Pak. J. Bot., 57(5): DOI: http://dx.doi.org/10.30848/PJB2025-5(12)

ROLE OF BIO-INOCULANTS AND PLANT GROWTH REGULATORS ON GROWTH, PHYSIOLOGY AND YIELD OF CARROT (*DAUCUS CAROTA* L.) UNDER HEAT STRESS

RAMEEZ HUSSAIN[1], AAMIR ALI[1], NAIMA HUMA NAVEED[1], ABDUL MAJID[1], HAMID RAZA[1*], MUHAMMAD IMRAN[1], ANEELA NIJABAT[2] AND PHILIP W. SIMON[3]

[1]Department of Botany, University of Sargodha, Sargodha 40100, Pakistan
[2]Department of Botany, University of Mainwali, Punjab, Pakistan
[3]USDA-ARS, University of Wisconsin- Madison, USA
**Corresponding author's hamidraza78678600@gmail.com*

Abstract

Naturally plants exposed to unfavorable environmental conditions which drastically affects the plants. Among all abiotic stress, heat stress is considered a major threat towards the productivity of crops. To understand the effect of heat stress it is necessary to understand the molecular mechanism of heat stress of plants. This study was aimed to ascertain the effects of bio-inoculants and plant growth regulators on growth, physiology and yield of different carrot genotypes under heat stress conditions. A field experiment was carried out using randomized complete block design at Noor-Pur-Thal, Punjab during the growing season. The outcomes of recent investigation revealed that elevated temperatures had a detrimental effect on factors that contributed to crops growth, such as RD, RW, RL, LA, and various biochemical parameters. Significant decline was noted during exposure to heat stress, coupled with the increase in the activity levels of antioxidant enzymes. Highly significantly positive strong correlation was observed among the following parameters: RW vs RL, RW vs LA, RL vs RD, RL vs LA, PAL vs RS, PAL vs TS, PAL vs Prot, PAL vs FLA, TS vs RS, TS vs Prot, TS vs FLA, RS vs Prot, RS vs FLA, Prot vs FLA, Prot vs CARO, and FLA vs CARO under all diverse applied treatments of both PGR and BI. The peak specialized potentials of biochemical attributes in mgg^{-1} of tissue under the applications of HS+BI (T_5) were 0.01, 0.11, 10.45, 8.25, and 41.77 for PAL, POD, TS, RS, and Prot, accordingly. The applications of BI and PGR to plants experiencing heat stress enhanced growth, accelerating the development of yield contributing factors and biochemical parameters, thereby ultimately increasing the final crops yield. The utilization of both PGR and bio-inoculants proved advantageous for plant growth in the experiencing of heat stress, with bio-inoculants exhibited a greater impact on plant growth and development than PGR.

Key words: Heat stress, Carrot, Genotypes, Bio-fertilizers, Morphological traits, Biochemical parameters.

Introduction

Vegetables are among the most essential components of the human diet, providing a rich source of proteins, carbohydrates, fats, vitamins, and minerals. They are vital to human nutrition and serve as significant contributors to the sustenance of growers through their production, marketing, and processing. In recent times, the adoption of advanced management techniques and applications has led to a steady increase in vegetables production. Apiaceae family is a key group of plants that includes several important vegetables and flavor crops, such as carrot, celery, cilantro, parsnip, and fennel. *Daucus carota* is an important biennial root vegetable belongs to genus "*Daucus"* which contains 25 species and is currently estimated to be the largest genus in the family "Apiaceae". It is cultivated globally for its edible roots, which are of significant nutritional and medicinal value, serving as a major source of carbohydrates, proteins, sodium, fiber, and a wide range of pharmacologically active compounds (Kadluczka & Grzebelus, 2022).

Recently, there has been a significant rise in the production and consumption of *Daucus carota*, driven by growing awareness of their health benefits. It is also very rich source of carotene (Vitamin A) and antioxidant compounds which make it best source for antimelignant medicines. *Daucus carota* is ranked third among the root vegetables in global production. In Pakistan, carrots are cultivated over an area of 13.9 thousand hectares, yielding a total production of 242.3 thousand tonnes. The Pakistan average yield for carrot is only 17.5 tonnes ha^{-1}, which is quite low compared to other advanced countries of the world, such as Belgium (47.64 tonnes ha^{-1}), Denmark (44.29 tonnes ha^{-1}), United Kingdom (44.28 tonnes ha^{-1}) (Ashraf *et al*., 2018). Similarly, India and China, which share some similar climatic conditions, have average yields of 30 and 38.54 tonnes ha^{-1}, respectively.

The yield of carrot variety T-29 in Pakistan is much lesser than its potential i.e. 22000 kg h^{-1}. There are several reasons for the low yield of carrot in Pakistan. Among them, the most important are non-availability of high quality seed (Noor *et al*., 2020), inadequate technical expertise in production methods (Muhie *et al*., 2024), and effect of biotic (disease like pest and smut) and abiotic stress (heat, drought, and salt stress) that hugely affect the crops and yield (Choudhary *et al*., 2021).

Abiotic stresses (heat, salt, and drought) in crop species are of great significance and their severity may result in the yield reduction by bringing changes at morphological, physiological and biochemical level. With the Rapid increase in industrialization and global warming, heat stress becomes the most important factor that effects the growth of plants leading to reduction of yield. This increase in temperature has become major global issue. In particular, the earliest flowering species in spring are the most sensitive to high temperature. Heat stress directly alters photosynthetic acclimation, physiological processes and indirectly changes the pattern of plant growth and development (Hemantaranjan *et al*., 2018). In different studies, high temperature has been reported to cause decrease in growth, transpiration, respiration and photosynthesis that ultimately results in economic loss of farmer (Malhi *et al*., 2021).

The need for increasing agricultural productivity and quality has led to an excessive use of chemical fertilizers, creating serious environmental pollution. Generally, most carrot farmers use inorganic fertilizers for higher yield. The use of inorganic fertilizers as a source of nutrient has however, been associated with human health problems and environment degradation. In addition, the rising costs of inorganic fertilizers have made them too expensive to most resource-poor small scale growers (Aryal *et al*., 2021).

The use of biofertilizers and biopesticides is an alternative for sustaining high production with low ecological impact. Agrochemical fertilizers have adversely effected the environment and no longer able to sustain the productivity. In order to combat the harmful effects of synthetic fertilizers, it is essential to adopt a system of organic farming especially in vegetables. Bio-fertilizers are microbial preparations containing, primary beneficial role in famishing a proper rhizosphere for plant growth. Thus, they cause minerals solubilization and facilitate their uptake (Kalia & Kaur, 2019).

Manures and composts can provide significant quantity of nutrients and have a considerable effect on increasing the fertility of soil for a long time. These may have direct and indirect effect on plant growth documented increased corn yield and improved soil properties for several years after application of compost and manures (Oyetunji *et al*., 2022). Organic manure and compost increases soil organic carbon, improves soil moisture and soil N, P, K, Mg and Ca. Indirect effects involve improvement of soil properties and direct effects require uptake of humic substance into the plant tissue resulting in various biochemical changes. The organic fertilizers main constituent is humic acid. Its effect on the increase of crop yield is more significant than chemical fertilizer and manure. Its application is totally safe, harmless and pollution free (Tiwari *et al*., 2023). Further studies on these aspects are still needed in Pakistan. However, there is a critical need to increase the availability of bio-fertilizers and transfer technology to formers. Keeping in view these facts, the present study work was undertaken to study the effect of bio-inoculants and plant growth regulators against heat stress in carrot as well as the effect of BI and PGR on morphological, physiological, and biochemical characterization of carrot under heat stress.

Material and Methods

To characterize and assess the role of PGR and BI on various carrot varieties exposed to heat stress (HS) conditions, an experimental field test was undertaken during the period of 2022 to 2023 in Noor-Pur-Thal, Khushab, Pakistan, where weather patterns consist of severe summer heat, often exceeding 40°C, and mild winters, with January temperatures ranging from 10 to 15°C. The region receives an annual rainfall of approximately 300 to 500 mm, primarily during the monsoon season from July to September. Throughout the experimental period, daily maximum and minimum temperatures, average relative humidity levels, total precipitation, soil moisture measurements, and daily sunlight exposure were recorded. The details regarding the utilized materials and the methodologies employed throughout the experimentation and analysis are outlined below:

Germplasm details

Source of genotype: Seeds for various carrot genotypes, including accessions USDA 8, USDA 13, Ames 27397, and local varieties such as T-29 (cultivated) and GB (wild relative), were obtained from the gene bank of USDA, ARS, University of Wisconsin-Madison, USA.

Selection of genotypes: In the first year i.e. 2021-2022, response of 100 genotypes was checked against heat stress and the genotypes were categorized into different classes according to their response. Then to check the effect of bio fertilizers and PGR each genotype of each representative class was taken into consideration i.e. highly tolerant (HT), moderately tolerant (MT), susceptible (S), local wild (GB) and local cultivated (T-29) and their detail is given in (Table 1).

Table 1. Genotype and their response.

Sr. No.	Genotype	Response
1	USDA 8	HT
2	USDA 13	MT
3	Ames 27397	S
4	Local cultivated (**T-29)**	T
5	Local Wild (**GB**)	T

Experimental details

Experimental design: The field experiment was designed in randomized complete block design (RCBD) with three replicates of each genotype. A plot of 3×3 feet was used for the sowing of one replicate of each genotype. Distance of 5 feet was maintained from plant to plant in each replicate of all genotypes.

Sowing details: Seeds of different carrot accessions were sown in October 2022 at study area and were irrigated after 21 days of sowing. After first irrigation, specified treatments of BI and PGR were applied to plants in each replicate. Harvest of vegetative part (root) was carried out in January 2023 at the end of growing season.

Bio-fertilizers and plant growth regulators: Isolates of bacterial strains were obtained from Institute of Agricultural Sciences, University of the Punjab, Lahore.

Preparation of biofertilizer inoculum: A consortium of PGR isolated from rhizosphere of desert soil, mixed with maize straws as carrier material. Equal quantity (100 gm) of bio-inoculants was applied for each of five selected genotypes under both conditions i.e. normal and stress after 15 days of germination.

Treatment schedule: Six treatments of bio fertilizers and plant growth regulators were applied and detail is given as under.

T_0 = Control
T_1 = Plants inoculated with Bio fertilizer
T_2 = Plants inoculated with PGR
T_3= Plants exposed to Heat stress
T_4 = Application of Bio fertilizer + Heat stress
T_5 = Plants inoculated with PGR + Heat stress

Tunnel formation: Polythene plastic tunnel was formed with the help eucalyptus shoots in order to apply heat stress on genotypes under study. Maximum temperature was recorded up to 35°C with the help of thermometer.

Weeds and pest control: For weeds and insect control, weedicide and pesticide were applied on the plants so that damage to the crop may be reduced. Pre emergence and post emergence weedicides were applied as well as pesticide was sprayed at the time of umbel formation to manage the attack of pests on the umbels.

Harvesting of carrot roots: Carrots were harvested during January, 2023 for the morphological and biochemical analysis while seeds were collected during the month of June to July, 2023 for the conservation of germplasm.

Collection of data

Morpholological parameters

Root length: Root length (RL) was measured in cm with the help of meter rod. Three carrots were randomly selected for each treatment and RL was measured from reduced stem to tip of carrot. Average root length was measured in cm.

Root weight: Root weight was measured by electrical balance.

Root diameter: Root diameter was measured for each three randomly selected genotypes with the help of Varner calipers in cm.

Leaf area: It was recorded with the help of leaf area meter in cm^2.

Biochemical characterization of carrot: Quantitative estimation of biochemical contents (polyphenol oxidase, phenylalanine ammonia lyase, peroxidase, total soluble sugars, reducing sugars, total soluble proteins, carotenoids, and flavonoids) of investigated carrot genotypes by using UV-1100 Absorption spectrophotometer at Hi tech. Lab, University of Sargodha.

Extraction procedure: 1g leaves of carrot genotypes were weighed with electrical balance and crushed into fine powder with the help of pestle mortar using Liquid Nitrogen. Then, 10 ml of 0.02 M phosphate buffer having 7.0 pH was added to that powder and slurry was formed that was transferred to 1.5 ml eppendroff tubes. To separate the supernatant for analysis eppendroff tubes were centrifuged at 12000 rpm for 10 minutes in centrifuge (MIKRO 120 Hettich Zentrifugen model number 0004108-02-00). The supernatant removed by using micropipette and transferred to another eppendroff tube for biochemical analysis.

Total sugar contents: A protocol given by Yemm & Willis (1954) was used to check the total sugar contents.

Reducing sugars: A procedure given by Asad *et al*., (2015) was used to find out the quantity of reducing sugars.

Total soluble proteins: Biuret method described by Roensen & Johnson (1961) was used for the estimation of total soluble protein contents.

Calculation for proteins
To calculate the total soluble protein content following formula was used.

$$\text{Protein content (mg/gm of tissue)} = \frac{\text{CV x TE}}{\text{E.U x WT x 1000}}$$

Where;
CV = Standard curve value **TE**= Total extract
E.U. = Extract used (ml) **WT** = Weight of tissue

Activity of phenylalanine ammonia lyase (PAL): To check the activity of PAL protocol of Zucker (1965) and modified by Faiz *et al*., (2022) was used.

Activity of polyphenol oxidase (PPO): A method described by Decker (1977) was used for the study of PPO activity.

Estimation of peroxidases: Activity of peroxidase was estimated at wavelength of 470 nm according to protocol described by Faiz *et al*., (2022).

Calculation of enzymes

$$\textbf{Amount of enzyme (mg/gm of tissue)} = \frac{\textbf{OD x DF}}{\textbf{E.U x 1000}}$$

Where;
O.D. = Optical density, **DF** = Dilution factor, **E.U =** Extract used (ml)

$$\text{Specific activity of enzyme (units/mg of proteins)} = \frac{\text{Amount of enzyme}}{\text{Amount of soluble protein}}$$

Flavonoids: To analyze the total flavonoids in carrot, method described by Zhishen *et al*., (1999) was used.

Statistical analysis

Statistical analysis was performed by using statistic software Statistica, XL-stat, statistix, and MS excel. Analysis of variance was used to check the effect of treatments on studied genotypes, which correlation and principle component analysis were used to determine the interaction between studied parameters and treatments applied.

Results

Descriptive statistics of traits under study: Effect of all treatments of bio-inoculants, PGR and heat stress on

genotypes (cultivated and wild) and their response on different attributes under heat stress along with descriptive statistics has been given in (Table 2). Data showed that all the morphological parameters were negatively affected by heat stress (T_3) i.e., mean of root weight was 7.6593 g, root length 6.8133 cm, root diameter 1.73 cm and leaf area 392.62 cm^2, respectively. A decrement of -24.51660589%, -17.85266458%, - 4.525386313% and -1.155560031% under heat stress (T_3) over control conditions (T_0) was recorded. With the application of PGR (T_1) an increase in yield contributing factors over control was recorded i.e. root weight was 11.555 g which was 30.28481325 % increase over control (T_0). Similar trend was noticed for root length, root diameter and leaf area i.e. 9.7393 cm, 1.8927 cm, 402.62 cm^2 which were 26.4769713%, 8.758278146 % and 2.698824299 % higher compared to their respective controls (T_0). As far as the effect of the application of bio-inoculants (T_2) was concerned, means of root weight, root length, root diameter and leaf area were 13.22 g, 10.49 cm, 1.9707 cm, 407.93 cm^2, respectively which were 30.28481325 %, 26.4769713 %, 8.758278146 %, 2.698824299 % increased over control (T_0). When plants grown under heat stress were treated with bio-inoculants (T_5) maximum increase was recorded compared to plants growing under heat stress (T_3) without any application of bio-inoculants or PGR. The maximum value of root weight, root length, root diameter and leaf area under heat stress after the application of bio-inoculants (T_5) was 10.88 cm, 8.44 cm, 1.84 cm and 400.68 cm^2, respectively. Heat stress drastically affected the morphological parameters. All the parameters under investigation had maximum value under normal conditions (control) when treated with bio-inoculants (T_2) while minimum were recorded in control (T_0) when there was no application of plant growth regulators and bio-inoculants. The results of yield contributing (morphological) parameters exhibited that bio-inoculants were more efficient growth promoter than PGR in all genotypes growing under all treatments. The maximum specific activity of per-oxidase was 6.31 mg g^{-1} tissue, when plants growing under normal condition (control) were treated with PGR (T_1). The level of peroxidase increased gradually under heat stress and the maximum specific activity of peroxidase was recorded 7.29 mg g^{-1} tissue, with the application of PGR on plants growing under heat stress (T_4). After application of bio-inoculants to plants growing under normal conditions (T_2), maximum specific activity of phenylalanine ammonia lyase was 0.01 mg g^{-1} tissue, polyphenol oxidase was 0.12 mg g^{-1} tissue, total soluble sugars were 9.37 mg g^{-1} tissue, reducing sugars were 6.73 mg g^{-1} tissue and total soluble proteins contents were 36.58 mg g^{-1} tissue. Under heat stress conditions, after treatment of bio inoculant (T_5), the maximum specific activities of phenylalanine ammonia lyase, polyphenol oxidase, total soluble sugars, reducing sugars and total soluble proteins were 0.01 mg g^{-1} tissue, 0.11 mg g^{-1} tissue, 10.45 mg g^{-1} tissue, 8.25 mg g^{-1} tissue and 41.77 mg g^{-1} tissue, respectively (Fig. 1). Application of plant growth regulators results in an increase level of flavonoid and carotenoid contents. When plants growing under normal condition were treated with plant growth regulators (T_1), maximum flavonoid and carotenoids contents were 24.70 mg g^{-1} tissue and 23.88 mg g^{-1} tissue, respectively. However, their contents were increased under heat stress conditions. After the application of plant growth regulators to plants growing under heat stress (T_4), maximum level of flavonoids and carotenoids was 32.64 mg g^{-1} tissue and 31.54 mg g^{-1}, respectively showing that plant growth regulators showed their effect more on the concentration of flavonoid and carotenoid contents compared to bio-inoculants. It was inferred that application of bio-inoculants appeared to had more pronounced effect to combat heat stress because its effect on biochemical changes was less compared to PGR (T_4) under heat stress.

Table 2. Descriptive statistics of traits under study.

Stats.	Treat	RW	RL	RD	LA	POD	PAL	PPO	TS	RS	Prot	FLA	CARO
Minimum	C (T_0)	8.1	7.1	1.72	390.4	4.09	4.00E-04	0.078	6.08	4.2	6.09	5.28	0.171
	PGR (T_1)	9.7	8.56	1.81	393.4	4.15	1.40E-03	0.078	6.24	4.5	6.59	6.88	0.892
	BI (T_2)	11.4	9.1	1.83	397.2	4.15	4.00E-04	0.078	6.6	4.8	6.68	6.78	0.195
	H (T_3)	5.4	5.6	1.61	387.5	4.177	4.00E-04	0.081	6.28	4.77	6.99	6.079	0.872
	PGR+H (T_4)	7.1	6.5	1.65	390.5	4.18	5.00E-04	0.092	6.68	5.3	7.39	7.38	0.110
	BI+H (T_5)	9.34	6.99	1.74	393.5	4.17	5.00E-04	0.019	6.98	5.44	7.69	6.85	0.929
Mean	C (T_0)	10.147	8.294	1.812	397.21	5.922	5.23E-03	0.103	8.259	5.632	28.676	19.382	19.012
	PGR (T_1)	11.555	9.739	1.892	402.62	6.308	8.02E-03	0.107	9.092	6.450	34.654	24.701	23.881
	BI (T_2)	13.22	10.49	1.970	407.93	6.198	6.52E-03	0.119	9.374	6.734	36.576	23.15	21.6
	H (T_3)	7.659	6.813	1.73	392.62	6.906	6.27E-03	0.106	8.939	6.556	34.053	27.5	25.845
	PGR+H (T_4)	8.931	7.445	1.792	395.93	7.294	5.91E-03	0.118	10	7.481	39.104	32.643	31.541
	BI+H (T_5)	10.88	8.444	1.842	400.68	7.117	8.91E-03	0.111	10.477	8.249	41.771	30.603	28.918
Standard Error	C (T_0)	0.310	0.198	0.015	1.264	0.329	9.68E-04	5.94E-03	0.500	0.276	6.056	2.587	4.614
	PGR (T_1)	0.317	0.254	0.014	1.505	0.300	1.62E-03	6.97E-03	0.659	0.403	7.350	3.495	5.711
	BI (T_2)	0.305	0.234	0.027	2.119	0.294	1.17E-03	0.010	0.707	0.456	7.939	3.139	5.245
	H (T_3)	0.336	0.254	0.022	1.018	0.378	1.26E-03	5.87E-03	0.643	0.406	7.234	4.153	6.090
	PGR+H (T_4)	0.324	0.216	0.022	1.067	0.446	1.66E-03	7.23E-03	0.738	0.478	8.355	4.919	7.359
	BI+H (T_5)	0.276	0.271	0.016	1.290	0.411	1.91E-03	9.16E-03	0.790	0.613	8.813	4.524	6.775
Maximum	C (T_0)	12.3	9.6	1.94	406.7	7.8	9.70E-03	0.147	12.1	7.4	62.01	32.08	46.403
	PGR (T_1)	13.2	12.4	1.99	412.6	7.3	0.02	0.158	13.45	9.102	73.09	42.18	57.783
	BI (T_2)	14.9	12.01	2.22	426.3	7.28	0.011	0.216	14.12	10.99	75.5	38.68	52.173
	H (T_3)	10.5	9.8	1.88	400.2	8.4	0.013	0.139	13.89	9.78	70.09	46.88	60.213
	PGR+H (T_4)	11.8	9.1	1.95	405.4	9.24	0.016	0.192	15.023	10.04	79.09	56.08	71.573
	BI+H (T_5)	12.8	10.34	1.94	410.2	8.9	0.019	0.171	16.03	12.25	82.47	52.38	66.793

RW= Root weight (g), RL= Root length (cm), RD= Root diameter (cm), LA= Leaf area (cm2), POD= Peroxidase (mg g^{-1} tissue), PAL= Phenylalanine ammonia lyase (mg g^{-1} tissue), PPO= Polyphenol oxidase (mg g^{-1} tissue), TS= Total soluble sugars (mg g^{-1} tissue), RS= Reducing sugars (mg g^{-1} tissue), Prot= Total soluble proteins (mg g-1 tissue), FLA= Flavonoids (mg g^{-1} tissue), CARO= Carotenoids (mg g^{-1} tissue), C= Control, PGR= Plant growth regulators, BI= Bio-inoculants, H= Heat stress

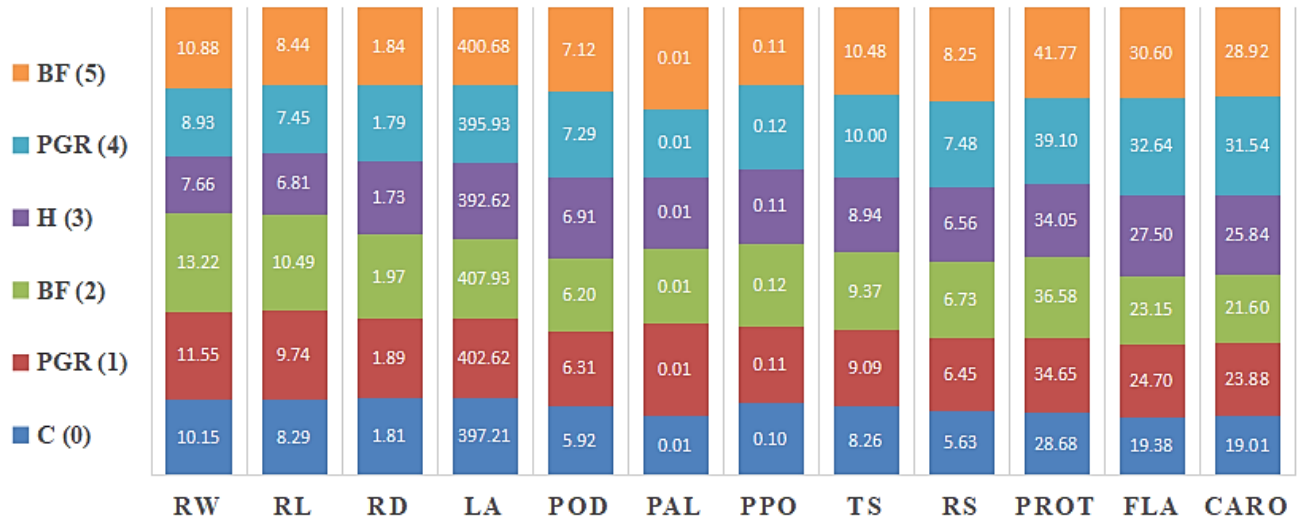

Fig. 1. Average responses of studied attributes under six treatments.

Mean squares of the studied parameters along with LSD based grouped means: Analysis of variance was carried out for randomized complete block designed (RCBD) experiments to check the effect of treatments (BI and PGR) on the all studied morphological and biochemical characters. (Table 3) depicted that different sources of variation like genotypes and treatments had significant effect on all traits under study. Effect of genotypes and treatments on all the morphological and biochemical characters was found to be highly significant at $p \leq 0.001$ reflecting that each genotype responded differently to treatment. After each treatment all parameters were significantly altered showing their effectiveness on the traits. It is also obvious from the results that the interaction between genotypes and treatments was significant for all parameters under study at $p \leq 0.001$ except for root length and Leaf area index for which $p>0.05$, however all replicates were found to be highly non-significant except for leaf area in which $p \leq 0.05$. The mean of genotypes and treatments were grouped into different classes on the basis of least significant difference (LSD) and means followed by different letters in the same column differed significantly from each other at p= 0.05. On the basis of least significant difference (LSD) means of all five studied genotypes were classified into 51 classes while 58 classes were generated for means of treatments. In case of genotypes, means of yield contributing factors i.e. root weight, root length, root diameter and leaf area index were classified into 3, 3, 5 and 3 classes, respectively whereas the biochemical characters i.e. peroxidase, phenyl alanine ammonia lyase, reducing sugars, total soluble proteins, flavonoids and carotenoids, were categorized into 5 classes except polyphenol oxidase and total soluble sugars for which 4 classes were generated. Placement of mean into different classes showed that each genotype responded differently to all variables under study. On the basis of critical value of comparison all mean of treatments were clustered into different letters which differed significantly from each other. Yield contributing parameters i.e. root weight, root length, root diameter and leaf area index were placed into 6, 5, 6, and 5 classes respectively while classes obtained for the means of treatment of biochemical attributes namely peroxidase, phenyl alanine ammonia lyase, polyphenol oxidase, total soluble sugars, reducing sugars, proteins, flavonoids and carotenoids were divided 5, 5, 3, 6, 4, 6, 6 and 6 groups, respectively reflecting that all parameters were significantly affected by different treatments.

Pearson correlation between traits under study: Table 4 reflects the correlation between morphological and biochemical traits of genotypes under study. The correlation between root weight and biochemical characters i.e. peroxidase (r= -0.179), phenylalanine ammonia lyase (r= -0.165), total sugars (r= -0.092), reducing sugars (r= -0.127) and total soluble proteins (r-0.229) was negative and non-significant except polyphenol oxidase (r= 0.211), flavonides (r= -0.366*) and carotenoids (r= -0.406*). However, there was negative and significant correlation of root weight with flavonides and carotenoids. Root length and root diameter exhibited negative correlation with all biochemical attributes except polyphenol oxidase. Negative and non-significant correlation was drawn among root diameter and all biochemical parameters i.e. peroxidase, phenylalanine ammonia lyase, total soluble sugars, reducing sugars and total soluble protein contents. Leaf area exhibited positive and significant correlation with polyphenol oxidase only and negative and non-significant with phenylalanine ammonia lyase, total soluble sugars, reducing sugars and total soluble proteins.

Discussions

The production of carrot (*Daucus carota* L.) is drastically effected under heat stress and recent global climate changes have made this situation more alarming. In Pakistan, Thal desert, like the other deserts is facing problem of heat stress. In present study under heat stress conditions factors responsible for the decline of crop yield was recorded i.e. root diameter, root length, root weight and leaf area. Nijabat *et al*., (2020) studied the effect of heat stress on root diameter of carrot and reported that root diameter decreased under heat stress. Similarly, Shehzad *et al*., (2020) reported a decrease in root diameter as a result of high temperature. In present study a decreasing trend of root diameter was recorded. Under heat stress -4.52% decreases was recorded at 35°C (Tables 2 & 3) over control.

Table 3. Mean squares of the studied parameters along with LSD based grouped means.

SOV	df	RW	RL	RD	LA	POD	PAL	df	PPO	TS	RS	PROT	FLA	CARO
Reps	2	4.7ns	0.27ns	0.01ns	274.254**	0.02697ns	0.00000131ns	2	0.0002616^{ns}	0.17152^{ns}	0.01397^{ns}	2.76^{ns}	3.555^{ns}	1.738^{ns}
Gen ID	4	17.95***	12.4787***	0.03685***	302.937***	37.9948***	0.0005525***	4	0.01401***	136.393***	55.2483***	18277.9***	4530.67***	11189.7***
Treat	5	57.75***	28.4704***	0.10513***	442.494***	4.6372***	0.00002869***	5	0.00066**	9.349***	12.2993***	306.8***	361.56***	322.3***
Gen ID*Treat	20	0.94*	0.3141ns	0.00695*	17.275ns	0.6058***	0.00002026***	20	0.00052**	1.094***	1.195***	55.5***	44.46***	56.2***
Error	60	0.537	0.2698	0.00363	16.752	0.0589	1.45E-06	60	0.0002	0.232	0.1808	0.5	0.53	0.4
Gen ID	**RW (Mean)**		**RL**	**RD**	**LA**	**POD**	**PAL**	**PPO (Mean)**		**TS**	**RS**	**PROT**	**FLA**	**CARO**
Local wild	11.79^{a}		9.8961^{a}	1.8756^{ab}	406.31^{a}	7.4483^{b}	0.0017^{d}	0.1347^{b}		8.135^{c}	5.8644^{d}	7.218^{e}	13.693^{d}	0.802^{e}
USDA 8	10.49^{b}		7.8178^{c}	1.8083^{cd}	400.14^{b}	7.8706^{a}	0.0139^{a}	0.1464^{a}		13.39^{a}	9.4086^{a}	72.655^{a}	44.008^{a}	41.239^{b}
USDA 13	9.66^{c}		8.1383^{c}	1.7806^{d}	397.94^{bc}	6.4978^{d}	0.0076^{c}	0.0939^{c}		7.858^{c}	6.299^{c}	22.246^{c}	29.082^{c}	22.732^{c}
Ames 27397	10.79^{b}		8.7511^{b}	1.8883^{a}	397.11^{c}	4.1854^{e}	0.0009^{e}	0.0815^{d}		6.528^{d}	4.9376^{e}	9.397^{d}	6.675^{e}	2.401^{d}
T-29	9.2^{c}		8.0856^{c}	1.8472^{bc}	395.98^{c}	7.1211^{c}	0.01^{b}	0.0993^{c}		10.874^{b}	7.7436^{b}	67.513^{b}	38.192^{b}	58.49^{a}
LSD	**0.489**		**0.3463**	**0.0402**	**2.729**	**0.1618**	**8.04E-04**	**0.009489**		**0.3213**	**0.2835**	**0.4823**	**0.4847**	**0.4143**
Treat	**RW (Mean)**		**RL**	**RD**	**LA**	**POD**	**PAL**	**PPO (Mean)**		**TS**	**RS**	**PROT**	**FLA**	**CARO**
C (T_0)	10.14^{d}		8.294^{c}	1.812^{cd}	397.21^{c}	5.9227^{e}	0.00523^{d}	0.1038^{b}		8.259^{e}	5.632^{d}	28.676^{f}	19.382^{f}	19.012^{f}
PGR (T_1)	11.55^{b}		9.739^{b}	1.8927^{b}	402.62^{b}	6.3087^{d}	0.00802^{b}	0.1072^{b}		9.093^{cd}	6.4507^{c}	34.654^{d}	24.701^{d}	23.881^{d}
BI (T_2)	13.22^{a}		10.49^{a}	1.9707^{a}	407.93^{a}	6.198^{d}	0.00652^{c}	0.1194^{a}		9.375^{c}	6.7343^{c}	36.576^{c}	23.15^{e}	21.6^{e}
H (T_3)	7.65^{f}		6.813^{e}	1.73^{e}	392.62^{d}	6.9065^{c}	0.00627^{c}	0.1065^{b}		8.94^{d}	6.556^{c}	34.053^{e}	27.5^{c}	25.845^{c}
PGR+H (T_4)	8.93^{e}		7.445^{d}	1.792^{d}	395.93^{c}	7.2947^{a}	0.00591^{cd}	0.1189^{a}		10^{b}	7.4815^{b}	39.104^{b}	32.643^{a}	31.541^{a}
BI+H (T_5)	10.88^{c}		8.445^{c}	1.8427^{c}	400.68^{b}	7.1173^{b}	0.00891^{a}	0.1112^{ab}		10.477^{a}	8.2493^{a}	41.771^{a}	30.603^{b}	28.918^{b}
LSD	**0.535**		**0.3794**	**0.044**	**2.9895**	**0.1773**	**8.81E-04**	**0.0104**		**0.3519**	**0.3105**	**0.5283**	**0.531**	**0.4539**

Means followed by different letters in the same column differ significantly from each other at p=0.05

RW= Root weight (g), RL= Root length (cm), RD= Root diameter (cm), LA= Leaf area (cm2), POD= Peroxidase (mg g^{-1} tissue), PAL= Phenylalanine ammonia lyase (mg g^{-1} tissue), C= Control, PGR= Plant growth regulators, BI= Bio-inoculants, H= Heat stress, PPO= Polyphenol oxidase (mg g^{-1} tissue), TS= Total soluble sugars (mg g^{-1} tissue), RS= Reducing sugars (mg g^{-1} tissue), Prot= Total soluble proteins (mg g-1 tissue), FLA= Flavonoids (mg g^{-1} tissue), CARO= Carotenoids (mg g^{-1} tissue), C= Control, PGR= Plant growth regulators, BI= Bio-inoculants, H= Heat stress

Table 4. Pearson correlation between traits under study.

Variables	RW	RL	RD	LA	POD	PAL	PPO	TS	RS	Prot	FLA	CARO
RW	**1**											
RL	**0.901*****	**1**										
RD	**0.749*****	**0.830*****	**1**									
LA	**0.869*****	**0.852*****	**0.727**	**1**								
POD	-0.179	-0.228	-0.305	0.1	**1**							
PAL	-0.165	-0.316	-0.255	-0.122	**0.567****	**1**						
PPO	0.211	0.102	-0.082	**0.484****	**0.710*****	**0.384***	**1**					
TS	-0.092	-0.29	-0.177	-0.004	**0.734*****	**0.830*****	**0.612*****	**1**				
RS	-0.127	-0.321	-0.173	-0.013	**0.734*****	**0.810*****	**0.561****	**0.940*****	**1**			
Prot	-0.229	**-0.371***	-0.158	-0.188	**0.559****	**0.845*****	0.344	**0.916*****	**0.871*****	**1**		
FLA	**-0.366***	**-0.472****	-0.342	-0.219	**0.743*****	**0.863*****	**0.394***	**0.872*****	**0.893*****	**0.901*****	**1**	
CARO	**-0.406***	**-0.450***	-0.232	-0.336	**0.535****	**0.775*****	0.138	**0.768*****	**0.758*****	**0.929*****	**0.901*****	**1**

Values in bold are different from 0 with a significance level p=0.05

This reduction in diameter might be due to heat stress which inhibits early root growth and causes oxidative damage in root tissue by elevating the level of ROS. This increase in ROS results in the lipid peroxidation, disruption of membrane integrity and elevates antioxidant enzyme levels which ultimately reduce the root diameter. Rosenfeld *et al*., (1998) demonstrated the effect of heat stress on root length, according to them root length was decreased under high temperature. Results of present study showed that root length of carrots decreases under heat stress conditions and decrease was -17.82% compared to root length harvested under natural conditions (Table 2 & 3). This may be due to the fact that under heat stress, soil receives direct sunlight due to which moisture contents in the soil decreases which also affects the uptake of mineral nutrients ultimately resulting in the reduction of root length. Root weight depends upon root diameter and root length so reductions of either in diameter or length ultimately lead to decrease root weight i.e. final yield of crop. In present study root weight decreases under heat stress conditions compared normal conditions. In present investigation data presented in Table 2 & 3 showed that root weight was decreased under heat stress and decrease of -24.51% was recorded over control. Almost similar findings were also reported by Rosenfeld *et al*., (1998) that root weight of carrot decreased at higher temperature i.e. 15, 18 and 21°C which strengthened our results. Similarly, reports of Gonzalez *et al*., (2009) also corroborates with our findings that root weight of carrot decreases under high temperature conditions i.e. 35°C. This reduction may be attributed that under heat stress transpiration rate increases leading to excessive loss of water which ultimately affect the rate of photosynthesis as well as disturb the source-sink relationship leading to yield loss i.e. loss in weight. These all parameters are directly linked to leaf area because decrease in leaf area resulting in the decrease of the photosynthesis process which ultimately reduce the root diameter, root length and finally yield (root weight).

The effect of high temperature (heat stress) on leaf area was reported by Ahamed *et al.,* (2010) in wheat and Al-busaidi *et al.,* (2012) in *Jatropha curcas*. According to them, under the influence of high temperature significant decrease in leaf area was recorded over control. In present study reduction of -1.15% in leaf area was recorded under heat stress over control. Similar findings were also reported by Kesici *et al*., (2013) that leaf area under heat stress decreases which further strengthen our results. The decrease in leaf area was due to that, under heat stress plants had loosely arranged mesophyll cells in leaves, poorly developed vascular bundles and open stomata. Due to opening of stomata transpiration rate increases and RWC of plant decreases which ultimately lowers the photosynthesis.

Adeleye *et al*., (2010) reported that application of organic manure resulted in an increase in root diameter of carrot. The results of present investigation shows that root diameter increases after the application of bio-fertilizer and plant growth regulator i.e., 8.75% over control (Tables 2 & 3). Similar findings were reported by Jeptoo *et al*., (2013) who used applications of bio-slurry manure and obtained larger root diameter compared to untreated one which were in accordance with the findings of present study and reinforced our results. An increase in root diameter after the application of organic manures is due to the fact that they have been recognized to enhance soil water holding ability and to decrease soil bulk density, hence, better root growth and expansion can be achieved.

Panwar *et al*., (2002) demonstrated that root weight increased after the treatment of bio-fertilizers compared to untreated plants which clearly strengthened our findings. Moreover, Kiran *et al*., (2019) also reported that root weight in radish increased with the application of bio-fertilizers which also supported our results. Under heat stress, in response of ABA special proteins are produced, called Dehydrin proteins which protect the plants by stabilizing cell membranes, osmotic adjustment, free ion sequencing, and control solute concentration. Saidi *et al*., (2020) reported the higher level of total soluble protein contents present in wet samples of carrot compared to dry samples. A significant increase in the total soluble protein contents was recorded i.e. 34.053 mg g^{-1} tissue which was an increase of 20.8467% over control (Table 2 & 3). The findings recorded by Duc *et al*., (2018) demonstrated that total soluble protein contents increased as a result of drought heat stress in tomato plants which was in accordance to our results. This increase in the total soluble protein contents might be due to the increase production of heat shock proteins which ultimately enhanced the total soluble protein contents in the plant. It is also reported by several workers that total soluble protein contents decreases as a result of heat stress. This decrease might be due to protein denaturation and inhibition of the protein synthesis at higher temperatures, since the injury from high temperature has often been attributed to the denaturation of proteins. It is also obvious from data presented in Table 2

& 3 that application of bio-fertilizer and plant growth regulator play a supplementary role and increases the total soluble protein contents which was 22.46% more over treatment T_3 (Heat stress).

It was noticed that specific activity of phenylalanine ammonia lyase increases under heat stress and increase of 0.00627% was recorded compared to control (Tables 2 & 3). Vidhyasekaran *et al*., (2002) reported the relative increase in phenylalanine ammonia lyase synthesis in resistant cultivars of mungbean in comparison to control which was in accordance with our results. Similar results were reported by Zhang *et al*., (2003) that activity of phenylalanine ammonia lyase increases with the application of bio-fertilizer which strengthen the findings of present investigation. Increased activity of PAL in response to thermal stress is considered as the main acclamatory response of cells to pathogen attack and heat stress.

Rivero *et al*., (2001) investigated the effect of heat stress on the specific activity of polyphenol oxidase in water melon and reported an increase level of polyphenol oxidase compared to control which was in agreement with our findings. The results of Ramazani & Zabet (2022) also support the outcomes of present study. They reported that activity of PPO was increased in Triticale in the presence of heat stress and by the application of PGR and bio fertilizers. Increase in peroxidase activity during stress is often associated with a progressive incorporation of phenolic compounds within the call wall. Peroxidases also catalyze rapid H_2O_2 dependent cross linking of cell wall proteins. Kaur *et al*., (2018) reported that specific activity of peroxidase enzymes in seedlings of Brassica species was increased under heat stress condition. These results also reinforced by the reports of Rached-Kanouni & Alatou (2013) that specific activity of peroxidase was increased when plants were exposed to high temperature compared to normal condition. Sassi-Aydi *et al*., (2014) stated that total soluble sugar contents was increased under osmotic stress for osmotic adjustment in many legumes and non-legumes which further strengthened our results. This increment in total soluble sugar contents was due to inhibition of sucrose synthase or invertase activities.

According to our results, under heat stress and after the application of treatments (bio-fertilizers and plant growth regulators) carotenoids contents increased compared to control which were 35.94% and 22.03%, respectively. The outcomes of present were in conformity with the reports of Merghany *et al*., (2008) that carotenoid contents in carrot roots was enhanced under the heat stress as well as under the beneficial influences of compost, microorganisms and the highest levels of mineral fertilization. These results might be due to the improvement of photosynthesis as a result of the positive effects of bio-fertilizer and plant growth regulator used for vegetative growth of carrot. In this scenario there is dire need to introduce high temperature resistant varieties to increase the production. Nowadays excessive use of synthetic fertilizers to enhance productivity is not only deteriorating the quality of soil but also causing environmental pollution and hazard to health. Therefore, the use of bio-fertilizers for organic farming is gaining popularity among growers due to their eco-friendly behavior.

Conclusions and Future prospects

It is evident from the present study that heat stress has negative impact on the yield contributing (morphological) factors which reduce the final yield. It also enhances the level of antioxidant enzymes which provide defensive mechanism to plants. After the application of bio-inoculants and plant growth regulators an increase was recorded in the morphological characters which is a positive sign towards crop yield. Therefore, in present study bio-inoculants and plant growth regulators were proved to be effective against heat stress by minimizing the effect of heat stress resulting in the increase of the yield. It is a dire need to introduce bio-inoculants and plant growth regulators on the commercial scale so that the pollution caused by synthetic fertilizers can be minimized. It is indispensable to recognize appropriate organics and bio-inoculants for improving biochemical and physiological attributes of carrot.

Acknowledgment

The authors extend their gratitude to the Department of Botany, University of Sargodha, for providing the necessary resources and support to conduct this research. This manuscript constitutes a portion of Rameez Hussain's M.Phil. thesis.

References

Ashraf, M., G. Sadique, S. Ahmad and M. Ahmad. 2018. Development of tractor operated carrot digger and its performance evaluation. *J. Agric. Res*., 56(3).

Aryal, J. P., T.B. Sapkota, T.J. Krupnik, D.B. Rahut, M.L. Jat and C.M. Stirling. 2021. Factors affecting farmers' use of organic and inorganic fertilizers in South Asia. *Environ. Sci. Pollut. Res.*, 28(37): 51480-51496.

Adeleye, E.O., L.S. Ayeni and S.O. Ojeniyi. 2010. Effect of poultry manure on soil physico-chemical properties, leaf nutrient contents and yield of yam (*Dioscorea rotundata*) on alfisol in southwestern Nigeria. *J. Am. Sci.*, 6(10): 871-878.

Ahamed, K.U., K. Nahar, M. Fujita and M. Hasanuzzaman. 2010. Variation in plant growth, tiller dynamics and yield components of wheat (*Triticum aestivum* L.) due to high temperature stress. *Adv. Agric. Bot*., 2(3): 213-224.

Al-Busaidi, A., M. Ahmed and J. Chikara. 2012. The impact of heat and water stress conditions on the growth of the biofuel plant *Jatropha curcas*. *Int. J. Environ. Stud.*, 69(2): 273-288.

Asad, S. A., A. Tabassum, A. Hameed, A. Afzal, S.A. Khan, R. Ahmed and M. Shahzad. 2015. Determination of lytic enzyme activities of indigenous Trichoderma isolates from Pakistan. *Braz. J. Microbiol*., 46: 1053-1064.

Choudhary, V., K. K. Kumar, B. H. Sahu, S.K. Muthappa and A.D.A. Das. 2021. Agronomic innovations in biotic stress management and its combined effect with abiotic stresses in crop production. *Indian. J. Agron.*, 66(5): 237-257.

Decker, A. 1977. Enzymes reagents and related enzyme biochemical. (Eds.). Worthington enzyme manual. Worthington Biochemical Corporations Freehold, New Jersey, USA. pp. 74-76.

Duc, N.H., Z. Csintalan and K. Posta. 2018. Arbuscular mycorrhizal fungi mitigate negative effects of combined drought and heat stress on tomato plants. *Plant Physiol. Biochem*., 132: 297-307.

Faiz, S., A.A. Shah, N.H. Naveed, A. Nijabat, N.A. Yasin, A.I. Batool and A. Ali. 2022. Synergistic application of silver nanoparticles and indole acetic acid alleviate cadmium induced stress and improve growth of *Daucus carota* L. *Chemosphere*, 290: 133200.

Gonzalez, M.V., V.O. Sandras, M.A. Equiza and J.A. Toqnetti. 2009. Suboptimal temperature favours reserve formation in biennial carrot (*Daucus carota*) plants. *Plant Physiol*., 137: 10-21.

Hemantaranjan, A., C. Malik and A.N. Bhanu. 2018. Physiology of heat stress and tolerance mechanism-an overview. *J. Plant. Sci. Res.*, 33(1): 55-68.

Jeptoo, A., J.N. Aguyoh and M. Saidi. 2013. Improving carrot yield and quality through the use of bio-slurry manure. *Sustain. Agric. Res*., 2(1).

Kadluczka, D. and E. Grzebelus. 2022. Comparative fruit morphology and anatomy of wild relatives of carrot (Daucus, Apiaceae). *Agriculture*., 12(12): 2104.

Kalia, A. and H. Kaur. 2019. Nano-biofertilizers: Harnessing dual benefits of nano-nutrient and bio-fertilizers for enhanced nutrient use efficiency and sustainable productivity. *Nanoscience for Sustainable Agriculture*, 51-73.

Kaur, H., G. Sirhindi, R. Bhardwaj, M.N. Alyemeni, K.H.M. Siddique and P. Ahmad. 2018. 28-homobrassinolide regulates antioxidant enzyme activities and gene expression in response to salt-and temperature-induced oxidative stress in *Brassica juncea*. *Sci. Rep*., 8(1): 8735.

Kesici, M., H. Gulen, S. Ergin, E. Turhan, I.P.E.K. Ahmet and N. Koksal. 2013. Heat-stress tolerance of some strawberry (*Fragaria ananassa*) cultivars. *Notulae Bot. Hortic. Agrobot*., 41(1): 244-249.

Kiran, M., M.S. Jilani, K. Waseem, M.S. Khan, F. Haq, M.A. Nadim and S. Shaheen. 2019. Integrated use of organic and inorganic fertilizers on the growth and yield of radish. *Sarhad J. Agric*., 35(3): 933-941.

Muhie, S.H., F. Akele and T. Yeshiwas. 2024. Economic feasibility of carrot (*Daucus carota* L) production in response to different seed priming techniques under deficit irrigation. *Sci. Hortic.*, 325: 112662.

Merghany, M.M., H.A. Mahmoud, M.M. Shahien and M.A.A. El-Sayed. 2008. Productivity improvement of carrot (*Daucus carota*) under north sinai conditions. *J. Plant Prod*., 33(12): 8777-8797.

Malhi, G.S., M. Kaur and P. Kaushik. 2021. Impact of climate change on agriculture and its mitigation strategies: A review. *Sustainability.*, 13(3): 1318.

Nijabat, A., A. Bolton, M. Mahmood-ur-Rehman, A.I. Shah, R. Hussain, N.H. Naveed and P. Simon. 2020. Cell membrane stability and relative cell injury in response to heat stress during early and late seedling stages of diverse carrot (*Daucus carota* L.) germplasm. *Hortscience*, 55(9): 1446-1452.

Noor, A., K. Ziaf, M.A. Ghani, C.M. Ayub, I. Ahmad and M. Amjad. 2020. Plant spacing effects on seed yield and quality of carrot cultivar T-29. *Pure Appl. Biol*., *(PAB)*., 9(4): 2563-2570.

Oyetunji, O., N. Bolan and G. Hancock. 2022. A comprehensive review on enhancing nutrient use efficiency and productivity of broadacre (arable) crops with the combined utilization of compost and fertilizers. *J. Environ. Manage*., 317: 115395.

Panwar, J.D.S. and O. Singh. 2002. Response of *Azospirillum* and *Bacillus* on growth yield of wheat under field conditions. *Indian J. Plant Physiol*., 5: 108-110.

Pendharkar, M.B. and P.M. Nair. 1975. Induction of phenylalanine ammonia lyase (PAL) in gamma irradiated potatoes. *Radiation Botany.*, 15(2): 191-197.

Rached-Kanouni, M. and D. Alatou. 2013. Change in activity of antioxidative enzymes in leaves of *Acacia retinodes, Biota orientalis* and *Casuarina equisetifolia* under heat stress condition. *Eur. Sci. J*., 9(24): 402-410.

Ramazani, S.H.R. and M. Zabet. 2022. Triticale (X Triticosecale Wittmack): Role and Responses Under Abiotic Stress. In Sustainable Remedies for Abiotic Stress in Cereals (pp. 209-228). Singapore: Springer Nature Singapore.

Rivero, M.R., J.M. Ruiz, P.C. Garcia, L.R. Lopez-Lefebre, E. Sanchez and L. Romero. 2001. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *J. Plant Sci*., 160: 315-321.

Roensen, D. and D.B. Johnson. 1961.Estimation of protein in cellular material. *Nature*., 91(2): 492-493.

Rosenfeld, A.H., H. Akbari, J.J. Romm and M. Pomerantz. 1998. Cool communities: Strategies for heat island mitigation and smog reduction. *Energy Build*., 28(1): 51-62.

Saidi, R., M. Hamdi and H. Bouallagui. 2020. Hyperthermophilic hydrogen production in a simplified reaction medium containing onion wastes as a source of carbon and sulfur. *Environ. Sci. Pollut. Res.*, 27: 17382-17392.

Sassi-Aydi, S., S. Aydi and C. Abdelly. 2014. Inorganic nitrogen nutrition enhances osmotic stress tolerance in *Phaseolus vulgaris*: lessons from a drought-sensitive cultivar. *HortScience*., 49(5): 550-555.

Shehzad, M.A., F. Nawaz, F. Ahmad, N. Ahmad and S. Masood. 2020. Protective effect of potassium and chitosan supply on growth, physiological processes and antioxidative machinery in sunflower (*Helianthus annuus* L.) under drought stress. *Ecotoxicol. Environ. Saf*., 187: 109841.

Tiwari, J., A.L. Ramanathan, K. Bauddh and J. Korstad. 2023. Humic substances: Structure, function and benefits for agroecosystems-A review. *Pedosphere*., 33(2): 237-249.

Vidhyasekaran, P., A. Ramanathan, L. Rathinakumar and R. Samiyappan. 2002. Induction of defense-mechanisms in mungbean suspension cultured cells by an elicitor from *Macrophomina phaseolina*. Acta Phytopathol. *Entomol. Hung*., 37(1-3): 91-98.

Yemm, E.W. and A. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J*., 57(3): 508.

Zhang, L., H. Meakin and M. Dickinson. 2003. Isolation of genes expressed during compatible interactions between leaf rust (*Puccinia triticina*) and wheat using cDNA-AFLP. *Mol. Pl. Pathol*., 4(6): 469-477.

Zhishen, J., T. Mengcheng and W. Jianming. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*., 64(4): 555-559.

Zucker, M. 1965. Sequential induction of phenylalanine ammonia-lyase in cell suspension culture of Barley. *Plant Physiol.*, 5(2): 822-827.

(Received for publication 23 February 2024)