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AMELIORATIVE EFFECT OF BRASSINOSTEROIDS AND BIOCHAR ON MORPHO-PHYSIOLOGICAL ATTRIBUTES OF TOMATO (LYCOPERSICON ESCULENTUM MILL.) UNDER INDUCED DROUGHT STRESS

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Abstract
Plant growth and development are impeded by abiotic stresses that interfere with physiological processes. This study aimed to assess the distinct and combined effects of biochar and brassinosteroids (BRs) on Rio Grande and Amitabh-004 tomato plants under water deficit stress. Results showed that biochar has porous morphology with significant water absorption and retention capability as inveterate by SEM. EDX of biochar illustrated the existence of various essential elements including carbon (75.04%), oxygen (13.57%), nitrogen (5.66%), potassium (2.43%), and calcium (1.52%). Similarly, Co-application of biochar and BRs quenched the drought stress by triggering antioxidant-enzymes including POD, APX, CAT and SOD accompanied with photosynthetic pigments boosting and amplification of osmolytes including proline, sugar and proteins for the better survival of plants under stress. Furthermore, HPLC of Gallic acid was identified at the retention time of 2.307 with peak area 63.25 and Vitamin-C at 3.15 retention times with peak area of 34.27 in all treatments of both varieties supplemented with different stresses along with biochar and BRs. Furthermore, Drought-stress significantly affects the agronomic feature of tomato plants such as leaf area, fresh biomasses of leaf, root and shoot, root length. Physicochemical characteristics i.e., pH, soil moisture, and percent field capacity are also adversely affected by water deficit stress. The principal component of physiological attributes was found to have 20.51% followed by 17.93%, 14.70%, 11.68% and 10.69%. Conclusively, the study emphasizes the potential of biochar and BRs to alleviate drought stress and enhance physio-biochemical attributes, thereby promoting improved plant growth under water deficit conditions.

Keywords: Biochar, Antioxidant enzymes, osmolytes, Brassinosteroids**,** Drought

1. Introduction
Numerous environmental stressors, including light, heat, salt, drought, cold, heavy metals, and pesticides, can affect a plant's ability to function physiologically and biochemically in its native habitat. Plants respond differently to certain environmental pressures, which improves their ability to

withstand harm and live in stressful environments. The impact of climate variations and environmental contamination induces stress in plants (Fang et al., 2019). Plant responses to environmental challenges involve molecular and cellular pathway modifications for sustained growth (Sharma et al., 2019). Drought deficit stress poses a significant threat to global agricultural systems (Dansih et al., 2020). Under stress, plants activate antioxidant systems, including enzymes like ascorbate peroxidase, peroxidase, catalase, glutathione reductase, and superoxide dismutase, to regulate reactive oxygen species and maintain redox homeostasis (ElSayed et al., 2019). Brassinosteroids (BRs) play a crucial role in enhancing stress tolerance, regulating various developmental processes, and responding to abiotic stressors at the molecular level (Sharma et al., 2019; Li et al., 2016). BRs positively impact plant development, influencing processes such as cell elongation, leaf expansion, photo-morphogenesis, flower development, stomata regulation, male sterility, and stress resistance (Hafeez et al., 2021). Recognized as a new class of plant hormones, BRs also enhance plant tolerance to abiotic stresses like dry seasons, temperature extremes, salinity, calcium nitrate, and heavy metal toxicity (Li et al., 2016; Wei et al., 2015). Development of water deficit resistant variety through biotechnology and plant breeding, combined with natural resources management likewise a favorable and efficient way to enhance the productivity of crops and water use effectiveness against dry season and water deficiency. Applying BRs topically to plants' leaves can boost photosynthesis, which is definitely associated with stress tolerance (Ahammed et al., 2020). It has also been observed to improve pepper plant resistance to chilling (Yang et al., 2019), heavy metal stress (Soares et al., 2020), and low temperatures in Brassica campestris (Zhao et al., 2019). Drought-like effects appear in the cotton mutant pag 1 that lacks BR (Chen et al., 2019). Biochar, a carbon-rich organic material derived through the pyrolysis of plant material in the absence of oxygen at elevated temperatures, has been explored for its agricultural benefits (Hafeez et al., 2021). Its supplementation in soil has shown positive effects, including enhanced organic carbon content, pH adjustment, increased electrical conductivity (EC), cation exchange capability (CEC), improved phosphorus (P) accessibility, and overall enhancement of nitrogen availability due to its high organic content and essential elements (Albert et al., 2021). The addition of biochar (BC) to soil has been documented to improve the structural resilience of soil aggregates, reducing the risks of compaction and pathogenic influences. Biochar's absorptive and permeable characteristics, along with its extensive surface area, provide a conducive habitat for soil microorganisms and contribute to pH regulation. These attributes are key factors in enhancing both the physical and chemical properties of the soil, fostering optimal conditions for plant growth (Zafar-Ul-Hye et al., 2021). Focusing on vegetables, the tomato (Lycopersicon esculentum Mill.), a member of the Solanaceae family, holds significant agricultural importance globally, ranking as one of the most vital vegetables after potatoes (Jangid and Dwivedi et al., 2017). Tomatoes are rich sources of essential components such as vitamin C and E, lycopene, β-carotene (carotenoids), and flavonoids, contributing to their nutritional value (Petruccelli et al., 2015).Previous work on tomatoes elucidates that humidity shortage in the root region decreases size of leaf, chlorophyll concentration, and photosynthetic activity (Athar and Ashraf 2005). Water stress usually affects crops grown in rain-fed conditions at various stages which cause a decrease in productivity (Ali et al., 2011). To understand the effect of biochar and BRs on tomato (Lycopersicon esculentum Mill.) under stress the tomato plant are subjected to different duration (5 and 10 Days) of drought under control condition with supplementation of biochar before cultivation and exogenous application of BRs at vegetative stage. In the present investigation the photosynthetic pigments, protein, osmolytes contents and antioxidant activities were assessed in both varieties of tomato under stress as well as normal situation (control).

2. MATERIAL AND METHODS

2.1 Experimental layout

During the crop growing season of 2021, the experiment was carried out in the Department of Botany, University of Malakand, Pakistan, which is located at latitude 34.66925′ N, longitude 72.06124′ E, and height 2349 feet. The seeds of the two native tomato varieties (Rio Grande and Amitabh-004)

that were obtained from the Agricultural Research Institute in Tarnab, Peshawar, Pakistan (Lycopersicon esculentum Mill.). The seeds were sterilised with 50% ethanol and 5% chlorox before being sown, and they were then thoroughly cleaned three times using distilled water. The sterilised seeds were grown in plastic pots with a 3:1:1 ratio of soil, charcoal, and sand. The experiment was set up as follows and carried out in triplicate in a control setting using Complete Randomised Block Design (CRBD):

C= Control
T1= 05ddrought,
T2= 05ddrought+biochar,
T3= 05ddrought+BRs,
T4= 05ddrought+biochar+BRs,
T5= 10ddrought,
T6= 10ddrought+biochar,
T7= 10ddrought+BRs,
T8= 10ddrought+biochar+BRs.

All the plants were watered and maintained till stability. The control plants were watered daily. The pots were subjected to different duration of drought (5 and 10 days). Brassinosteroids foliar spray application was carried out on the third day of drought induction.

2.2. Biochar Preparation

Biochar from Quercus baloot residues was produced under controlled supply of oxygen in pyrolysis furnace. The practice was sustained till a dark materials developed following the methodology of Fahad et al., (2019).

2.3. SEM and EDX anlsysis for soil and biochar

The powdered biochar was screened to produce a finely ground powder, and then the Spi coating module was used to cover it in gold for SEM study. A scanning electron microscope (JSM-5910-JEOL-JAPAN) was used to analyse the structural and morphological aspects of biocahr, and energy dispersive X-ray (EDX) spectroscopy was used to analyse the elements in the soil and the powdered biochar.

Agronmic features

Morphological features, including fresh and dry biomass of leaf and roots, it's percent moisture content, root and shoot length, were analyzed following the methods detailed by Ullah et al., (2016). Percent Plant Height Stress Index (%PHSI) and Leaf Area Ratio (LAR) were determined using the precise procedures outlined by Shah et al., (2017). The root/shoot ratio (RSR) was calculated with precision, adhering to the protocols established by Asch et al., (2005) and Sainju et al., (2017) through the specified equation.

LAR= leaf area (cm^2)/ final dry weight

$$\text{PSHI (\%)} = \frac{\text{Stressed plant height}}{\text{Unstressed plant height}} \times 100$$

RSR=Root dry mass/Shoot dry mass

$$\text{\% moisture content of leaves} = \frac{\text{Leaves Fresh weight} - \text{Leaves dry weight}}{\text{Fresh weight of Leaves}} \times 100$$

2.3. Physio-biochemicals analysis of plants

2.3.1. Protein Contents of Leaves

The protein content was analyzed employing the procedure outlined by Lowery et al., (1951), with BSA serving as the reference standard. Fresh leaves were finely pulverized using a pestle and mortar, with the addition of one milliliter of phosphate buffer at a pH of 7.5. Resulting mixture was

centrifuged at 3000 rpm for ten minutes. For volume determination, 0.1 milliliter of supernatant was combined with 1 milliliter of distilled water in a test tube. Reagents C and D were incrementally added after shaking the reaction mixture for ten minutes. Following a 30-minute incubation period, the OD of each sample was determined at 650 nm. BSA, or bovine serum albumen, served as the reference standard for calculating the protein concentration. Protein content was estimated using

$$\text{Protein Content}\frac{\text{mg}}{\text{g}} = \frac{\text{Absorbance} \times \text{Dilution Factor} \times \text{K Value}}{\text{Weight of Sample}} \quad \text{Eq:1}$$

Sample Wt.= 0.1g K Value=19.6 Dilution Factor=10

2.3.2. Sugar Estimation

The standard Dubos et al., (1956) methodology was used in order to determine the sugar content of fresh leaves. Fresh sample of leaves (0.5 g) was ground up in 10 millilitres of distilled water with the help of pestle and mortar to form homogenate. After centrifuging the homogenate for five minutes at 3000 rpm, the supernatant was removed. One millilitre of 80% (w/v) phenol was added to a test tube holding 0.1 millilitre of supernatant, and the mixture was left to stand at room temperature for four hours. After incubation, 5 millilitres of concentrated H_2SO_4 were added, and at 420 nanometers, the optical density (OD) was determined.

Sugar Concentration (mg/g) = Absorbance × K value × Dilution factor/ Wt. of sample…..Eq:2

Sample weight = 0.5g K value = 20 Dilution factor = 10

2.3.3. Estimation of Photosynthetic Pigments

Chlorophyll contents were assessed by following Zou et al., (2017) procedure. A 1ml crude sample was mixed with 80% acetone and placed at room temperature in a dark chamber. The supernatant of sample was collected in a clean test tube after centrifugation for 5min at 2000rpm. Absorbance of chlorophyll a, b and carotenoid were estimated at 645nm, 663nm and 470nm respectively, against acetone (80%) blank following the below mentioned formulas;

Chlorophyll ‘a’ (mg/mL) = $12.7*A_{663} - 2.69*A_{645}$……Eq:3

Chlorophyll ‘b’ (mg/mL) = $22.9*A_{645} - 4.68*A_{663}$…… Eq:4

Total chlorophyll (mg/mL) = (Chlorophyll ‘a’ + Chlorophyll ‘b’)

$$\text{Total carotenoid content} = \frac{1000\text{A}470 - 2.27(Chl\ \text{a}) - 81.4(Chl\ \text{b})}{227} \times 100 \quad \text{Eq:5}$$

Whereas A= absorbance

2.3.4. Leaves Proline Estimation

Proline concentration was quantified using the procedure outlined by Bates et al., (1973). Leaves were finely ground to produce a homogenate in 10 ml of 3% sulphosalicylic acid. The homogenate was subjected to filtration, and 2 milliliters of the resulting filtrate were combined with 4 milliliters of glacial acetic acid and 4 ml of 20% ninhydrin. Following one hour of heating at 100 ˚C, the mixture was further combined with 4 ml of toluene. Proline content was determined by measuring the absorbance at 520 nm.

$$\text{Proline Content } (\mu g/g) = \frac{\text{Dilution Factor} \times \text{K Value} \times \text{Absorbance}}{\text{Fresh weight of sample}} \quad \text{Eq:6}$$

Dilution Factor=2 K Value=17.52 Fresh weight of sample= 0.5g

2.4. Antioxidant enzymes activities

2.4.1. Peroxidase (POD) activity

Peroxidase activity was precisely measured following the procedure outlined by Chance & Maehly (1955). A homogenate was created by crushing 0.5 g of leaf sample in 2 ml of Morpholino Ethane Sulphonic acid (MES). The mixture underwent centrifugation at 2000 rpm for 20 minutes. Subsequently, 0.2 ml of the supernatant was extracted, and a mixture of 1.3 ml of MES, 0.1 ml of

phenyl diamine, and 1 ml of 30% H_2O_2 was added. The resulting mixture was vigorously agitated, and the absorbance at 485 nm was recorded.

$$POD = \frac{\text{Change in OD}}{\text{Time taken}} \times 1/EC \times TV/UV \times 1/FW \times 1000 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq:7}$$

2.4.2. Superoxide Dismutase (SOD) activity

The Beauchamp and Fridovich (1971) approach was used to measure SOD activity. To generate a homogenate, a fresh sample was chopped up in six millilitres of phosphate buffer. After centrifugation at 3000 rpm for 15 minutes, 0.1 ml of the supernatant was taken off. The recovered supernatant was then supplemented with 150 µl riboflavin, 5 ml methionine, and nitro-blue tetrazolium (NBT). Next, each sample's absorbance was identified at 560 nm.

$$SOD \left(\frac{\mu}{gFW}\right) = \frac{\text{OD control} - \text{OD test}}{\text{OD control}} \times 1/50 \times Vt/SQ \times FW \ldots \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq:8}$$

2.4.3. Ascorbate Peroxidase (APX) activity

Salimi et al., (2016)'s technique was used to the investigation of APX concentration. After being crushed in phosphate buffer, samples of fresh leaves were centrifuged. Ascorbic acid, H2O2, and ETDA were added to the supernatant. First reading was recorded at 290nm and then second was measured after 60s at the same OD. The content of ascorbate peroxidase was expressed as Unit/g f.w.

2.4.4. Catalase (CAT) activity

The approach outlined by Tyburski et al., (2009) was used to investigate the activity of the catalase (CAT) enzyme. After being homogenised, fresh leaves were spun up in buffer. H_2O_2 and phosphate buffer were added to supernatant. Initial reading was measured at 240nm the noted on the same OD after 60 second.

2.5. HPLC profiling

HPLC profiling was carried out on 15 samples in agreement with the previously stated protocol using an Agilent 1260 system, (Zeb, 2015). A roughly 1 g sample of powdered tomato leaf was digested using a 1:1 (20 mL) methanol and water combination. The mixture was heated in a water bath for one hour at 70 C before being centrifuged for ten minutes at 4000 rpm. The sample (2 mL) was then put into HPLC vials and labelled with the proper codes after being filtered with Whatman filter paper. Sample retention durations were compared to known standards to identify phenolic compounds.

3. RESULTS

Physicho-chemical charachteristics of rhizospheric soil

The results shown in Table 1 show that the maximum pH has been confirmed in the Rio Grande variety's T4 (05drought+BC+BRs), T8 (10drought+BC+BRs), and T2 (05drought+BC) as well as in Amitabh-004. On the other hand, reports of a drop in pH have been made for T5 (10drought) and T3 (05drought+BRs), two tomato cultivars. As compared to T5 (10drought) and T3 (05drought+BRs), the findings of T4 (05drought+BC+BRs) and T8 (10drought+BC+BRs) in both types are more successful, indicating that biochar-accompanied BRs play a compensatory role in improving soil pH under water shortage stress. As shown in Table 1 the maximum moisture content and percentage field capacity of soil have been measured in the Rio Grande variety and Amitabh-004a during treatments C (Control), T2 (05drought+BC), and T6 (10drought+BC). On the other hand, in T1 (05drought), T3 (05drought+BRs), and T5 (10drought), in both tomato types, the percentages of MC and FC of soil drop. The outcome showed how important biochar and BRs are to improving the pH, %MC, and %FC of soil under drought stress.

Table 1. Effect of biochar on germination rate, soil pH, % moisture content and % field capacity of soil of tomato (Lycopersicon esculentum Mill.) under induced drought stress.

Treatment	Germination Rate		Soil pH		%MC		% FC	
	V1	V2	V1	V2	V1	V2	V1	V2
C	4.33±0.33	3.33±0.33	6.67±0.28	5.97±0.09	18.77±2.12	22.00±1.33	26.10±3.19	28.21±2.18
T1	3.67±0.88	4.33±0.88	6.70±0.38	7.10±0.87	13.50±1.08	11.20±2.38	15.61±1.46	12.61±3.00
T2	5.00±0.58	5.00±0.58	7.17±0.32	7.03±0.09	17.13±2.25	11.53±2.22	20.68±3.36	17.04±2.78
T3	4.33±0.88	4.00±0.58	6.47±0.32	6.53±0.15	14.03±1.63	12.53±1.27	16.32±2.26	14.33±1.63
T4	4.00±0.58	3.67±0.33	7.30±0.15	6.53±0.30	15.00±0.47	14.13±2.45	16.28±0.64	16.46±3.39
T5	3.00±0.58	4.33±0.33	6.17±0.03	6.47±0.29	13.33±0.99	13.93±0.26	15.38±1.33	16.19±0.35
T6	6.67±0.67	4.33±0.67	6.53±0.20	6.07±0.12	16.70±1.97	14.97±0.12	18.62±2.74	17.60±0.17
T7	3.00±1.53	4.00±0.58	6.67±0.03	6.87±0.37	15.60±1.85	13.93±0.62	18.48±2.59	16.28±0.78
T8	5.67±0.88	5.00±0.58	7.20±0.06	7.13±0.58	16.07±1.40	15.40±0.49	17.74±1.96	15.87±0.63

Agronomic study of experimental plants

The analysis in Table 1 shows that, for both types, the highest germination rates were observed in T6 (10drought+BC) and T8 (10drought+BC+BRs), whereas the lowest germination rates were recorded in T1 (05drought), T5 (10drought), and T7 (10drought+BRs). As shown in Tables 2 and 3, the maximum root length, fresh, dry biomass, and percent moisture content of the leaf and root were recorded in T0 (Control) and T8 (10drought+BC+BRs), whereas T3 (05drought+BRs), T5 (10drought), and T4 (05drought+BC+BRs) for both types revealed a reduction in leaf fresh biomass. Maximum shoot height, fresh biomass, and dry biomass in T8 (10drought+BC+BRs), T7 (10drought+BRs), and T6 (10drought+BC) are all shown in Table 4. Shoot height decreased in T1 (05drought) and T3 (05drought+BRs). For all kinds, the maximum moisture content of the shoot was recorded in T2 (05drought+BC) and T3 (05drought+BC), whereas the percentage of moisture content (%MC) dropped in T5 (10drought), T7 (10drought+BRs), and T8 (10drought+BC+BRs). The findings demonstrated that biochar and BRs treatments, namely T8 (10drought+BC+BRs) and T6 (10drought+BC), had favourable benefits on a number of growth metrics in both the Rio Grande and Amitabh-004 tomato cultivars when subjected to drought stress.

Regression analysis

Regression is applied for assessing the correlation among morphological traits. The results shown in (Table 5) clarify that a significant relationship exists among agronomic characters.

Table 2. Effect of biochar and BR on root length, root fresh, dry biomass and % moisture content of tomato (Lycopersicon esculentum Mill.) under induced drought stress.

Treatment	Root length		Root Fresh Biomass		Root Dry Biomass		% MC	
	V1	V2	V1	V2	V1	V2	V1	V2
C	7.57±0.64	6.27±0.47	0.43±0.01	0.33±0.04	0.12±0.02	0.15±0.02	72.31±0.01	54.55±0.06
T1	6.57±0.93	6.57±1.01	0.36±0.03	0.25±0.01	0.16±0.02	0.14±0.03	58.54±0.04	36.84±0.04
T2	5.33±0.78	7.97±1.00	0.39±0.08	0.34±0.13	0.13±0.04	0.14±0.00	41.07±0.02	58.42±0.09
T3	6.50±1.32	7.77±0.26	0.37±0.08	0.42±0.18	0.12±0.01	0.19±0.03	65.05±0.07	54.76±0.12
T4	5.33±0.32	6.50±0.78	0.38±0.02	0.43±0.12	0.20±0.03	0.21±0.04	40.00±0.01	58.46±0.09
T5	7.27±1.21	7.03±0.44	0.28±0.05	0.34±0.06	0.12±0.05	0.18±0.03	61.29±0.02	37.62±0.05
T6	7.00±1.31	8.43±1.53	0.48±0.09	0.47±0.10	0.21±0.05	0.17±0.01	56.25±0.13	48.57±0.10
T7	6.97±1.25	7.73±0.62	0.27±0.01	0.34±0.10	0.12±0.01	0.16±0.01	53.25±0.01	52.48±0.09
T8	7.57±0.79	8.93±0.92	0.46±0.07	0.33±0.08	0.20±0.05	0.15±0.02	60.04±0.10	54.08±0.06

Table 3. Effect of biochar and BR on leaf area, fresh and dry biomass of leaf and % moisture content of Lycopersicon esculentum Mill. under induced drought-stress stress.

Treatment	Leaf Area		Fresh Biomass of Leaf		Dry Biomass of Leaf		% MC	
	V1	V2	V1	V2		V1	V2	V1
C	202.93±9.93	184.37±13.79	0.81±0.14	0.70±0.03	0.53±0.01	0.44±0.01	68.22±7.87	65.07±1.30
T1	168.74±12.41	174.60±14.38	0.74±0.01	0.68±0.37	0.40±0.03	0.33±0.07	56.04±3.55	57.85±10.74
T2	157.01±20.05	149.74±24.53	0.65±0.02	0.53±0.41	0.35±0.02	0.28±0.16	66.42±2.01	58.65±6.96
T3	144.45±16.37	152.16±33.93	0.51±0.06	0.46±0.30	0.27±0.01	0.20±0.12	61.20±2.29	60.71±1.14
T4	146.64±14.11	142.13±48.31	0.58±0.07	0.52±0.42	0.47±0.04	0.43±0.16	58.68±5.43	63.07±3.67
T5	117.92±27.93	153.64±42.26	0.48±0.29	0.76±0.19	0.31±0.16	0.46±0.04	43.98±1.02	52.14±1.50
T6	190.03±20.87	162.03±42.06	0.62±0.23	0.59±0.30	0.41±0.15	0.37±0.04	61.44±4.77	54.00±0.12
T7	158.13±36.63	154.06±6.88	0.58±0.10	0.52±0.04	0.29±0.04	0.20±0.03	49.71±3.36	51.90±2.71
T8	192.82±33.49	176.49±38.85	0.72±0.27	0.86±0.27	0.43±0.04	0.54±0.07	67.69±0.31	65.99±4.28

Table 4. Effect of biochar and BR on Shoot Height, shoot fresh biomass, shoot dry biomass and %moisture content of tomato (Lycopersicon esculentum Mill.) under induced drought stress.

Treatment	Shoot height		Shoot Fresh Biomass		Shoot dry Biomass		% MC	
	V1	V2	V1	V2	V1	V2	V1	V2
C	28.70±3.10	24.37±1.78	3.39±0.71	2.84±0.85	0.59±0.26	0.14±0.03	82.51±5.61	92.20±10.47
T1	24.60±3.21	26.00±7.40	2.75±0.91	2.56±1.15	0.54±0.22	0.64±0.38	80.48±4.08	75.03±4.26
T2	26.47±3.80	28.83±6.83	1.80±0.51	2.86±2.59	0.39±0.02	0.27±0.18	95.19±0.69	94.37±4.54
T3	23.13±0.84	23.87±4.48	1.13±0.48	1.09±0.56	0.27±0.01	0.29±0.19	93.22±8.74	73.70±5.20
T4	28.30±2.34	32.53±4.62	2.77±0.73	3.18±0.70	0.17±0.05	1.11±0.57	93.74±0.33	65.13±11.82
T5	27.13±1.13	27.87±1.67	2.79±0.43	2.21±0.03	0.28±0.32	0.77±0.19	63.68±5.38	65.31±8.83
T6	32.47±2.01	28.97±6.85	4.12±0.38	2.44±0.80	0.76±0.51	0.68±0.31	70.15±9.56	72.17±8.29
T7	30.17±2.59	24.20±2.82	2.88±0.47	1.54±0.68	0.85±0.33	0.31±0.13	70.40±7.84	79.87±4.03
T8	33.60±5.98	32.37±5.54	2.44±0.97	3.70±0.73	0.80±0.70	0.51±0.83	67.17±8.89	40.27±8.78

V1=Rio grande V2=Amitabh-004, C=Control daily watering, T1=05ddrought, T2=05ddrought+BC, T3=05ddrought+BRs, T4=05ddrought+BC+BRs, T5=10ddrought, T6=10ddrought+BC, T7=10ddrought+ BRs, T8=10ddrought+BC+ BRs

Table 5. Linear regression model of morphological characters evaluated in L. esculentum Mill.after induction of 5 and 10days stress

LA	**SL**	**RL**	**RFW**	**RDW**	**LFW**	**LDW**	**SFW**	**SDW**
r=.048***	r=.238 ***	r=.387***	r=.082***	r=.205***	r=.226***	r=.153***	r=.052***	r=.335***
R^2=.002	R^2=.057	R^2=.150	R^2=.007	R^2=.042	R^2=.051	R^2=.024	R^2=.003	R^2=.112

Note: LA=Leaf Area, SL=Shoot Length, RL=Root Length, RFW=Root Fresh Weight, RDW=Root Dry Weight, LFW=Leaf Fresh Weight, SFW= Shoot Fresh Weight, SDW= Shoot Dry Weight, *** ($p \leq 0.001$), ** ($p \leq 0.01$), * ($p \leq 0.05$), r=regression, R^2=regression square

Pearson Coefficient Correlation of Agronomic Traits of Tomato Varieties

Correlation of agronomic traits showed that LA is significantly correlated with RL, RFW, RDW, LFW, LDW, SL, SFW and SDW at $p \leq 0.01$ while least significant with MCR at $p \leq 0.05$ and non-significant with MCL and MCS (Table 6). Similarly, RL is significantly correlated with RFW, LFW and LDW at $p \leq 0.01$ but non-significant with RDW, LDW, MCL, MCS and least significant with MCR, SFW and SDW at $p \leq 0.05$. a significant correlation of RFW with RDW, LFW, LDW and SL at $p \leq 0.01$ and $p \leq 0.05$. RDW had a significant correlation LDW and LFW while no-significant MCR, MCL, SL, SFW, SDW and MCS at $p \leq 0.01$ and $p \leq 0.05$. MCR is significantly correlated at $p \leq 0.01$ and $p \leq 0.05$ with LFW, LDW, and SL but non-significantly correlated with MCL, SFW, MCS and SDW. Likewise, LFW had significant correlation with LDW, SL, SFW, and SDW at $p \leq 0.01$ and $p \leq 0.05$ while non-significant with MCL and MCS. In the same way LDW is correlated significantly at $p \leq 0.01$ and $p \leq 0.05$ with MCL, SL and SFW. SL is significantly correlated with SFW, SDW but negatively correlated with MCS. SFW had significant correlation with SDW and non-significant with MCS. SDW is significantly correlated with MCS at $p \leq 0.01$ and $p \leq 0.05$.

Table 6. Pearson correlation of agronomic traits of tomato varieties under drought stress

	LA	RL	RFW	RDW	MCR	LFW	LDW	MCL	SL	SFW	SDW	MCS
LA	1											
RL	.429**	1										
RFW	.464**	.388**	1									
RDW	.392**	.263	.344*	1								
MCR	.326*	.300*	.916**	.061	1							
LFW	.684**	.449**	.544**	.307*	.447**	1						
LDW	.682**	.217	.464**	.372**	.335*	.615**	1					
MCL	.006	.153	.023	.033	.038	.235	.382**	1				
SL	.595**	.364**	.344*	.155	.300*	.640**	.482**	.028	1			
SFW	.516**	.309*	.240	.029	.243	.502**	.467**	.042	.783**	1		
SDW	.352**	.318*	.258	.103	.230	.364**	.256	.063	.635**	.556**	1	
MCS	.149	.240	.261	.084	.242	.155	.147	.101	-.245	.051	.728**	1

Key: ** significant at $p \leq 0.01$, * significant at $p \leq 0.05$

Note: LA=Leaf Area, RL=Root Length, RFW=Root Fresh Weight, RDW=Root Dry Weight, MCR=Moisture Content of Root, LFW=Leaf Fresh Weight, LDW=Leaf Dry Weight, MCL=Moisture Content of Leaf, SL=Shoot Length RFW=Shoot Fresh Weight, RDW=Shoot Dry Weight, MCS=Moisture Content of Shoot.

3.1. Morpho-elemental analysis of soil and biochar via SEM and EDX spectroscopy

Morphological analysis of biochar revealed that, biochar have highly porous structure confided through SEM as shown in (Figure-1). The pores were spherical to polygonal in shape. Energy dispersive X-ray spectroscopy was used to identify the main components of the soil. The percentages of these elements are shown in Table 7 and are as follows: carbon (12.28%), oxygen (30.60%), silicon (29.85%), iron (8.32%), and calcium (8.87%). As shown in Figure 2, spectroscopic peaks were produced using a full scale of 3001 cts and a cursor set at 15.124 Kev. With energy dispersive X-ray spectroscopy, the powdered biochar's elemental analysis for important element concentrations was also carried out. Table 8 shows the concentrations of carbon (75.04%), oxygen (13.57%), nitrogen (13.57%), and potassium (2.43%) that were found during the study. As shown in Figure 3, spectroscopic peaks were produced using a full scale of 2807 cts and a cursor set at 15.049 Kev.

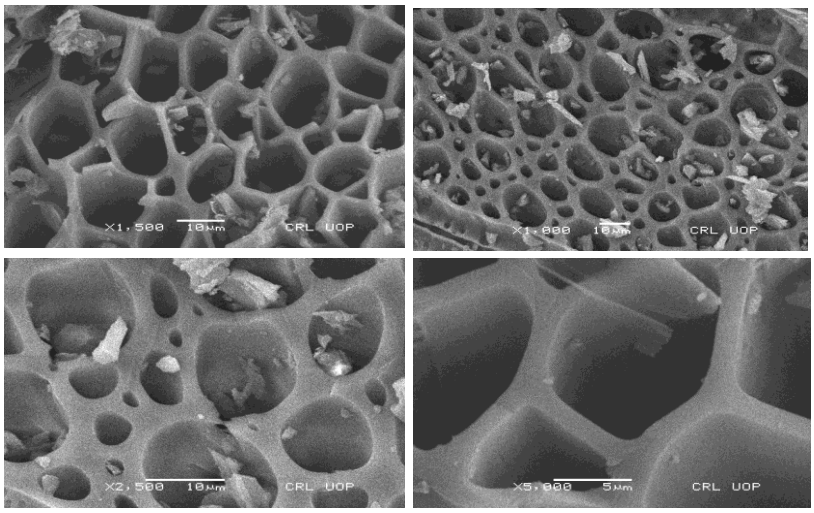

Figure-1. Morphological structure of biochar under scanning electron microscope.

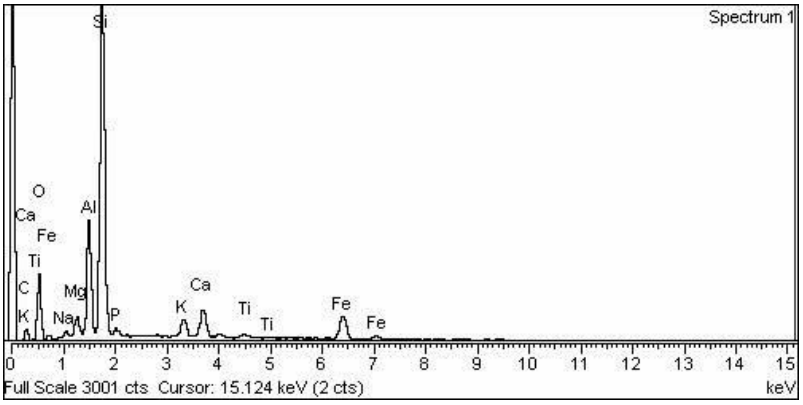

Figure-2. Elemental analysis of soil achieved via EDX spectroscopy.

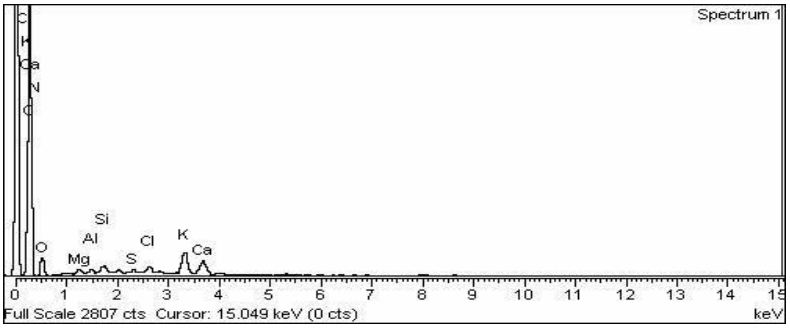

Figure-3. Elemental analysis of biochar via EDX spectroscopy

Table-7. Elemental analysis of soil via EDX spectroscopy

Element	Weight%	Atomic%
C	12.28	21.42
O	30.60	40.07
Na	0.68	0.62
Mg	1.63	1.41
Al	9.15	7.10
Si	29.58	22.06
P	0.98	0.66
K	2.35	1.26
Ca	3.87	2.02
Ti	0.55	0.24
Fe	8.32	3.12

Table-8. Elemental analysis of biochar via EDX spectroscopy

Element	Weight%	Atomic%
C	75.04	81.56
N	5.66	5.27
O	13.57	11.07
Mg	0.33	0.18
Al	0.24	0.11
Si	0.42	0.19
S	0.20	0.08
Cl	0.60	0.22
K	2.43	0.81
Ca	1.52	0.50

3.2. Physiological study of the experimental plant

The comprehensive physio-biomolecules estimation were accomplished for tomato under induced drought stress. The results elucidate remarkable differences among these varieties with application of biochar and brassinosteroids under induced water stress (Figure-4).

3.3. Photosynthetic Pigments.

To evaluate the consequences of natural stresses photosynthetic pigments are often analyzed in plants, as variation in pigments content are associated with visible outcome of plants inefficiency, disorder and reduction in photosynthetic activities.

3.3.1. Chlorophyll a contents (mg/g)

Chlorophyll a content was analyzed in tomato varieties i.e. Rio Grande and Amitabh-004 at vegetative stage under induced drought stress with co-application of biochar and BRs (Figure-5). Maximum chlorophyll "a" contents has been reported in control treatment (C) of Rio Grande as well as Amitabh-004 as displayed in the results. Whereas; the concentration of chlorophyll "a" in control is significantly similar with T4 (05ddrought+BC+BRs), T8 (10ddrought+BC+BRs) and T6 (10ddrought+BC) at $P \leq 0.05$ both varieties. The least chlorophyll "a" content in T1 (05ddrought) followed by T5 (10ddrought) and T2 (05ddrought+BC) has been noted in Amitabh-004 at $P \leq 0.05$. The results elucidate that application of biochar and brassinosteroids improve the level of chlorophyll under stress.

3.3.2. Chlorophyll b Contents (mg/g)

Comparison were made for chlorophyll "b" concentration in the selected tomato varieties including Rio Grande and Amitabh-004 at vegetative stage under stress (Figure-5). High concentration of

Chlorophyll "b" contents has been recorded in control treatment (C) of Rio Grande as well as Amitabh-004 as displayed in the results. Whereas; chlorophyll "a" concentration in T3 (10ddrought+ BRs) is significantly similar with T5 (05ddrought), T8 (10ddrought+BC+BRs) at p≤0.05 in both varieties at vegetative stage. The reduction of chlorophyll "b" content in T7 (10ddrought+BRs) followed by T6 (10ddrought+BC) and T5 (10ddrought) has been reported in Amitabh-004 at p≤0.05 revealed that under induced water stress biochar and BRs perform supportive role in the amelioration of chlorophyll b concentration.

3.3.3. Total Chlorophyll Content (mg/g)

The increase in total chlorophyll contents has been noted in control treatment (C) of Rio Grande as well as Amitabh-004 as displayed in the results. Whereas; total chlorophyll concentration in control treatment (C) is significantly similar with T8 (10ddrought+BC+BRs) and T6 (10ddrought+BC) p≤0.05 in both varieties. The decline in total chlorophyll content in T3 (05ddrought+BRs) followed by T2 (05ddrought+BC) and T1 (05ddrought) has been reported in in both varieties at P≤ 0.05 elucidate encouraging role of biochar and BRs in amelioration of entire chlorophyll contents.

3.3.4. Carotenoids Content (mg/g)

Assessment of carotenoid content (mg/g) in leaf samples of tomato varieties i.e. Rio Grande and Amitabh-004 were conducted at vegetative stage under water deficit stress with amendment of biochar and BRs (Figure-5). Higher carotenoids concentration has been recorded in T8 (10ddrought+BC+BRs) of Rio Grande as well as Amitabh-004 as displayed in the results. Whereas; carotenoids concentration in T8 (10ddrought+BC+BRs) is significantly similar with T6 (10ddrought+BC), T4 (05ddrought+BC+BRs) at P≤ 0.05 in both varieties at growing stage. The decrease in carotenoids contents in T5 (10ddrough) followed by T7 (10ddrought+BRs) and T1 (05ddrought) has been reported in Amitabh-004 at P≤ 0.05. The finding revealed the improving role in carotenoid contents with biochar amendment and BRs foliar spray under stress.

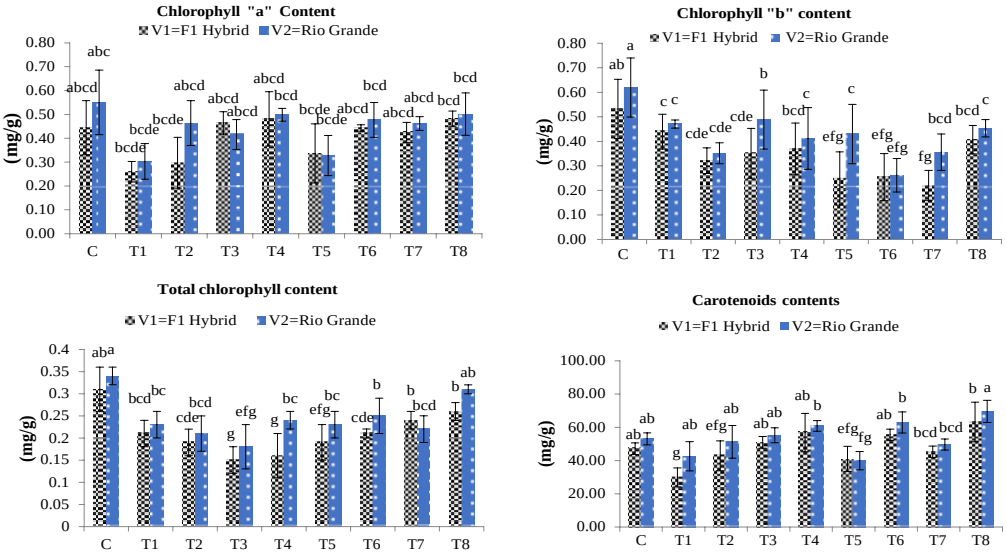

Figure-5. Photosynthetic pigments of the selected tomato varieties under induced water deficit stress after application of biochar (BC) and brassinosteroids (BRs) at vegetative stage.

C=Control (untreated), T1=05ddrought, T2=05ddrought+biochar, T3=05ddrought+BRs, T4=05ddrought+biochar+BRs, T5=10ddrought, T6=10ddrought+biochar, T7=10ddrought+BRs, T8=10ddrought+biochar+BRs. All such means having same English letter are non-significantly different at P≤ 0.05.

3.4. Osmolytes analysis

Osmolytes includes low molecular weight molecules such proline, soluble sugar along with proteins will be study. To evaluate the impact of environmental stresses, the measurement of osmolytes and protein are very essential and also used as a selectable marker for stress tolerant varieties.

3.4.1. Proline content (µg/g)

Enhanced proline content has been found in T8 (10ddrought+BC+BRs) of both varieties as displayed in the results (Figure-6). Whereas; the concentration of proline in T6 (10ddrought+BC) is significantly similar with T7 (10ddrought+ BRs) and T5 (10ddrought) at vegetative stage at P≤ 0.05. The minimum proline content in Control (daily watering) followed by T1 (05ddrought) and T2 (05ddrought+BC) has been noted in Amitabh-004 as well as Rio Grande at P≤ 0.05. The investigation displays that under induced water stress biochar and BRs play ameliorative role in the proline content.

3.4.2. Proteins Content (µg/g)

Proteins content (mg/g) in leaf samples of tomato varieties i.e. Rio Grande and Amitabh-004 were evaluated at vegetative stage with amendment of biochar and BRs (Figure-6). Maximum proteins concentration has been verified in T8 (10ddrought+BC+BRs) treatment of Rio Grande as well as Amitabh-004 as presented in the results. Whereas; protein content in T8 (10ddrought+BC+BRs) is significantly similar with T4 (05ddrought+BC+BRs) and T7 (10ddrought+BRs) at at vegetative stage p≤0.05 in both varieties. The decrease in protein content in T6 (10ddrought+BC) followed by T3 (05ddrought+BRs) and T2 (05ddrought+BC) has been reported in Amitabh-004 at P≤ 0.05 clarify that biochar and BRs can't play encouraging role in improvement of proteins content of plants exposed to water deficit stress.

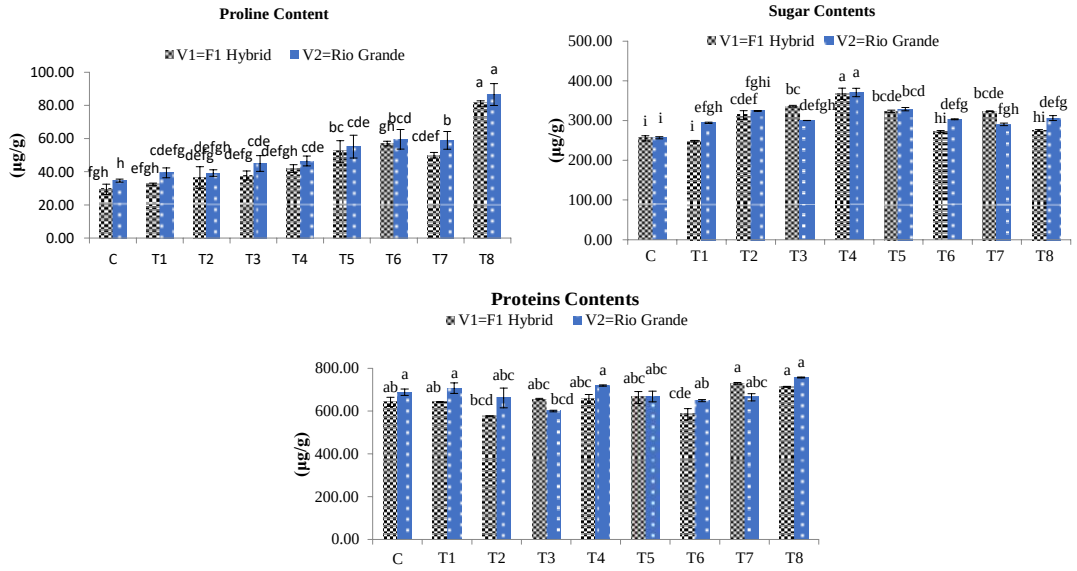

Figure-6. Osmolytes analysis of the selected tomato varieties under induced water deficit stress after application of biochar (BC) and brassinosteroids (BRs) at vegetative stage.

C=Control-daily watering, T1=05ddrought, T2=05ddrought+biochar, T3=05ddrought+BRs, T4=05ddrought+biochar+BRs, T5=10ddrought, T6=10ddrought+biochar, T7=10ddrought+BRs, T8=10ddrought+biochar+BRs. All such means having same English letter are non-significantly different at P≤ 0.05.

3.5. Antioxidant enzymes activities

The fluctuations in antioxidant enzyme activities reveal the impact of environmental stresses on plant metabolic activities and its response to stresses.

3.5.1. Superoxide dismutase (OD/min/g f. w)

Analysis of superoxide dismutase (SOD) activity were performed for selected plant samples at vegetative stage collected from designed experiment for both varieties of tomato i.e. Rio Grande and Amitabh-004 under induced drought stress with co-application of biochar and BRs (Figure-7). Maximum SOD activity has been recorded in control treatment (C) of Rio Grande as well as Amitabh-004 as presented in the results. Whereas; superoxide dismutase activity of control is considerably alike with T8 (10ddrought+BC+BRs), T6 (10ddrought+BC) and T1 (05ddrought) at $p \leq 0.05$ in both varieties. The minimum SOD activity in T7 (10ddrought+BRs) followed by T2 (05ddrought+BC) and T4 (05ddrought+BC+BRs) has been recorded in Amitabh-004 at $P \leq 0.05$ revealed that biochar and BRs perform optimistic role in enhancement of superoxide dismutase (SOD) activities of under stress of water.

3.5.2. Catalase (OD/min/g F.W)

Comparison of catalase activity were made for collected plant samples at vegetative stage for the designed experiment of both varieties i.e. Rio Grande and Amitabh-004 under induced drought stress (Figure-7). Maximum CAT activity has been recorded in T7 (10ddrought+BRs) and T1 (05ddrought) of Amitabh-004 as shown in the results. Whereas; CAT activity of T8 is significantly similar with T4 (05ddrought+BC+BRs), T2 (05ddrought+BC) and T6 (10ddrought+BC) at $p \leq 0.05$ in Rio Grande variety at vegetative stage. The lowest CAT activity in T6 (10ddrought+BC) followed by T3 (05ddrought+BRs) of Amitabh-004 and also in control (05ddrought+BC+BRs) of Rio Grande has been recorded at $P \leq 0.05$ revealed that biochar and BRs carry out a constructive role in augmentation of catalase under water deficit stress.

3.5.3. Peroxidase (OD/min/g f. w)

Comparative evaluation of POD activity (Unit/Min/g f. w) were made for selected plant samples collected from planned experiment at vegetative stage for both varieties of tomato i.e. Rio Grande and Amitabh-004 under induced water deficit stress with amendment of biochar and BRs (Figure-7). Maximum POD activity has been recorded in T8 (10ddrought+BC+BRs) and T4 (05ddrought+BC+BRs) of Rio Grande as depicted in the results. Whereas; APOX activity of T8 (10ddrought+BC+BRs) is significantly similar with control (untreated) and T1 (10ddrought) at $P \leq 0.05$ in Rio Grande variety at vegetative stage. The reduce POD activity in T6 (10ddrought+BC) followed by T7 (10ddrought+BRs), T4 (05ddrought+BC+BRs) and control (daily watering) of Amitabh-004 has been recorded at $P \leq 0.05$ indicated that biochar and BRs play affirmative role in augmentation of POD activity under induced water deficit stress.

3.5.4. Ascorbate peroxidase (OD/min/g f. w)

Comparative Assessment of APOX activity (Unit/min/g f. w) were carried out for selected plant samples at vegetative stage for the designed experiment of selected variety under induced drought stress with co-application of biochar and BRs (Figure-7). Maximum APOX activity has been recorded in control (daily watering) and T1 (05ddrought) of Rio Grande as displayed in the results. Whereas; APOX activity of control was significantly similar with T8 (10ddrought+BC+BRs) and T6 (10ddrought+BC) at $P \leq 0.05$ in Rio Grande variety at vegetative stage. The decrease APOX activity in T7 (10ddrought+BRs) followed by T2 (05ddrought+BC) and T4 (05ddrought+ BC +BRs) of Amitabh-004 has been recorded at $P \leq 0.05$ revealed that biochar and BRs played a helpful role in improvement of APOX activity under induced water deficit stress.

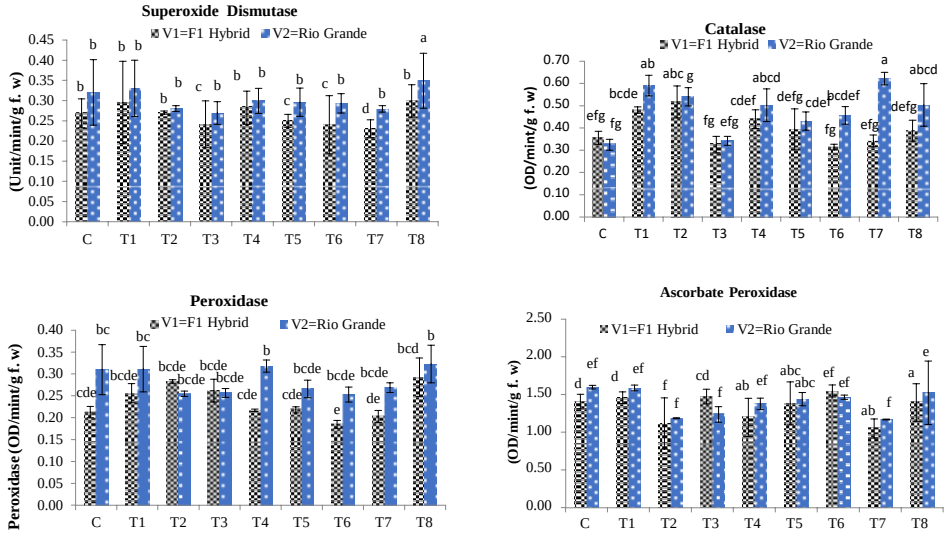

Figure-7. Antioxidant enzymes analysis of the selected tomato varieties under induced water deficit stress after application of biochar (BC) and brassinosteroids (BRs) at vegetative stage. C=Control-daily watering, T1=05ddrought, T2=05ddrought+biochar, T3=05ddrought+BRs, T4=05ddrought+biochar+BRs, T5=10ddrought, T6=10ddrought+biochar, T7=10ddrought+BRs, T8=10ddrought+biochar+BRs. All such means having same English letter are non-significantly different at $P \leq 0.05$.

3.6. HPLC profiling of crude extract of tomato

In order to determine various compounds in the extract of tomato varieties were subjected to HPLC analysis. Figure-10 indicated that the retention time and peak area of the compounds were correlated with standard chromatogram. The Gallic acid was identified at the retention time of 2.307 with peak area 63.25 and Vitamin-C at 3.15 retention time with peak area of 34.27 in all treatments of both varieties supplemented with different stresses along with biochar and BRs. The chromatograms of all treatments reflected similarity in peaks with the peaks of standard irrespective of the stress duration.

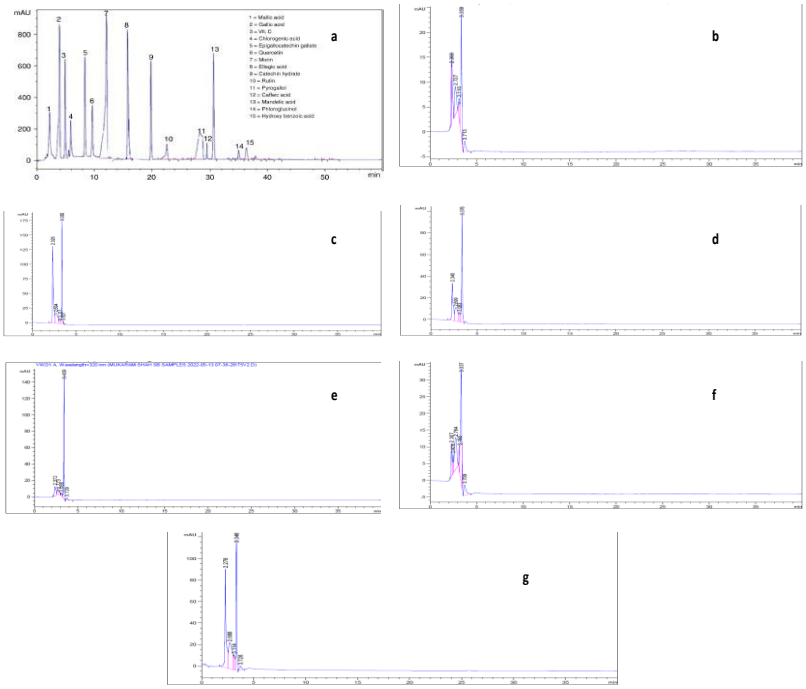

Figure-10. HPLC chromatogram of (a) Standard, (b) T1 Rio Grand variety (c) T2 Rio Grand variety (d) T2 Amitabh-F1 variety (e) T5 Amitabh-F1 variety (f) T6 Amitabh-F1 variety (g) T8 Rio Grand variety

3.7. Principal Component Analysis (PCA)

PCA analysis was carried out for analysing principal components of morphological traits under drought stress. The first principal component was found to have 40.57% followed by 13.01%, 12.04%, 9.82% and 9.31% (Table 9). The minor variation may be attributed to duration of stress incident. In the PCA-biplot the morphological faeature deflected towards right, while the plant numbers deflected towards the left against the morphological features (figure 8). Principal components of physiobiochemical were analysed under drought stress using PC-ORD. It is contemporary approach to assess the principal components of plants to screen and select most sucessful varity of plants among various kinds. Five major clusters in the PCA-biplot ordination recognised here showing a clear defelction of between the phytochemical (right) and plant numbers (left) as shown in (Fig 9). The first principal component was found to have 20.51% followed by 17.93%, 14.70%, 11.68% and 10.69% (Table 10) . The slight variation may be credited to duration of stress incident. Physio-biochemicals are the key attribute which effect under the influence of stress and are significant in determination of plant varieties.

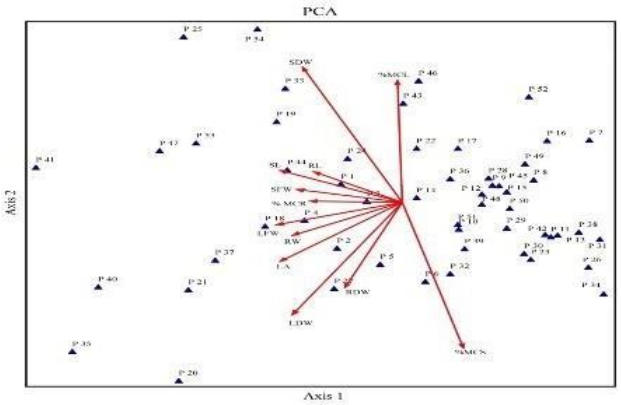

Figure-8. PCA of morphological attributes of tomato under drought stress.

Note; SDW (Shoot Dry Weight), SL (shoot Length), SFW (Shoot Fresh Weight), %MCR (%MoistureContent of Root), LFW (Leaf Fresh Weight), RW (Root Weight), RL(Root Length), SL (Shoot Length) LA (Leaf Area), LDW (Leaf Dry Weight), RDW (Root Dry weight), %MCS (%Moisture content of Shoot), %MCL (%Moisture content of Leaf).

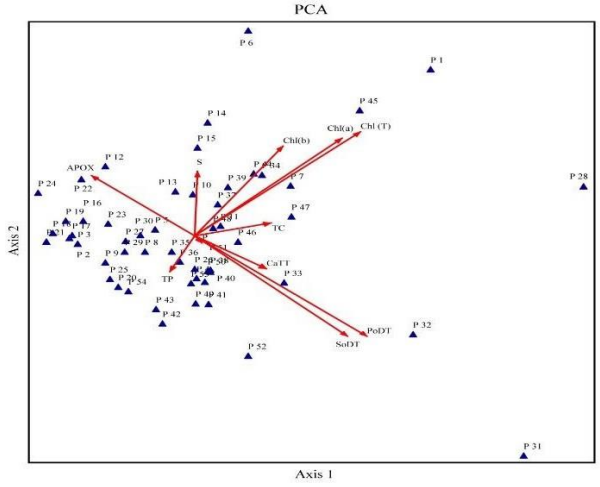

Figure-9. PCA of Physio-biochemical attributes of tomato leaf under stress.

Note; SG (Sugar), Chl(b) (Chlorophyll-b), Chl(a) (Chlorophyll-a), TCC (Total Chlorophyll contents), TC (Total Carotenoid), PT (Protein), CT (Catalase), PoDT (Peroxidase Total), SODT (Superoxide Dismutase Total), TP (Total Proline), APOX (Ascorbate Peroxidase).

4. DISCUSSION

Adequate availability of water is vital for metabolism, diversity, optimum growth and efficiency of plants. So reduction in productivity may be influenced by alteration in physiological and chemical reaction of plants under drought stress. The current investigations shows that drought stress sensitivity or tolerance of tomato variety might not only in variation in the physiological process like accumulation of osmolytes, proteins and photosynthetic pigments but also fluctuations in antioxidant enzymes activities.

The present finding declares that the soil pH decrease with incidence and duration of stress. Maximum soil pH was noted in the soil of Rio Grande and Amitabh-004 varieties of tomato but soil pH was reduced with an increase in duration of water stress which is ameliorated by BC amendment accompanied with BRs spray (Table 1). The decline in soil pH is due to water shortage and an increase of soil electrolytes. The soil that has been enhanced with biochar could serve as a mechanism to preserve soil pH under pressure. Our study is consistent with the findings of Mannan et al., (2021), who noted that biochar is known to have an impact on the soil's physicochemical characteristics and to boost crop yield in soil amended with biochar when drought stress is prevalent.

The assessment of percent field capacity showed that %FC is directly linked with water deficit stress. The highest percentage of %FC was found in the soil containing biochar and BRs when compared to the untreated condition. This indicates that the combination of BRs and biochar helps to improve the percent FC of soil under water deficiency stress. As to the findings of Romdhane et al., (2018), biochar has the potential to enhance maize's resistance to drought by modifying its physicochemical qualities and water content. The application of biochar from Vitis vinifera L., a carbon-based modification with a significant bulk density, improved soil cation exchange capacity, and high organic carbon contents, was found to significantly increase the growth of sorghum plants (Videgain-Marco et al., 2020). Our results are consistent with their findings.. Other researchers, like Sun, He et al., (2017), who investigated across the physical-chemical characteristics of biochar made from apricot and platane wood, also reported similar results. They suggested that biochar could hold water and a number of vital nutrients in the soil, change the pH of the soil to an alkaline state, and control the electrical conductivity (EC) of the soil. These results validate the present work. The present works are by Lalay et al., (2022) who suggested that the concentration of elements obtained

Drought stress markedly influences the morphological traits of plants such as plant height, fresh biomass of leaf, shoot and root, rooting density and general appearance of plant. Maximum leaf area was recorded in Rio Grande and Amitabh-004 varieties of tomato but leaf area declined with an increase in duration of water stress which is ameliorated by of BC and BRs supplementation (Table 2). The decline in leaf area is a mechanism for the escape of water shortage; it causes reduction of transpiration rate and possible way to conservation of water loss. Our results align with those of Minhas et al., (2020), who found that applying sugarcane bagasse significantly enhances maize's agronomic characteristics and attributed this to the soil's increased organic matter content. The addition of biochar increases the availability of phosphorus and strengthens the ability to exchange potassium.

The findings in Tables (2, 3 & 4) indicate that fresh biomass of leaves, shoots, and roots decreaseswith increasing water stress duration during the vegetative phases in all tomato cultivars. Rio Grandeand Amitabh-004 variety showed the greatest reduction in leaf and shoot biomass throughout the vegetative stage (Table 2, 3 & 4). The maximum decreases in fresh biomass with an increase in drought stress were recorded in Amitabh-004 variety. Rio Grande indicates maximum increase in fresh biomass as compared to Amitabh-004 (Table 2, 3 & 4) with the amendment of biochar and application of BRs. An increase in fresh biomass under stress with application of biochar and BRs shows to induce tolerance mechanism and enhance the productivity of plants under unfavorable conditions. Amendment of biochar application is a modern mechanism that might be used for the escape of plants from stress. The fresh weight of shoot, shoot height, leaf area and number, and relative water content were decreased with various level of drought stress (Kusvuran and Dasgan 2016). The outcomes of Romdhane et al., (2018) support our findings that biochar may effectively

increase maize's resistance to drought. This is probably because treated soils have better water concentration and physicochemical qualities; hybrids that can withstand drought should benefit the most because of their longer roots and higher transpiration rates. According to Table 4, our research indicates that applying BRs and biochar during the vegetative stage can lessen the negative impacts of drought stress on shoot height.. In addition to adding additional nutrients and improving the soil's ability to hold water, biochar as a soil supplement also increases the amount of organic carbon that is available to plants (Khan & Awan et al., 2021; Khan & Haidar et al., 2021).). Danish and Zafar-ul-Hye (2019) suggest that due to its effective water-holding ability, biochar's porous surface area acts as a home for PGPR colonies and protects them from desiccation.

The aforementioned properties of biochar and BRs could be helpful to increase the above-mentioned attributes of L. esculentum L. under water deficit stress. The germination potential, germination rate, shoots and root fresh biomass, and shoot and root length reduce under drought stress (Liu et al., 2015). It is noteworthy that the pH of soil decreases with intensity of water stress and biochar maintain the pH of soil at neutral range during stress condition as shown in (Table 1). pH is one of the important factors for nutrients availability to plants and any change in pH can alter the accessibility of nutrients leading to adverse effects on plants. Our findings are consistent with those of Sun et al., (2017), who evaluated woody biochar and reported that it raises soil pH, increases soil's capacity to store water, and modifies soil EC. A similar finding was made by Albert et al., (2021), who reported that the presence of organic matter and nutrients in biochar increases EC, alters pH, increases organic carbon (C), safeguards phosphorus accessibility, increases cation-exchange capacity (CEC), and enhances total nitrogen (TN) availability. It has been reported that applying biochar improves soil core tensile strength and lowers the risk of soil compaction. According to Zafar-Ul-Hye et al., (2021), the major force behind improving the soil's physicochemical qualities and plant growth is biochar's significant characteristics, such as porosity, vast surface area, providing home for soil microorganisms, and pH adjustment property.

The soil and plants treated with biochar and BRs showed maximum moisture content compared to un-treated as depicted in table (1, 2, 3, & 4). Soil treated with biochar contain greater content of moisture that could be due to water holding characteristics of biochar and that might be available for physiological processes and also improving the moisture content of the root, leaf, and shoot of the plant. According to Awad et al., (2017), who conducted similar research, biochar can improve carbon sequestration and provide favourable conditions for improved plant development by supplying essential nutrients and maintaining moisture around the roots. According to research by Romdhane et al., (2015) and Joseph et al., (2015), biochar's high porosity/surface area increases the soil's ability to store water, while unstable organic material and BC bond soil particles develop stable macro-collections linked to improvements in soil structure. The findings shown in Table (1) indicate that the percent field capacity and percent germination rate were increased when biochar and BRs were applied combined. According to Amendola et al., (2017), enhanced root growth and specific root length (finer roots, which are significantly involved in water absorption) are two ways that soil amended with BC under drought conditions will improve water use efficiency, soil nutrient availability, and maize productivity. BC can also improve soil nutrient quantity, which could enhance plant growth and productivity under stress situations (Gavili et al., 2019). The potassium (K) content of BC may enhance plant activities under stress (Tanure et al., 2019; Zahoor et al., 2017).

In addition to its ability to hold water, the biochar amendment provided mineral nutrients that may enhance plant development. The studies conducted by Danish et al., (2020) are consistent with our findings, as they also observed elemental peaks in biochar that closely align with the keV values in our data. Our current research aligns with the conclusions of Lalay et al., (2022), who suggested that the elemental contents and carbon stock determined through Energy Dispersive X-ray (EDX) analysis provide valuable insights into the elemental contents and their availability to crops, potentially influencing growth and production outcomes. The current studies are consistent with the findings of Srinivasan and Sarmah (2015), who used scanning electron microscopy (SEM) to analyse the specific structure in a prepared biochar sample and suggested that SEM figures are important for identifying

the structure and demonstrating its hydrophilic and hydroprotectant nature. In their SEM analysis of biochar, Lalay et al., (2022) observed that the material had a smooth morphology, a porous structure, and polygonal forms. The author proposed that the porous form of biochar enhances soil's ability to retain water and draws in more bacteria and fungus, among other microorganisms. The results of current study reflect the significant variations in biochemical and physiological processes of selected varieties of tomato. At vegetative stage, water deficit stress produced maximum increase in the chlorophyll contents of tomato varities i.e. Rio Grande and Amitabh-004 with amendment of biochar and BRs. Our findings are correlated with the Hafez et al., (2021) who documented that biochar and glycine betaine application improve the photosynthetic pigments (Chlorophyll a, b and carotenoids) and physiological features in rice. The results obtained are consistent with the investigation of Lalarukh et al., (2022) who reported that drought stress reduce pigments such as Chlorophyll a, b and total chlorophyll contents and morphological features and increased oxidative stress indicators but combined supplement of biochar and BRs were potent in ameliorating photosynthetic pigments, growth features and ion accumulation by wheat plants. Consequently, the co-application of biochar and BRs is crucial to enhance drought stress tolerance in plants. Shah et al., (2019) verified that phytohormones like auxins, ABA, Jasmonic acid, ethylene, gibberellins and brassinosteroids take part in regulation of drought stress tolerance by regulating the protective response of plants against environmental stresses.

Higher carotenoids content was recorded in Rio Grande as well as Amitabh-004 at vegetative stage as displayed in results. The maximum carotenoid contents in the leaf revealed the tolerant variety under stress condition because it may be way for escape from stress condition. The finding achieved are correlated with the results of Lalay et al., (2021) who proposed that the application of biochar and PGPR enhanced the photosynthetic pigments such as chlorophyll, carotenoid and anthocyanin contents and antioxidant enzymes like CAT (catalase) and APX (ascorbate peroxidase) activity. Our results are comparable with the investigation of Ullah et al., (2020) who reported that biochar and PGPR supplementation increase chlorophyll and carotenoid contents. Our outcomes are similar with the finding of Hashmi et al., (2019) who revealed that under nutritional stress biochar improve the synthesis and content of anthocyanin (carotenoid) in Pisum sativum L. Similar investigation were determined by Danish and Zafa-ul-Hye (2019) who suggested that coupled use of PGPR and biochar increase anthocyanin content of plants and enhanced plant growth. Estimation of proline content for certain samples of tomato varieties i.e. Rio Grande and Amitabh-004 showed that proline contents increase at vegetative stage under induced drought stress accompanied with biochar and BRs. The increase in proline content considered is one of the important strategy for plant to overcome stress and survive in hostile condition. The existing findings are similar with Hafeez et al., (2017) who documented that sugar and proline concentration increases significantly in soybean under drought stress and biochar mitigate the influence of water deficit stress on soybean. Our investigations are supported by Zaid et al., (2020) who recorded that proline contents and antioxidant enzymes activities up-regulate differentially in Mentha arvensis L. under Cd stress with supplementation of plant growth regulators. In our results the proline concentration increases considerably under drought stress and then decline with combined application of biochar and BRs at vegetative stage. Similar finding was recorded by Lalay et al., (2021) that proline content increases during water deficit stress but reduce with co-application of biochar and plant growth promoting rhizobacteria (PGPR). Our investigations are co-related with Danish et al., (2020) who proposed that the combined supplementation of PGPR and biochar decreases the concentration of proline of plants subjected to drought stress.

The results of current study indicated that sugar conents increase significantly with short term exposure to stress but decline with increase in severity and duration of drought stress in both varieties of tomato along with biochar and BRs application. The accumulations of sugar are essential for membrane stability and functions of protein and also act as osmo-protectant and turgidity of cell. Our results are in accordance with Nafees et al., (2021) that osmolyte such as soluble sugar, proline and glycine betaine content increases with co-application of biochar and growth promoting rhizobacteria in Vicia faba under drought stress. Ahmad et al., (2020) evaluated that sugar perform a crucial role

in the photosynthetic capacity, maintenance of cellular organization and detoxification of ROS by performing as metabolic signals in the stress situation. Osmolytes accumulation enhance the capacity of cell to adjust with in stress condition by increasing the osmotic potential and pressure of cell. They are highly soluble organic substances stored in different parts of the plants in response to adverse environmental condition that lead to retaliate osmotic stress (Ozturk et al., 2021). The outcomes of present study indicated that there is significant increase in protein content with increase in water deficit stress duration in all treatment as compare to control (untreated) in both varieties of tomato at vegetative stage as shown in the results. Our investigations are in accordance with Khan et al., (2020) who reported that the external use of epibrassinolids and water deficit stress impacted positively the accumulation of osmolytes like proline, sugar and protein contents. The current findings are in line with Zong et al., (2018) who reported that application of 28-homobrassinolide decline accumulation of soluble protein, non-reducing sugars improve soluble sugar in juncea plantlets. Our investigations are in correlation with Lalarukh et al., (2021) who recorded that biochar and BRs application showed considerable positive impact on plants under water deficit stress via decreasing MDA, oxidative stress indicators and increasing protein contents in wheat. At vegetative stage the different response shown by different antioxidant enzymes i.e. SOD, POD, CAT, APOX with the increase in drought stress duration as displayed in results. Biochar along with glycine betaine changed the antioxidant enzymes activity like catalase, peroxidase, ascorbate peroxidase and also reflect the importance and ecofriendly sustainable application strategy of biochar and glycine betaine to enhanced the plant tolerance in all plants and especially in rice and cereal crops to abiotic stresses. The present study is in accordance with Nazir et al., (2021) who recorded that the application of EBL and H_2O_2 on distinct way increase photosynthetic productivity, adjust chloroplast structure, anatomical adaptation, cell sustainability and proline and antioxidant enzyme production that improved resilience of tomato to Cu stress. The results reported here are consistent with the findings made by Nazir et al., (2019), who postulated that a rise in hydrogen peroxide (H2O2) and brassinosteroids (BRs) strengthens plants' antioxidant capacity by increasing enzyme activity for the detoxification of reactive oxygen species (ROS). In particular, under nickel stress, this results in enhanced activity of SOD, CAT, GPOX, and catalase in Solanum lycopersicum L. as well as proline accumulation. Similar to this, it has been found that applying biochar and jasmonic acid together might mitigate the detrimental effects of salinity, improving plant development and the levels of chlorophyll a and b in Vicia faba L. (Nahhas et al., 2021). According to the results, which are consistent with those of Nahhas et al., (2021), biochar and jasmonic acid dramatically reduce the activities of SOD, CAT, and glutathione reductase in Vicia faba L. under salt stress. According to Sanchez-Rodriguez et al., (2012), who used HPLC to quantify the phenolic compounds in cherry tomatoes, grafting on rootstocks that are suited to withstand water stress might be a useful technique to enhance crop quality in situations when mild water stress is artificially induced. Meulebroek et al., (2012) observed increased amounts of lycopene, β-carotene, and α-tocopherol in Red-Ripe tomatoes grown under varying salt stress levels after quantifying liposoluble antioxidants using U-HPLC-MS/MS. Furthermore, Sancho et al., (2011) studied the effects of ripeness stages on the content of bioactive compounds in papaya fruits using HPLC-DAD-MS/MS-ESI. They identified and quantified the amount of phenols, carotenoids, and vitamin C in papaya (Carica papaya L., cv. Maradol) fruit. Prior studies used typical HPLC as the basis for the chromatographic separation of carotenoids and tocopherols, which led to lengthy analytical periods (>30 min) (Zhang et al., 2010).

10. CONCLUSION

The inhibitory influence of drought stress on tomato at vegetative stage can be ameliorated through biochar amendment and foliar application of brassinosteroids particularly in sensitive varieties. Significant variation in responses to water deficit stress shown by selected varieties owing changes in biochemical and physiological mechanism. The treatment T8, T6, T2 and T4 elucidated that that maximum raise in sugar and proline contents, POD, CAT, SOD, and APX activity indicated by Rio Grande variety. On vegetative and physio-biochemical basis Rio Grande variety were determined

more tolerant to water deficit stress and reactive to foliar presentation of brassinosteroids and biochar amendment while Amitabh-004 was sensitive to drought and least responsive to biochar and brassinosteroids application. Antioxidant enzymes and proline can serve as functional indicator for opting drought tolerant accession. The results shown that application of brassinosteroids and biochar sufficient role separately but their combined applications are more significant than separate use. Moreover, the current varieties are not enough for drastic condition; advancement is indispensable in both physiological as well as genetic engineering basis.

Statements and Declarations

Declaration of competing of interests

All authors declare that they have no financial or non-financial competing interests.

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Authors contribution

Conceptualization: [Ali Hazrat]; Methodology: [Jehan Zada]; Formal analysis and investigation: [Muhammad Nafees, Ikram Ullah and Zahid Ullah]; Writing - original draft preparation: [Jehan Zada and Muhammad Nafees]; Writing - review and editing: [Muhammad Nafees and Sami Ullah]; Supervision: [Ali Hazrat and Sami Ullah]

Data Avalibility Statement

The data from the study will be provided upon reasonable request.

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