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Biochar Application to Soil and Seed Pre-Soaking on Growth, Yield and Physiological Response of *Solanum melongena* L. Under Induced Abiotic Stresses

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Abstract

Abiotic stressors are one of the major impediments to plant growth, which results in a large loss of yield and productivity for plant producers. The objective of this research was to comprehend the remediation potential of wood-chip biochar and gallic acid by perusing its effectiveness on biomass, yield, anatomical, physiological and antioxidant enzyme activities of *Solanum melongena* L under salinity and boron stress. Pot experiment was designed with biochar (5 g kg^{-1}) amendment to soil and seed pre-soaking with gallic acid (2 mM) under induced salinity (NaCl 120 mM) and boron stress (25 mg kg^{-1} boric acid). Results evaluated that salinity and boron negatively affect plant growth, yield, photosynthetic pigments, and antioxidant defense system. However, biochar and gallic acid treatment under stress-enhanced germination percentage (100–73%), total biomass (TB = 1.83), absolute growth rate (AGR), relative water content (RWC) 115%, plant height stress tolerance index (PHSTI), plant dry mass stress tolerance index (DMSTI) 36% and decreased mean germination time. Likewise, both varieties produce highest fruit yield such as flower number (FLN = 7.3 per plant), fruit number per plant (FRNP = 7.0), fruit size (FRS = 7.5 cm), fresh fruit weight per plant (FRFWP = 28.4 g) and fruit dry weight per plant (FRDWP = 24.6 g) in growth regulators under stress. Leaf chlorophyll 'a', 'b' content, soluble sugar, protein, superoxidase dismutase (SOD), peroxidase (POD) increased rapidly whereas malondialdehyde (MDA), hydrogen peroxide ($H_2O_{2)}$ and glycine betaine dramatically lowered in biochar and gallic acid treatments. Anatomical traits showed that stomata size and density were increased ($p < 0.05$) with growth stimulators under combined abiotic stress. In conclusion, the application of biochar and gallic acid may be a promising strategy to reduce the negative impacts of abiotic stressors by improving the biomass, yield, physiological, and antioxidant defense of *Solanum melongena*.

Keywords Growth · Agronomy · Anatomy · Physiology · Antioxidant enzymes · *Solanum melongena* L

Introduction

Egg-plant (*Solanum melongena* L.) is the second most valuable fruit in the family Solanaceae after tomato with different shapes, color and size, low production cost, widely grown and consumed in Southeast Asia and southern parts has increased its popularity in Pakistan (Diaz-Perez and Eaton 2015). Over the world its cultivation area is about 1.86 million hectares (FAO 2018) whereas in Pakistan, brinjal occupies an area of 9044 hectares with an average yield of 88,148 tons hectares (Habib et al. 2015). It is deemed to be rich in fibers and help in regulating sugar level in blood. The phenolics and anthocyanins produced by eggplant inhibits the enzymes involved with type 2 diabetes, response to environmental stresses to generate defense system against infection caused by pathogens and ultraviolet radiation (Nino-Medina et al. 2017; Abbas et al. 2021).

Global climate change is presently deemed as the most destructive hazard to the natural world, getting a substantial consideration from researchers, farmers, and programmed makers due to its major impact on farming. The problem has gotten worse as a result of continual increases in the complexity and unpredictability of environmental conditions as well as global climate change (Uddin et al. 2021; Rodriguez et al. 2007). Such worse environmental factors

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included abiotic stressors regarded as ultimate constraint to crop yield. Among these stressors salinity is the utmost acute environmental stress presently influencing the agriculture (1128 million hectares land), a foremost hazard to the world food security (Ullah et al. 2022; Wicke et al. 2011).

A high boron concentration reduces plant chlorophyll contents, net absorption rate, lipid oxidation, membrane permeability, and antioxidant enzymes. Hydrogen peroxide (H_2O_2), a harmful cytotoxic chemical that acts as an intermediary signaling molecule to affect the expression of genes relevant to antioxidant defense systems, is a secondary messenger in stress-response indicators created by redox reactions in plants (Xing 2018). The lethal levels of boron can cause the generation of reactive oxygen species (ROS), like hydrogen peroxide ((H_2O_2), superoxide (O_2^-), and hydroxyl radicals ($^-$OH) which can significantly stimulate oxidative stress in plants (Metwally et al. 2018). Upregulation of antioxidant defense mechanism in the plant cell by both non-enzymatic antioxidants (ascorbate, carotenoids, alpha-tocopherol, and glutathione), and enzymatic antioxidants such as peroxidase (POD), superoxide dismutase (SOD), mechanisms to decompose H_2O_2 into useful water and oxygen (O_2) takes place in plant tissues/cells. These enzymatic and non-enzymatic antioxidants increase the ability of plants to withstand salt stress by oxidizing co-substrates (Landi et al. 2019; Yang et al. 2019; Eraslan et al. 2007).

Biochar a black biomass of carbon, low-cost porous pyrogenous matter formed at high temperature from organic wastes (crop deposit, animal, or poultry manure etc.) under zero or inadequate oxygen environments in a closed furnace at ≤ 700 °C through pyrolysis (Lutfunnaha et al. 2021). In recent years, biochar has gained significant attention for its use in combating global climate change through the sequestration of atmospheric CO2 into soil. In addition to its appropriate carbon content, biochar also has the potential to be more effective at supplying plants with nutrients and minerals in highly weathered, nutrient-poor, and degraded soils than in well-structured, high-quality nutrient-rich soils (Chaganti et al. 2015; Tsai et al. 2012).

Gallic acid (GA) used in phytoextraction of different metals by chelating mechanism helps the solubilization of Ni, Zn, and Cd from contaminated soil (Volf et al. 2012). Maintenance of crop capacity to high growth rate, RWC, and photosynthetic capability was estimated in gallic acid treated plants under induced abiotic stress in *Glycine max* L. (Yildiztugay et al. 2017). GA-induced tolerance in rice seedlings against NaCl stress was examined by enhancing the activities of H_2O_2-scavenging enzymes such as POD, SOD, CAT, and APX, hence protect cell membranes from oxidative damages caused by ROS (Ozfidan-konakci et al. 2015). Currently, there is no published scientific report on the possible preventive roles of gallic acid and biochar on individual and combined effect of salinity and boron stress in egg-plant. So, the present study was conducted to explore the germination, agronomic and physio-biochemical attributes of egg-plant under induced abiotic stressors.

Materials and Methods

Biochar Preparation and Physico-Chemical Analysis Through SEM and EDX

Biochar produced from hardwood of *Vachellia nilotica* L. was prepared in furnace with a thermocouple at average temperature of 500–550 °C for 24–48 h by pyrolysis. Well powdered biochar was analyzed with exposure to a gold glaze put on Spi coating segment for morphological characteristics using scanning electron microscope (JSM5910-JEOL-JAPAN) following the procedure of Lalay et al. (2021). Furthermore, energy dispersive X-ray spectroscopy (INCA200/Oxford instruments, U.K) was used for elemental analysis including total carbon (C), oxygen (O), nitrogen (N), sulphur (S), chlorine (Cl), calcium (Ca), magnesium (Mg), aluminum (Al), silicon (Si), iron (Fe), cupper (Cu) and zinc (Zn). Biochar suspension with water (1:10 w/v) was used to determined pH by the method of Li et al. (2016). Cation exchange capacity (CEC) of biochar was measured through ammonium acetate method following the standard protocol modified by Thomas et al. (1982). Electrical conductivity (EC) of biochar was evaluated by the methodology of Gaskin et al. (2008).

Site Description and Seed Sterilization

Pot experiments were performed at University of Peshawar (34° 1′ 33.3012" N and 71° 33′ 36.4860" E.), KPK Pakistan lies in Iranian plateau with 513 mm of mean annual rainfall in 2021. Soil texture was determined by hydrometer method established by Gee and Bauder (1986) whereas, EC of the saturated soil was measured at 25 °C by calibrated EC meter (BANTE, DDS-12DW, China). Exchangeable sodium percentage of soil was measured by using the methodology of Page et al. (1982). Soil and farmyard elemental analysis i.e. calcium (Ca), aluminum (Al), potassium (K), silicon (Si), oxygen (O), iron (Fe) and zinc (Zn) were accessed by energy dispersive X-ray spectroscopy. The seeds of two varieties (Neelam & BSS 513) of *Solanum melongena* L. were collected from National Institute of Food and Agriculture (NIFA) Pakistan, and were surface sterilized with 0.1% mercuric chloride solution and 70% ethanol for five minutes followed by thorough washing with deionized water (Warwate et al. 2017).

Experimental Design and Growth Conditions

Pots were carefully kept at a nursery in a complete randomized block design (CRBD) at a space of 5 cm away from each other with a net plot size of 3.0 × 2.0 m for proper air passage and were kept safe from rain. The experiment was carried out in green house with 2 × 2 × 2 (two varieties, two level of abiotic stress, treated and non-treated soil with biochar and seed with gallic acid). Both varieties of eggplant with 12 treatments in triplicates was divided into three groups. Group-1 was taken as control (untreated by stresses), Group-2 was primed with 2 mM solution of GA solution (3, 4, 5-triphydroxyl-benzoic acid) before sowing for 24 h (Yildiztugay et al. 2017), and Group-3 was treated with biochar mixed in ratio of 5:1 g kg^{-1} soil at the time of sowing. Treatments were designed as;

T1 = Control (Untreated).
T2 = 25 mg kg-1 Boric acid.
T3 = 120 mM NaCl.
T4 = 25 mg kg-1 Boric acid + 120 mM NaCl.
T5 = 5 g kg-1 Biochar.
T6 = 5 g kg-1 Biochar + 25 mg kg-1 Boric acid.
T7 = 5 g kg-1 Biochar + 120 mM NaCl.
T8 = 5 g kg-1 Biochar + 25 mg kg-1 Boric acid + 120 mM NaCl.
T9 = 2 mM Gallic acid.
T10 = 2 mM Gallic acid + 25 mg kg-1 Boric acid.
T11 = 2 mM Gallic acid + 120 mM NaCl.
T12 = 2 mM Gallic acid + 25 mg kg-1 Boric acid + 120 mM NaCl.

Before seed sowing, 72 pots were well ploughed and filled with 2 kg silt clay (1:2) soil along with farmyard manure. Ten seeds of each variety were sown in earthen pots of 18 cm top and bottom diameter and 20 cm height placed 10 cm apart in triplicates. Pots were thinned after a week of seed germination and 5 healthy seedlings were maintained. Data counted for germination parameters was taken from 1st day to 10th day before application of stress. After 15 days of post germination, plants were subjected to salinity stress of 120 mM NaCl solution (Ozfidan-konakci et al. 2015) and boron stress by applying boric acid powder of 25 mg kg^{-1} soil (Metwally et al. 2018). After induced stress on 25th day, plants were up-rooted for the measurements of agronomic studies such as roots were gently removed and then washed distilled water to remove adhered dust particles. After absorbing moisture from the root surface, the fresh weights of root and shoot was measured. Shoot and root length, leaf area via dry weights were determined after drying in an oven at 30 °C for 72 h until the weight became constant whereas undamaged fresh leaf of plants per treatment in replicates were collected and stored in refrigerator at 4 °C of photosynthetic pigments, osmoprotectants, and plant antioxidant enzymes through spectronic UV-1700 Shimadzu, Japan. Some of the fresh leaves from each treatment were morphological studies through SEM for the analysis of stomatal physiology. The crop was then left until flowering and fruit formation. After day 75 of germination, harvesting was carried out with the collection of purple color fruits for each replication and were weighed for fresh and then oven-dried to a constant dry weight for 72 h at 30 ℃. Best precautions were carried out throughout the experiment including weeding approximately once a week and no pest or disease were seen in the experimental period.

Determination of Germination and Growth Parameters

Germination Energy (GE)

Number of germinated seeds were recorded on daily basis according to the formula given by Shumaila and Ullah (2020).

$$\mathrm{GE} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \frac{X_3 - X_2}{Y_3} + \cdots + \frac{X_n - X_{n-1}}{Y_n} \quad (1)$$

Here X_1 to X_n (emerged seeds on day first till nth day), Y_1 to Y_n (days count from number sowing nth count).

Final Germination Percentage (FG%)

Final germination percentage was defined by the method of Al-Ansari Ksiksi (2018).

$$\mathrm{FG\%} = \frac{\text{Total number of germinated seeds at end}}{\text{Number of initial seeds}} \times 100 \quad (2)$$

Timson Germination Index (TGI)

Timson germination index was evaluated by following the standard formula of Al-Ansari and Ksiksi (2018).

$$\mathrm{TGI} = \frac{\sum G}{\sum T} \quad (3)$$

"G" (seed germination percentage), "T" (time of germination).

Coefficient of Velocity of Germination (CVG)

The CVG described the speed of seed germination. The value will be found highest when the required for germination is lower. If all the seeds emerged on first day then the

highest CVG value (100%) could be possible (Shumaila and Ullah 2020).

$$\mathrm{CVG} = \frac{N_1 + N_2 + N_3 + \cdots + N_X}{100} \quad (N_1T_1 + N_2T_2 + N_3T_3 + \cdots + N_XT_X) \tag{4}$$

"*N*" (number of seeds germinated), "*T*" (number of days).

Mean Germination Time (MGT)

Mean germination time denotes the minimum time for germination. Lower rate of mean germination time will give the highest population germinated (Shumaila and Ullah 2020)

$$\mathrm{MET} = \frac{\sum fx}{\sum f} \tag{5}$$

where "*f*" is the number of seeds germinated on day *x*.

Germination Index (GI)

Germination index gives reported highest to the seeds germinated on first day while minimum to those germinated later (Shumaila and Ullah 2020).

$$\mathrm{GI} = \left(10 \times n_1\right) + \left(9 \times n_2\right) + \left(8 \times n_3\right) + \cdots + \left(1 \times n_{10}\right) \tag{6}$$

where n_1, n_2... n_{10} (number of germinated seeds on first, second day till day10th) while 10, 9... and 1 (weights of the number of germinated seeds).

Germination Rate Index (GRI)

Germination rate index suggests the percentage of germination calculated by the proposed formula of Shumaila and Ullah (2020).

$$\mathrm{GRI} = \frac{G_1}{1} + \frac{G_2}{2} + \frac{G_3}{3} + \ldots + \frac{G_X}{X} \tag{7}$$

"G_1" (germination percentage × 100), "G_X" (final germination percentage × 100).

Time to 50% Germination (T50%)

Time to 50% germination was counted from day of seed sowing until 50% of seed has been emerged by the formula represented by Vujosevic et al. (2018)

$$\mathrm{T}_{50} = t_i + \frac{N/2 - n_i}{n_j - n_i}(t_j - t_i) \tag{8}$$

"*N*" denotes the final germinated seeds number, n_j and n_i represents the cumulative germinated seeds by adjacent counts at times t_j and t_i, respectively, such as $n_i < N/2 < n_j$

Absolute Growth Rate (AGR)

Ghule et al. (2013) represented the formula for calculating the absolute growth rate that denotes increase in rate of growth variable during time "*t*" and was calculated through differential coefficient of '*W*' with time '*t*'. Height and mass of the crop was measured two times (vegetative and flowering stage) during growing season and then the mean value for each treatment was measured.

$$\mathrm{AGR} - \mathrm{I}\ (\mathrm{plantheight}) = \frac{H_2 - H_1}{t_2 - t_1} \tag{9}$$

$$\mathrm{AGR} - \mathrm{II}\ (\mathrm{plantdrymatter}) = \frac{W_2 - W_1}{t_2 - t_1} \tag{10}$$

where H_1 and H_2 are the heights (cm) of plant measured at t_1 and t_2 from soil level to the tip of the plant whereas; and W_1 are W_2 are the dry weight (g) of plant from time t_1 and t_2, respectively. Time was expressed in days.

Relative Growth Rate (RGR)

The data obtained by plant biomass separated into root, stem and leaves after harvest and were used to measure the relative growth rate indicated the rate of growth per unit dry weight (Ghule et al. 2013). Dry mass of the plant was measured two times (vegetative and flowering stage) after oven-drying at 30 °C for 72 h.

$$\mathrm{RGR} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} \tag{11}$$

where '$\log_e$' is the natural logarithms whose calculated value is 2.3026. the parameter was measured in g/plant/day.

Crop Growth Rate (CGR)

It was determined by following the standard formula represented by Kul et al. (2021)

$$\mathrm{CGR} = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{1}{\mathrm{land\ area}}(g/m^2/d) \tag{12}$$

where, W_1 and W_2 are dry weights of plant taken at time t_1 and t_2, respectively.

Relative Water Contents (RWC)

Relative water contents calculated by estimating the fresh leaf weight in grams and then kept it in oven for drying for 72 h. However, leaf saturated weight was determined by soaking in distilled water for 18 h (Kul et al. 2021).

$$\mathrm{RWC} = \frac{W_f - W_d}{W_s - Wd} \times 100 \tag{13}$$

where, "W_f" denotes leaf fresh weight, (W_d) its dry weight and (W_s) is the leaf saturated weight.

Total Biomass

Total biomass of the plant was calculated by the dry mass of root, stem and leaf after drying at 30 °C for 72 h in oven.

Stress Tolerance Index

Stress tolerance index is a useful tool for determining the high yield and stress tolerance potential of genotypes. Stress tolerance indices for different growth parameters were calculated using following formulae (Amin et al. 2014).

$$\text{Plant height stress tolerance index (PHSTI)} = \frac{\text{Height of stress plant}}{\text{Height of control plant}} \times 100 \tag{14}$$

$$\text{Drymatter stress tolerance index(DMSTI)} = \frac{\text{Weight of stressplant}}{\text{Weight ofcontrolplant}} \times 100 \tag{15}$$

Stomata Index (SI)

Stomata index was calculated by the method of Dubberstein et al. (2021).

$$\mathrm{SI} = \frac{S}{S+E} \times 100 \tag{16}$$

Determination of Yield Parameters

Crop yield was determined by collecting the fruits after 75 days of germination period for fresh weight analysis and then oven dried at 30 °C for 72 h in oven. Yield parameters included;

1. Flower number per plant.
2. Fruit number per plant.
3. Fruit size.
4. Fruit fresh weigh.
5. Fruit dry weight.

Size of the fruit was measured with the help of digital vernier caliper at three different positions and measurement was the average of three fruits per plant. Fruits were kept for 72 h in oven at 30 ℃ for dry mass analysis.

Physiological and Biochemical Attributes

Determination of Chlorophyll Content

Chlorophyll contents were evaluated by the standard methodology of Zou et al. (2017). Fresh leaves (0.2 g) were grounded in mortar and pestle in 80% acetone and incubated for 24 h in the dark followed by centrifugation at 2000 rpm. The absorbance was recorded at 649 nm for chlorophyll "a" content, and 663 nm for chlorophyll "b" content through spectrophotometer against 80% acetone blank.

$$\text{Chlorophyll} - a = (\text{mg/gr fresh weight}) = [19.3\,(\text{A663}) - 0.86\,(\text{A645})]\,v/100w$$

$$\text{Chlorophyll} - b = (\text{mg/gr fresh weight}) = [19.3\,(\text{A645}) - 3.6\,(\text{A663})]\,v/100w$$

Determination of Soluble Sugar Content (SSC)

Soluble sugar content was determined by a modified protocol of Bouzroud et al. (2018) with little modifications. Fresh leaves of 0.5 g were grounded with 10 ml deionized water followed by centrifugation at 10,000 × g for 10 min and homogenized by adding 1 ml of 30% phenol. Take 0.1 ml supernatant and samples were incubated for 20 min. Concentrated sulphuric acid of 5.0 ml of was mixed with the samples after incubation and optical density were recorded through UV detector at 420 nm.

Determination of Glycine Betaine Content (GB)

Glycine betaine content (GB) content in leaf was extracted and quantified by using the methodology of Shah et al. (2021). In detail, 0.5 g frozen leaf was chopped in 10 ml distilled water. The reaction buffer for measuring GB was filtered and the filtrate was diluted with 2 ml H_2SO_4 solution. After the homogenate was centrifuged at 10,000 × g for 20 min, cold potassium-iodide iodine (KI–I_2) was mixed with the supernatant. 1 ml supernatant was collected and measured for its optical density at 365 nm. The parameter was examined using the protocol of Shah et al. (2021).

Determination of Soluble Protein Content (SPC)

Soluble proteins in leaves were calculated by the standard method of Lowery et al. (1951). Fresh leaves (0.5 g) were chopped in 1 ml phosphate buffer solution (pH 7.0) and homogenate for 10 min. About 0.1 ml extract was taken followed by the addition of distilled water to make a total volume of 10 ml. The solution was then mixed with 1 ml reagent including [0.1 N sodium hydroxide, 0.75 g sodium carbonate, 0.37 g sodium potassium tartrate in 40 ml deionized water]. The solution was shacked for 15 min followed bt addition of 0.1 ml folin phenol reagent and incubated for 30 min. Absorbance of the samples were calculated at 650 nm using spectrophotometer.

Determination of Hydrogen Peroxide Content (H_2O_2)

Hydrogen peroxide content was analyzed using the methodology proposed by Shah et al. (2021). 0.5 g of frozen leaf sample was chopped in 5 ml trichloro acetic acid (TCA) followed by centrifugation for 15 min. The supernatant (0.5 ml) was mixed with 0.5 ml phosphate buffer and 1 ml potassium iodide (KI) reagent. H_2O_2 level was calculated using a standard curve calculated at 390 nm.

Determination of Malondialdehyde Content (MDA)

Malondialdehyde content (MDA) was determined using the methodology of Yin et al. (2022) with some modifications. Fresh leaf material (0.25 gm) was chopped in 3 ml 1.0% (w/v) trichloro acetic acid (TCA). The reaction mixture was spun in centrifuge machine at 10,000 rpm for 20 min. 1 ml supernatant was mixed with 4 ml of 0.5% (w/v) 2-thiobarbituric acid (TBA). The mixture was heated at 95 °C for 1 h and the samples were then cooled in ice bath. Optical density was measured at 532 nm.

$$\text{MDA}\ (\mu\text{mol/L}) = 6.45 \times (\text{A532} - \text{A600}) - 0.56\ \times \text{A450}$$

Determination of Antioxidant Enzymes

Peroxidase (POD) and superoxide dismutase (SOD) activity levels was examined according to the proposed methods of Ma et al. (2017). 0.5 g fresh foliar material were grounded and homogenized with 2 ml solution contained [0.2 ml phosphate buffer solution (pH 7.0), 12.5 g (PVP), 4.6 g ethylene diamine tetra acetic acid (EDTA) followed by the addition of 125 ml deionized water and centrifuged at 10,000 rpm for 20 min. Total reaction mixture (3 ml) included 0.1 ml supernatant, 0.1 ml phenyl diamine (36 mg in 4 ml deionized water), 1.3 ml methyl ethyl sulphonic acid (MES), (970 mg MES dissolved in 50 ml deionized water), and a single drop of H_2O_2 (0.3% v/v). Absorbance of samples was observed for 3 min at 485 nm by spectrophotometer.

SOD activity was assayed by measuring the inhibition of nitro blue tetrazolium reduction. The reaction mixture of 3 ml for SOD content in 0.5 fresh leaves included 0.1 ml supernatant, 0.72 ml nitroblue tetrazolium (NBT) contained 1.89 mg NBT mixed with 30 ml deionized water, methionine 0.72 ml (58 mg methionine added in 30 ml deionized water), 0.72 ml EDTA (1.1 mg EDTA dissolved in 30 ml deionized water) and riboflavin 0.72 ml (0.02 mg riboflavin in 30 ml deionized water) followed by 30 min incubation period in dark. One unit of SOD activity was defined as an absorbance change of 1 per min in OD_{560nm} causes inhibition of the photo reduction of NBT by 50%.

Determination of Leaf Anatomy Through SEM

One leaf per replicate was harvested and immediately submerged into liquid nitrogen for 1 min followed by 30 s submersion in methanol and 1 min submersion in hexamethyl disilazane. For leaf surface anatomy, leaves were mounted on SEM cylinder specimen mounts (JSMIT100-JEOL-JAPAN, aluminum, grooved edge, Ø32 mm). Leaves were oriented so that the adaxial surface could be examined. Mounted leaves were coated using JEOL-EC-32010CC Coating System for 30 nm of carbon. A JEOL scanning electron microscope was used to examine leaf surface anatomy including stomata size and density of each variety at high 20 kV, working distance ranging from 15 to 16 mm, magnification range from 2.20 kx to 5.00 kx with a specimen stage $T = -10° - +90°$ and $R = 360°$, respectively.

The standard methodology of Liu et al. (2021) was applied with minor modifications for leaf anatomical studies through electron microscopy. On 15th day of salinity and boron stress treatment, mature leaves were randomly obtained from each treatment and peeled carefully at about 1 mm^2 size and preserved in formalin acetic alcohol solution (90% ethanol, 5% formalin, 5% acetic acid) at 4 °C until dehydration. The cell structure of the samples was mounted in Canada balsam and the images were taken using Nikon Eclipse E600 microscope (DS-U3, Nikon, Japan). Leaf pieces from three separate plants/treatment were obtained and the values were expressed in micrometers. The trichome size was measured by microscope graticules, and the values were the mean of 3 measurements.

Statistical Analysis

The statistical analysis was a factorial design with induced salinity and boron stress. The analyses were performed in triplicate ($n = 3$) for various parameters including germination, agronomic, anatomical, physiological, and biochemical attributes were analyzed by Statistix 10 and IBM SPSS Statistics 22 (SPSS Inc, Chicago IL). Three-way ANOVA at significance

difference ($p \leq 0.05$) for all measurements, mean separation and standard deviations were compared by Tukey's multiple range test at $p \leq 0.05$ for each variety separately. Pearson correlation (R) was measured by the same software.

Results

Physico-Chemical Analysis of Soil and Biochar

In the present research work, precise surface morphological studies of soil and biochar was investigated SEM/EDX, which was processed to determine the alterations in surface forms and quantify chemical analysis. SEM micrographs revealed large sized and many pores biochar component with several cracks which enhance soil water holding capacity, araciality of carbon content in soil environment and inviting more microbes including fungi, ascomycetes and algae that help in soil fertility. Furthermore, EDX probe of SEM for elemental analysis of well fined biochar were in weigh percentage including maximum carbon content (56.33%), followed by oxygen (35.34%), silicon (3.12%), calcium (1.36%), iron (1.34%), aluminum (1.06%), nitrogen and magnesium (0.63%), chlorine (0.10%), copper (0.06%), zinc (0.04%) and sulphur content (0.01%) were determined (Table 1). Similarly, SEM showed integrated soil structure and profile with small pore, sphere like with rough surface. Physico-chemical measurement of soil through EDX probe

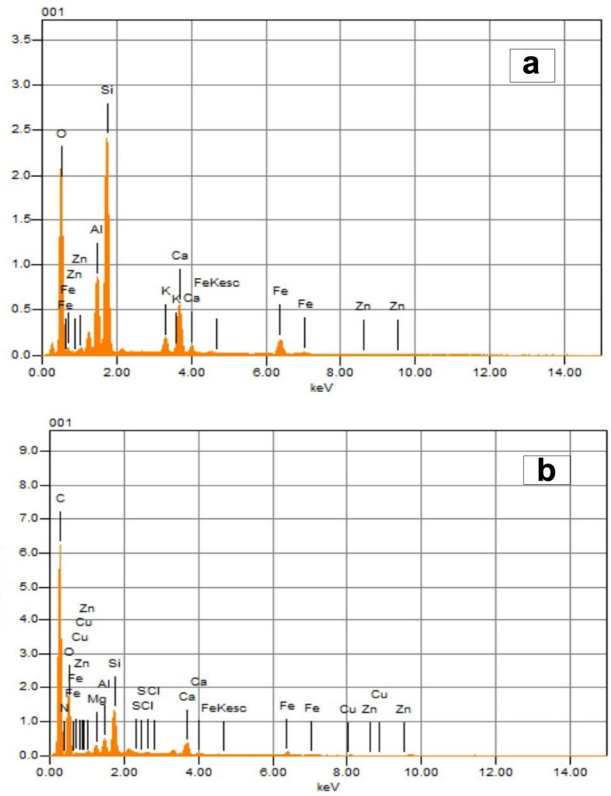

Fig. 1 Elemental analysis of **a** soil sample and **b** biochar obtained through energy dispersive X-ray analysis (EDX)

Table 1 Elemental analysis of powdered sieved biochar and soil sample obtained through EDX

Elements	Symbols	Biochar		Soil		Farmyard manure
		Mass %	Atom %	Mass %	Atom %	
Carbon	C	56.33	65.30	0.00	0.00	29.32 g kg^{-1}
Nitrogen	N	0.63	0.62	0.00	0.00	1.94 g kg^{-1}
Oxygen	O	35.34	30.76	54.86	71.26	–
Magnesium	Mg	0.63	0.36	0.00	0.00	3.19 cmol kg^{-1}
Aluminum	Al	1.06	0.54	7.46	5.75	–
Silicon	Si	3.12	1.54	20.2	14.9	–
Sulphur	S	0.01	0.00	0.00	0.00	–
Chlorine	Cl	0.10	0.04	0.00	0.00	–
Calcium	Ca	1.36	0.47	7.86	4.07	35.85 cmol kg^{-1}
Iron	Fe	1.34	0.03	6.91	2.57	–
Cupper	Cu	0.06	0.01	0.00	0.00	–
Zinc	Zn	0.04	0.01	0.20	0.06	–
Potassium	K	0.00	0.00	2.47	1.31	2.14 cmol kg^{-1}
Phosphorus						57.24 mg kg^{-1}
	Total	100	100	100	100	
Biochar	EC = 6.7 ds/m	pH = 9.5	Bulk density = 0.48 g/cm^3		Cation exchange capacity = 5.1 cmol/kg	
Soil	EC = 1.9 ds/m	pH = 6.2	ESP = 7.5–8.1%			
Farmyard manure		pH = 6				

(Fig. 1; Table 1) presented large content of oxygen (54.86%) followed by silicon (20.2%), calcium (7.86%), aluminum (7.46%), iron (6.91%) and potassium (2.47%), respectively.

Germination Parameters

Seed germination is a vital process in plant life begins with phenomena of imbibition following the activation of biochemical stage, process of cell division, cell elongation and its differentiation (Awatif and Alaaeldin 2017). However, the unavailability of soil moisture content enough for imbibition of seed and activation of metabolic events delay the initiation of seedling germination and retarding the rate of germination for a tolerant crop (Ma et al. 2016). The results in Table 2, revealed that all the traits were affected by the experimental factors and there was completely non-significant difference between control and seeds primed with 2 mM gallic acid solution and soil provided with 5 g kg^{-1} biochar except for TGI which was found significant in both varieties ($p < 0.05$) under growth stimulators. In the present study, the finding represented that imbibition of seeds with gallic acid in T12 before sowing has greatly enhanced timson germination index (0.91 ± 0.08), germination index (41.0 ± 3.7), germination rate index (1.48 ± 0.2), germination energy percentage (1.23 ± 0.05) time to 50% germination (3.27 ± 0.56) and reducing mean germination time (6.42 ± 0.36) in variety Neelam as compared to control seedlings without gallic acid priming. However, germination energy (GE) of seedlings under T2 and coefficient of velocity of germination (CVG) under T10 in gallic acid primed has been determined highest. This suggested the more potential role of gallic acid pretreatment to seeds initiated some metabolic processes for promoting early germination efficiency. However no significant differences were found among the parameter as reported in supplementary Table 1.

Similarly in variety BSS 513, timson germination index (1.10 ± 0.0) was obtained highest at T7 when the soil was amended with biochar supply soluble nutrient to germinating for activating some metabolic processes to initiate seedling formation early. In detail, maximum germination rate index (1.87 ± 0.7) and germination energy percentage (1.22 ± 0.02) has been calculated under treatment T8 in combined boron sand salinity stress whereas coefficient of velocity of germination (111 ± 6.90) was noticed highest under biochar addition to soil (T7) as compared to control, clearly suggested that biochar increased soil moisture content, physical and chemical status that indirectly enhanced seeds viability for germination. Consequently, regarding under control condition (T1) germination index (51.3 ± 2.0), germination energy (1.26 ± 0.02), time to 50% germination (3.64 ± 0.8) was increased and decreasing mean time to seedling germination (5.86 ± 0.24) was evaluated (Table 1). Results suggested that both the varieties given different responses to gallic acid and biochar treatment proposed the viability of varieties to germination indices. However, some of the treatments T7, T11 reported decreased seedlings germination indices but recorded non-significant as compared to control and growth regulators treatment mentioned in ANOVA supplementary Table 1, where interactions between genotype, between treatment, growth regulator, T x G, G x GR, T x GR, and G x T x GR were non-significant ($p > 0.05$) respectively.

Growth Parameters

Plant growth parameters (Table 3) are of significant measures to observe the effect of abiotic stresses. Salt stress (NaCl) alone or in combination with boron stress considerably suppressed leaf RWC in variety Neelam (47%) and BSS 513 (55%). However, applied biochar treatment to soil significantly ($p < 0.05$) enhanced leaf RWC by 115% and 66% reporting the ability of biochar to conserve water in soil and thus enhance the vigor and viability of plant under stress. Similarly, RGR (0.880) and CGR (0.69) was non significantly reduced by boron stress with gallic acid pretreated seeds in variety Neelam as compared to control (T1). However, in variety BSS 513 it was found a little bit decreased under salinity stress by applied biochar as compared to combined effect of salt and boron stress where the same traits were measured highest thus declaring the stress modulating ability of biochar amendment to soil. Plant biomass was obtained greater at significant level ($p < 0.05$) by applying biochar application in Neelam variety (1.443 ± 0.3) and gallic acid pretreatment to seed in variety BSS 513 (1.8367 ± 0.19). However, the biomass of different organs as root, stem and leaf biomass was also examined. Increased salinity stress affected the total dry mass of plant reducing biomass production to 0.453 ± 0.08 in variety Neelam. On the other hand, boron stress effectively reduced the biomass in BSS 513 (0.356 ± 0.11) more significantly without the growth regulators ($p < 0.001$). Such results reported the affective role of biochar and gallic acid enhancing total biomass of plant, respectively.

Rendering to absolute growth rate of plant with respect to plant height (AGR-I) and its dry mass (AGR-II) was found highest by biochar amendment under boron stress (0.15 ± 0.04, 0.038 ± 0.05) and decreased non significantly when salinity stress (T2) was applied in variety Neelam. Furthermore, gallic acid primed seeds reported increased plant height (0.20 ± 0.05) and dry mass in T10 (0.036 ± 0.180) by alleviating the adverse effect of boron stress in BSS 513 and found lower under combined salt and boron stress. These results successfully representing the positive and distinguishing effect of both varieties under different growth regulator treatments and under different stress situations. The enhancement in plant height stress tolerance index (PHSTI) was highly significant ($p < 0.05$) and more pronounced by

Table 2 Effect of biochar (5 g kg $^{-1}$) and gallic acid (2 mM) on germination indices of *Solanum melongena* L. under induced abiotic stresses

Varieties	Treatments		TGI	GI	GRI	GE	GE%	MGT	T50%	CVG
Neelam	T1	Control (Untreated)	0.91 ± 0.14a	36.30 ± 4ab	1.33 ± 0.1ab	1.20 ± 0.04a	90.0 ± 14.10ab	6.92 ± 0.34a	2.83 ± 0.47a	62.600 ± 12a
	T2	25 mg kg^{-1} Boric acid	0.83 ± 0.09a	29.0 ± 10ab	1.16 ± 0.2ab	1.23 ± 0.05a	83.30 ± 9.40ab	7.60 ± 0.97a	3.16 ± 0.94a	62.6 ± 5.40a
	T3	120 mM NaCl	0.86 ± 0.18a	39.0 ± 8.0ab	1.38 ± 0.2ab	1.19 ± 0.03a	83.3 ± 23.0ab	6.47 ± 0.22a	3.02 ± 1.31a	56.3 ± 13.0a
	T4	25 mg kg^{-1} Boric acid + 120 mM NaCl	0.89 ± 0.14a	34.0 ± 9.0ab	1.29 ± 0.2ab	1.19 ± 0.04a	86.6 ± 12.0ab	7.18 ± 0.88a	2.95 ± 0.64a	65.0 ± 14.0a
	T5	5 g kg^{-1} Biochar	0.83 ± 0.09a	27.6 ± 4.0ab	1.09 ± 0.1ab	1.15 ± 0.02a	83.3 ± 9.40ab	7.69 ± 0.12a	2.500 ± 0.0a	64.00 ± 6.3a
	T6	5 g kg^{-1} Biochar + 25 mg kg-1 Boric acid	0.81 ± 0.04a	30.6 ± 6.0ab	1.16 ± 0.1ab	1.17 ± 0.01a	83.3 ± 4.70ab	7.32 ± 0.81a	2.88 ± 0.54a	61.00 ± 7.1a
	T7	5 g kg^{-1} Biochar + 120 mM NaCl	0.71 ± 0.24a	27.30 ± 13ab	1.04 ± 0.4ab	1.13 ± 0.06a	73.3 ± 24.0ab	7.50 ± 1.02a	3.00 ± 0.70a	53.30 ± 16a
	T8	5 g kg^{-1} Biochar + 25 mg kg^{-1} Boric acid + 120 mM NaCl	0.73 ± 0.20a	26.6 ± 214ab	1.08 ± 0.5ab	1.16 ± 0.12a	73.3 ± 20.0ab	7.59 ± 0.91a	3.270 ± 1.0a	56 ± 12.00a
	T9	2 mM Gallic acid	0.76 ± 0.12a	26 ± 7.480ab	1.04 ± 0.2ab	1.15 ± 0.06a	76.6 ± 12.0ab	7.65 ± 0.66a	2.94 ± 0.31a	58.30 ± 0.1a
	T10	2 mM Gallic acid + 25 mg kg-1 Boric acid	0.81 ± 0.14a	25.6 ± 4a.0b	1.05 ± 0.1ab	1.18 ± 0.09a	80.0 ± 14.0ab	7.73 ± 0.72a	2.69 ± 0.27a	62.30 ± 14a
	T11	2 mM Gallic acid + 120 mM NaCl	0.62 ± 0.24a	20.0 ± 5.65b	0.810 ± 0.2b	1.13 ± 0.07a	60.00 ± 8.10b	7.29 ± 1.16a	2.84 ± 0.34a	46.00 ± 20a
	T12	2 mM Gallic acid + 25 mg kg^{-1} Boric acid + 120 mM NaCl	0.91 ± 0.08a	41.0 ± 3.7ab	1.48 ± 0.2ab	1.19 ± 0.02a	90.0 ± 8.10ab	6.42 ± 0.36a	3.7 ± 0.56a	60.0 ± 9.00a
BSS 513	T1	Control (Untreated)	1.00 ± 0.00a	51.3 ± 2.0ab	1.74 ± 0.0ab	1.26 ± 0.02a	100.0 ± 0.0ab	5.86 ± 0.24a	4.54 ± 0.48a	58.6 ± 2.40a
	T2	25 mg kg^{-1} Boric acid	1.00 ± 0.00a	42.6 ± 5.3ab	1.53 ± 0.1ab	1.20 ± 0.03a	96.6 ± 0.00ab	6.73 ± 0.53a	3.570 ± 1.0a	67.3 ± 5.30a
	T3	120 mM NaCl	0.96 ± 0.04a	38.3 ± 4.4ab	1.41 ± 0.1ab	1.18 ± 0.03a	96.6 ± 4.10ab	7.04 ± 0.54a	3.27 ± 0.81a	68.0 ± 2.00a
	T4	25 mg kg^{-1} Boric acid + 120 mM NaCl	0.94 ± 0.04a	43.0 ± 7.0ab	1.51 ± 0.1ab	1.20 ± 0.05a	100 ± 0.00ab	6.57 ± 0.21a	3.60 ± 0.64a	63.3 ± 5.00a
	T5	5 g kg^{-1} Biochar	0.93 ± 0.04a	36.6 ± 1.2ab	1.35 ± 0.0ab	1.20 ± 0.02a	93.30 ± 4.7ab	7.06 ± 0.56a	2.95 ± 0.82a	66.0 ± 7.00a
	T6	5 g kg^{-1} Biochar + 25 mg kg-1 Boric acid	0.91 ± 0.03a	35.0 ± 4.0ab	1.32 ± 0.10a	1.16 ± 0.04a	93.30 ± 4.7ab	7.23 ± 0.37a	3.08 ± 0.00a	67.6 ± 49.0a
	T7	5 g kg^{-1} Biochar + 120 mM NaCl	1.10 ± 0.00a	36.0 ± 3.7ab	1.87 ± 0.7ab	1.19 ± 0.03a	100 ± 0.00ab	7.40 ± 0.69a	2.50 ± 0.90a	111 ± 6.90a
	T8	5 g kg^{-1} Biochar + 25 mg kg^{-1} Boric acid + 120 mM NaCl	1.00 ± 0.00a	40.0 ± 6.9ab	1.47 ± 0.1ab	1.22 ± 0.02a	100 ± 0.00ab	7.0 ± 0.590a	3.14 ± 0.61a	70 ± 71.00a
	T9	2 mM Gallic acid	0.83 ± 0.04a	37.6 ± 4.1ab	1.52 ± 0.3ab	1.18 ± 0.0a	83.3 ± 9.0ab	6.46 ± 0.14a	3.21 ± 0.00a	100 ± 4.40a
	T10	2 mM Gallic acid + 25 mg kg-1 Boric acid	0.93 ± 0.04a	40.0 ± 0.8ab	1.40 ± 0.1ab	1.18 ± 0.03a	93.3 ± 4.7ab	6.70 ± 0.31a	2.50 ± 1.00a	62.6 ± 2.80a
	T11	2 mM Gallic acid + 120 mM NaCl	0.91 ± 0.80a	39.0 ± 3.5ab	1.39 ± 0.1ab	1.19 ± 0.01a	90 ± 8.1.1ab	6.66 ± 0.16a	3.64 ± 0.82a	60.0 ± 5.80a
	T12	2 mM Gallic acid + 25 mg kg^{-1} Boric acid + 120 mM NaCl	0.86 ± 0.90a	36.6 ± 5.4ab	1.31 ± 0.1ab	1.17 ± 0.03a	86.6 ± 9.4ab	6.78 ± 00.24	3.35 ± 0.80a	58.6 ± 5.40a

Values are mean ± S.D. of plants from each treatment

TGI timson germination index, *GI* germination index, *GRI* germination rate index, *GE* germination energy, *GE%* germination energy percentage, *MGT* mean germination time, *T50* time to 50% germination, *CVG* coefficient of velocity of germination

Table 3 Effect of biochar (5 g kg $^{-1}$) and gallic acid (2 mM) on growth parameters of *Solanum melongena* L. under induced abiotic stresses

Varieties	Treatments	RWC	RGR	CGR	AGR-I	AGR-II	TB	PHSTI	DMSTI
Neelam	T1	Control (Untreated)	56 ± 28.1ab	0.080 ± 0.03a	0.69 ± 0a	0.130 ± 0.05bc	0.0380 ± 0.0a	0.94 ± 0ab	32 ± 0a	0.81 ± 0a
	T2	25 mg kg^{-1} Boric acid	49 ± 7.90b	0.080 ± 0.04bc	0.63 ± 0bc	0.140 ± 0.02bc	0.0350 ± 0.1bc	0.550 ± 06b	− 53 ± 4e	− 68 ± 22abc
	T3	120 mM NaCl	47 ± 18.0b	0.088 ± 0.01a	0.69 ± 0a	0.12 ± 0.03abc	0.0360 ± 0.1ab	0.453 ± 08b	− 40 ± 10de	− 158 ± 33bc
	T4	25 mg kg^{-1} Boric acid + 120 mM NaCl	84 ± 11.9ab	0.082 ± 0.02bc	0.64 ± 0bc	0.140 ± 0.02bc	0.0350 ± 0.0bc	0.980 ± 0b	− 8 ± 4bc	1.300 ± 0a
	T5	5 g kg^{-1} Biochar	115 ± 2.6ab	0.083 ± 0.03abc	0.65 ± 0abc	0.140 ± 0.02bc	0.036 ± 0.1abc	1.443 ± 0a	36 ± 0a	9.300 ± 4a
	T6	5 g kg^{-1} Biochar + 25 mg kg-1 Boric acid	95 ± 16.9ab	0.084 ± 0.02ab	0.65 ± 0ab	0.15 ± 0.04abc	0.0380 ± 0a	0.696 ± 0b	− 13 ± 4bcd	− 111 ± 34abc
	T7	5 g kg^{-1} Biochar + 120 mM NaCl	131 ± 18.1a	0.082 ± 0.07bc	0.64 ± 0bc	0.120 ± 0.02bc	0.035 ± 0.1bc	0.77 ± 0ab	− 18 ± 2bcd	− 167 ± 41c
	T8	5 g kg^{-1} Biochar + 25 mg kg^{-1} Boric acid + 120 mM NaCl	92 ± 3.6ab	0.085 ± 0.08ab	0.66 ± 0ab	0.130 ± 0.03bc	0.036 ± 0.2ab	0.793 ± 0b	− 29 ± 6cde	− 92 ± 29abc
	T9	2 mM Gallic acid	103 ± 22.8ab	0.083 ± 0.0abc	0.64 ± 0abc	0.15 ± 0.04abc	0.036 ± 0.0abc	1.10 ± 0ab	29 ± 0a	0.900 ± 0a
	T10	2 mM Gallic acid + 25 mg kg-1 Boric acid	56 ± 19.3ab	0.081 ± 0.04bc	0.63 ± 0bc	0.130 ± 0.02bc	0.035 ± 0.2bc	0.84 ± 0ab	− 2 ± 1b	− 28 ± 13ab
	T11	2 mM Gallic acid + 120 mM NaCl	72 ± 10.3ab	0.082 ± 0.04bc	0.64 ± 0bc	0.140 ± 0.03bc	0.035 ± 0.1bc	0.523 ± 0b	− 7 ± 1bc	− 114 ± 13abc
	T12	2 mM Gallic acid + 25 mg kg^{-1} Boric acid + 120 mM NaCl	58 ± 8.7ab	0.083 ± 0.0abc	0.64 ± 0abc	0.140 ± 0.02bc	0.036 ± 0.0abc	0.713 ± 0b	− 1.4 ± 1b	− 83 ± 10abc
BSS 513	T1	Control (Untreated)	60 ± 17.6ab	0.083 ± 0.02abc	0.65 ± 0abc	0.16 ± 0.02abc	0.036 ± 0.1abc	1.61 ± 0ab	34 ± 0a	1.250 ± 0a
	T2	25 mg kg^{-1} Boric acid	66 ± 23.6ab	0.084 ± 0.03ab	0.65 ± 0ab	0.15 ± 0.04abc	0.036 ± 0.2ab	0.356 ± 0d	− 48 ± 8bc	− 463 ± 103b
	T3	120 mM NaCl	55 ± 13.7ab	0.083 ± 0.05abc	0.65 ± 0abc	0.170 ± 0.00ab	0.036 ± 0.0abc	0.87 ± 0bcd	− 61 ± 10bc	− 107 ± 13a
	T4	25 mg kg^{-1} Boric acid + 120 mM NaCl	59 ± 8.4ab	0.0780 ± 0.0c	0.610 ± 0c	0.12 ± 0.020bc	0.034 ± 0.0c	0.60 ± 0 cd	− 89 ± 29c	− 190 ± 39a
	T5	5 g kg^{-1} Biochar	61 ± 17.8ab	0.083 ± 0.05abc	0.65 ± 0abc	0.14 ± 0.020bc	0.036 ± 0.2abc	1.19 ± 0cde	31 ± 0a	1.00 ± 0a
	T6	5 g kg^{-1} Biochar + 25 mg kg-1 Boric acid	47 ± 35.1b	0.082 ± 0.05bc	0.64 ± 0bc	0.15 ± 0.03abc	0.035 ± 0.3bc	0.91 ± 0cde	− 50 ± 6bc	− 53 ± 2.20a
	T7	5 g kg^{-1} Biochar + 120 mM NaCl	58 ± 15.5ab	0.083 ± 0.07abc	0.64 ± 0abc	0.15 ± 0.02abc	0.036 ± 0.2abc	0.85 ± 0bcd	− 44 ± 5b	− 57 ± 27.0a
	T8	5 g kg^{-1} Biochar + 25 mg kg^{-1} Boric acid + 120 mM NaCl	43 ± 31.7b	0.084 ± 0.06ab	0.660 ± 0ab	0.130 ± 0.10bc	0.036 ± 0.1ab	0.693 ± 0 cd	− 21. ± 2b	− 118 ± 32.0a
	T9	2 mM Gallic acid	55 ± 25.4ab	0.081 ± 0.01bc	0.64 ± 0bc	0.110 ± 0.01c	0.035 ± 0.0bc	1.34 ± 0abc	36 ± 0a	1.100 ± 0a
	T10	2 mM Gallic acid + 25 mg kg-1 Boric acid	51 ± 22.0b	0.083 ± 0.02abc	0.64 ± 0abc	0.20 ± 0.050a	0.036 ± 0.1abc	0.366 ± 0d	− 32 ± 4b	− 429 ± 1.20b
	T11	2 mM Gallic acid + 120 mM NaCl	45 ± 41.2b	0.084 ± 0.09ab	0.66 ± 0ab	0.12 ± 0.02bc	0.036 ± 0.4ab	1.21 ± 0cde	− 34 ± 6b	− 54 ± 89.0a
	T12	2 mM Gallic acid + 25 mg kg^{-1} Boric acid + 120 mM NaCl	73 ± 14.8ab	0.084 ± 0.01ab	0.65 ± 0ab	0.14 ± 0.02	0.036 ± 0.1ab	1.8367 ± 0a	− 31 ± 17b	13 ± 14.0a

Values are mean ± S.D. of plants from each treatment

RWC relative water content, *RGR* relative growth rate, *CGR* crop growth rate, *AGR* absolute growth rate, *PHSTI* plant height stress tolerance index, *DMSTI* dry mass stress tolerance index

Table 4 Effect of biochar (5 g kg^{-1}) and gallic acid (2 mM) on fruit yield of *Solanum melongena* L. under induced abiotic stresses

Varieties	Treatments		FLN	FRNP	FRS (cm)	FFWP (g)	FDWP (g)
Neelam	T1	Control (Untreated)	6.33 ± 0.5abc	5.33 ± 2.3ab	3.74 ± 0.4ab	12.32 ± 0.31ab	7.051 ± 0.64cd
	T2	25 mg kg^{-1} Boric acid	5.0 ± 2.0abc	5.00 ± 3.6ab	3.45 ± 0.11b	7.7333 ± 0.90c	2.720 ± 1.660e
	T3	120 mM NaCl	5.1 ± 0.7abc	5.66 ± 1.1ab	2.26 ± 1.19b	9.823 ± 1.88ab	4.346 ± 2.06de
	T4	25 mg kg^{-1} Boric acid + 120 mM NaCl	6.0 ± 4.3abc	6.33 ± 1.1ab	1.97 ± 0.72b	7.2367 ± 0.25c	2.30 ± 0.900e
	T5	5 g kg^{-1} Biochar	7.33 ± 0.57a	5.33 ± 2.5ab	7.44 ± 6.64b	12.64 ± 1.95ab	7.80 ± 2.04cd
	T6	5 g kg^{-1} Biochar + 25 mg kg-1 Boric acid	5.6 ± 4.0abc	5.00 ± 3.0ab	3.96 ± 0.12b	14.90 ± 0.20ab	10.16 ± 0.1bc
	T7	5 g kg^{-1} Biochar + 120 mM NaCl	7.33 ± 1.15a	6.00 ± 1.0ab	3.32 ± 0.61b	12.19 ± 2.75ab	7.376 ± 2.6cd
	T8	5 g kg^{-1} Biochar + 25 mg kg^{-1} Boric acid + 120 mM NaCl	6.33 ± 0.5ab	7.00 ± 1.00a	4.45 ± 1.11b	19.47 ± 3.720a	14.70 ± 3.72a
	T9	2 mM Gallic acid	5.6 ± 0.5abc	5.00 ± 1.0ab	3.84 ± 0.54b	16.5 ± 3.340ab	11.60 ± 3.3ab
	T10	2 mM Gallic acid + 25 mg kg-1 Boric acid	5.0 ± 1.0abc	4.0 ± 1.73ab	4.0 ± 0.33ab	12.12 ± 3.21ab	7.276 ± 3.1cd
	T11	2 mM Gallic acid + 120 mM NaCl	5.3 ± 0.5abc	3.66 ± 1.52b	3.08 ± 0.44b	6.8667 ± 1.22c	4.91 ± 1.72de
	T12	2 mM Gallic acid + 25 mg kg^{-1} Boric acid + 120 mM NaCl	6.66 ± 1.5a	4.33 ± 1.1ab	3.53 ± 0.25b	10.23 ± 1.75ab	5.546 ± 1.7de
BSS 513	T1	Control (Untreated)	3.6 ± 1.1abc	1.66 ± 0.57b	6.89 ± 0.6bc	22.3 ± 2.27bcd	16.5 ± 2.1cde
	T2	25 mg kg^{-1} Boric acid	3.0 ± 1.0abc	1.33 ± 0.57b	5.04 ± 0.6de	17.633 ± 2.13e	12.16 ± 2.36e
	T3	120 mM NaCl	1.33 ± 0.5 cd	1.00 ± 0.00b	4.51 ± 0.59e	20.26 ± 1.0cde	15.00 ± 1.0de
	T4	25 mg kg^{-1} Boric acid + 120 mM NaCl	1.33 ± 0.5 cd	1.00 ± 0.00b	4.77 ± 0.65e	7.7333 ± 0.90f	2.366 ± 0.90f
	T5	5 g kg^{-1} Biochar	2.6 ± 0.5abc	1.66 ± 0.57b	7.56 ± 0.4ab	27.20 ± 2.30ab	22.46 ± 2.4ab
	T6	5 g kg^{-1} Biochar + 25 mg kg-1 Boric acid	3.3 ± 0.5abc	2.66 ± 0.57b	7.86 ± 0.9ab	25.70 ± 1.75ab	21.1 ± 1.5abc
	T7	5 g kg^{-1} Biochar + 120 mM NaCl	3.3 ± 0.5abc	2.00 ± 1.73b	7.4 ± 0.59ab	22.9 ± 0.95bcd	17.5 ± 1.10bc
	T8	5 g kg^{-1} Biochar + 25 mg kg^{-1} Boric acid + 120 mM NaCl	2.6 ± 0.5abc	5.66 ± 4.61a	7.13 ± 0.5ab	23.6 ± 2.43abc	19.2 ± 1.0bcd
	T9	2 mM Gallic acid	1.6 ± 0.5abc	1.66 ± 0.57b	5.96 ± 0.6 cd	28.40 ± 1.270a	22.93 ± 1.05a
	T10	2 mM Gallic acid + 25 mg kg-1 Boric acid	3.3 ± 1.1abc	2.00 ± 1.00b	7.6 ± 1.14ab	25.53 ± 2.15ab	19.7 ± 1.6bcd
	T11	2 mM Gallic acid + 120 mM NaCl	2.6 ± 0.5abc	1.33 ± 0.57b	8.21 ± 0.71a	22.06 ± 1.9bcd	16.2 ± 2.4cde
	T12	2 mM Gallic acid + 25 mg kg^{-1} Boric acid + 120 mM NaCl	1.00 ± 0.0d	1.12 ± 0.3b	7.78 ± 0.1ab	18.33 ± 0.82de	24.6 ± 2.19de

Values are mean ± S.D. of three plants from each treatment

FLN flower number, *FRN* fruit number per plant, *FRS* fruit size, *FFWP* fruit fresh weight per plant, *FDWP* fruit dry weight per plant

biochar application in Neelam variety (36.60 ± 0.0) and gallic acid (36.30 ± 0.0) in BSS 513. The non-significant decrease in stress-tolerance capability of *Solanum melongena* L. under stress treatment which were greatly decreased by applied growth regulators as compared to control without growth stimulators suggesting the semi-tolerance ability of the specie under induced abiotic stress. Contrarily, dry mass stress tolerance index (DMSTI) of plant was significantly ($p < 0.05$) enhanced in biochar treatment (T5) in Neelam variety (9.300 ± 4.70) and observed minimum under salinity stress in gallic acid presoaked seeds. However, in variety BSS 513 highest DMSTI was recorded in T12 (13.00 ± 14.0) where gallic acid pretreatment alleviate the toxicity of both combined boron and salt stress while minimum value has been reported under combined stresses without growth regulators ($p < 0.05$). Results undoubtedly presented the crucial role of biochar and gallic acid by enhancing the stress tolerance index of the specie to give more height and biomass production. ANOVA results in supplementary Table 1, revealed that all the interaction between genotype, between treatments, between growth regulators and their relations with each other T x G, G x GR, T x GR, and G x T x GR were found non- significant ($p < 0.05$) for the measured traits except PHSTI and DMSTI that indicated positive and significant interactions.

Yield Parameters

Crop yield must be increased markedly over the upcoming years to keep pace with global food demand led by growing population. Abiotic stresses decrease growth and yield of crop by affecting plant metabolism. Results obtained from the designed experiment (Table 4) showed that flower number (FN) per plant measured enhanced under biochar

treatment (T5) to 7.33 ± 0.57 in Neelam variety and reduced by boron stress (T10). Control condition favored maximum flower number (3.6 ± 1.1) in BSS 513 while combine boron and salt stress decreased FLN non significantly. The highest fruit number per plant (FRNP) accessed when plant was exposed to combined salinity and boron stress under treatment T12 in both varieties (7.0 ± 1.0, 5.6 ± 4.6) with biochar application in Neelam and gallic acid presoaked seed in BSS 513. Based on these findings, the growth regulators have an affective potential in stimulation of various mechanisms that increase plant yield. Nonetheless, biochar amendment to soil upgrades the fruit size (FRS) taken in centimeters under control treatment (7.44 ± 6.6) in Neelam variety and decreased in T4. Likewise, large sized fruits in BSS 513 were found under boron stress with biochar formation (T6) whereas salinity stress decrease the size of fruit, respectively. Fruit weight less than 10 g is of no economic importance. Plants of both varieties responded differently to biochar and gallic acid such as soil treated with biochar under combined salt and boron stress (T8) increased fruit fresh weight per plant (FFWP) and fruit dry weight per plant (FDWP) by 19.47 ± 3.72 and 14.70 ± 3.72 g in Neelam. On the other hand, variety BSS 513 represented increase in FFWP by 28.40 ± 1.27 under gallic acid treatment (T9) and FDWP in combined born and salt stress by 24.6 ± 2.19 with same growth regulator. However, at combined abiotic stresses (T4), minimum FFWP (7.23 ± 0.25) and FDWP (2.30 ± 0.90) in Neelam variety has been found in comparison to control treatment. An effective decrease in FFWP (7.73 ± 0.9) and FDWP (2.36 ± 0.9) assumed under interactive effect of salt and boron stress, respectively. It has been proposed that improvement in fruit yield of plant by biochar and gallic acid might be correlated with enhancement in leaf chlorophyll content and thus improving plant performance under suboptimal growth conditions. There was no significant treatment effect found on egg-plant fruit number, fresh weight, dry weight as compared to control as shown by statistical data (Supplementary Table 1).

Physiological and Biochemical Attributes

Apparent morphological and physiological attributes of egg-plant are related to its ability in maintaining cellular metabolism, water uptake and oxidative stress under abiotic stresses lowered the physical injuries and physiological disturbance need to be quite understood. The present results of photosynthetic components including chlorophyll 'a' and chlorophyll 'b' reported maximum efficiency of both the pigments (chlorophyll 'a' and chlorophyll 'b') in control (T1) followed by gallic acid treatment along with salinity stress (T11) in variety Neelam (Fig. 2) while in variety BSS 513, changes in the photosynthetic pigments were markedly observed under biochar along with combined salinity and boron stress (T8) respectively. However, a noticeable lowest content of chlorophyll capacity observed in T8 (variety Neelam) and T3, T4 in variety BSS 513. Besides, the addition of biochar and GA alleviated the damage of salinity and boric acid stress on photosynthetic capacity such that the values reached the control. F-ratio in supplementary Table 1, showed that both chlorophyll 'a' and 'b' significant approach in terms of growth regulators (GR) and interactions between G x T x GR at $p < 0.05$.

In salt and boric acid, soluble sugar content increased and was measured to a greater extent in gallic acid than biochar. (Fig. 2). In other words, gallic acid treatment with salinity and boric acid stress (T12) in both varieties increased sugar content and decreased in salinity stress in variety Neelam whereas in contrast with variety BSS 513, sugar content has been found reduced in boric acid stress, respectively. GA as compared to biochar treatment to salinity and boric acid stressed plants more effective in enhancing the sugar content. The interactions were found significant ($p < 0.05$) between genotype and between growth regulators (Supplementary Table 1). Also, highest value of soluble protein content (Fig. 2) of leaf treated with biochar in combined salinity and boric acid stress observed in variety Neelam. Besides, the addition of gallic acid increased protein content after salinity exposure in variety BSS 5113. Moreover, decreased in protein level measured in control (T1) in both varieties clearly suggested the best significant ($p < 0.001$) interactions between all treatments and application of growth regulators (Supplementary Table 1).

The influence of biochar and gallic acid on oxidative damage caused by salinity and boron stress was studied by detecting GB content (Fig. 3). In results, biochar application significantly increased GB under boron stress alone compared to control (untreated) in Neelam. However, exposure to gallic acid followed by biochar greatly reduced GB in combined salinity and boron stresses. In contrast, the BSS 513, maximum GB content was recorded by gallic acid application under born stress (T10) while significant reduction in GB level has been observed in combined salinity and born stresses (T12). ANOVA, supplementary Table 1, showed that growth regulators interactions were non-significant but were found more significant ($p < 0.001$) and positive when interacted with applied stress (G x T x GR).

Key Stress Indicators Analysis (H_2O_2 & MDA)

H_2O_2 content in the leaves of egg-plant treated with boric acid and combined abiotic stress salinity stress (T2 & T4) increased than control and decreased with gallic acid with similar stress treatments (T12) in variety Neelam. In contrast, the BSS 513 revealed low level of H_2O_2 through gallic acid application under salinity stress followed by biochar

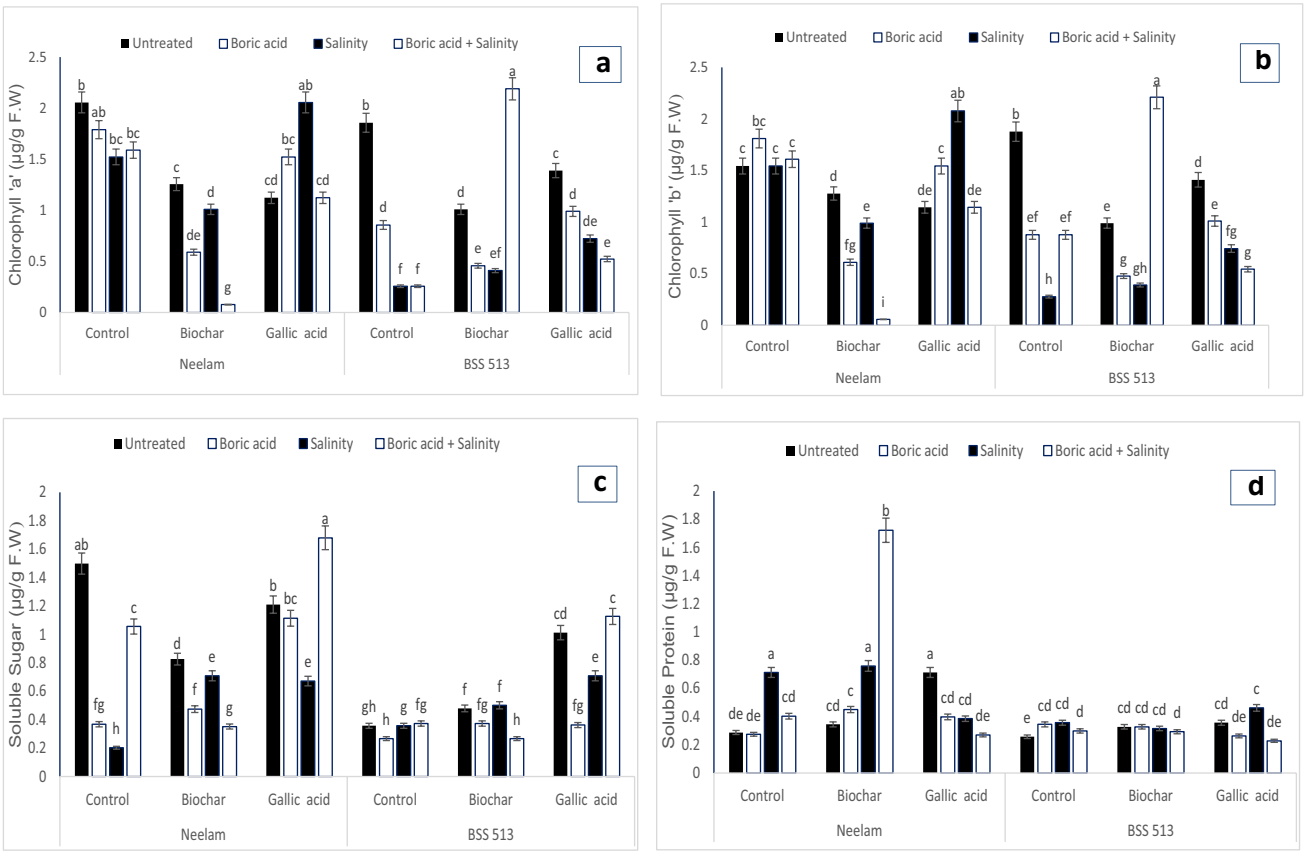

Fig. 2 Role of biochar (5 g kg^{-1}) and gallic acid (2 mM) on **a** chlorophyll 'a' **b** chlorophyll 'b'**c** soluble sugar and **d** soluble protein in foliar (mean ± standard) under induced salinity (120 mM NaCl) and boron (25 mg $kg.^{-1}$) stress. Vertical bars stand for standard errors with least significance difference among mean values at $p < 0.05$

addition to soil with similar stress condition. Moreover, biochar and gallic acid application with their interaction to combined salinity and boron stresses (G x T x GR) decreased H_2O_2 accumulation non-significantly in both varieties (Fig. 3; Supplementary Table 1). Our current experiment study proposed that lipid peroxidation (MDA) of leaf was affected by salt interaction (Fig. 3) that amplified MDA content that was more pronounced in the absence of growth regulators in both varieties. An effective decrease in the trait was assumed with gallic acid primed seedlings followed by biochar amendment under interactive salt and boron stress in variety Neelam and salinity stress in BSS 513. Present results marked that salinity stress might have caused toxic injury on membranes and was well alleviated by gallic acid pre-soaking technique. In contrast, a non-significant increase by biochar application to soil was measured under salinity (T7) than control condition in BSS 513. Besides this, gallic acid successfully decreased the level of MDA over induced boron stress alone (T10). Results in supplementary Table 1, declared significant ($p < 0.05$) F-ratio with respect to interactions between genotype, between treatment and their interaction with each other (G x T x GR).

Antioxidant Enzymes (POD, SOD)

The scavenging activity of antioxidant enzymes in leaves was examined to show the impact of gallic acid and biochar in reducing salinity and boric acid stress (Fig. 4). The total SOD activity was significantly highest than compared to control in Neelam variety with biochar treatment under boron followed by boric acid stress while reduced level determined in control. Additionally, in variety BSS 513, a considerable increase in SOD activity was seen after gallic acid treatment under salinity and then boric acid treatment. Similar to this, spectroscopic examination revealed that the Neelam variety's leaves with gallic acid displayed the highest POD activity up to a substantial level under combined boric acid and salt stress. The reduced POD level was noticed in salinity stress with gallic acid application non-significantly. In detail, enhancement in POD content of BSS 513 variety exhibited with gallic acid alone followed by biochar with mutual salinity and boric acid stresses. Results proposed that both the varieties respond differently under distinct growth regulators. However, both regulate the ability of plant antioxidant systems to scavenge free radicals. Results showed

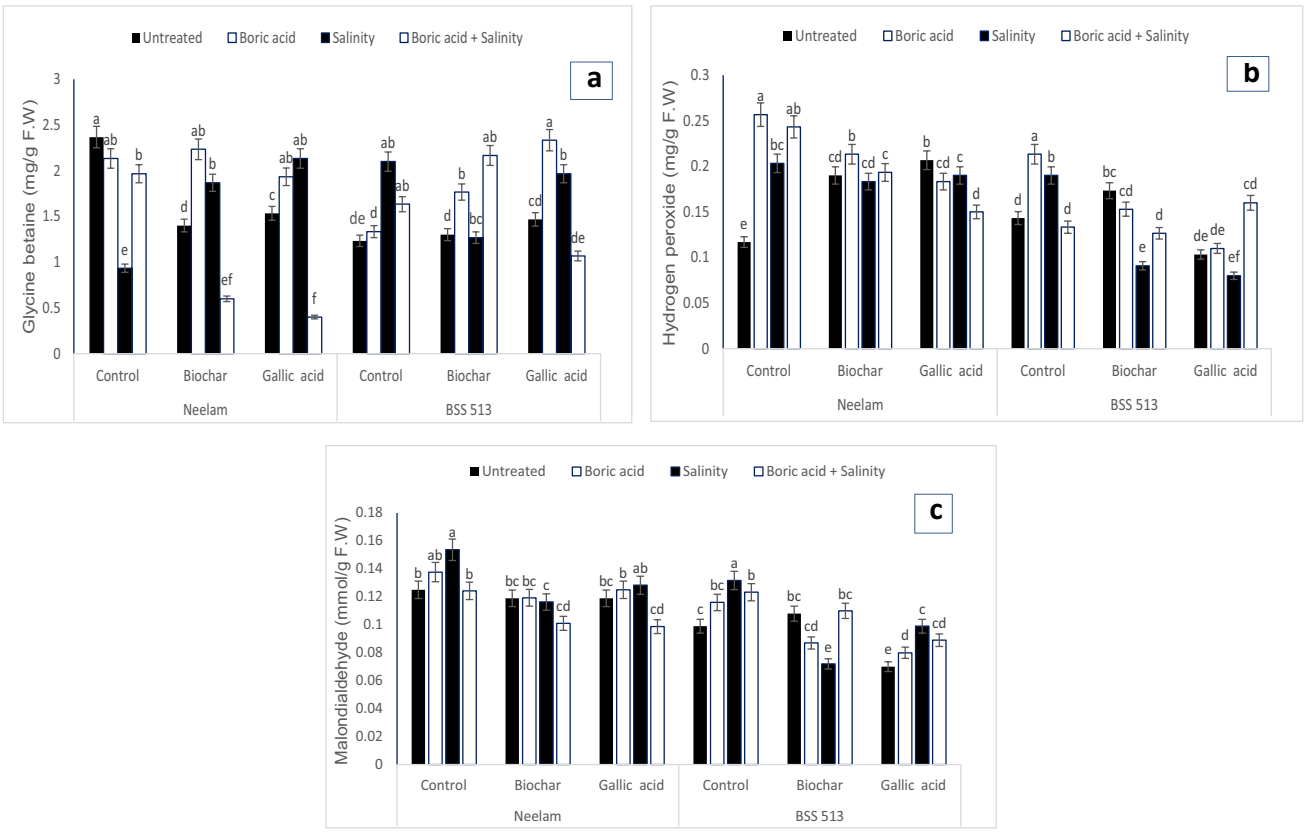

Fig. 3 Role of biochar (5 g kg^{-1}) and gallic acid (2 mM) on **a** glycine betaine **b** hydrogen peroxide **c** malondialdehyde in foliar (mean ± standard) under induced salinity (120 mM NaCl) and boron (25 mg $kg.^{-1}$) stress. Vertical bars stand for standard errors with least significance difference among mean values at $p < 0.05$

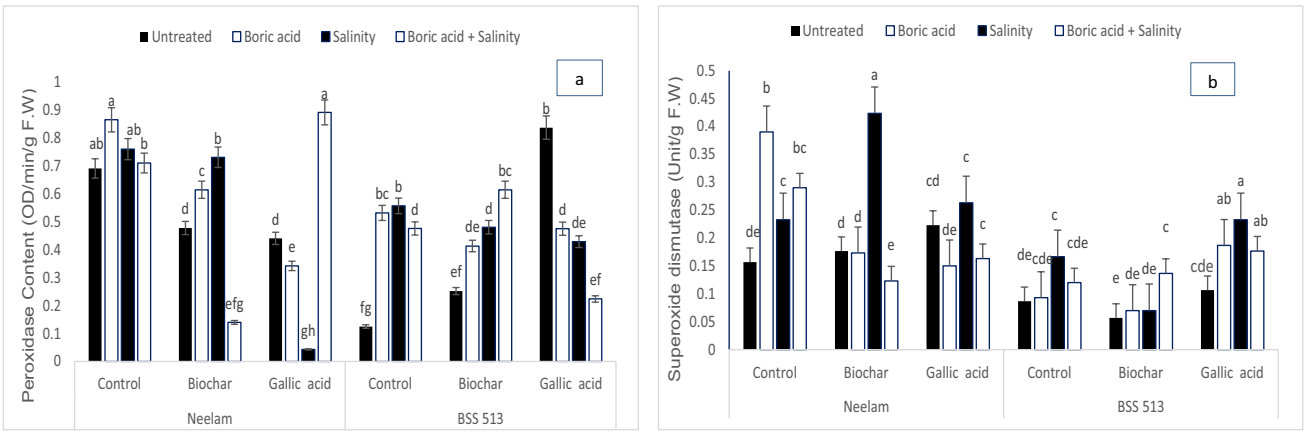

Fig. 4 Role of biochar (5 g kg^{-1}) and gallic acid (2 mM) on **a** peroxidase and **b** superoxide dismutase in foliar (mean ± standard) under induced salinity (120 mM NaCl) and boron (25 mg $kg.^{-1}$) stress. Vertical bars stand for standard errors with least significance difference among mean values at $p < 0.05$

that plants become more resilient to environmental challenges when their antioxidant enzyme activity is elevated in stressful conditions. However, ANOVA in supplementary Table 1, proposed markedly significant results in terms of G x T x GA, T x GR, G x GR, between treatment at $p < 0.05$, respectively.

Leaf Surface Study Through SEM and Electron Microscopy

Plants alter their stomata closure to minimize water loss and a moderate absorption of CO_2 for changing circumstances (Richardson et al. 2017). Leaf structure analysis (Fig. 5a–d; Table 5) obtained from the micrographs of SEM (JSMIT100-JEOL-JAPAN), showed that gallic acid treatment (T10) significantly increased stomata length and width of 240 µm and 165 µm under salinity stress in Neelam variety and under combined salt and boron stress (T12) by 143 µm and 177 µm in variety BSS 513. In detail, the variation in stomata density (STD) has been calculated maximum by 181 per mm^2 in Neelam and 244 per mm^2 in BSS 513 when gallic acid and biochar treatment was applied under control. However, both stresses in combine form decreased the trait more negatively in treatment T4. Contrarily, the biochar treatment to soil enhanced leaf stomata size and density in both varieties significantly $p < 0.05$ ranges from 28.9 µm to 35.4 µm whereas lowered up to 18 µm by combined salt and boron stresses (T4) without the amendment of soil with biochar and seed priming with gallic acid in Neelam variety and under treatment T12 in BSS 513 with application of growth regulators.

A rise in density and size of trichome may contribute a significant role in stress tolerance mechanism of plant. Nonetheless, results (Fig. 6a–d; Table 5) showed a noticeable increase in trichome length by biochar application under boron stress (T6) was ranging from 3125 µm in Neelam variety and 2401 µm with soaked seeds by gallic acid in BSS 513 thus decreasing the harsh effects of combined stresses (T12). In detail, an increased level has been noticed under boron stress that led to enhance in trichome size by biochar treatment as compared to control. Data clearly confined the adverse effect of combined stress condition on leaf photosynthetic aperture under untreated growth regulator or biochar applications. Nonetheless, stomata density showed a noticeable increase by biochar application under boron stress (T6) was ranging from 3125 µm in Neelam variety and 2401 µm with soaked seeds by gallic acid in BSS 513 thus decreasing the harsh effects of combined stresses (T12). The data for stomata and trichome analysis was confirmed by the evaluations via F-ratio analysis of all the traits with significant level at $p < 0.05$ in terms of interactions between treatment, between genotype, between growth regulators and between G x T, G x GR, T x GR, G x T x GR respectively (supplementary Table 1).

Correlation and Regression Analysis

Regression and correlation (Supplementary Table 2, 3) exemplified a positive and significant ($p < 0.05$) relation between the chlorophyll 'b' content of leaf in both varieties of *S. melongena* L. via growth regulators was $r = 0.12$, SSC and SPC were calculated $r = 0.34$ whereas as SOD, H_2O_2 and MDA measured $r = 0.67$, $r = 0.41$, $r = 0.33$ respectively. However, a non-significant and negative relation between chlorophyll 'a' content, POD and GB has been observed. Contrarily, by applied abiotic stresses, all the traits were responded negatively and non-significant except for the SPC, SOD, H_2O_2. Correlation analysis of physiological and biochemical attributes determined a positive and significant correlation between chlorophyll and SPC, SOD correlate with SPC at $p < 0.01$ whereas, GB also evaluated significantly in correlation with SPC ($p < 0.05$). However, MDA and H2O2 significantly correlate with antioxidant enzymes SOD, POD and SPC at $p < 0.05$, respectively.

Discussion

Biochar Analysis

The present study was conducted to investigate the effects biochar and gallic acid on salt and boron stress mitigation in *Solanum melongena* L. Biochar, a carbon rich organic compound generally when added to soil increase its pH, EC, ion exchange capacity, many organic compounds including cellulose, lignin, and enhance crop yield. This high carbon content is due to elevated temperature of pyrolysis procedure leading to fix the carbon content enhanced while oxygen decreased (Hao et al. 2013). SEM/EDX results showed more cracks and porous nature of biochar along with high EC, pH, and carbon content as compared to oxygen. Our investigation is in lineage to the results of Hao et al. (2013) and Mendez et al. (2013) who worked and analyzed that large pore with cracks generated due to high temperature pyrolysis that enhance carbon stability in soil. Ahmad et al. (2012) work was also in agreement to our results who examined the decrease oxygen/carbon ration with high temperature hence results in hydrophilic surface of biochar. Elemental analysis via EDX exposed element content and nutrients to our crops may be a useful for plants in time of growth and yield. Thus, biochar is a promising technique to improve soil physical structure, fertility, nutrients availability seed germination and growth (Nafees et al. 2021).

Germination, Growth and Yield Parameters

Compared to control, pretreatment gallic acid and biochar amendment to soil heightened seed germination energy, velocity of germination, germination percentage and emergence percentage. This might be due to best water holding capacity of biochar for better germination or mineral nutrients composition in biochar that released in soil thus maintaining best level of fertility to stimulate growth

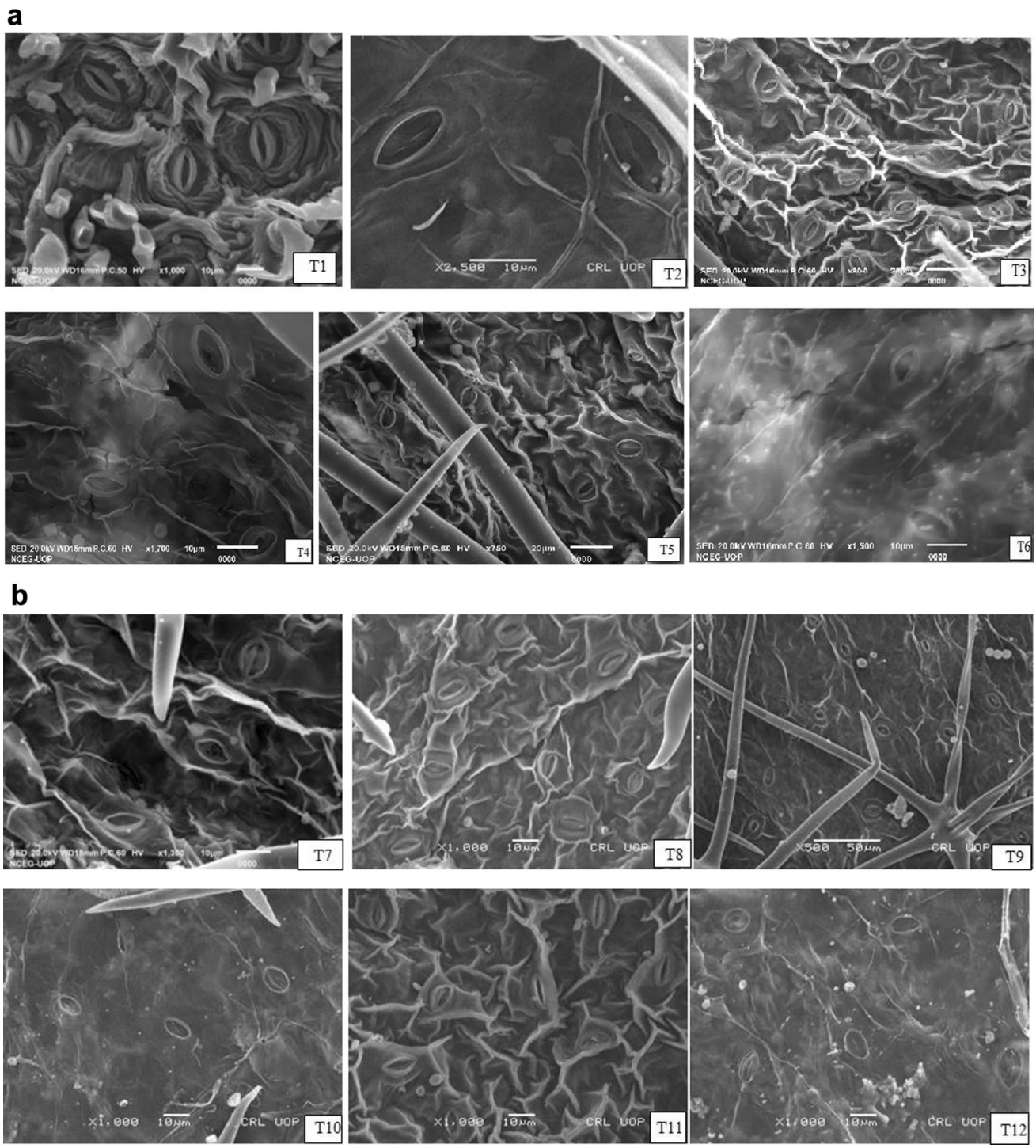

Fig. 5 **a** Changes in leaf stomata length (STL)), stomata width (STW), stomata density (STD) of *Solanum melongena* L. in variety Neelam through SEM under induced salinity (120 mM NaCl) and boron (25 mg kg^{-1}) stress. **b** Changes in leaf stomata length (STL)), stomata width (STW), stomata density (STD) of *Solanum melongena* L. in variety Neelam through SEM under induced salinity (120 mM NaCl) and boron (25 mg kg^{-1}) stress. **c** Changes in leaf stomata length (STL)), stomata width (STW), stomata density (STD) of *Solanum melongena* L. in variety BSS 513 through SEM under induced salinity (120 mM NaCl) and boron (25 mg kg^{-1}) stress. **d** Changes in leaf stomata length (STL)), stomata width (STW), stomata density (STD) of *Solanum melongena* L. in variety BSS 513 through SEM under induced salinity (120 mM NaCl) and boron (25 mg kg^{-1}) stress

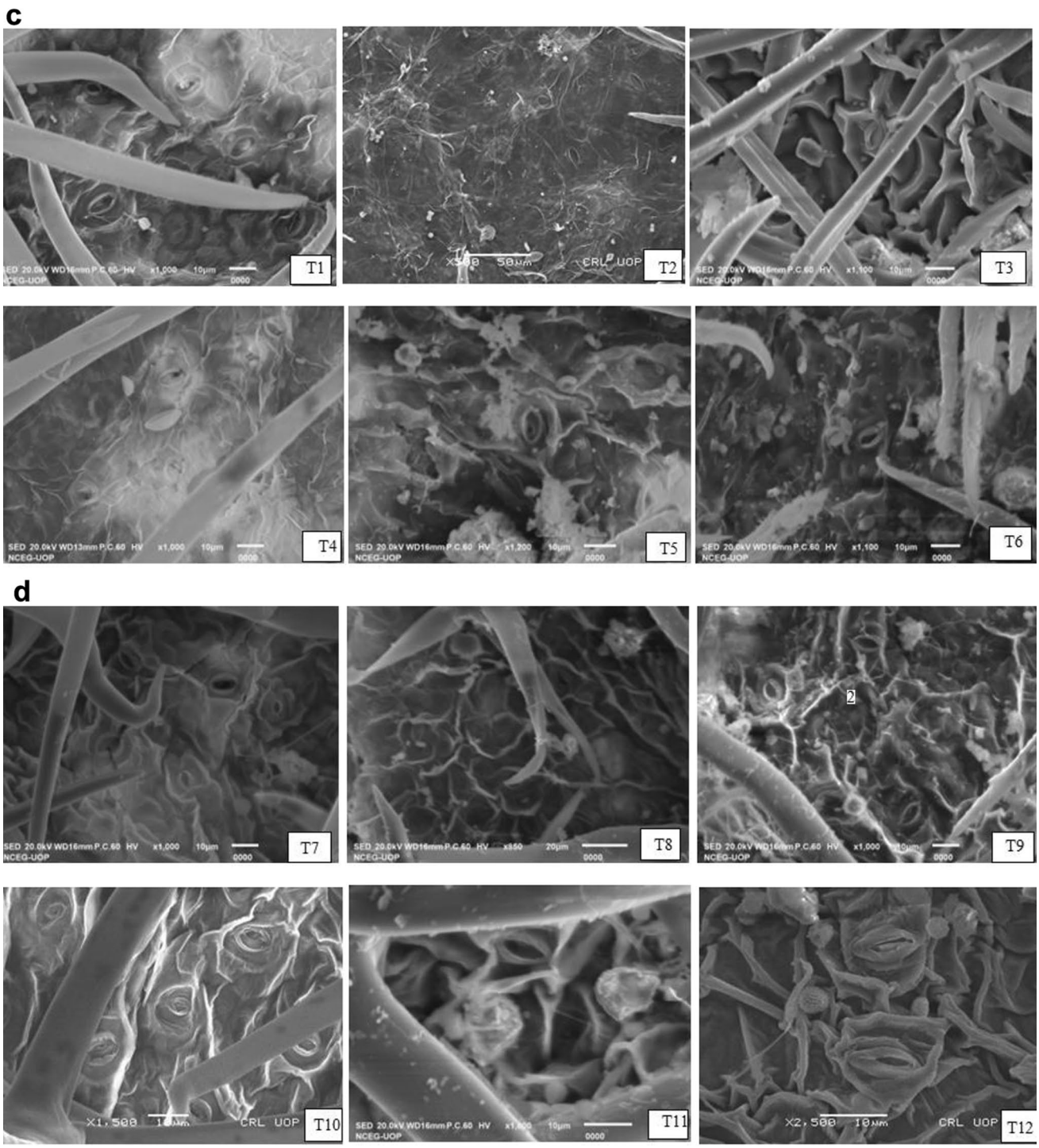

Fig. 5 (continued)

parameters. Our investigations are in linked with studies of Ali et al. (2021) who reported that biochar addition into the soil increased germination parameters. Similar results were determined by Zhang et al. (2021); Bua et al. (2020). Similarly, maximum T50% and decreased the time for seedling germination (MGT) has been reported by gallic acid pre-treated seeds. Although, native bacteria and soluble plant growth regulators present in biochar also have an advantage to better germination of seeds (Page-Dumroese et al. 2015). Adetunjia et al. (2021) investigated that gallic acid proved to be more effective for cabbage and lettuce seed germination.

Table 5 Effect of biochar (5 g kg^{-1}) and gallic acid (2 mM) on leaf anatomical characteristics of *Solanum melongena* L. under induced abiotic stresses

Treatments		STL (µm)		STW (1 mm)		STD (µm)		TL (µm)	
		Neelam	BSS 513	Neelam	BSS 513	Neelam	BSS 513	Neelam	BSS513
T1	Control (Untreated)	157 ± 2.64bc	26.667 ± 5.5d	112 ± 2.6bc	17.66 ± 1.5ef	136 ± 4.0bc	204 ± 4bc	270.0 ± 15c	612 ± 135bcd
T2	25 mg kg^{-1} Boric acid	111 ± 6.51cde	25.33 ± 2.5de	89 ± 6.5bcd	15.66 ± 2.1 fg	108 ± 4.0 cd	176 ± 4de	336.00 ± 6c	778 ± 55bcd
T3	120 mM NaCl	27.66 ± 4.04 g	24.33 ± 5.5de	58 ± 9.10ef	19.66 ± 5.6ef	96.0 ± 4.0d	156 ± 8 fg	311 ± 227a	776 ± 145bcd
T4	25 mg kg^{-1} Boric acid + 120 mM NaCl	38.6 ± 4.06 fg	32.6 ± 2.5cde	24.0 ± 6.2hi	22.66 ± 1.5de	76.0 ± 4.0e	134 ± 6hi	622 ± 85bc	558 ± 80 cd
T5	5 g kg^{-1} Biochar	22.0 ± 4.58 g	19.0 ± 2.60ef	19.6 ± 3.2hi	17.33 ± 3.2ef	168 ± 4.0ab	244.0 ± 4a	236 ± 19c	288 ± 25.0d
T6	5 g kg^{-1} Biochar + 25 mg kg-1 Boric acid	225 ± 54.85a	24.33 ± 3.1de	140 ± 32.1ab	17.14 ± 1.5ef	133 ± 12bc	188 ± 4 cd	3125 ± 289a	274 ± 27.0d
T7	5 g kg^{-1} Biochar + 120 mM NaCl	73 ± 15.27def	49.333 ± 7.5c	50.0 ± 0.0 fg	29.0 ± 6.0 cd	110 ± 8.3 cd	165 ± 6ef	1417 ± 384bc	553 ± 80 cd
T8	5 g kg^{-1} Biochar + 25 mg kg^{-1} Boric acid + 120 mM NaCl	66. ± 15.23ef	70.33 ± 15.0b	40.0 ± 17gh	55.33 ± 5.5bc	128 ± 4bcd	112 ± 4jk	1287 ± 712bc	619 ± 73bcd
T9	2 mM Gallic acid	19.0 ± 3.610f	71.333 ± 20ab	15.6 ± 1.5 h	68.33 ± 19ab	181.0 ± 6.1a	208 ± 12b	873 ± 881bc	845 ± 43bcd
T10	2 mM Gallic acid + 25 mg kg-1 Boric acid	240 ± 38.68a	39.66 ± 5.5 cd	165 ± 19.0a	26.3 ± 2.1 cd	124 ± 4bcd	141 ± 6gh	2102 ± 374ab	1427 ± 647b
T11	2 mM Gallic acid + 120 mM NaCl	189 ± 53.7ab	31.33 ± 4.5cde	78 ± 38cde	24.6 ± 8.1 cd	116.0 ± 4 cd	129 ± 6hi	1415 ± 130bc	1278 ± 84bc
T12	2 mM Gallic acid + 25 mg kg^{-1} Boric acid + 120 mM NaCl	193 ± 21.0ab	143.50 ± 26a	124 ± 30abc	77.66 ± 40a	112.0 ± 4 cd	112 ± 4jk	1676 ± 861abc	2401 ± 417a

Values are mean ± S.D. of three plants from each treatment

STL stomata length, *STW* stomata width, *STD* stomata density, *TL* trichome length

In addition, salt stress is known to decrease RWC (Irshad et al. 2021). Our results indicated significant improvement in RWC of plant under salt stress that are well in line with the results obtained by Abbas et al. (2021) who worked on quinoa and told that high RWC with biochar addition enhance RWC of plant in saline soil. Farhangi-Abriz and Torabian (2018) also proposed highest RWC under saline stress in bean seedlings. Similarly, a non-significant reduction in RGR and CVG by employed stress with treated and untreated condition believed that both gallic acid and biochar unwarily improve in regulating such traits as varieties respond tolerantly. According to Parkash and Singh (2021) said that egg-plant is moderately sensitive to salinity stress such that the dry mass was lowed and was non-significantly different as compared biochar treated plants under same stress. In our research studies, a non-significant enhancement in AGR-I and AGR-II in biochar amendment treatment with its co-attributes under salinity. On contrary, studies by Usman et al. (2016) on tomato and Akhtar et al. (2015) on wheat, it was found that salinity stress decreased the plant height, leaf area while biochar addition enhanced significantly. Parkash and Singh (2020) also proposed enhancement occurred in plant height and leaf surface area with biochar amended treatments than the control (non-biochar). Loss in plant biomass under salinity and boron stress was significantly enhanced under growth stimulators. Similarly, PHSTI and DMSTI were accelerated due to biochar formation under stress condition suggested the ability of biochar by mitigating the adversities of saline stress. Our results confined the property gallic acid by enhancing crop yield are in lineage with finding of Gharib et al. (2018) who reported that GA up to 150 ppm concentration significantly enhanced 100-seed weight in grams of cowpea plants more than control treatment.

Physiological and Biochemical Characteristics

The decrease in chlorophyll content is directly correlated with a decrease in the photosynthetic activity of plants. Torabian et al. (2019) explained that there is reduction in ROS and DPPH activity in leaf cells due to applied biochar formation and thus enhanced chlorophyll amount in saline soil.

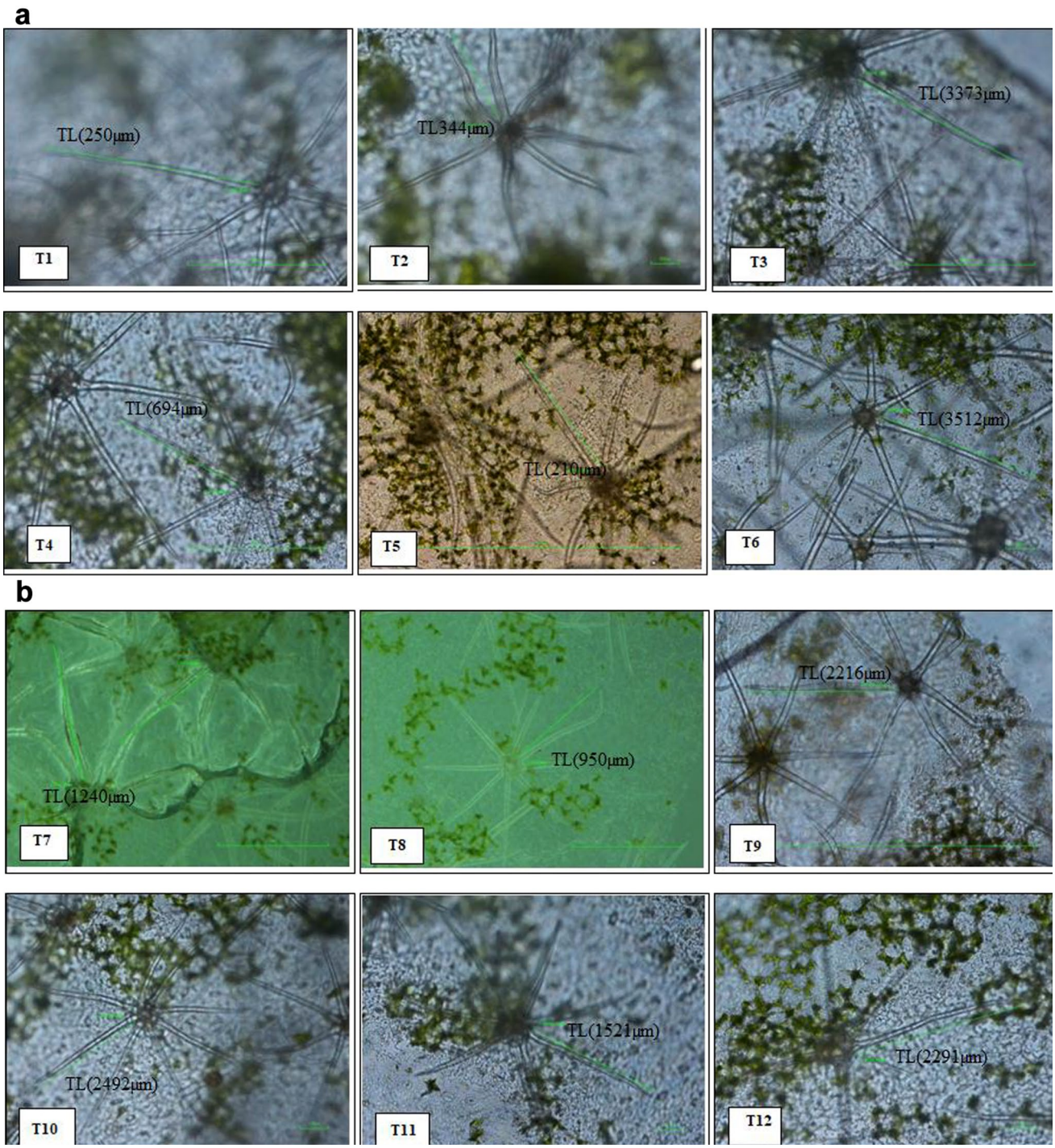

Fig. 6 **a** Changes in trichomes length (TL) of *Solanum melongena* L. in variety Neelam through light microscopy under induced salinity (120 mM NaCl) and boron (25 mg kg^{-1}) stress. **b** Changes in trichomes length (TL) of *Solanum melongena* L. in variety Neelam through light microscopy under induced salinity (120 mM NaCl) and boron (25 mg kg^{-1}) stress. **c** Changes in trichomes length (TL) of *Solanum melongena* L. in variety BSS 513 through light microscopy under induced salinity (120 mM NaCl) and boron (25 mg kg^{-1}) stress. **d** Changes in trichomes length (TL) of *Solanum melongena* L. in variety BSS 513 through light microscopy under induced salinity (120 mM NaCl) and boron (25 mg kg^{-1}) stress

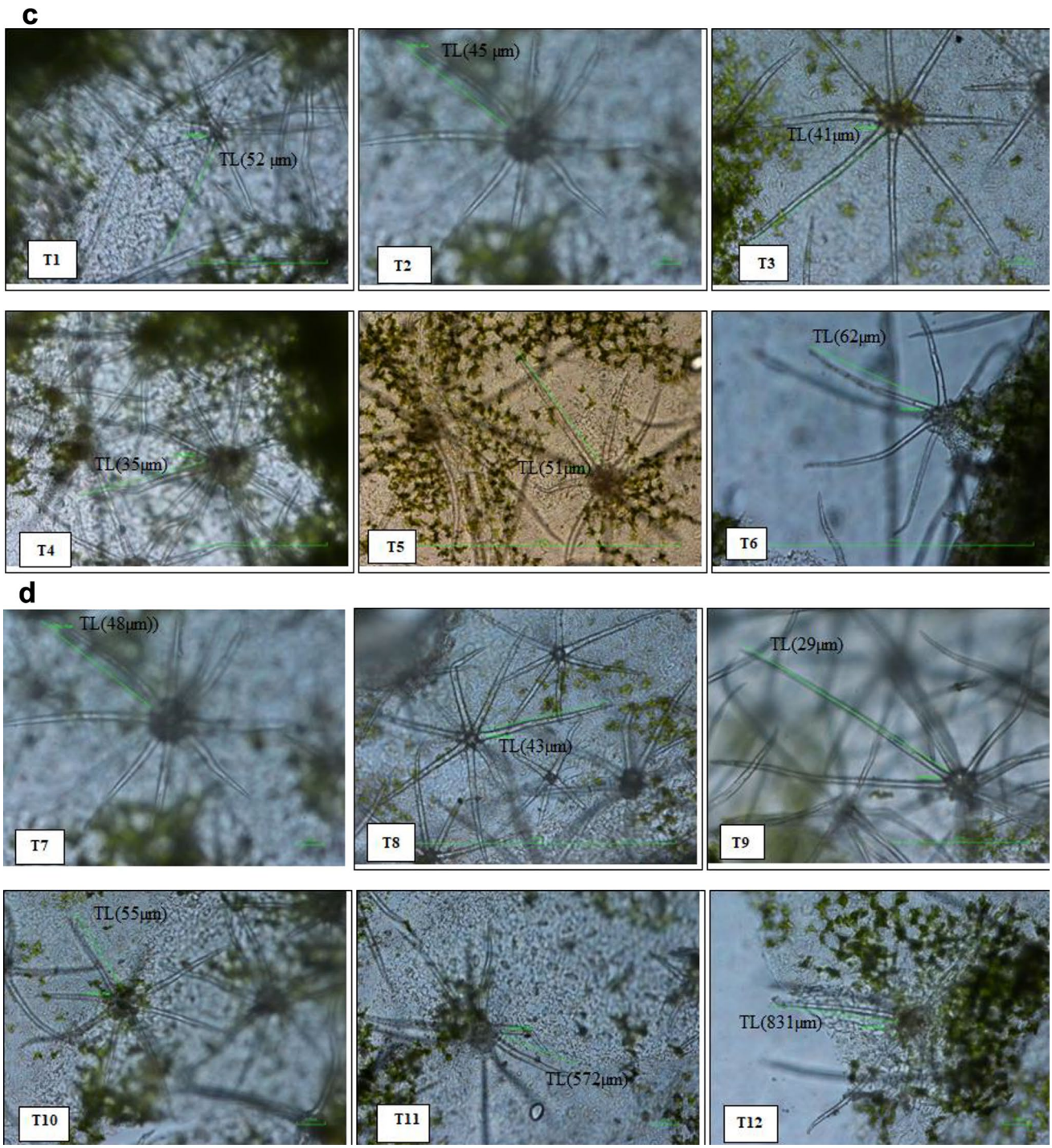

Fig. 6 (continued)

Biochemical results reported enhancement in chlorophyll 'a' and chlorophyll 'b' content by biochar addition to soil and gallic acid successfully withhold the advertises of both stresses and improved photosynthetic pigments. Our results are in line with the findings Ran et al. (2020) proposed significant increase in chlorophyll under salinity stress. Results agree with the investigation of Klein et al. (2015); and Farghaly et al. (2021), who said that gallic acid increase the photosynthetic capacity of tomato callus under boron stress. A small content of free amino acid and proline buildup in cytoplasm can rapidly reach a great level and play a more effective role in cell osmotic potential (Farhangi-Abriz and

Torabian 2017). Our results are linked to the evaluation of Kiani et al. (2021) worked in wheat and *Aegilops cylindrica* accompanied more chlorophyll under salt stress.

Accumulation of osmolytes under stress condition counterbalance osmotic pressure and perform significant role in redox-regulation via their capability to scavenge oxy-free radicals for most advantageous level for cellular actions (Hisyam et al. 2017). Furthermore, increase in soluble sugar content in salinity and boron stress increased in gallic acid treatment while non-significant enhancement in biochar amendment proved that plant required SSC for osmotic adjustment under abiotic stress could be further explained by the investigation of Farhangi-Abriz and Torabian (2017) in bean seedlings where no applicable changes observed in sugar concentration under salt stress. Similar results are indicated by experiment of Kanwal et al. (2018) in wheat under salinity stress and soil biochar formation. Glycine betaine (GB) and proline are the most important organic osmolytes and stress marker that accumulate in different plant species in response to environmental stresses. A remarkable increase in GB obtained under stress condition and reduced by biochar and GA application.

The dismutation of O^{-2} into H_2O_2 and oxygen by SOD whereas H_2O_2 into H_2O through POD plays a function in cellular protecting against ROS and is a vital step in defending the cells. (Farhangi-Abriz and Torabian 2017). Higher SOD and POD activity in boron and salt indicated that gallic acid inhibit the oxidative injury that may takes part in mechanism of biomolecules synthesis by scavenging the ROS (Rosa et al. 2022). The result agrees with work of Ozfidan-konakci et al. (2015) where increased in SOD activity in *Oryza sativa* under gallic acid subjected to salt stress observed. Gallic acid presoaking treatment led to SOD activity enhancement in wheat seedlings (Bhardwaj et al. 2017). Sgherri et al. (2003) proposed a model that explain the enhancement in POD activity via gallic acid treatment under salinity stress. Ozfidan-konakci et al. (2015) also reported that gallic acid alone or under stress condition did not change POD activity.

Lipid peroxidation is associated with plant stress tolerance mechanism that arising from oxidative stress conditions. In this study a significant decrease in MDA content by both stresses in pre-treatment gallic acid has been observed that proposed same finding investigated by Yildiztugay et al. (2017), gallic acid application caused a decline in lipid peroxidation on membranes under temperature stress. Our findings are in accordance with the findings of Yetişsin and Kurt (2019) in maize seedlings supplemented with gallic acid cause remarkable decrease in MDA contents thus indicating a protective effect on membranes under cupper stress. Shao et al (2005) investigated reduced levels of MDA in pre-treatment with gallic acid confined the indication of high membrane stability. Similarly, gallic acid treatment reduced formation of hydrogen peroxide in stress condition were consistent with previous results representing gallic acid pre-treatment appeared to be more effective by decreasing H_2O_2 content in wheat cultivars under stressed conditions (Bhardwaj et al. 2017). Gallic acid enhanced plant tolerance capability to salt stress by reducing H_2O_2 and protect the cell membrane from oxidative in sunflower damage (Saidi et al. 2021). Phenolic compounds perform a critical role in the defense opposed to biotic and abiotic stresses (Sieminska-Kuczer et al. 2022). These secondary metabolites take effect of antioxidants in numerous plants tissues by alleviating the oxidative stress by scavenging ROS.

Anatomical Characteristics

Anatomical investigations of leaves showed that, they were sensitive to induced salt stress as compared to boron stress. As leaf plasticity, changes in morphological structure led to modulation in both physiological and biochemical attributes in plants (Liu et al. 2021). In the present experiment work, the SEM analysis showed an increase in stomata size and density by gallic acid and biochar in soil while decreased under both stresses. Our results are in alignment to the previous work on tomato plants carried out by Akhtar et al. (2014), who found that both stomatal aperture and density reduce under salinity stress be likely to decline such variables. Similar finding obtained by Nafees et al. (2022) and Akhtar et al. (2015), who found that biochar incorporation rises both stomatal aperture and density involving reduced stress level experienced by the plants. Contrarily, trichomes are epidermal cells development in aerial parts of plant importantly help in response to abiotic stress (Zhao et al. 2016). According to results obtained by light microscopy, the increased trichome length under both stresses individually and combinedly signifies by growth regulator and biochar amendment. These findings agree with the work of Khanam et al. (2018) who described that growth regulator enhanced trichome diameter in Mentha piperata L. under salt stress. These outcomes may be well recognized with aid of Passinho-Soares et al (2017) reported that growth regulators influenced quantitative and qualitative profiles of the distribution of trichomes on the leaf surface. Bose et al. (2013) found growth regulators have immense role in trichomes development.

Conclusion

Conclusively, it has been anticipated that the world's changing climate would cause abiotic stressors that will disrupt physiological and biochemical processes of plants, as a result, diminish the yield of our valuable commercial crops including *Solanum melongena* L. in Pakistan. Our findings showed that abiotic stress adversely effected the germination

and agronomic attributes of the aforesaid plant along with the architecture of leaves by adversely lowering stomatal size and trichomes, which are subsequently favorably and dramatically improved by the applied growth regulators, notably gallic acid as compared to biochar. It is further added that, the amount of key stress indicator molecules including MDA and H_2O_2 were enhanced in all non-biochar and gallic acid treatments which were adversely amended by amount of these molecules. In conclusion, both the growth stimulators alleviated the negative effects of salt and boron stress in egg-plant seedlings by improving the plant growth, yield parameters, anatomy by adjusting some physiological, biochemical and antioxidant defense mechanisms.

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Author Contributions SU conceived and designed the experiments. Shumaila performed the experiments, analyzed the data, and wrote the paper. MN edited and reviewed the paper.

Declarations

Conflict of interest All authors contributed equally and declare no conflict of interest. The paper is our original research work and is not published elsewhere.

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