analysed to asses the overall growth and development of the tomato. Growth attributes of the harvested plants including plant height (PH), root length (RL), shoot length (SL), root fresh weight (RFW), root dry weight (RDW), plant fresh weight (PFW), and plant dry weight (PDW) were evaluated for each treatment. RL, SL, and PH were measured using a measuring tape, while fresh and dry weights were

determined using an electronic balance. For dry weight measurements, plant samples were oven-dried at 70 °C for 48 h, following the procedure described by Murtaza et al. (2024) [46].

Physiological parameters

Measurement of chlorophyll contents

For the determination of chl a, chl b, and carotenoids, the procedure of Zeeshan et al., (2020) [47] was used. To assess RWC, the methodology of Farhangi-Abri et al. (2023) [48] was followed. Relative electrolytic leakage (REL) was assessed through the standard protocol of Karimi et al., (2024) [49]. The RWC was calculated using the following,

$$RWC (\%) = [(FW - DW)/(TW - DW)] \times 100 \quad (1)$$

Where FW means fresh weight of sample, TW shows the weight of turgid and DW represent the dry weight of sample.

Biochemical parameters

Assessment of sugar and proline concentration

The procedure of Khan et al. (2021) [50] was followed to assess the total soluble sugar of the tomato plant leaves of individual treatment using the following equation.

$$\text{Sugar Content} = K \text{ value} \times \text{dilution factor} \times \text{absorbance/sample weight} \quad (2)$$

Where the *k* value is 20.

A proline contents of plants were measured following the previous standard protocol of Hafeez et al. (2017) [51] by using the below given formula.

$$\text{Proline Content} = k \text{ value} \times \text{dilution factor} \times \text{absorbance/fresh weight of sample} \quad (3)$$

k has a value of 17.52, with a sample weight of 0.5 g and a dilution factor of 2.

Measurement of antioxidant enzyme assays

To assess the antioxidant defense system of the tomato in a DS, the activities of the superoxide dismutase

Table 1 Experimental treatments for drought stress induction, Biochar and melatonin application in *Sesbania*

Treatment	Drought Stress	Biochar application	Auxin Application
T1	40%	No application	No application
T2	No stress	No application	No application
T3	40%	3% (W/W)	No application
T4	40%	3% (W/W)	10 ppm
T5	40%	3% (W/W)	20 ppm
T6	40%	3% (W/W)	40 ppm

(SOD), catalase (CAT), and peroxidase (POD), were assessed [52].

Assessment of oxidative stress markers

The H₂O₂ concentration was determined following previous standard protocol [53]. For MDA determination the methodology of Nasiri et al., (2024) [53] was followed.

Data analysis

The experimental data were assessed with the help of SPSS version 20.0. Analysis of variance (ANOVA) was conducted to evaluate the effects of different treatments, and the Least Significant Difference (LSD) test at a significance level of $p < 0.05$ was applied to identify statistically significant differences.

Results

Characteristic of soil and Biochar

PBC applied to soil was prepared from grass feedstock by pyrolysis where the raw material is decomposed thermochemically at a temperature of 450 °C in an anaerobic environment in a vacuum furnace. Biochar was produced of grass feedstock. The basic properties of BC and soil is presented in Table 2 after their analysis.

Characterization of fungus (*Penicillium citrinum*)-charged biochar (PBC)

The scanning electron microscopy image of BC revealed a rough and porous surface morphology (Fig. 1A), which may facilitate fungal attachment and growth. The *P. citrinum* strain (Fig. 1B) adhered effectively to the BC, with most hyphae colonizing or spreading across its surface (Fig. 1C). It is likely that some hyphae penetrated through the pores present on the surface of biochar. The successful colonization of the microbial strain can be accredited to the roughness of the biochar.

Physiological and morphological attributes

The subsequent physiological and morphological attributes helped us in determining the response of tomato under various conditions.

Table 2 Characteristics of the soil and pure Biochar

Characteristics	Biochar	Soil
Ash concentration	1.18%	–
pH	9.71	7.9
Texture	–	Silty clay
Organic matter (g kg ⁻¹)	84.33	6.1
Total C (g kg ⁻¹)	61.5	–
Total P (g kg ⁻¹)	1.33	1.11
Total N (g kg ⁻¹)	9.33	1.87
Total K (g kg ⁻¹)	12.4	8.22
CEC (cmol kg ⁻¹)	11.66	7.11
E.C (dS/m)	–	1.233

Root length, shoot length, and plant height under drought stress

The findings revealed the lowest RL (7.11 cm) was observed in T1 but the remarkable increase in tomato RL in DS with the application of PBC and IAA was significantly enhanced (11.11 cm) at 3% of PBC and 40 ppm of IAA in DS condition, in comparison to control plants. The SL of uninoculated plants (T1) was also significantly declined under DS (12.22 cm). By PBC and IAA inoculation, an enhanced plant shoot length (17.88 cm) was examined at 3% of PBC and 40 ppm of IAA in DS condition, then control (Fig. 2). Evidently, the combined application of PBC and IAA increased RL and SL when subjected to DS soil as in comparison to the control (Fig. 2).

Regarding plant height, the treatment of DS had a huge effect on the growth of tomato. The lowest plant height, 19.33 cm, was recorded in T1, with the application of PBC the PH was increased to 34.11 cm in T3 whereas the highest PH of 38.12 cm was observed in the joined application of PBC and IAA under DS. Significant differences were found among all treatment groups in comparison to the control plants. The synergistic application of melatonin and biochar likely played an important part in boosting plant height and alleviating the negative impacts of DS (Fig. 2).

Effect of PBC and IAA on the leaf width (cm) of tomato

Under drought stress (T1), the leaf area of tomato plant typically decreases due to reduced cell expansion and accelerated senescence. However, the application of PBC and IAA has been shown to mitigate these effects. Biochar enhances soil texture, water holding capacity, and nutrient accessibility, producing a more favorable rhizosphere environment under limited moisture. Meanwhile, auxin, a key plant growth hormone, promotes cell elongation and division, enhancing leaf expansion. The application of PBC and IAA in different concentrations has influenced the leaf width of the tomato. The control plants have leaf width of 31.33 cm² was lowest among all the treatments. T6 with PBC, and IAA particularly at higher concentrations, resulted in a notable increase (40.77 cm) in leaf width (Fig. 3). The alliance of PBC and IAA led to synergistic effects, where the leaf width was more noticeable in comparison to control groups. At lower concentrations of IAA, the increase was less noticeable, but at higher concentrations, the plants exhibited substantial growth, declare that IAA perform a part in regulating leaf growth.

Effect of PBC and IAA on tomato root fresh and dry weight, shoot dry and fresh weight, and plant fresh and dry weight under DS

The treatment of biochar and auxin has a positive effect on the shoot, root, and overall plant fresh and dry weight, especially under stress conditions such as drought. When

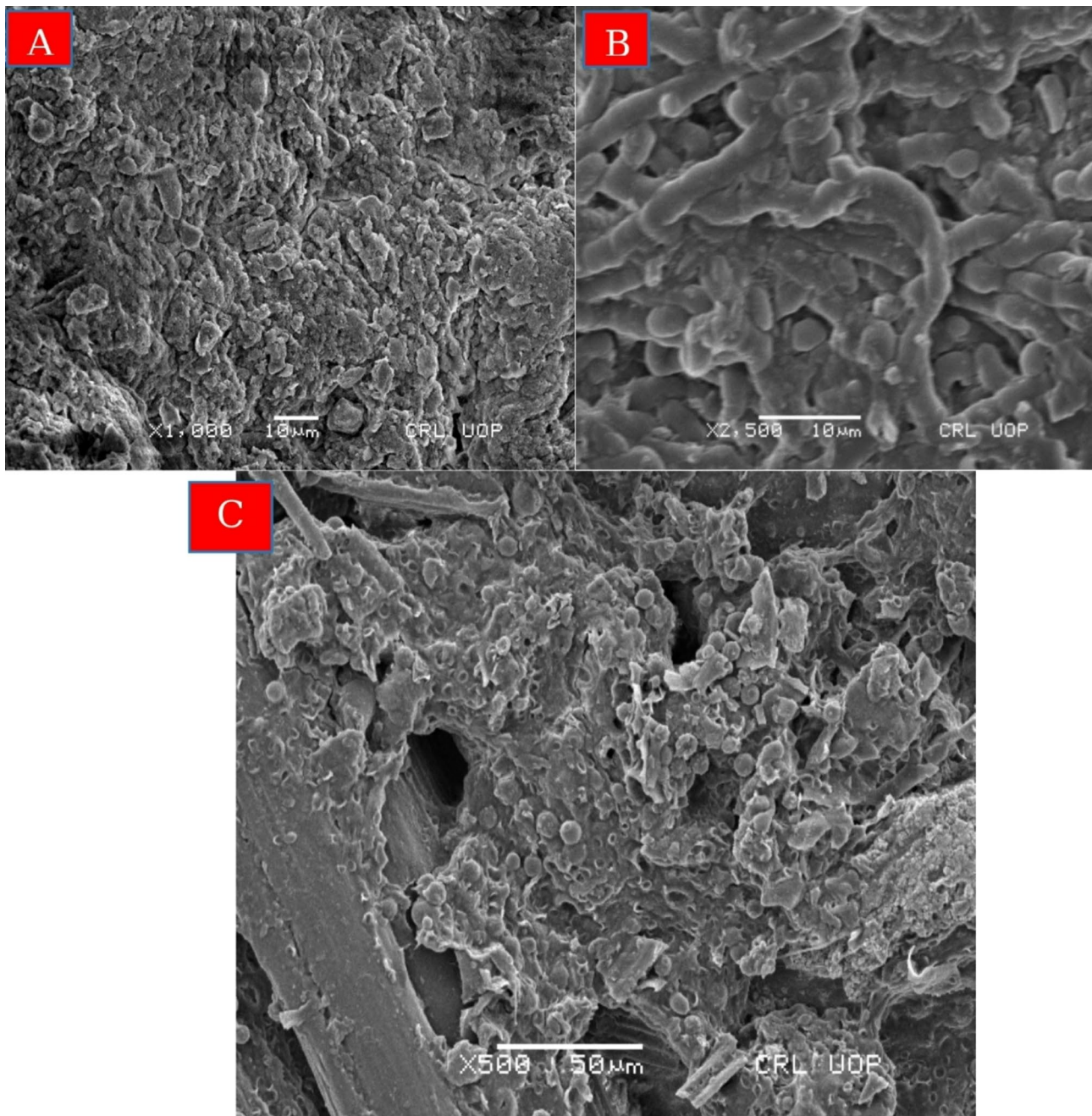


Fig. 1 SEM micrographs of pristine biochar (A), *P. citrinum* (B), and *P. citrinum* loaded biochar (C)

applied together, PBC and IAA create a synergistic effect, leading to significant increases in both fresh and dry weights of roots, shoots, and whole plants, reflecting improved plant vigor and biomass production. The RFW, SFW, PFW were 7.31 cm, 11.56 cm, and 17.55 cm, while RDW, SDW and PDW were 6.99 cm, 10.33 cm, and 16.11 cm in the PBC and IAA, independent treatments (T1), respectively. With the application of PBC the RFW, SFW, PFW were 8.44 cm, 14.44 cm, and 20.55 while RDW, SDW and PDW were 7.66 cm, 12.88 cm, and 18.55 cm in

the PBC (T3) application only. The co-application of PBC and IAA treatments, increased the RFW, SFW, PFW were 8.98 cm, 15.66 cm, and 22.44 cm while, the RDW, SDW and PDW were 8.11 cm, 13.33 cm, and 19.77 cm in T4. Similarly, RFW, SFW, PFW were 9.11 cm, 16.11 cm, and 24.88 while RDW, SDW and PDW were 8.66 cm, 14.55, and 21.66 cm, in T5 respectively. The highest increase in RFW was 9.5 cm, SFW was 17.11 cm, PFW was 25.44 cm, RDW was 9.01 cm, SDW was 15.99 cm, and PDW was 22.45 cm in T6. These whole results are depicted in Fig. 4.

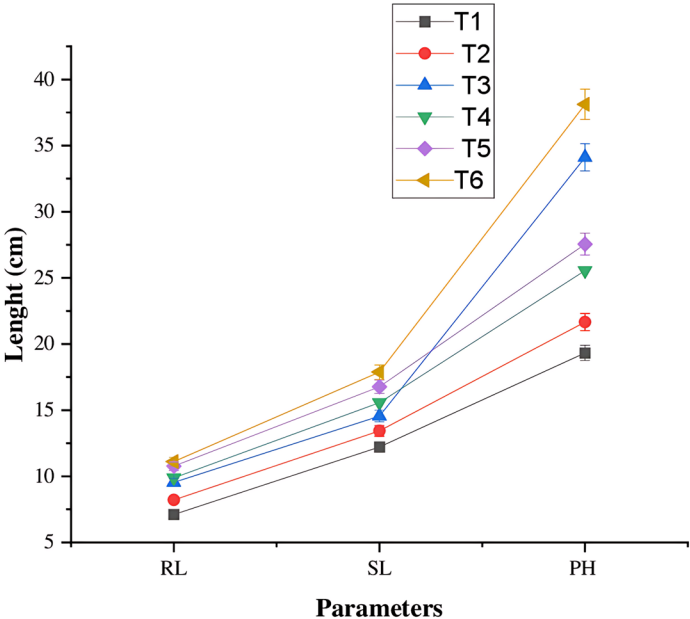


Fig. 2 Effects of PBC and IAA on RL, SL and PH of Tomato in DS

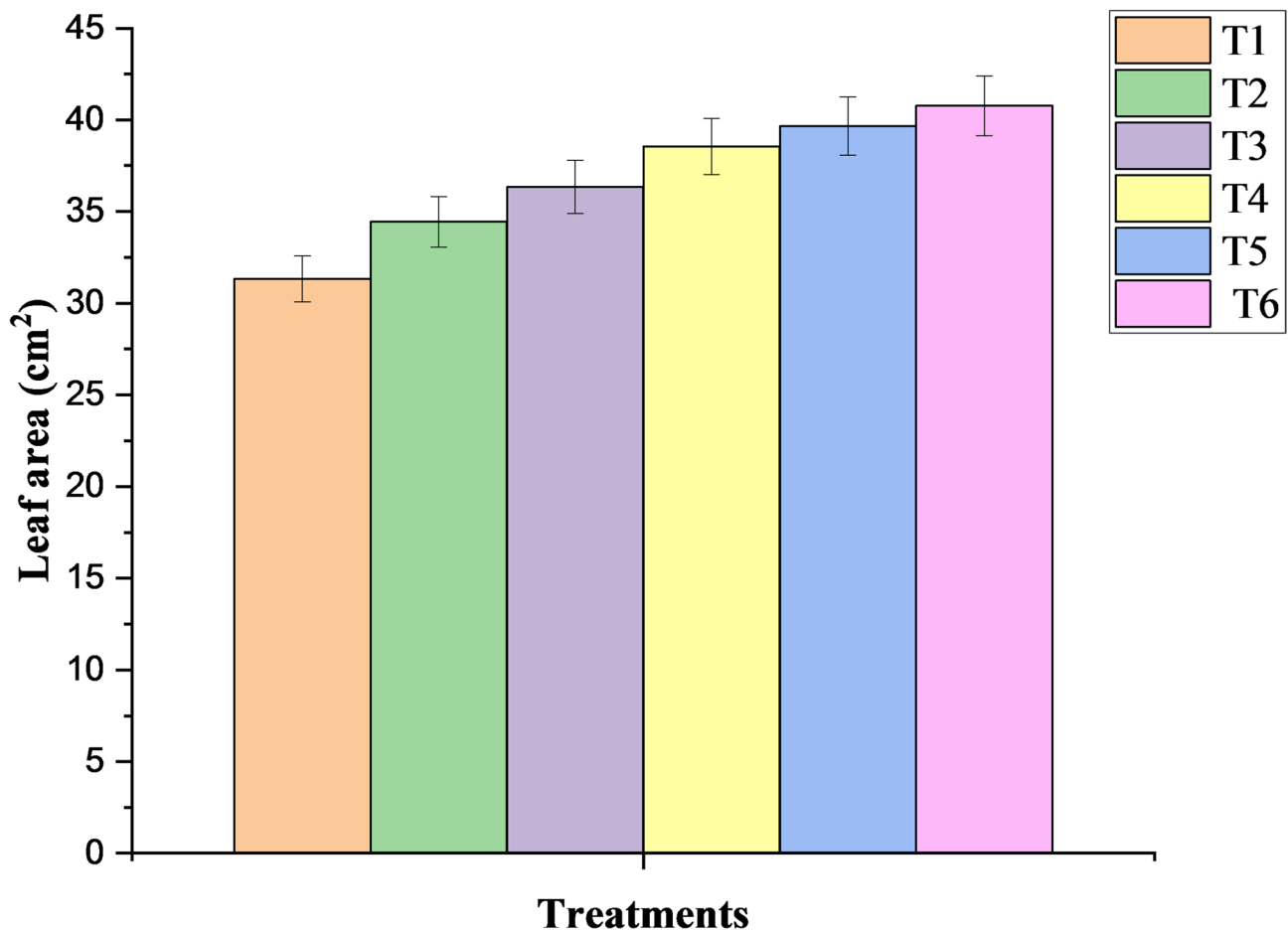


Fig. 3 Comparison of leaf area in control and PBC and IAA treated tomato plant samples in DS

PBC enhances soil aeration, water-holding capacity, promoting healthier and more extensive root systems. This, in turn, supports greater shoot development and biomass accumulation. IAA further contributes by stimulating root initiation, elongation, and shoot growth through its role in cell division and expansion.

Biochemical parameters

TSS, and proline concentrations

The amendment of PBC and IAA sustained better sugar, and proline concentration in amended plants in comparison to the untreated plants (Fig. 5). These findings confirm the positive role of our treatments. The lowest sugar ($24.88\mu\text{mol g}^{-1}\text{FW}$), and proline ($110.11\mu\text{mol g}^{-1}\text{FW}$) was observed in T1. The dose dependant increases in TSS, and proline concentration was observed with the maximum amount of $45.33\mu\text{mol g}^{-1}\text{FW}$ and $160.77\mu\text{mol g}^{-1}\text{FW}$ was observed at 3% of PBC and 40 ppm of IAA (T6) respectively.

Effect on carotenoid and chlorophyll contents

The effects of PBC and IAA under ordinary and drought stress on photosynthetic pigments are depicted in Fig. 6.

The application of PBC and IAA under water stress increased the chl a, chl b and carotenoids contents as compared to controls. The lowest contents of chl a ($5.88\text{ mg g}^{-1}\text{FW}$), chl b ($3.11\text{ mg g}^{-1}\text{FW}$) and carotenoids ($6.11\text{ mg g}^{-1}\text{FW}$) were observed in T1 under water limited control. The application of PBC enhances the chl a ($6.99\text{ mg g}^{-1}\text{FW}$), chl b ($4.44\text{ mg g}^{-1}\text{FW}$) and carotenoids contents ($7.44\text{ mg g}^{-1}\text{FW}$). With the addition of IAA along with PBC further increase the plant growth. The dose dependant increases the chl a, chl b and carotenoids contents were observed with the highest amount of the chl a was $9.99\text{ mg g}^{-1}\text{FW}$, chl b was $4.88\text{ mg g}^{-1}\text{FW}$ and carotenoids contents was $8.88\text{ mg g}^{-1}\text{FW}$ was noted in T6.

Effect of PBC and IAA on oxidative stress markers

EL and RWC (%)

Electrolyte leakage, which identify the membrane damage, was also greatly effected by the amendments (Fig. 7). Control plants (T1) exhibited a mean EL of $50.33 \pm 1.12\%$ and $46.11 \pm 1.13\%$ respectively. PBC application alone reduced EL to $40.11 \pm 1.12\%$ individually without

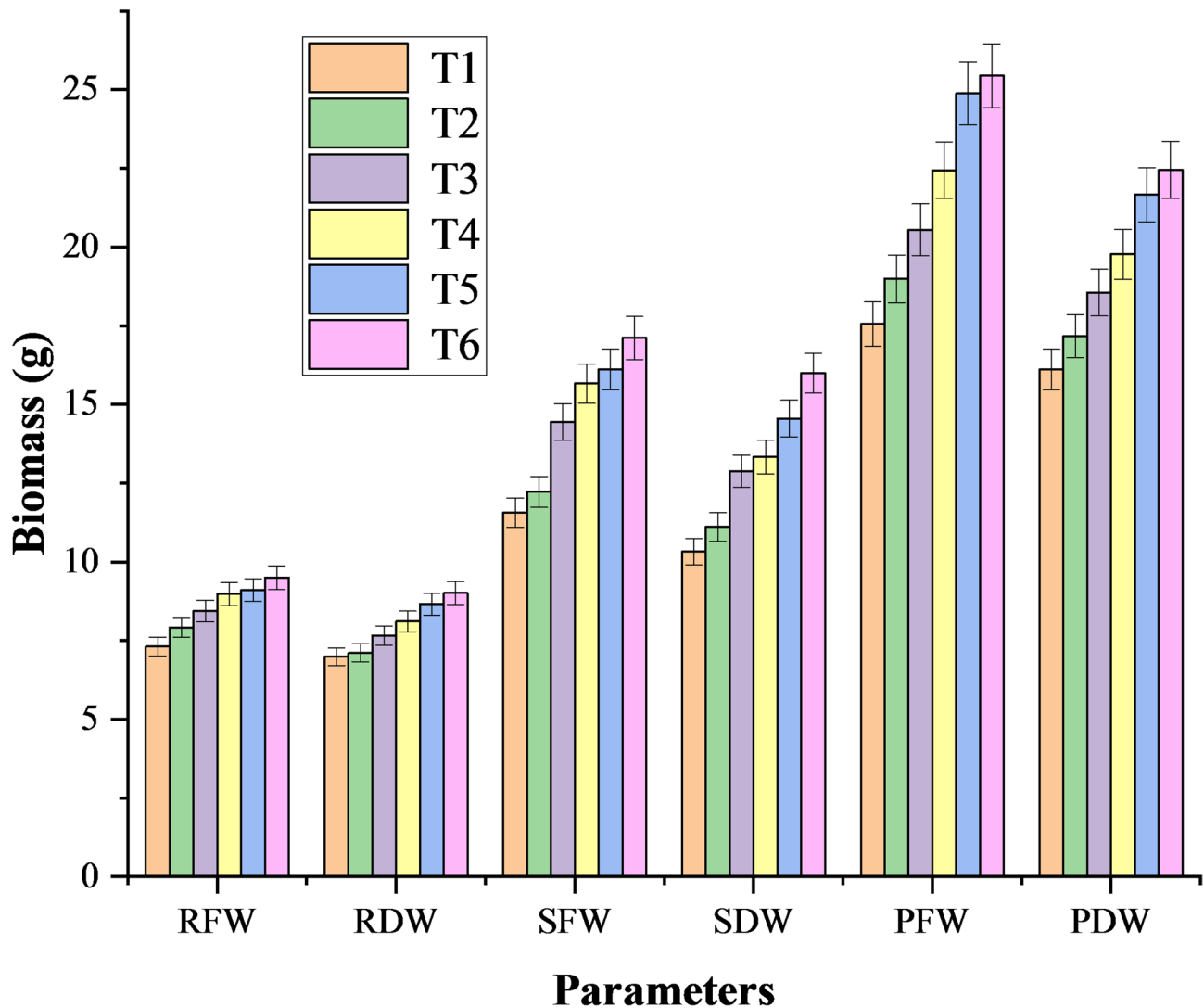


Fig. 4 Effects of PBC and IAA on Fresh and Dry weight of root, shoot and plant of tomato in DS soil

application of IAA. The joined application of PBC and IAA resulted in decrease in leakage to $35.66 \pm 1.11\%$ at 3% of PBC and 10 ppm of IAA, $30.44 \pm 1.13\%$ at 3% of PBC and 20 ppm of IAA, and $26.99 \pm 1.10\%$ at 3% of PBC and 40 ppm of IAA under stressed environments. Drought stress markedly increased electrolyte leakage to $50.33 \pm 1.12\%$.

RWC of the tomato plants reduced significantly in drought stressed treated plants ($39.77 \pm 0.90\%$). The application of PBC significantly maintained RWC ($42.66 \pm 1.20\%$) under drought stressed conditions (Fig. 7). With the addition of IAA further increase in RWC was noted. The combination of IAA and PBC (T6) increased and the highest RWC was $51.88 \pm 1.60\%$ at 3% of PBC and 40 ppm of IAA under stressed conditions.

H₂O₂ content ($\mu\text{mol/g FW}$)

DS increased oxidative stress markers. As a marker, elevated levels of hydrogen peroxide can indicate oxidative stress, which may be linked to various diseases and cellular damage. Application of PBC alone did not significantly decrease H_2O_2 content, which was $9.44 \mu\text{mol/g FW}$, while the synergistic application of PBC and IAA resulted in decrease of the H_2O_2 content under non-stressed conditions. The lowest concentration H_2O_2 was $6.11 \mu\text{mol/g FW}$ in T6, when we have applied 3% of PBC and 40 ppm of IAA (Fig. 8). DS significantly increased H_2O_2 content to $11.33 \mu\text{mol/g FW}$. Moreover, both PBC and IAA amendments under DS effectively alleviated this increase.

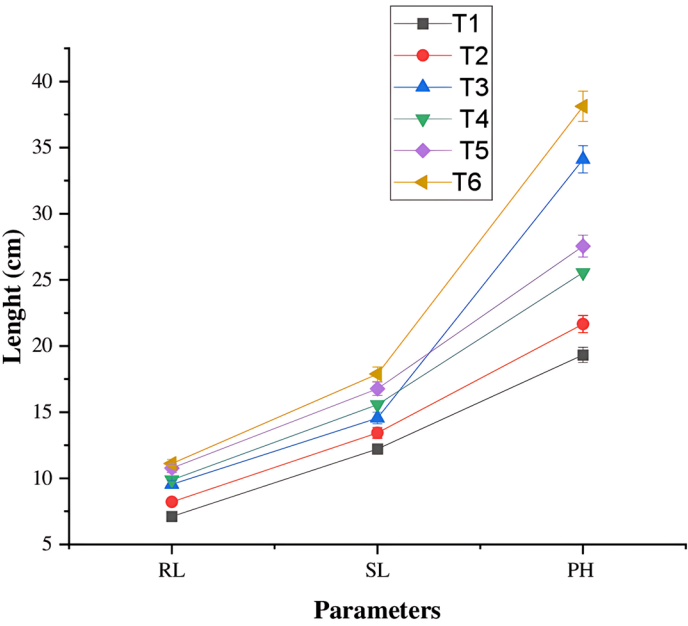


Fig. 5 Effects of PBC and IAA and on total soluble sugar, and proline, contents of tomato in DS conditions

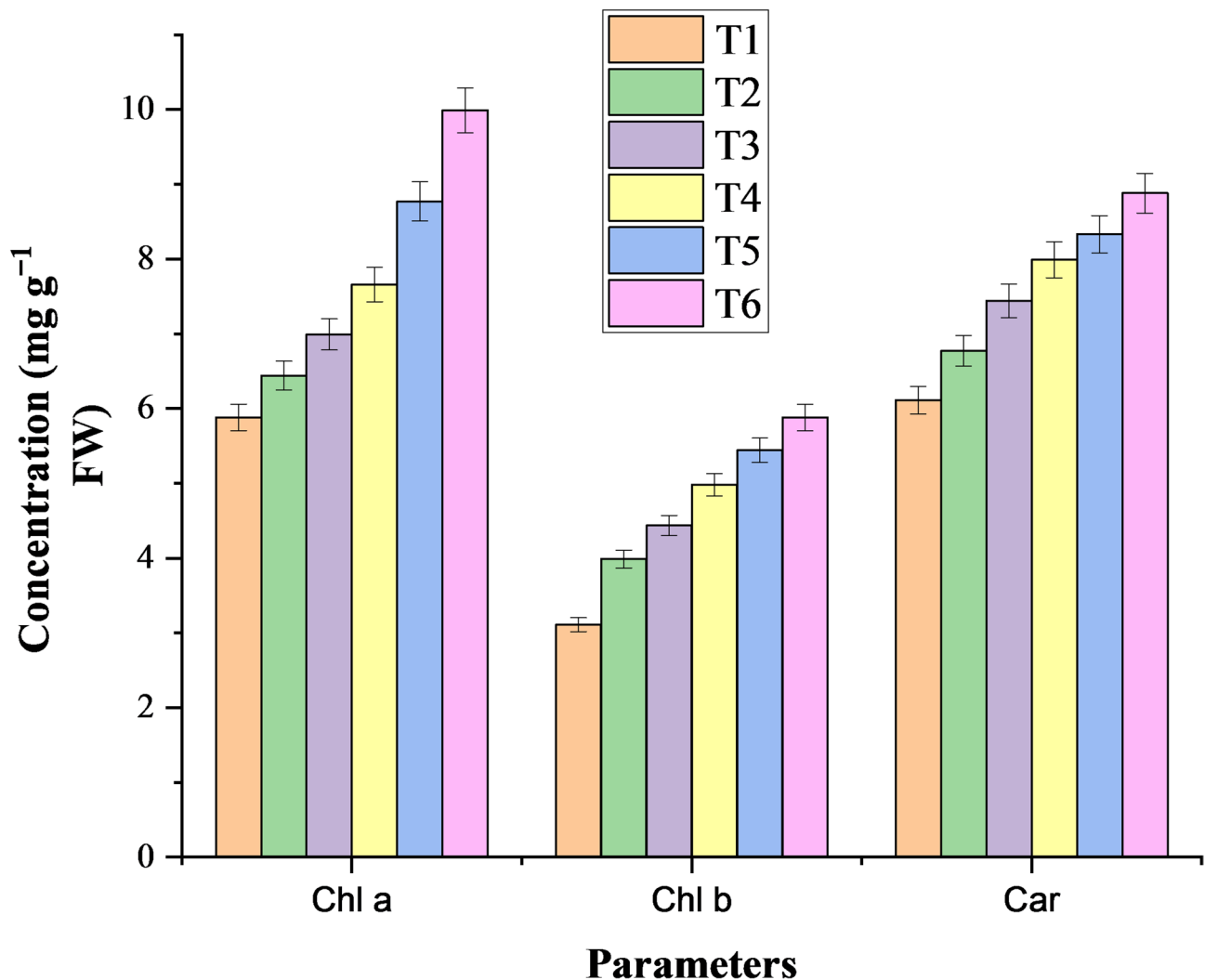


Fig. 6 Effects of PBC and IAA on Chl a, Chl b, and Carotenoids concentration of tomato plants in DS

Malondialdehyde content

MDA concentration in tomato plants, work as a marker for lipid peroxidation, was significantly effected by DS (Fig. 9). Control tomato plants had a mean MDA concentration of $0.98 \pm 0.50 \mu\text{mol/g FW}$. Treatment of PBC reduced MDA concentration to $0.66 \pm 0.19 \mu\text{mol/g FW}$, and IAA application further decreased it to $0.51 \pm 0.44 \mu\text{mol/g FW}$ at 10 ppm, $0.44 \pm 0.67 \mu\text{mol/g FW}$ at 20ppm, and $0.29 \pm 0.20 \mu\text{mol/g FW}$ at 40ppm. The joined use of PBC and IAA resulted in the minimum $0.29 \pm 0.10 \mu\text{mol/g FW}$ content in T6. The decrease in MDA content indicating decline in lipid peroxidation.

Antioxidant enzymes

Enzymatic antioxidant activities varied notably in response to different treatments under saline environment. Antioxidants enzymes perform an important role

in DS by inhibiting the oxidation. In the current study, integration of PBC and IAA protected the tomato from oxidative stress through boosting activity of POD, CAT, and SOD under SS (Fig. 10). POD, CAT, and SOD activities were prominently increased in T3, T4, T5, and T6, in comparison to T1 and T2. Here the findings were very interesting, in T6 (3% of PBC and 40 ppm of IAA) found that antioxidant enzymes activities in T6 clearly enhanced. The maximum values of SOD ($3.88 \text{ mmol g}^{-1} \text{ FW}$), POD ($7.66 \text{ mmol g}^{-1} \text{ FW}$) and CAT ($3.91 \text{ mmol g}^{-1} \text{ FW}$) were observed in the T6.

Correlation study of growth, physiological, and biochemical attributes in tomato under DS after PBC and IAA treatments

Figure 11 demonstrates the Pearson correlation matrix for growth and physiological parameters of tomato plants

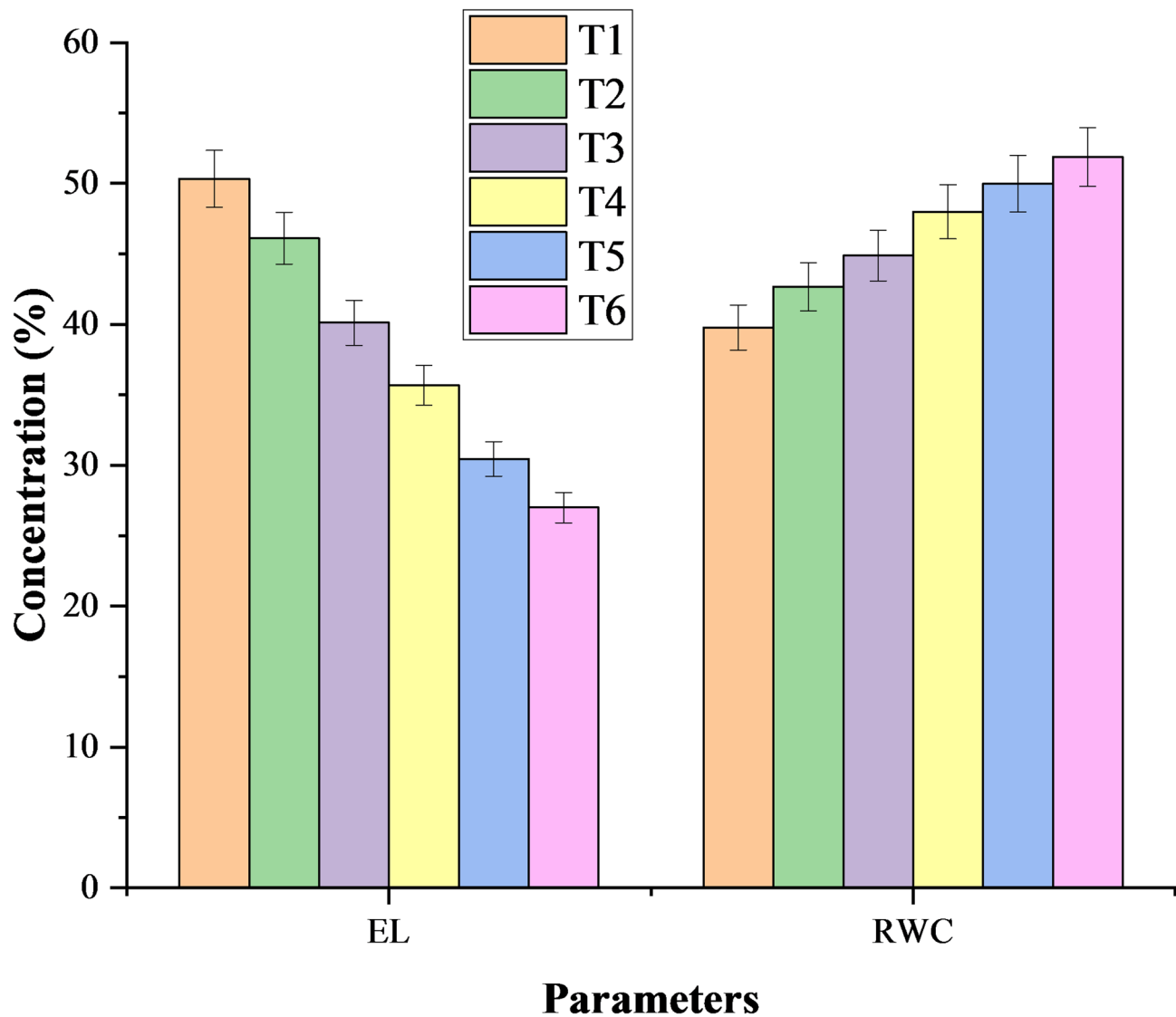


Fig. 7 Effects of PBC and IAA on EL and RWC, tomato in drought stressed soil

amended with PBC and IAA under water stress. Positive correlations (red colour) were examined among growth parameters, such as RL, SL, PH, RFW, SFW, PFW, RDW, SDW, PDW, proline, TSS, RWC, chl a, chl b, and carotenoids. Conversely, oxidative stress markers such as H_2O_2 , and MDA, EL exhibited strong negative correlations with RL, SL, PH, RFW, SFW, PFW, RDW, SDW, PDW, proline, TSS, RWC, chl a, chl b, and carotenoids, propose reduced oxidative harm. Antioxidant enzyme (POD, SOD, and CAT) activities positively correlated with RL, SL, PH, RFW, SFW, PFW, RDW, SDW, PDW, proline, TSS, RWC, chl a, chl b, and carotenoids. These findings highlight the combined effect of PBC and IAA in enhancing growth, alleviating oxidative stress, and increasing drought tolerance in tomato plants.

Correlation network among morphological, physiological, and biochemical parameters

The network plot illustrates the complex interrelationships among various biochemical, physiological, and morphological parameters of tomato under drought stress after the treatment of PBC and IAA (Fig. 12). Each node represents a specific trait such as RL, SL, PH, RFW, SFW, PFW, RDW, SDW, PDW, Chl a, Chl b), Carotenoid, POD, SOD, CAT, Proline, EL, RWC, H_2O_2 , and MDA. The dense web of blue lines (edges) connecting the nodes signifies significant correlations among these traits, suggesting that changes in one parameter likely influence others. Central nodes like SFW, SL, SOD, and RL are highly interconnected, indicating their potential roles as key indicators of overall plant health. This comprehensive

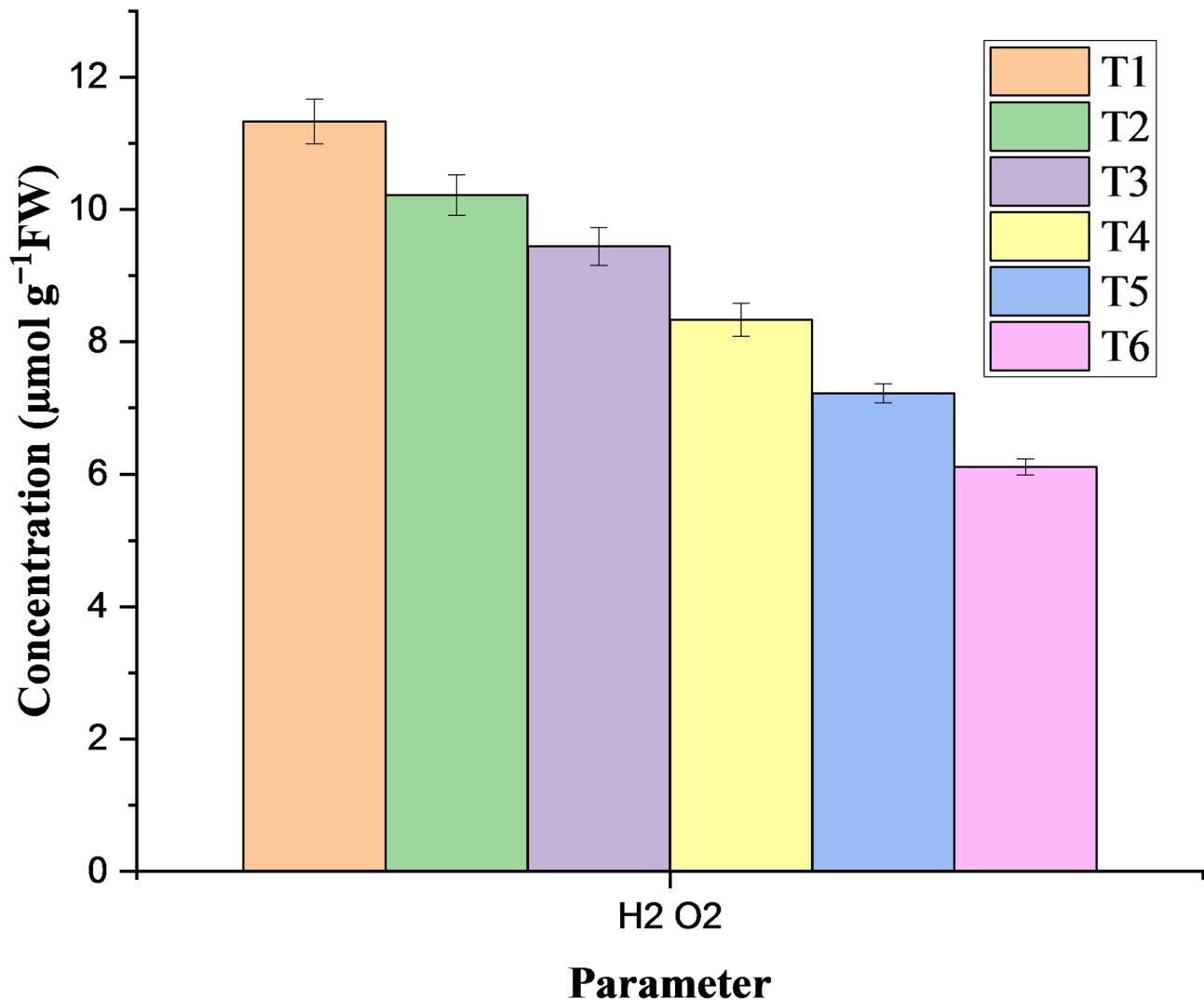


Fig. 8 Effects of PBC and IAA on H₂O₂ concentration of tomato in DS soil

correlation network reflects a tightly coordinated physiological response, useful for identifying critical traits contributing to stress tolerance.

Discussion

Sufficient water availability is vital for plant metabolism, optimal growth, and overall efficiency [54]. A decline in productivity can result from disruptions in the physiological and biochemical processes of plants under DS. Drought-induced stress poses a major challenge, particularly in developing countries where agriculture is a primary livelihood. As a form of abiotic stress, drought can severely impact plant growth, morphology, and various physiological and biochemical traits. When such stress occurs during the early stages of plant development, it can significantly reduce crop yield.

DS markedly effects the morphological attributes of plants like plant height, fresh and dry biomass of plants,

shoot and root, and general plant morphology [55]. The combined application of PBC and IAA demonstrated a significant positive impact on root length, shoot length, and overall plant height in tomato subjected to drought stress. This synergistic effect can be attributed to the complementary roles of PBC and IAA in enhancing plant physiological functions. PBC improves soil physical properties, enhances water retention, and increases nutrient availability, thereby facilitating better root development and stress tolerance [56]. Simultaneously, IAA acts as a key plant hormone regulating cell elongation, root initiation, and shoot growth, which is particularly crucial under abiotic stress conditions like drought [56]. The enhanced root length supports greater water and nutrient uptake, while improved shoot growth reflects better above-ground biomass accumulation and hormonal balance. These findings are aligned with the previous work of other researchers. Maximum leaf area was recorded in

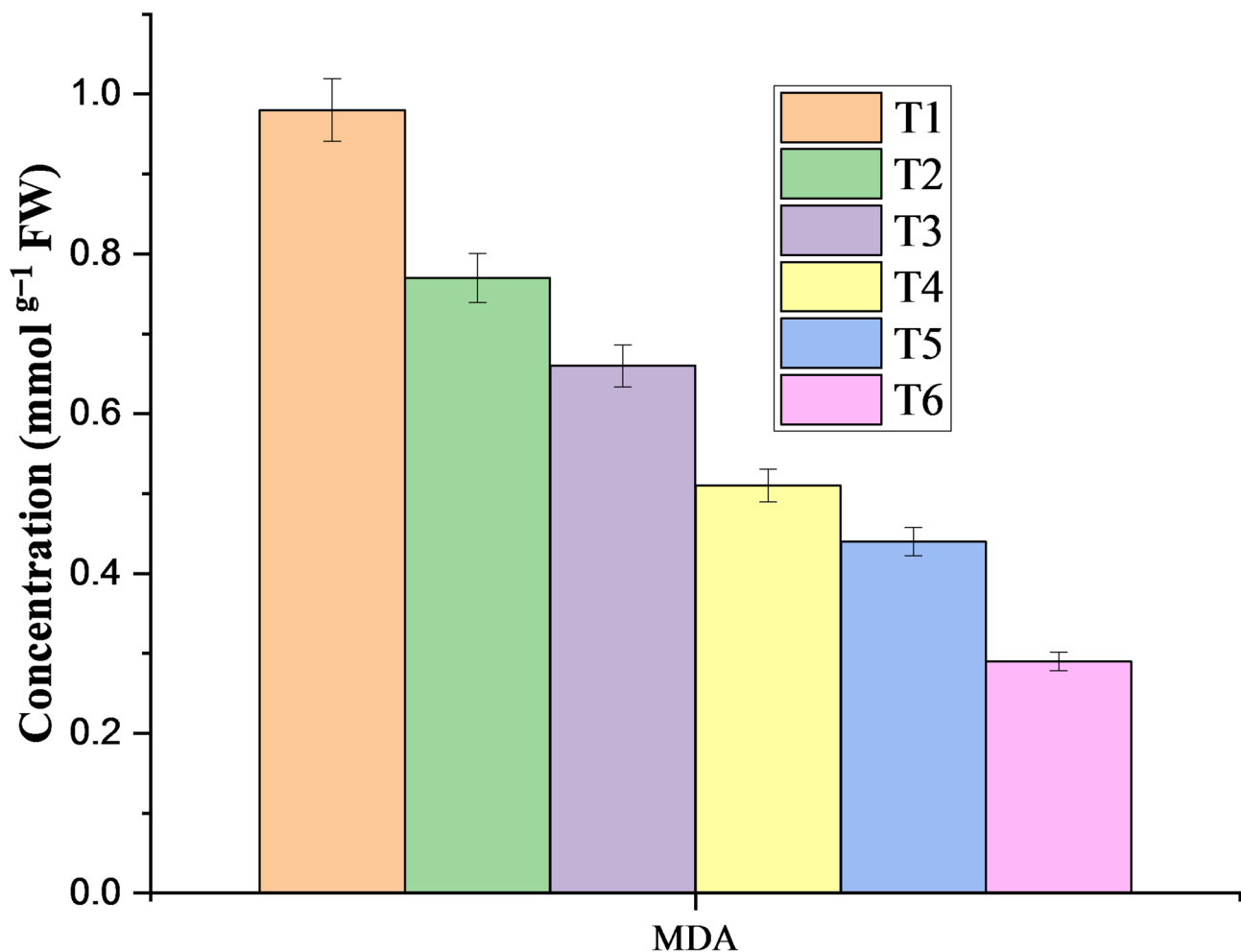


Fig. 9 Effects of PBC and IAA on MDA of tomato plants in drought stress

T6 of tomato which is ameliorated by of PBC and IAA application (Fig. 3). Our findings are consistent with those of Soussani et al. (2025) [57], who reported that the application of sugarcane biochar significantly improved the agronomic traits of maize, attributing these effects to an increase in soil organic matter content.

Figure 4 illustrates that the fresh and dry mass of roots, shoots, and whole plants increased with rising concentrations of IAA during the vegetative stages across all tomato cultivars. The observed increase in fresh biomass under drought conditions following IAA application suggests the activation of tolerance mechanisms, thereby improving plant productivity under stress. Biochar application is a promising amendment strategy that can help plants escape or mitigate stress effects. As previously reported by Akram et al. (2024) [58], drought stress significantly reduces the fresh weight of roots, shoots, and whole plants. Our results are line up with the findings of Hygienus et al. (2025) [59], who established that biochar enhances drought resistance in maize, likely due to improvements in soil

water retention and physicochemical properties. Based on Fig. 4, our study concludes that the joint application of IAA and biochar (PBC) during the vegetative stage effectively alleviates drought-induced reductions in biomass. Supporting this, Zada et al. (2024) [60] reported that biochar's high porosity and water-holding capacity help protect plants from desiccation.

Leaf chlorophyll (chl a and chl b), and carotenoids content reduced in DS tomato plants in comparison to stressed free plants. DS decrease the rate of photosynthesis in plants. The reduction in photosynthesis under DS has been credited to stomatal closure and a decrease in chl a, chl b, and carotenoids formation [61]. The application of PBC and IAA, however, reduced the losses. The increase in the photosynthetic pigments may be attribute to the PBC, is rich of the nutrients like nitrogen which may release from PBC and becomes the part of treated plant and leads to enhance in the contents of the chl a, chl b, and carotenoids, which subsequently rise the photosynthetic rates by providing some protection against DS. This was aligned with the with the previous work

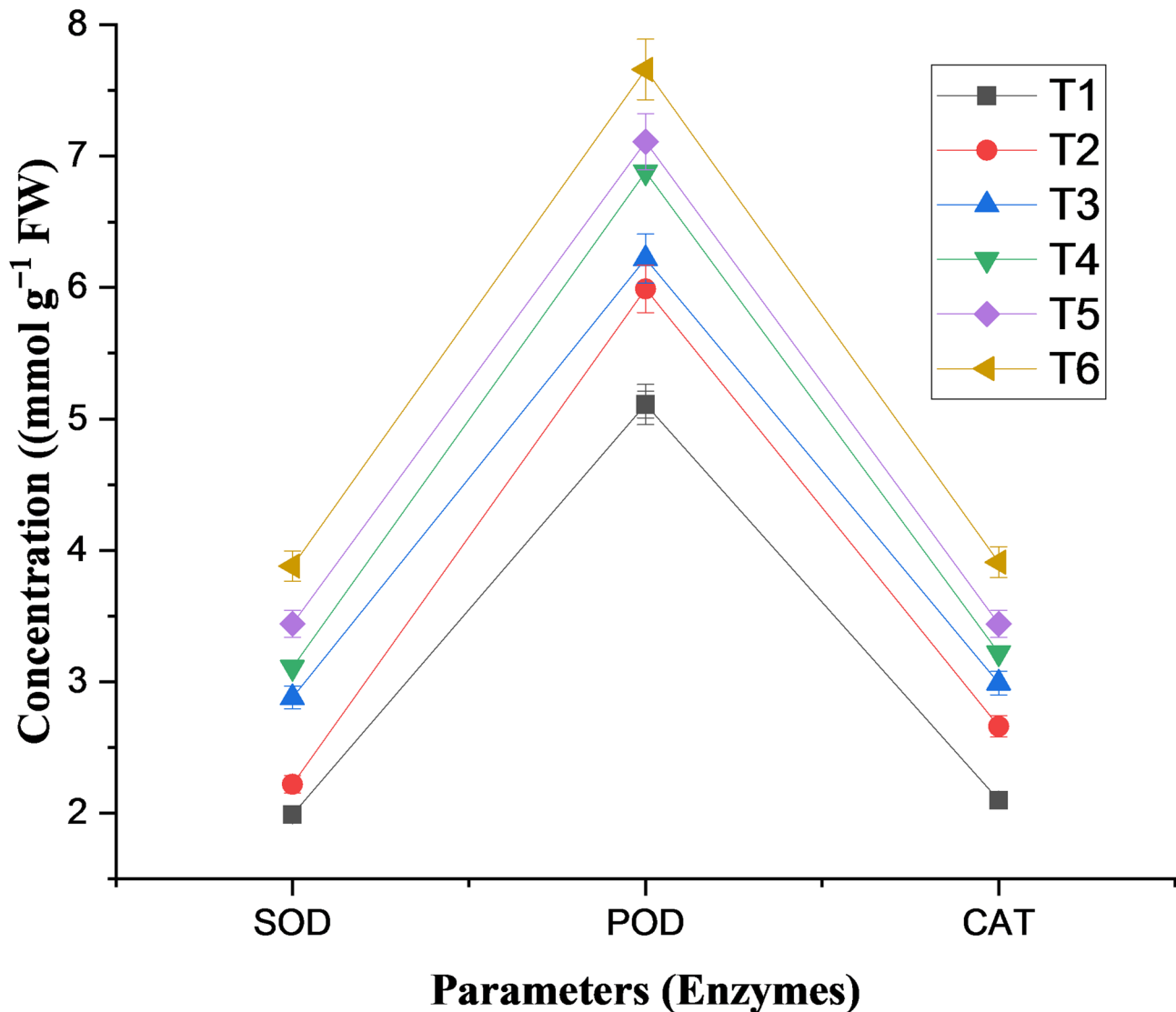


Fig. 10 Effects of PBC and IAA on POD, SOD, and CAT of tomato in DS

[62]. Additionally with increase in the concentration of the IAA the concentration of the photosynthetic varies which are align with the work of previous researcher. Enhanced photosynthetic pigment concentration and improved antioxidant defenses collectively supported better growth and stress resilience, aligning well with previous research [63].

Under drought conditions, this synergistic treatment of PBC and IAA has been observed to significantly increase TSS, RWC, and proline, in tomato plants. TSS act as osmo-protectants, proline serves as a compatible solute protecting cellular functions, and improved water content helps maintain turgor and metabolic activity. Together, these changes contribute to better drought tolerance and sustained growth. These findings are supported by studies such as Katal et al. (2024) [64], who reported that biochar and foliar growth regulators can

enhance drought resilience in tomato by improving osmolyte accumulation and water relations. Increasing leaf proline sugar and water contents plays a vital role in defending plants from severe desiccation under DS. Mushtaq et al. (2024) [65] found significant increases in proline and soluble sugar contents in drought-stressed tomato plants treated with biochar and IAA, indicating improved physiological adaptation to water deficit. These findings are relevant to the previous work [66]. DS markedly increased the accumulation of MDA and H_2O_2 in plants, highlighting the severity of oxidative damage under such conditions. However, the application of PBC and IAA under water-deficit conditions resulted in significantly lower MDA levels in shoots, which corresponded with reduced H_2O_2 content and enhanced total phenolic content and antioxidant activity. This suggests that these treatments helped protect membrane systems from lipid

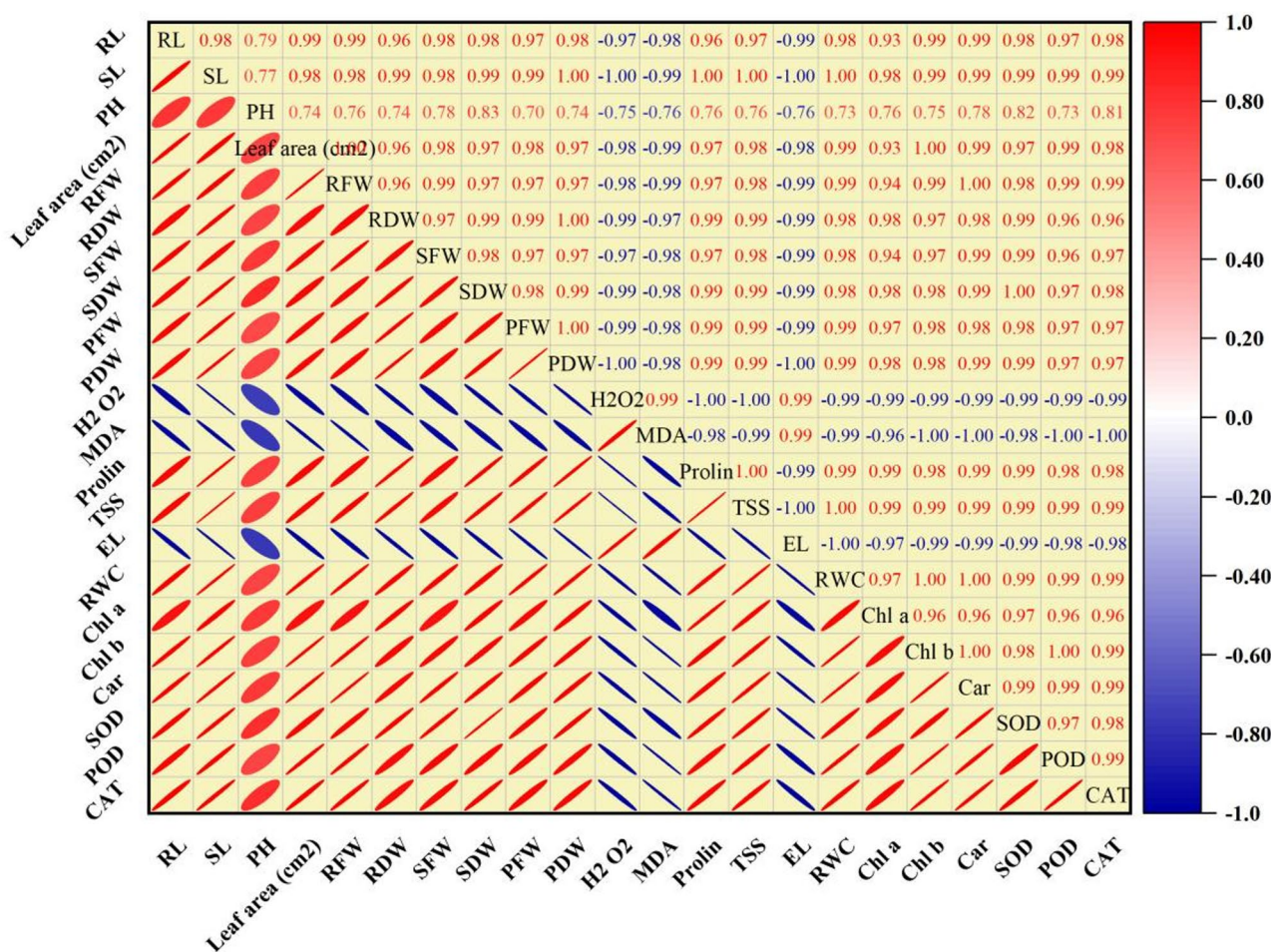


Fig. 11 Pearson correlation for various growth parameters of tomato in response to PBC and IAA treatment in SS. Intensity of blue colour represent negative correlation while red colour denotes positive correlation

peroxidation. In contrast, control plants under drought stress exhibited higher MDA levels, indicating greater oxidative injury. These findings demonstrate that treated plants under drought stress experienced considerably less oxidative damage than untreated stressed plants. Our results are in strong agreement with those reported by Seymen et al. (2024) [67].

Osmotic stress leads to the accretion of ROS in plant cells, resulting in oxidative damage [68]. The joined application of PBC and IAA enhances the activity of antioxidant enzymes such as POD, SOD, and CAT, which play a vital role in scavenging ROS and mitigating oxidative stress (Raza et al., 2024) [69]. These enzymes help maintain cellular homeostasis by reducing oxidative damage and preserving membrane integrity, as indicated by decreased electrolyte leakage. This suggests an improved capacity of plant membranes to withstand stress. Additionally, IAA has been associated with strengthening the antioxidant defense system in plants (Ali et al., 2025) [71]. Similar findings have shown that IAA application boosts the activity of ROS-scavenging enzymes like SOD, CAT,

and POD, thereby enhancing stress tolerance and reducing cellular injury under osmotic conditions [72–74].

In the summary, the integration of PBC with exogenous application of IAA presents a synergistic strategy to enhance plant growth and mitigate drought-induced toxicity. Biochar serves as a porous carrier, supporting microbial colonization and improving soil physico-chemical properties such as water retention, aeration, and nutrient availability. When inoculated with *Penicillium citrinum*, a beneficial endophytic fungus, biochar enhances microbial-driven nutrient mobilization, especially phosphorus and other nutrients, which are crucial for plant metabolism under stress. Meanwhile, externally applied auxin stimulates root architecture, particularly lateral and adventitious root development, which allows better water and nutrient uptake under drought conditions. This dual approach significantly improves shoot and root length, and biomass by maintaining osmotic balance and promoting cell division and elongation [75]. The presence of fungal metabolites and auxin stimulates these antioxidant defenses, promoting redox homeostasis and

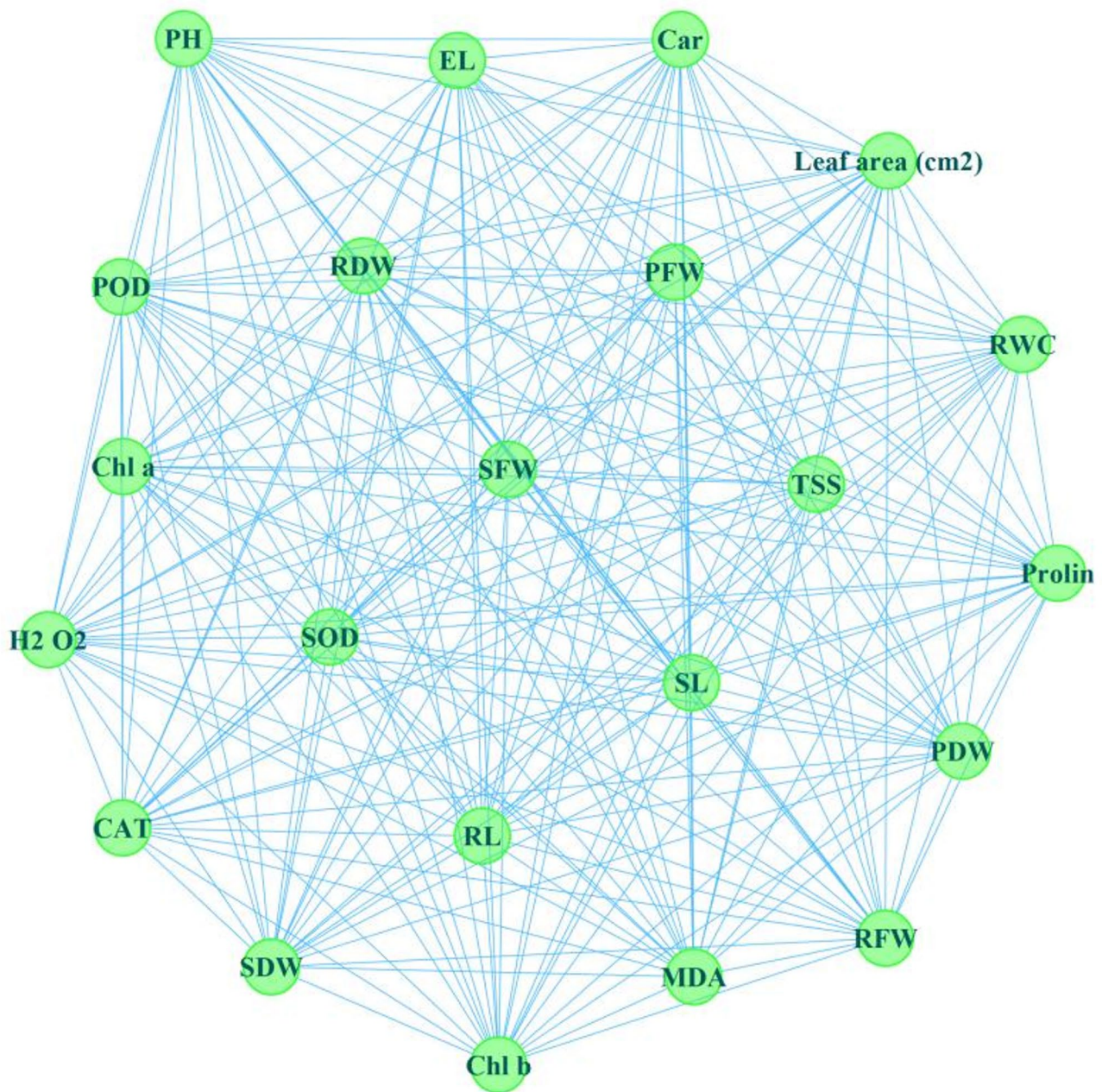


Fig. 12 The network plot illustrates the interrelationships among various morphological in tomato plants under DStress conditions

reducing lipid peroxidation in drought-stressed tissues. Study of Niaz et al. (2024) [76], support that microbial-activated amendments and hormonal treatments confer resilience by reinforcing the plant's physiological and biochemical defense network under water-limited environments. Moreover, the synergistic effect of PBC and IAA led to increased accumulation of osmo-protectants like proline and soluble sugars, enhanced antioxidant enzyme activities (SOD, CAT, POD), and reduced markers of oxidative damage such as MDA and H_2O_2 , thereby maintaining membrane stability and redox balance. These

findings suggest that the integrated use of biochar and IAA is an auspicious and sustainable strategy for improving drought tolerance in tomato plants, particularly in water-scarce agricultural systems.

Conclusion

Our findings conclude that the successful growth of *Solanum lycopersicum* can be achieved through the incorporation of suitable amendments that alleviate the hostile properties of drought. The current study demonstrated that the synergistic application of *P. citrinum*-loaded

biochar and auxin clearly enhances the growth, physiological, biochemical, and antioxidant responses of tomato plants under drought stress. The combined treatment improved key growth parameters, including root and shoot lengths, plant height, and leaf area, along with notable increases in biomass. Additionally, it boosted the levels of proline, chlorophyll, soluble sugars, carotenoids, and antioxidant enzyme activities (SOD, POD, CAT), while reducing such as H_2O_2 and MDA. These results indicate that PBC and IAA together offer a promising, eco-friendly strategy to reduce DS and improve crop performance. The findings support the potential of this combined amendment as a sustainable solution for enhancing plant resistance in DS. To effectively adapt to the changing global climate, our findings may contribute to the development of future strategies aimed at ensuring optimal growth and production of tomato under drought conditions, particularly in arid and semi-arid areas of Pakistan.

Abbreviations

BC	Biochar
PBC	Penicillium citrinum-Loaded Biochar
IAA	Auxin
RL	Root length
SH	Shoot length
PH	Plant height
FRW	Fresh root weight
FSW	Fresh shoot weight
FPW	Fresh plant weight
DRW	Dry root weight
DSW	Dry shoot weight
DPW	Dry plant weight
H_2O_2	Hydrogen peroxide
TSS	Total soluble sugar
PGRs	Plant growth promoters
SEM	Scanning electron microscope
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase
ROS	Reactive oxygen species

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Authors' contributions

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors are agreeing to publish the work in current form.

Competing interests

The authors declare no competing interests.

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