www.envsciarch.com

ENVIRONMENTAL SCIENCE ARCHIVES
ISSN: 2583-5092
Volume IV Issue 2, 2025

RESEARCH PAPER OPEN ACCESS

Received:
2025/09/04
Accepted:
2025/10/27
Published:
2025/11/16

Evaluation of Advanced Breeding Lines of *Brassica juncea* (L.) in Temperature Gradient Tunnels: Physiological and Biochemical Traits Influencing Yield

Pushp Sharma* and **Lovleen Brar**
Department of Botany, Punjab Agricultural University, Ludhiana, Punjab, India -141004
*Correspondence for materials should be addressed to PS (email: pushp20@pau.edu)

Abstract

High temperature is a prominent abiotic stress, affecting worldwide agricultural production, which requires rigorous screening in order to identify heat tolerant strains for crop productivity and sustainability. So, to investigate the effect of elevated temperature on physiological and biochemical traits of fourteen advanced Indian mustard (*Brassica juncea* L.) genotypes were sown under two conditions i) normal/ open field condition ii) Temperature Gradient Tunnels (TGT). Physiological and biochemical traits were assessed during the flowering stage. The SPAD value and photosynthetic pigments increased significantly under elevated temperature. Higher temperatures led to a decrease in leaf relative water content and leaf water retention, whereas relative saturation deficit and water saturation deficit increased. Increase in antioxidative enzymes (CAT, SOD and POX) and bio molecules (ascorbic acid and α-tocopherol) were also evident. Average temperature had a significant positive association with Chl b (0.58*), total Chl (0.79**), carotenoids (0.71**), but a significant negative association with CAT (-0.68**) under TGT. A strong relationship existed between seed yield and LRWC (R^2=0.429), RSD (R^2=0.407), WSD (R^2=0.429), POX (R^2=0.151) and MDA (R^2=0.245) under TGT. This study revealed JT-9 genotype as highly tolerant and JT-12, JA-53, and JD-6 were moderately tolerant with enhanced leaf photosynthetic pigments, stimulated redox homeostasis and increased activity of antioxidative enzymes and bio molecules, which conferred tolerance to heat stress.

Keywords: Antioxidative enzymes; Biomolecules; Indian mustard; Physiological parameters; Temperature gradient tunnel

Introduction

Plants are sessile and are continuously challenged by broad range of abiotic stresses. Among abiotic stresses, temperature stress is one of the major factors that alter physiological processes, specifically photosynthesis activity and membrane stability, thus leading to yield losses. Temperatures stress reduces photosynthesis, nutrient uptake and yield components (Ali et al., 2020) by ultra-structural alterations specifically the stability of the photosynthetic system, enzymatic activity, structural organization and physio-chemical properties of thylakoid membranes, stroma's chloroplast carbon metabolism and photochemical processes in thylakoid lamellae, the primary targets of high temperature damage which has direct or indirect impact on plant photosynthetic activities. Chlorophyll content is associated with assimilation and dry matter production.

Heat stress disrupts plant metabolism due to water availability, absorption, translocation of ions and organic solutes across the cell membrane. Raised temperature increases the evapotranspiration causing moisture deficits, thermal damages modulate the hydraulic conductance, thus affecting the water balance. The membrane becomes more permeable for

DOI: 10.5281/zenodo.17623718

water flow through the pores due to enhanced aquaporin activity from increased hydraulic conductivity (Martinez et al., 2009). Cell membrane is sensitive and changes in the ambient temperature disrupt the structure and functioning with alteration in the structures of membrane proteins, causing increased membrane permeability and electrolyte loss. Electrolyte leakage, an indicator to evaluate decreased membrane stability, malondialdehyde is used to detect membrane lipid peroxidation and changes in polypeptide pattern under stress are detected by SDS-PAGE. Relative water content and water retention of leaf are important parameters during heat and drought tolerance.

Oxidative stress is another metabolic change due to reactive oxygen species (ROS) like superoxide radical ($O^{2.-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$). High temperature disrupts the balance between ROS production and accumulation which results in oxidative damage (Chen and Yang, 2020) thereby leading to yield losses. To overcome these cellular damages, plants have antioxidative defence mechanisms which work efficiently to detoxify the toxic radicals. Non-enzymatic antioxidative biomolecules (ascorbic acid and a-tocopherol) along with antioxidant enzymes (catalase, superoxide dismutase and peroxidise) protect cell and sub-cellular systems from the adverse effects of the active oxygen radicals (Gill and Tuteja, 2010). As the ROS content increases, chain reaction starts in which SOD, a metalloenzyme catalyses the dismutation of $O_2^{\cdot-}$ radical to molecular O_2 and H_2O_2. The H_2O_2 is then detoxified either by catalase or peroxidase. Catalase (CAT) reduces H_2O_2 into H_2O and O_2, whereas peroxidase (POX) decomposes H_2O_2 by oxidation of a co-substrate such as phenolic compounds. Similarly, ascorbic acid serves as a reducing agent which helps in protecting the cell membrane from oxidative damage and α-tocopherols protect lipids and other membrane elements of chloroplasts/ other organelles by scavenging singlet oxygen and acting as an antioxidant (Das and Roychoudhary, 2014). Protection against oxidative stress is important in determining the survival of a plant under heat stress and antioxidant defense strength is correlated with the acquisition of thermo-tolerance.

Brassica juncea (Indian mustard) is rabi oilseed crop grown across the globe for edible oils, vegetable, fodder, condiments and also for its tolerance to stress conditions due to heat (Chug and Sharma, 2022), drought (Rhythm et al., 2022), salinity (KannuPriya et al., 2021) and low light stress (Kaur and Sharma, 2021). Owing to inter/mixed cropping with wheat or late sowing after cotton and rice exposes the mustard crop to high temperatures at the seed filling stage, leading to a significant decline in yield of almost all the *Brassica* species. The problem of heat stress is observed in all major mustard growing countries including China, Australia, Canada and Europe (Salisbury and Gurung, 2011). However, mechanism of high temperature tolerance studied in Indian mustard is limited. So, in the present study physiological and biochemical basis of thermo-tolerance was undertaken in fourteen advanced strains of Indian mustard. Crop damage taking place due to uncertain climatic conditions needs crucial improvement in approaches for advancement in food availability and crop production.

Materials and methods

Plant materials and stress treatments

Selected fourteen *Brasica juncea* genotypes consisted of introgression lines (JT-12, JT-128, JT-593, JT-575, JT-151, JA-53, JT-9, JA-24 and JT-77) released varieties (Giriraj, JD-6, PBR-378, PHR-126) and BPR-541-4 registered line for heat tolerance were procured from Oilseeds section, Punjab Agricultural University, Ludhiana (30°56′ N, 75°48′ E, at an elevation of 247 m above the sea level). During the two crop seasons 2017-18 and 2018-19 the crop was sown in randomized block design (RBD) in three replications under two environments i) normal/open field as control and ii) in temperature gradient tunnel (TGT) with 1.5 m row length and row-to-row distance of 30 cm. All the recommended agronomic and protection practices were followed to raise a healthy crop.

A set of five installed temperature, relative humidity, and radiation sensors, along with one sensor for radiation, were used to monitor the meteorological data inside the TGT. One set of these sensors was deployed outside the TGT to record of the relative humidity and ambient air temperature data. The data logger (Delta T Devices) automatically recorded the data at intervals of five minutes and logged it at intervals of 30 minutes. Photosynthetic pigments and biochemical parameters were assayed from 3rd and 4th fully opened leaf on main raceme at flowering stage (Fig.1). The flowering period during both the years in open field grown crop was about 3 to 4 weeks long while only of 2 weeks in TGT grown genotypes. The average maximum / minimum temperature for the first year

(2017-18) at flowering stage in the controlled condition was 21.7/8.8°C and in TGT was 29.5/14.3°C. During second year/2018-19 average maximum /minimum temperature in control was 18.4/6.1°C and in TGT was 25.0/12.1°C.

Fig. 1. Mustard (*Brassica juncea*) crop at flowering stage in TGT and control

Determination of photosynthetic pigments

Chlorophyll estimation was done by incubating 0.1 g of fresh leaf sample in 5 ml of dimethyl sulphoxide (DMSO) for 2hours at 65°C (Hiscox and Israelstam, 1979). The absorbance for mixture was recorded at 663 and 645 nm and for carotenoid content absorbance of the same mixture was read at 480 nm (Kirk and Allen, 1965).

$$\text{chl a (mg/g FW)} = 12.7 \times A_{(663)} - 2.69 \times A_{(645)} \times \frac{\text{Volume}}{\text{Weight (g)} \times 1000}$$

$$\text{chl b (mg/g FW)} = 22.9 \times A_{(645)} - 4.68 \times A_{(663)} \times \frac{\text{Volume}}{\text{Weight (g)} \times 1000}$$

$$\text{Total chl (mg/g FW)} = 20.2 \times A_{(645)} + 8.02 \times A_{(663)} \times \frac{\text{Volume}}{\text{Weight (g)} \times 1000}$$

$$\text{Carotenoids (mg/g FW)} = \frac{1000 \times A_{(480)} - 1.29 \times \text{chl a} - 53.78 \times \text{chl b}}{220} \times \frac{\text{Volume}}{\text{Weight (g)} \times 1000}$$

SPAD value

SPAD 502 system developed by Soil Plant Analysis Development Unit of Minolta Camera Cooperation, Japan was used to calculate chlorophyll content in leaves. Values were read from 3rd and 4th fully opened leaf of random 10 plants of each genotype average of which was SPAD value.

Determination of leaf water status

Five excised leaf discs were weighed for fresh weight (FW) and then were put in 10 ml distilled water for 4 hours at room temperature. After incubation turgid weight (TW), was recorded and discs were kept for drying in oven at 60°C for 2 days to record dry weight (DW). The calculation of leaf relative water content (LRWC), relative saturation deficit (RSD) and water saturation deficit (WSD) was calculated using formula given by Weatherley (1950) and Barrs (1968).

$$\text{LRWC (\%)} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100$$

$$\text{RSD (\%)} = \frac{\text{TW} - \text{FW}}{\text{TW}} \times 100$$

$$\text{WSD (\%)} = \frac{\text{TW} - \text{FW}}{\text{TW} - \text{DW}} \times 100$$

For leaf water retention (LWR) two equal sized 3rd 4th leaves were taken and weighed for FW, then kept at room temperature in dark for 4 hours to record weight and later both the leaves were dried in oven at 60°C for 2 days for dry weight (DW). The calculation of LWR was done using formula given by Sangakkara et al. (1966).

$$\text{LWR (\%)} = 1 - \left(\frac{\text{FW} - \text{weight after 4 hours}}{\text{FW}}\right) \times 100$$

Where, FW = fresh weight of leaf, TW= Turgid weight and DW= oven dried weight

Determination of membrane stability, injury and lipid peroxidation

0.1 g leaf sample in 10ml of distilled water was incubated for 4 hours at room temperature and electric conductivity was measured (C_1). Afterwards, the test tubes containing samples were kept in boiling water bath for 1 hour and electric conductivity was measured again after cooling (C_2). Membrane stability (MS %) and membrane injury (MI %) was calculated using formula given by Premchandra et al. (1990).

$$\text{MS (\%)} = 1 - \frac{C_1}{C_2} \times 100$$

$$\text{MI (\%)} = 100 - \text{MS}$$

Lipid peroxidation was determined was determined by estimating amount of malondialdehyde (MDA) content using Heath and Packer, (1968) method. To measure MDA, 0.2 g leaf sample was homogenized in 5% thiobarbutaric acid (2ml TBA), centrifuged and supernatant (1 ml) was combined with 1 ml thiobarbutaric acid- trichloroacetic acid (TBA-TCA) solution. Mixture was incubated in water bath for 30 minutes, left in ice bath to cool down and centrifuged again. Absorbance was measured at 532 nm and 600 nm. The results were expressed in unit µmol g^{-1} FW.

SDS-PAGE protein profiling

Following the Laemmli method (1970) sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE) was used to analyze the protein profiling pattern in leaf sample. The pellet was dissolved using the solubilisation buffer, which contains 0.5 mM Tris HCl (pH 6.8), 10% sodium dodecyl sulphate (SDS), and 2.5 ml tetra methyl ethylene diamine (TEMED). Then, Lowry et al. (1951) method was used to estimate the solubilised proteins.40 µg of protein were loaded onto each well of a vertical electrophoretic cell (Bio-Rad, USA) containing 30% (w/v) arylamide at a voltage of 100 V. The acrylamide gels were removed from the unit once electrophoresis was finished and the gel was thoroughly rinsed with distilled water. Gels were then left in staining solution (CBB-R-250) which was made by 10 ml acetic acid, 40 ml methanol and 125 mg commasie brilliant blue for the overnight staining of proteins. After the staining process gels were kept in distilled water for de-staining until the background of the gels turned stainless and protein bands were clearly visible. A broad range protein marker with a molecular weight reference range of 5.0–250.0 kDa was used.

Antioxidative enzyme activities

For assay of antioxidative enzymes, *viz.*, CAT, SOD and POX, the enzyme extract was prepared by homogenizing the leaf sample in 0.1 M sodium phosphate buffer (pH 7.5) under chilled conditions. The homogenate was centrifuged for 20 minutes at 10000 rpm at 4°C and supernatant obtained was used for enzymatic estimation. CAT (EC 1.11.1.6) activity was assayed from the rate of H_2O_2 decomposition as measured by decrease in absorbance at 240 nm (Chance and Maehley, 1955). One unit of CAT is defined as the amount of enzyme that decomposes 1 µmol of H_2O_2 per min at pH 7.0. SOD (EC 1.15.1.1) activity was measured using Marklund and Marklund method (1974), where 50% inhibition of auto-oxidation of pyragallol is considered as unit of enzyme activity and expressed as SOD unit EA min^{-1} g^{-1} FW. POX (EC 1.11.1.7) activity was quantified by calculating the rise in absorbance at 470 nm brought on by guaiacol (Shannon et al., 1966). One unit of POX is defined as the enzyme concentration that raises absorbance by 0.01 in one minute at room temperature and is measured as mmol min^{-1} g^{-1} FW.

Non-enzymatic antioxidative biomolecules activity

Ascorbic acid (AsA) was estimated using Roe and Kuether (1943) method. To measure AsA, 0.2 g of leaf sample was homogenized with 4 ml of trichloro-acetic acid (TCA) solution, centrifuged and supernatant was combined with 3 ml of TCA solution, recentrifuged, pooled the supernatant and total volume was made 5 ml of with TCA solution. From this 0.2 ml of supernatant was combined with 0.5 ml of 2, 4-di-nitrophenyl hydrazine (DNPH) and 2 drops of 10% thiourea. Mixture was incubated in boiling water for 30 minutes, left at room temperature to cool down. 2.5 ml of 80%

sulphuric acid (H_2SO_4) was added to the mixture and absorbance was recorded at 540 nm and calculated using standard value (0.04 mg = 0.826 OD) and expressed in mg g^{-1} DW. α-tocopherols (αTE) were estimated by Emmerie-Engel reaction (Rosenberg,1992).To measure α-TE 0.2 g of leaf sample was crushed in 3 ml of absolute alcohol and centrifuged. 1 ml of supernatant was combined with 1.5 ml of xylene and recentrifuged. Pooled supernatant was mixed with 1 ml of 2, 2 bipyridyl reagent and 1 ml of ferric chloride reagent. Absorbance was measured at 520 nm and calculated using standard value of αTE (0.36 mg = 0.680 O.D) and expressed in unit mg g^{-1} DW.

Statistical analysis

To test the significance of treatments and the relative performance of genotypes the data recorded in field and TGT were analysed statistically using Construction Plant Competence Scheme (CPCS1 2008) computer programme in a factorial randomized block design. Online statistical software OPSTAT was used to analyze Pearson's correlation between physiological, biochemical, seed yield (independent variable) with average temperature (two years pooled mean) (dependent variable). In Excel worksheet (2007), regression analysis between studied traits and seed yield was recorded using the data analysis tool.

Results

Photosynthetic pigments

Chlorophyll (a, b, total) and carotenoid content increased gradually in Indian mustard when subjected to elevated temperature conditions over controlled conditions (Table 1). Significant variations existed for photosynthetic pigments between environments and genotypes and their interactions were also statistically significant. Treatment mean indicated chl a increased by 32.7%, chl b by 35.0%, total chl by 36.7% and carotenoids by 31.6% when subjected to heat stress in TGT as compared to controlled conditions. Genotypic increase in chl a varied from (6.6–56.8%), chl b from (15.3-85.4%), total chl from (15.7-62.3%) and carotenoids from (7.7-15.4%). Maximum increase in chl a was in JT-77 (56.8%), chl b in JA-24 (85.4%), total chl in PBR-378 (62.3%) and carotenoid again in JT-77 (68.4%). Minimum increment was registered in chl a in PHR-126 (6.6%), chl b in JT-575 (15.3%), total chl in PHR-126 (15.7%) and carotenoid in PHR-126 (3.8%). Overall, JA-24 followed by JT-12 and PBR-378 registered maximum increase in photosynthetic pigments.

SPAD

At flowering stage, SPAD value was 10.3% higher when subjected to elevated temperature conditions as compared to open field grown cultivars. Significant variations existed between environment and genotypes and their interactions (E×G) were also significant (Table 1). Increase in SPAD values ranged from 7.7-15.4% under high temperature conditions. Overall, maximum increase in SPAD value was registered in JA-24 i.e. 15.4% whereas minimal increase of 7.7% was observed in PBR-378 and BPR-541-4.

Leaf water status

Leaf water status measured in different forms revealed, reduced leaf relative water content (LRWC), leaf water retention (LWR) while relative saturation deficit (RSD) and water saturation deficit (WSD) increased when subjected to elevated temperature conditions over control (Table 2). Significant variation was noticed between genotype and environment for leaf water status. Interactions (E×G) for studied traits were also statistically significant. Treatment mean indicated LRWC decreased by 11.6%, LWR by 7.3% whereas RSD increased by 44.1% and WSD by 37.8% when grown at elevated temperature in TGT compared to controlled conditions. Decline in LRWC ranged from 2.1-30.0% and LWR from 0.7-14.0% while increase in RSD was from 5.5-56.9% and WSD from 5.4-63.9% in stressed conditions. Reduction in LRWC was maximum in Giriraj (30.0%) and LWR in JA-53% whereas minimal decline was in JT-575 (2.1%) for LRWC and in JT-9 (0.7%) for LWR. Maximum increase in RSD (56.9%) and WSD (63.9%) was noticed in Giriraj, while minimal decline in RSD (5.5%) and WSD (5.4%) was registered in JT-575. Overall, JT-9 suffered the least reduction in LRWC, LWR with minimal increase in RSD and WSD.

Membrane stability, injury and lipid peroxidation

Membrane stability decreased while membrane injury increased significantly when cultivars were subjected to elevated temperature (Fig 2). Significant variations were noticed for membrane stability and injury between the two environments, genotypes and their interactions (E×G) were also statistically significant. Membrane stability was reduced by 5.9% while membrane injury increased by 19.8% when exposed to heat stress as per treatment mean. Genotypic reduction in membrane stability varied from 0.6-23.2% and increase in membrane injury from 1.6-87.5%. Membrane stability was disrupted to maximum extent in JT-593 with maximum reduction of 23.2% and maximum increase of 87.5% in membrane injury. Minimum reduction (0.6%) in membrane stability and minimal increase (1.6%) membrane injury was registered in JT-9. JD-6, PBR-378 and JA53 thus implying higher membrane stability and least membrane injury compared to the rest of genotypes.

Table 1. Variation in photosynthetic pigments (mg g^{-1} FW) and SPAD values in control and temperature gradient tunnel (TGT) of B. *juncea* genotypes (pooled mean of two years)

Genotypes	Chlorophyll a		Chlorophyll b		Total Chlorophyll		Carotenoids		SPAD	
	Control	TGT	Control	TGT	Control	TGT	Control	TGT	Control	TGT
JT-12	1.06±0.08	1.62±0.06	0.23±0.04	0.32±0.04	1.28±0.10	1.94±0.08	0.31±0.02	0.48±0.02	41.1±0.8	47.5±1.8
JT-128	1.29±0.06	1.59±0.10	0.29±0.03	0.37±0.02	1.58±0.03	1.96±0.10	0.38±0.01	0.44±0.03	41.5±1.9	47.0±2.0
JT-593	1.36±0.08	1.56±0.09	0.30±0.04	0.39±0.05	1.63±0.08	1.95±0.04	0.38±0.03	0.45±0.03	41.8±0.4	45.2±1.5
JD-6	1.27±0.07	1.64±0.06	0.28±0.03	0.39±0.05	1.55±0.05	2.03±0.07	0.37±0.01	0.46±0.02	40.2±2.1	45.5±1.5
JT-575	1.32±0.09	1.69±0.08	0.35±0.04	0.41±0.03	1.67±0.05	2.09±0.07	0.37±0.02	0.46±0.02	43.6±1.2	48.4±0.7
JT-151	1.42±0.03	1.75±0.08	0.28±0.02	0.41±0.04	1.70±0.04	2.16±0.06	0.43±0.01	0.51±0.03	42.7±0.8	46.3±1.2
PBR-378	0.99±0.13	1.50±0.10	0.21±0.03	0.34±0.06	1.17±0.05	1.90±0.08	0.30±0.01	0.45±0.03	40.3±1.3	43.5±0.9
JA-53	1.11±0.15	1.60±0.10	0.26±0.02	0.31±0.03	1.33±0.07	1.95±0.05	0.34±0.02	0.48±0.05	43.4±2.5	48.5±1.9
JT-9	1.24±0.07	1.77±0.09	0.31±0.05	0.41±0.07	1.52±0.06	2.20±0.06	0.41±0.01	0.51±0.04	43.7±1.5	47.2±1.6
Giriraj	1.42±0.07	1.74±0.12	0.30±0.02	0.43±0.05	1.69±0.04	2.34±0.14	0.42±0.02	0.53±0.05	42.5±1.5	47.2±0.7
JA-24	1.30±0.12	1.88±0.12	0.24±0.03	0.45±0.02	1.54±0.12	2.33±0.14	0.38±0.04	0.55±0.05	44.3±0.6	48.3±1.4
JT-77	0.93±0.16	1.45±0.13	0.91±0.29	1.08±0.03	1.74±0.14	2.56±0.17	0.29±0.05	0.49±0.03	36.3±0.9	39.8±1.9
BPR-541-4	1.39±0.11	1.71±0.04	0.34±0.12	0.46±0.05	1.71±0.07	2.22±0.05	0.40±0.02	0.50±0.02	44.2±1.3	47.6±0.9
PHR-126	1.68±0.09	1.79±0.07	0.44±0.11	0.66±0.09	2.12±0.08	2.45±0.06	0.52±0.04	0.54±0.05	48.1±1.2	52.8±1.4
Mean	**1.25±0.09**	**1.66±0.09**	**0.34±0.06**	**0.45±0.05**	**1.57±0.07**	**2.14±0.08**	**0.37±0.02**	**0.49±0.03**	**42.2±1.3**	**46.5±1.4**
CD (*p*=0.05)	E=0.04, G=0.10, E×G=0.14		E=0.02, G=0.04, E×G=0.06		E=0.03, G=0.07, E×G=0.09		E=0.01, G=0.03, E×G=0.04		E=0.33, G=0.84, E×G=1.20	

Table 2. Variation in leaf water traits of B. *juncea* genotypes grown in control and temperature gradient tunnel (TGT) (pooled mean of two years)

Genotypes	Leaf relative water content (%)		Leaf water retention (%)		Relative saturation deficit (%)		Water saturation deficit (%)	
	Control	TGT	Control	TGT	Control	TGT	Control	TGT
JT-12	81.5±1.0	73.4±1.9	84.7±0.8	75.7±0.8	15.7±0.6	24.1±0.4	18.5±0.2	26.6±0.9
JT-128	80.9±2.2	73.2±1.5	83.5±2.0	76.9±1.1	16.7±0.6	24.0±0.2	19.1±1.1	26.8±0.5
JT-593	74.1±0.9	55.0±1.2	91.1±0.8	82.2±1.3	21.4±0.9	41.9±0.9	25.9±0.9	45.0±0.5
JD-6	77.0±1.3	56.7±1.6	88.0±0.9	79.2±1.9	19.9±1.1	39.2±0.2	23.0±0.3	43.4±0.6
JT-575	71.7±2.1	70.2±1.3	81.3±0.2	73.8±2.1	25.2±0.6	26.6±0.8	28.3±0.2	29.8±0.3
JT-151	77.1±1.1	72.7±1.4	78.5±1.7	74.7±0.3	20.0±0.7	23.9±0.1	22.9±0.1	27.3±0.4
PBR-378	77.4±1.3	68.1±1.7	74.1±0.5	69.3±1.8	19.5±0.4	28.8±0.7	22.6±0.3	31.9±0.7
JA-53	78.4±1.3	72.2±1.9	83.4±1.1	71.7±1.1	18.4±0.8	25.6±0.6	21.6±0.4	28.8±0.3
JT-9	73.9±1.4	70.9±1.4	70.4±1.5	70.0±1.3	22.6±0.6	25.8±0.2	26.1±0.8	29.1±0.4
Giriraj	76.8±1.8	53.8±1.4	74.8±1.2	73.1±1.7	20.4±0.3	43.0±0.3	23.2±0.6	46.2±0.4
JA-24	74.8±1.6	69.8±1.5	82.8±1.9	79.3±1.4	21.8±0.6	26.4±0.3	25.2±0.5	30.2±0.5
JT-77	72.6±2.0	67.3±0.9	77.8±0.6	76.5±1.4	23.8±0.2	29.3±0.4	27.5±0.7	32.7±0.9
BPR-541-4	74.8±0.9	72.0±1.8	76.2±0.7	69.0±1.3	21.2±0.5	25.0±0.7	25.2±0.9	28.1±0.7
PHR-126	84.2±1.5	82.4±0.9	94.9±.0.4	86.4±0.5	12.6±0.7	15.9±0.6	15.8±0.8	17.6±0.6
Mean	**76.4±1.5**	**67.6±2.0**	**81.0±1.0**	**75.1±1.3**	**20.3±0.6**	**29.3±0.5**	**23.6±0.6**	**32.5±0.6**
CD (*p*=0.05)	E=0.52, G=1.33, E×G=1.88		E=0.78, G=2.01, E×G=2.84		E=0.54, G=1.37, E×G=1.95		E=0.74, G=1.89, E×G=2.68	

Lipid peroxidation was detected by MDA content. Elevated temperature enhanced MDA content by 1.9 fold as compared to controlled conditions (Fig 2). Significant variations were observed for MDA between environment and genotypes and their interactions (E×G) were also statistically significant. Genotypic increase in MDA content varied from 1.3 to 2.5 fold. Maximum increment was noticed in JD-6 (2.5 fold) and least in Giriraj (1.3 fold).

SDS-PAGE protein profiling

Protein bands appeared in controlled conditions and under elevated temperature treatment differed in their molecular mass and intensity in B. *juncea* cultivars. In Fig. 3(a) control cultivars depicted 14 protein bands with molecular mass between 15 to 25 kDa. Each genotype showed two bands each. Genotype JT-12 band was of lower intensity as compared to other genotypes in control.

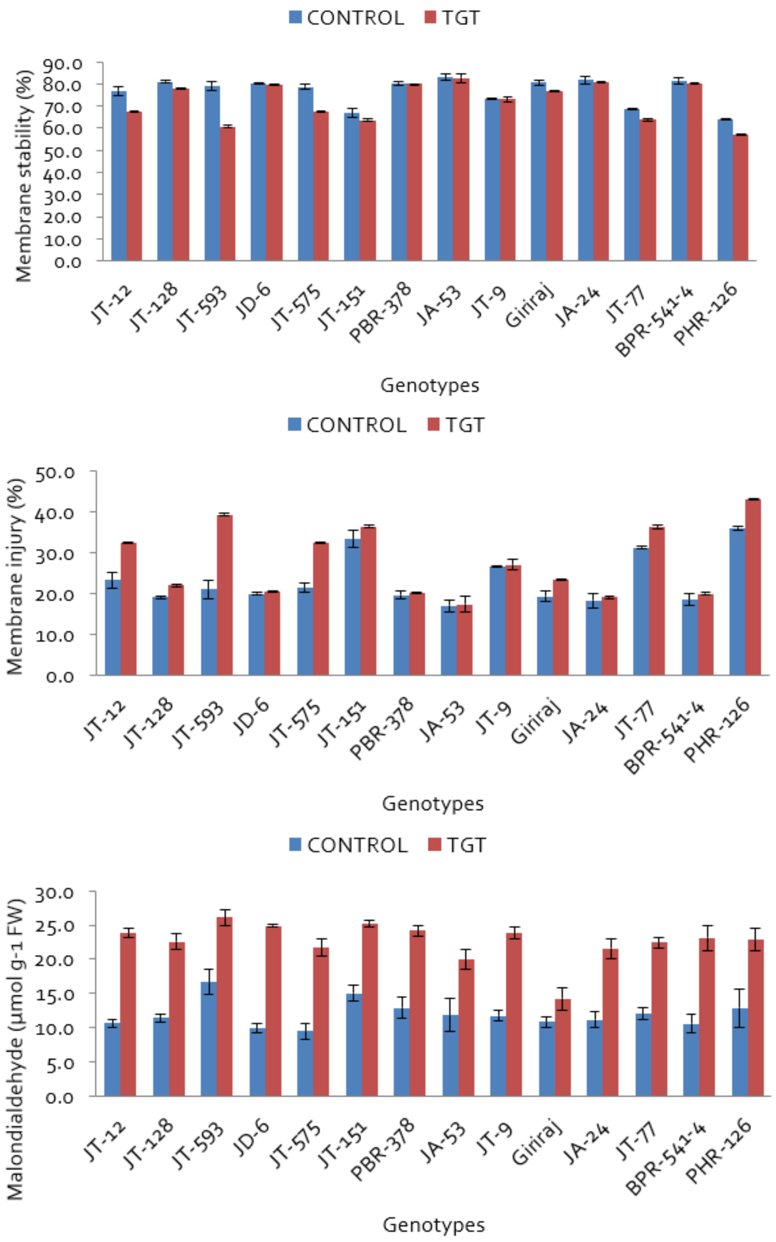
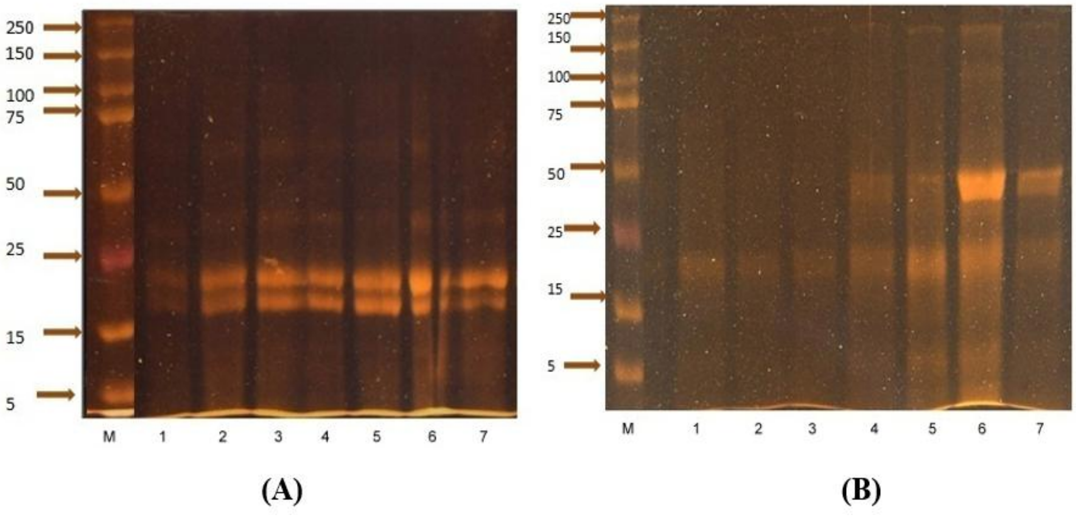


Fig. 2. Differential response of membrane stability, injury, and malondialdehyde in B. *juncea* under two environments



(A) (B)

Fig. 3. Banding pattern of leaf proteins of B. *juncea* genotypes grown in control (A) and TGT (B) (M) molecular marker, 1-JT-12, 2-JT-128, 3- JT-593, 4-JD-6, 5- JT-575, 6-JT-151, 7-PBR-378

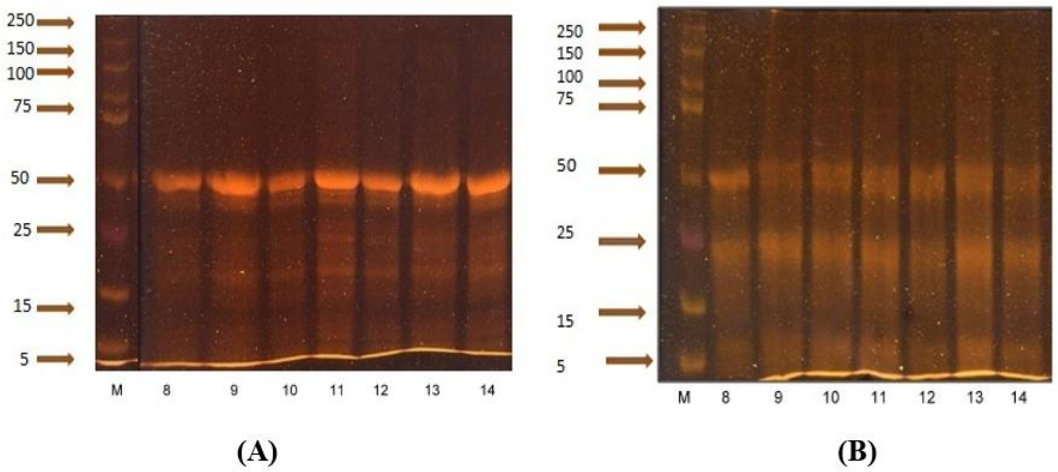


Fig. 4. Banding pattern of leaf proteins of B. *juncea* genotypes grown in control (A) and TGT (B) (M) molecular marker, (8) JA-53, (9) JT-9, (10) Giriraj, (11) JA-24, (12) JT-77, (13) BPR-541-4, (14) PHR-126.

In Fig.3 (b) stressed genotypes showed 11 different bands with molecular mass between 15 to 50 kDa. Cultivars 1 (JT-12), 2 (JT-128) and 3 (JT-593) showed single band of molecular mass between 15 to 25 kDa while genotypes 4 (JD-6), 5 (JT-575), 6 (JT-151) and 7 (PBR-378) showed 2 bands each with molecular mass ranged up to 50 kDa. These bands were of much lower intensity than bands depicted in 3 (a). Out of 11 prominent bands visible genotype 6 (JT-151) and 7 (PBR-378) were of high molecular mass i.e.50 kDa. In Fig. 4 (a) genotypes 8 to 14 under control condition showed more protein bands of higher intensity with molecular mass between 15 to 50 kDa however, dense bands were observed with molecular mass 50 kDa. Comparatively, in Fig. 4 (b) stressed genotypes 8 to 14 showed 14 different protein bands, two for each genotype with molecular mass 25 kDa and 50 kDa but these were of lower intensity than in 4 (a) in controlled genotypes.

Antioxidant enzymes activity

Thermo-tolerance in plants is associated with an enhancement in antioxidant capacity. Antioxidative enzymes activity was higher in all the studied genotypes/varieties when subjected to elevated temperature (Table 3). Significant differences existed for enzyme activity among environment and genotypes and their interactions were also significant. Treatment mean indicated an average increase of 1.7 fold in CAT activity, 1.1 fold in SOD activity and 1.6 fold in POX activity over control. Increase in CAT activity ranged from 1.1 to 2.9 folds, SOD from 1.0 to 1.5 folds and POX from 1.0 to 2.4 folds. Maximum increase in CAT activity was registered in JT-77(2.9 fold), SOD in JT-12 and JT-128 (1.5 fold) while POX in Giriraj (2.4 fold). However, minimal increase in CAT activity was in JA-24 (1.1%), SOD in JT-575, PHR-126 (1.0fold) and POX in PHR-126 (1.0 fold). Overall, maximum increase in antioxidative enzyme activity was registered in JT-128 and JT-9.

Table 3. Variation in antioxidative enzymes in *Brassica juncea* grown in control and temperature gradient tunnel (TGT) (pooled mean of two years)

Genotypes	**Catalase (mmoles min^{-1} g $^{-1}$FW)**		**Superoxide dismutase (EA min^{-1} g^{-1} FW)**		**Peroxidase (mmoles min^{-1} g^{-1} FW)**	
	Control	**TGT**	**Control**	**TGT**	**Control**	**TGT**
JT-12	250±2.3	514±1.7	92±0.9	139±0.8	0.75±0.1	1.22±0.2
JT-128	205±2.4	520±1.3	101±0.5	149±0.5	1.27±0.2	2.34±0.1
JT-593	228±1.3	356±1.2	126±0.4	135±0.2	1.42±0.3	2.41±0.1
JD-6	192±1.7	501±1.4	134±0.4	152±0.9	0.94±0.2	1.67±0.4
JT-575	237±2.2	416±1.7	132±0.2	135±1.1	0.87±0.2	1.56±0.4
JT-151	253±1.3	373±1.4	121±0.5	133±2.0	1.07±0.4	1.94±0.1
PBR-378	292±1.2	406±1.6	122±0.9	140±0.9	1.36±0.4	1.73±0.1
JA-53	201±1.5	378±1.8	127±0.3	142±0.6	1.44±0.4	1.75±0.2
JT-9	174±1.4	376±1.5	125±0.2	140±0.2	1.38±0.1	2.41±0.3
Giriraj	208±1.1	319±2.1	134±0.5	148±0.8	0.97±0.4	2.31±0.4
JA-24	328±2.3	370±1.7	125±0.5	135±0.6	1.14±0.3	1.56±0.4
JT-77	153±2.2	441±1.6	125±0.2	136±0.9	0.91±0.3	1.06±0.1
BPR-541-4	233±1.4	293±1.3	132±0.6	144±0.7	1.54±0.1	1.87±0.1
PHR-126	216±1.7	300±1.9	130±0.7	132±0.9	0.64±0.4	0.66±0.8
Mean	**229±1.7**	**405±1.6**	**123±0.5**	**141±0.8**	**1.16±0.3**	**1.83±0.3**
CD (*p*=0.05)	E=4.5, G=11.5, ExG=16.3		E=2.7, G=6.9, ExG=9.7		E=0.14, G=0.35, ExG=0.50	

Biomolecules

The tolerance mechanism also involves the production of several non-enzymatic molecules which scavenge reactive oxygen species and detoxify systems to prevent the damaging effects of ROS. Non-enzymatic antioxidative biomolecules increased significantly with an increase of 4.4 fold for ascorbic acid and 1.5 fold for α-tocopherols when subjected to elevated temperature in comparison with open field/control (Fig. 5).

Significant differences existed for biomolecules among environment and genotype and their interactions were also significant. Genotypic increase varied from 1.6 to 7.3 folds in ascorbic acid and from 1.3 to 2.1 folds in α-tocopherols. Minimal increase in ascorbic acid was registered in JT-151 (1.6 fold), α-tocopherols in JT-575, PBR-378 and JT77 (1.3 fold) whereas maximum increase in both ascorbic acid (7.3 fold) and α-tocopherols (2.1 fold) was recorded in JD-6.

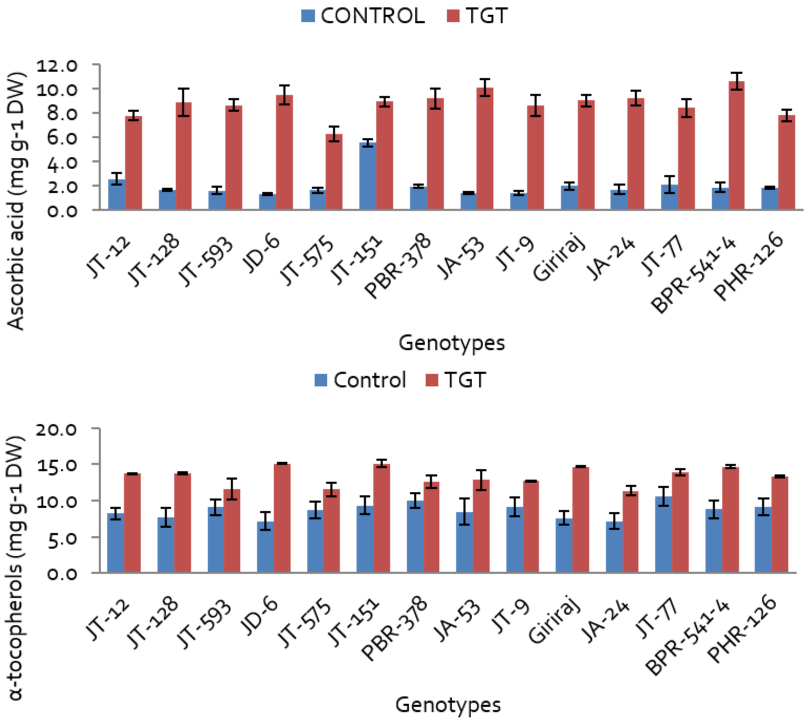


Fig. 5. Variation in non-enzymatic antioxidative biomolecules content under control and temperature gradient tunnel (TGT)

Correlation analysis

Correlation analysis revealed that all the studied physiological parameters exhibited positive association with average temperature except LRWC, LWR, MI under controlled condition except RSD, WSD and MI in TGT (Table 4). Significant associations with average temperature under temperature stress were registered with chlb (0.58*), total chl (0.79**), caro (0.71*) whereas under controlled condition positive significant association existed between average temperature and total chl (0.56*) only. A significant positive correlation under control and stress was noticed for chla with carotenoid (0.96**) (0.77**) and SPAD (0.79**) (0.73**), total chl with carotenoid (0.76**) (0.75**), SPAD with MS (0.64**) (0.57*) but negative significant association with MI (-0.64**) (-0.57*) respectively. LRWC had negative significant association with RSD (-0.99**) (-0.98**) and WSD (-0.99**). Similarly, under both the environments RSD had a positive significant association with WSD (0.94**) (0.98**) but significant negative association between MS and MI (-0.95**) (0.94**) respectively. In controlled condition total chl of genotypes had significant positive association with chl a (0.75**), carotenoid and SPAD (0.81**), LRWC significantly positively correlated with MS (0.67**) but significantly negatively associated with MI (-0.68**). RSD had significant negative correlation with LWR (-0.53*), MS (-0.69**) but positive with MI (0.70**). Similar significant negative association of WSD with MS (-0.67**) but association was positive with MI (0.68**). Under elevated temperature conditions total chl and chl b (0.81**) had positive significant association only. All the antioxidative enzymes were negatively correlated with average temperature under stressed conditions but association was positive under controlled condition except for POX.

	Chl a	Chl b	Total chl	Caro	SPAD	LRWC	RSD	WSD	LWR	MS	MI	CAT	SOD	POX	MDA	AsA	α- TE	SY	Avg temp
Chl a	1	-0.23	0.34	0.77^{**}	0.73^{**}	0.24	-0.25	-0.24	0.15	0.35	-0.36	-0.47	-0.17	0.33	-0.22	-0.03	-0.06	0.21	0.38
Chl b	-0.23	1	0.81^{**}	0.27	-0.40	0.11	-0.11	-0.12	0.28	-0.12	0.12	-0.10	-0.32	-0.52	-0.24	-0.18	0.13	0.25	0.58^{*}
Totalchl	0.75^{**}	0.47	1	0.75^{**}	0.38	0.14	-0.15	-0.15	0.25	0.15	-0.16	-0.45	-0.31	-0.40	-0.34	-0.11	0.16	0.29	0.79^{**}
Caro	0.96^{**}	-0.16	0.76^{**}	1	0.42	0.24	-0.25	-0.25	0.16	0.39	-0.39	-0.62^{*}	-0.33	-0.27	-0.40	0.07	0.08	0.31	0.71^{**}
SPAD	0.79^{**}	-0.43	0.42	0.81^{**}	1	0.47	-0.45	-0.46	0.28	0.57^{*}	-0.57^{*}	-0.37	-0.12	-0.14	-0.19	-0.14	-0.15	-0.28	0.20
LRWC	0.27	-0.28	0.17	0.33	0.34	1	-0.98^{**}	-0.99^{**}	0.42	0.35	-0.35	-0.15	-0.43	-0.52	0.20	-0.18	-0.09	-0.21	0.35
RSD	-0.30	0.27	-0.10	-0.35	-0.37	-0.99^{**}	1	0.98^{**}	-0.31	-0.34	0.34	0.13	0.43	0.52	-0.22	0.17	0.38	0.21	-0.35
WSD	-0.27	0.29	-0.26	-0.33	-0.35	-0.97^{**}	0.99^{**}	1	-0.23	-0.35	0.35	0.15	0.44	0.53	-0.21	0.19	0.29	0.23	-0.35
LWR	0.40	-0.04	0.36	0.30	0.28	0.48	-0.53^{*}	-0.48	1	0.11	-0.22	0.24	-0.31	-0.40	0.22	-0.33	-0.14	0.31	0.15
MS	0.42	-0.12	0.30	0.47	0.64^{*}	0.67^{**}	-0.69^{**}	-0.67^{**}	0.49	1	-0.94^{**}	-0.35	0.23	-0.28	-0.36	0.33	0.08	0.17	0.48
MI	-0.42	0.12	-0.30	-0.47	-0.64^{*}	-0.68^{**}	0.70^{**}	0.68^{**}	-0.49	-0.95^{**}	1	0.35	-0.23	0.28	0.35	-0.32	-0.08	-0.16	-0.49
CAT	0.07	-0.57^{*}	-0.31	-0.24	0.28	0.06	-0.16	-0.27	0.14	0.31	-0.22	1	0.36	-0.06	0.28	-0.22	0.11	-0.26	-0.68^{**}
SOD	0.35	0.18	0.42	0.35	0.24	-0.47	0.44	0.47	-0.27	0.41	-0.11	-0.14	1	0.44	-0.30	0.49	0.50	0.30	-0.32
POX	-0.19	-0.30	-0.41	-0.19	-0.15	-0.36	0.32	0.35	-0.45	-0.27	0.28	0.27	0.11	1	-0.10	0.34	-0.11	-0.11	-0.46
MDA	0.16	0.28	0.12	0.18	0.48	0.05	-0.12	-0.25	0.23	-0.21	0.21	0.19	-0.23	0.28	1	-0.06	-0.19	-0.43	-0.18
AsA	0.11	-0.03	0.39	0.14	-0.36	0.11	-0.28	-0.11	-0.15	-0.48	0.48	0.23	-0.23	-0.14	0.42	1	0.38	0.40	0.22
α- TE	-0.31	0.57^{*}	0.19	-0.20	-0.27	-0.23	0.19	0.23	-0.26	-0.20	0.20	-0.20	0.27	0.12	0.46	0.26	1	-0.20	0.44
SY	-0.36	0.45	-0.21	-0.38	-0.32	-0.37	0.39	0.38	0.35	-0.34	0.34	-0.17	0.14	-0.12	0.17	0.18	0.44	1	0.34
Avg temp	0.30	0.44	0.56^{*}	0.36	0.34	-0.26	0.44	0.17	-0.16	0.31	-0.31	0.27	0.48	-0.48	-0.36	-0.75	0.25	-0.16	1

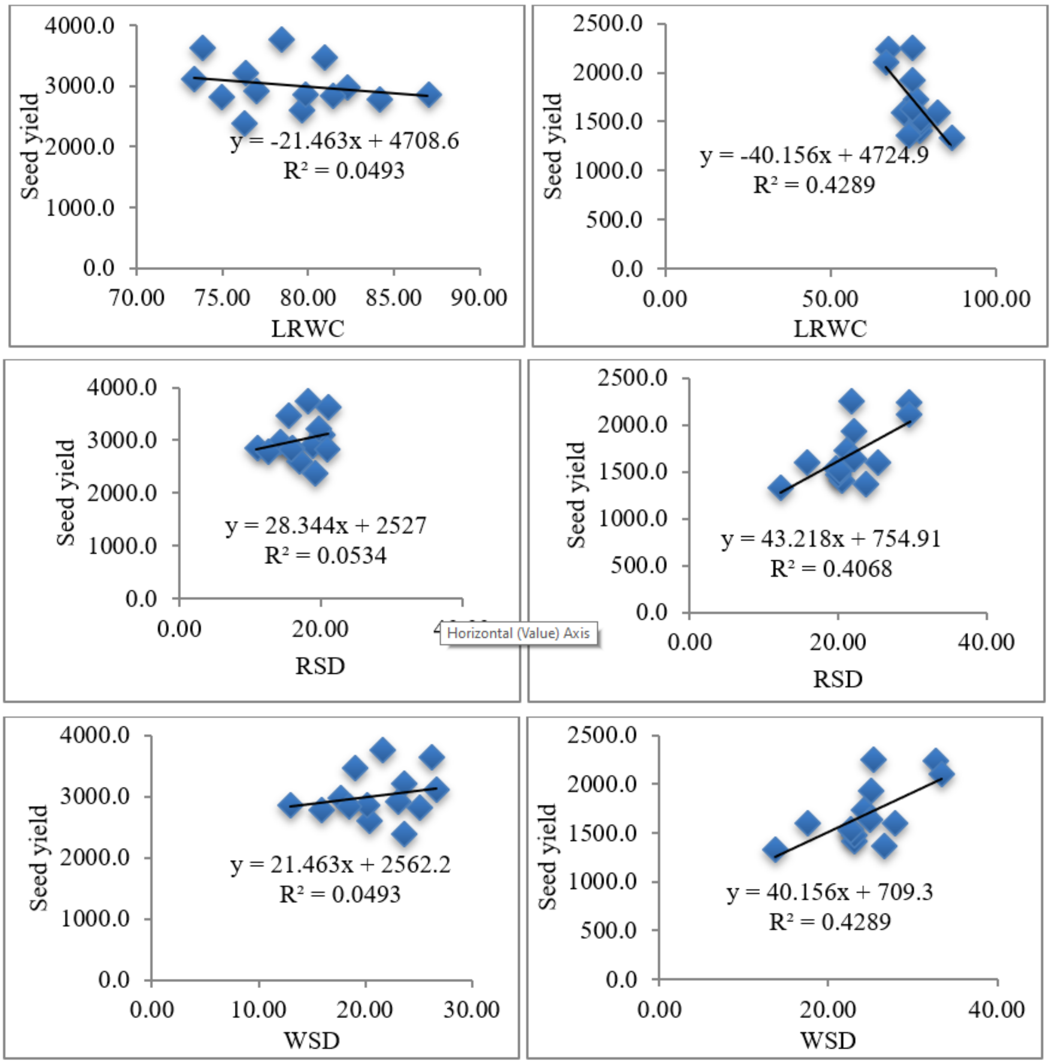
*Significant at 5%, **Significant at 1% chl a- chlorophyll a, chl b- chlorophyll b, Totalchl- Total chlorophyll, CAR- Carotenoids, LRWC- Leaf relative water content, RSD-Relative saturation deficit, WSD- Water saturation deficit, LWR-Leaf water retention, MS- Membrane stability, MI- Membrane injury, CAT- catalase, SOD- superoxide dismutase, POX- peroxidase, MDA- malondialdehyde, AsA- ascorbic acid, α- TE- α- tocopherols, SY- Seed yield, Avg temp- average temperature.

Table 4. Pearson's correlation coefficients of physiological and biochemical traits with average temperature under control (below the diagonal) and temperature gradient tunnel (TGT) (above the diagonal)

Non-enzymatic antioxidative molecules were positively associated with average temperature except MDA in stressed condition whereas correlation under controlled condition except α-TE. CAT (-0.68**) was negatively significantly correlated to average temperature under stressed conditions. Chl b was significantly negatively correlated to CAT (-0.57*) but significant positive to α-TE (0.57*).

Regression analysis

Regression analysis revealed that studied water status traits of the leaves i.e. LRWC (R^2= 0.429), WSD (R^2= 0.429), RSD (R^2=0.407) had strong relationship with seed yield under temperature stress as compared to controlled condition (Fig 6). Membrane stability (R^2=0.107) and injury (R^2= 0.107) had strong relationship with seed yield under controlled condition whereas MDA (R^2= 0.2454) under stressed condition (Fig 7). Strong relationship with seed yield was registered for SOD (R^2= 0.1165) under control and POX (R^2= 0.1508) under TGT (Fig 7). Seed yield had strong relationship with AsA (R^2= 0.1195) and α-TE (R^2=0.2115) under controlled condition only (Fig 8).



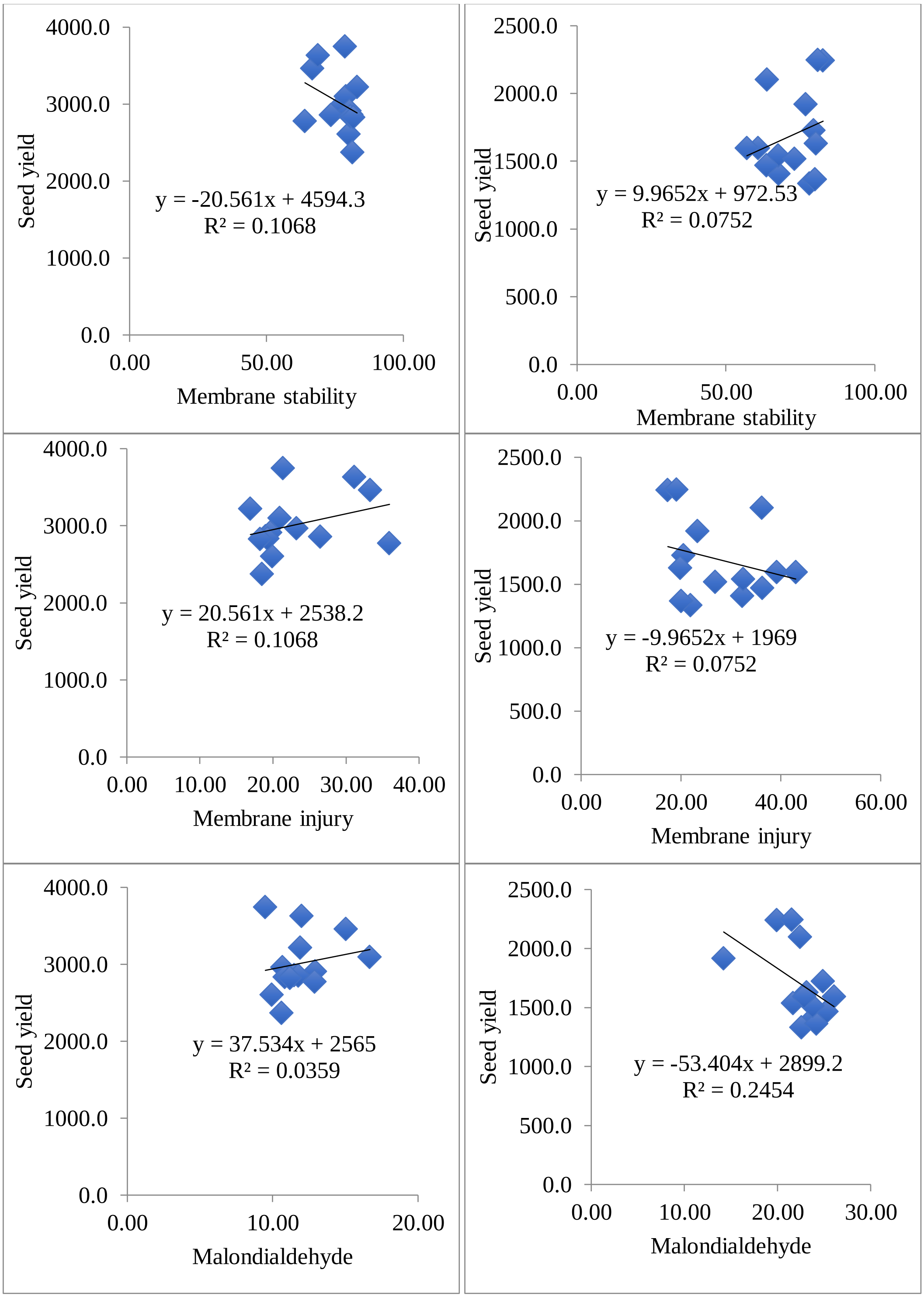
(A) **(B)**

Fig. 6. Relationship of leaf water traits with seed yield A: control and B: TGT

Discussion

Among abiotic stresses, temperature stress is the core reason that affects morphological, physiological and biochemical parameters of all *Brassica* species. In present study, photosynthetic pigments increased significantly. Increased chlorophyll content is plant's first reaction to heat stress as tolerance response according to Vuletic et al. (2022) and in tomato as reported earlier by Zhou et al. (2017). Heat stress increased cytokinin in leaves of *Arabidopsis* (Liu et al., 2020). This is due to cytokinin signal transduction genes which delays the leaf senescence by enhancing chlorophyll content and reducing sugar accumulation (Wu et al.,2021).

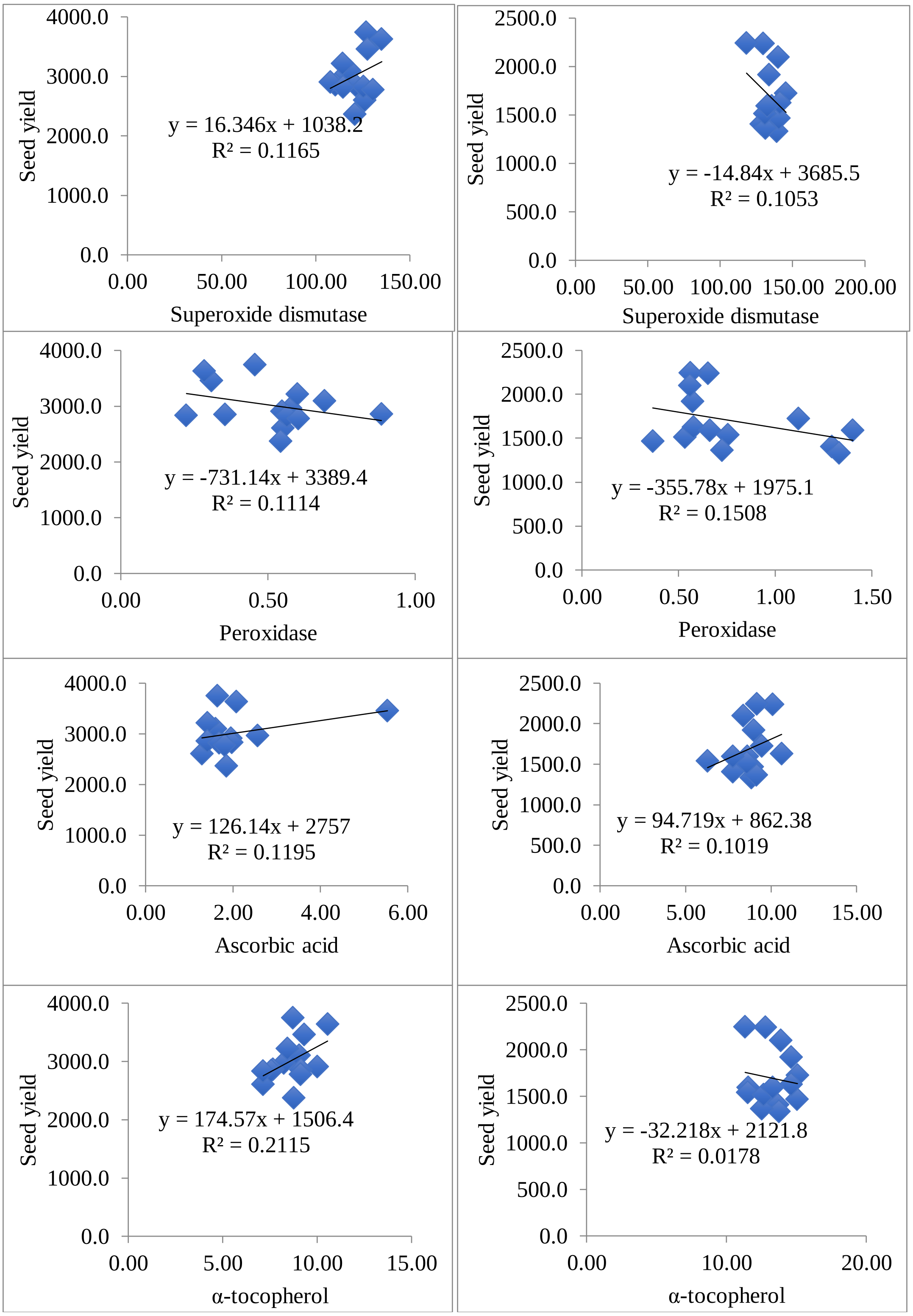
Our results are also consistent with Mishra and Chaturvedi (2019) and Samec et al. (2021) who reported increased photosynthetic pigments in *Brassica juncea* under different abiotic stresses. Increased SPAD value due to heat stress has been reported by Bhattarai et al. (2021) in tomato and Tang et al., (2018) in potato. Reduced relative water content, leaf water retention, membrane stability and increased relative saturation deficit, water saturation deficit and membrane injury was reported in *Brassica juncea* under terminal heat stress (Chugh and Sharma, 2020). In wheat, heat stress also significantly increased RSD (Kaur and Thind, 2016).



(A) **(B)**

Fig. 7. Relationship of membrane stability, injury and malondialdehyde with seed yield A: control and B: TGT

Similarly reduced membrane stability and increased membrane injury was observed earlier in B. *juncea* under temperature stress (Wilson et al.,2013). MDA is typically used to detect membrane lipid peroxidation which is a naturally occurring phenomenon in stressed plants, a self predictor of physiological status during plant development. Increased MDA content in *Brassica species* was reported due to water deficit (Rhythm et al., 2022; Ayyaz et al., 2021) heat stress (Wilson et al., 2013; Zou et al., 2017), temperature stress (Lee et al., 2020) and also because of late planting (Chugh and Sharma, 2020).



(A) **(B)**

Fig. 8. Relationship of antioxidative enzymes and non-enzymatic biomolecules with seed yield A: control and B: TGT

SDS- PAGE protein profile in our study revealed intense banding in controlled condition indicator of protein structural integrity while lower intensity protein bands in heat-stressed condition depicted denaturation of proteins and suppressed synthesis of normal proteins. Khani and Heidari (2008) detected protein accumulation by SDS-PAGE where some proteins were induced while others were diminished when maize was subjected to drought stress. SDS-PAGE analysis of leaf depicted lower expression of protein bands when rice was subjected to low temperature stress (Jan et al., 2015). Similarly, profiling by SDS-PAGE in wheat seedlings revealed suppressed synthesis of proteins and translation of new proteins when encountered by temperatures above the optimum growth temperature (Satbhai et al.,2016). In the present study increase in the activity of antioxidative enzyme was observed due to relatively higher temperatures. Previous studies have reported increased antioxidant enzyme activity to encounter oxidative stress caused by various abiotic stresses (Al-Issawi et al., 2016; Saleem et al., 2020). Our results are also consistent with that of Wilson et al. (2013). Studies from our laboratory (Rhythm et al., 2022; Chugh and Sharma, 2020) have also endorsed enhanced antioxidative enzyme activity in B. *juncea* on exposure to different abiotic stresses.

Similar trend has been reported in the literature in response to heat stress in Indian mustard (Rani et al., 2016; Mohan, 2017), *Brassica species* to salinity (Shariatinia et al., 2021), heat (Zou et al., 2017) and drought (Ayyaz et al., 2021). Significant changes in the content of biomolecules studied were evident in response to heat stress in the present study. Content of AsA and α-TE was enhanced in response to elevated temperature conditions. AsA acid serves as a reducing agent as it can donate electrons to both non-enzymatic and enzymatic reactions. It regenerates α-TE from tocopheroxy radical by reacting with H_2O_2, OH^-, and O^{2-} which helps in protecting cell membranes from oxidative damage (Das and Roychoudhury, 2014). Our results are in accordance with findings Rani et al. (2016), Chugh and Sharma (2020), where AsA increased significantly by high temperature stress. Similarly, AsA increased in *Brassica juncea* due to saline conditions (Kannu priya et al., 2021). By scavenging singlet oxygen and serving as an antioxidant, α-TE protects lipids and other membrane elements of chloroplasts (Das and Roychoudhury, 2014), including the structure and function of photosystem II (Kumar et al., 2021). In consistency with our results α-TE content was enhanced in B. *juncea* due to late planting (Chugh and Sharma, 2020) and saline conditions (Kannupriya et al., 2021).

Conclusion

Based on the studies identified JT-9 as highly tolerant and JT-12, JA-53 as moderately tolerant, which can be used as donors for breeding heat-tolerant varieties of mustard.

References

Ali S, Rizwan M, Arif MS, et al. (2020) Approaches in enhancing thermotolerance in plants: An updated review. Journal of Plant Growth Regulation 39:456–480.

Al-Issawi M, Rihan HZ, Al-Shmgani H, et al. (2016) Molybdenum application enhances antioxidant enzyme activity and COR15a protein expression under cold stress in wheat. Journal of Plant Interactions 11:5. DOI: 10.1080/17429145.2015.1129074.

Ayyaz A, Miao Y, Hannan F, et al. (2021) Drought tolerance in Brassica napus is accompanied with enhanced antioxidative protection, photosynthetic and hormonal regulation at seedling stage. Physiologica Plantarum 172:1133–1134. DOI: 10.1111/ppl.13375.

Barrs HD (1968) Determination of water deficits in plant tissues. In: Kozlowsky TT (ed) Water Deficits and Plant Growth. Academic Press, New York, pp. 235–268.

Bhattarai S, Joshua TH, Desire D, et al. (2021) Exploring morpho-physiological variation for heat stress tolerance in tomato. Plants 10:1–22. DOI: 10.3390/plants10020347.

Chance B and Maehley A (1955) Assay of catalases and peroxidases. Methods Enzymology 2:764–765. DOI: 10.1016/S0076-6879(55)02300-8.

Chen Q and Yang G (2020) Signal function studies of ROS, especially RBOH-dependent ROS, in plant growth, development and environmental stress. Journal of Plant Growth Regulation 39:157–171. DOI: 10.1007/s00344-019-09971-4.

Chugh P and Sharma P (2020) Evaluation of physiological and biochemical traits as screening technique for heat stress tolerance in Indian mustard [Brassica juncea (L.) Czern & Coss.] genotypes. Plant Cell Biotechnology Molecular Biology 21:64–81. DOI: 10.5433/PCBMB.21.5.64.

Chugh P, Sharma P, Sharma R, et al. (2022) Study on heat stress indices and their correlation with yield in Indian mustard genotypes under diverse conditions. Indian Journal of Genetics Plant Breeding 82:186–192. DOI: 10.31742/IJGPB.82.2.7.

Das K and Roychoudhury A (2014) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers Environmental Sciences 2:1–13. DOI: 10.3389/fenvs.2014.00053.

Gill SS and Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology Biochemistry 48(12):909–930. DOI: 10.1016/j.plaphy.2010.08.016.

Heath RL and Packer (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry Biophysics 125:189–198. DOI: 10.1016/0003-9861(68)90654-1.

Hiscox JD and Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany 57:1332–1334. DOI: 10.1139/b79-163.

Jan M, Shinwari SG, Khan M, et al. (2015) Consequences of short term temperature stress on physiological and biochemical aspects of rice (Oryza sativa L.). Scientific Agriculture 10:1–4.

Kannu Priya, Sharma P, Choudhary OP, et al. (2021) Regulation of salinity tolerance in Brassica juncea (L.) introgression lines: osmoprotectants, antioxidative molecules and ionic content. GSC Advance Research Reviews 6:116–131. DOI: 10.30574/gscarr.2021.6.3.0038.

Kaur A and Thind SK (2016) Relative water content and water saturation deficit in wheat (Triticum aestivum L) seedling under heat stress as affected by trehalose application. International Journal of Recent Scientific Research 7:115–118.

Kaur K and Sharma P (2021) Effect of low light stress on photosynthetic pigments and antioxidative enzymes in field grown Indian mustard (Brassica juncea L.) genotypes. Journal of Agricultural Science Technology 11:61–72. DOI: 10.17265/2161-6264/2021.02.002.

Kirk JTO and Allen RL (1965) Dependence of chloroplast pigment synthesis on protein synthesis: Effect of actidione. Biochemistry Biophysics Research Communication 21:523–530.

Kumar A, Parsad A and Sedlarova M (2021) Tocopherol controls D1 amino acid oxidation by oxygen radicals in Photosystem II. Proceedings of National Academy of Sciences USA 118(4):1–10. DOI: 10.1073/pnas.2019246118.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Lee JK, Woo SY, Kwak MJ, et al. (2020) Effects of elevated temperature and ozone in Brassica juncea L. growth, physiology, and ROS accumulation. Forests 11(1):68. DOI: 10.3390/f11010068.

Liu Y, Zhang M, Meng Z, et al. (2020) Research progress on the roles of cytokinin in plant response to stress. International Journal of Molecular Sciences 21:6574. DOI: 10.3390/ijms23115946.

Lowry OH, Rosenbrough NJ, Farr AL, et al. (1951) Protein measurement with folin phenol reagent. Journal of Biological Chemistry 193:265–275.

Marklund S and Marklund G (1974) Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 47:469–474. DOI: 10.1111/j.1432-1033.1974.tb03714.x.

Martinez-Ballesta MC, Perez LL, Muries B, et al. (2009) Climate change and plant water balance: The role of aquaporins—A review. In: Lichtfouse E (ed.) Climate Change, Intercropping, Pest Control and Beneficial Microorganism. Sustainable Agricultural Reviews:71–90.

Mishra SK and Chaturvedi M (2019) Effect of different environmental conditions on growth and production of mustard (Brassica juncea) in semiarid zone of Rajasthan. Journal of Pharmacognosy Phytochemistry SP3:69–71.

Mohammadkhani N and Heidari R (2008) Effects of drought stress on soluble proteins in two maize varieties. Turkish Journal of Biology 32:23–25.

Mohan N (2017) Biochemical and morpho-physiological changes in Indian mustard (B. juncea L.Czern & Coss.) under terminal heat stress. M.Sc. thesis. CCS HAU, Hisar, India.

Premchandra GS, Saneoka H and Ogata S (1990) Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soybean. Journal of Agricultural Science Cambridge 115:63–66. DOI: 10.1017/S0021859600073925.

Rani B, Kumari N, Pooja Jain, et al. (2016) Antioxidative system as influenced by high temperature stress in Brassica juncea (L) Czern & Coss. Current trends in biotechnology pharmacy 10:118.

Rhythm, Sharma P and Sardana V (2022) Physiological and biochemical traits of drought tolerance in Brassica juncea (L.) Czern & Coss. South African Journal of Botany 146:509–520. DOI: 10.1016/j.sajb.2021.11.019.

Roe JH and Kuether CA (1943) The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. Journal of Biological Chemistry 147:399–407. DOI: 10.1016/S0021-9258(18)72395-8.

Rosenberg HR (1992) Chemistry and physiology of vitamins. Interscience Publisher, New York, pp. 452–457.

Saleem MH, Rehman M, Fahad S, et al. (2020) Leaf gas exchange, oxidative stress, and physiological attributes of rapeseed (Brassica napus L.) grown under different light-emitting diodes. Photosynthetica 58:836. DOI: 10.32615/ps.2020.010.

Salisbury P and Gurung A (2011) Final report on oilseed Brassica improvement in China, India and Australia. Australian Centre for International Agricultural Research, Canberra, Australia, p. 9.

Samec D, Ida L and Salopek-Sondi B (2021) Salinity stress as an elicitor for photochemical and minerals accumulation in selected leafy vegetables of Brassicaceae. Agronomy 11:361. DOI: 10.3390/agronomy11020361.

Sangakkara HR, Hartwig UA and Nosberger J (1996) Response of root branching and shoot water potential of Phaseolus vulgaris L. to soil moisture and fertilizer potassium. Journal of Agronomy and Crop Science 177:165–168. DOI: 10.1111/j.1439-037X.1996.tb00234.x.

Satbhai R, Kale A and Naik R (2016) Study on effect of high temperature stress in wheat genotypes using SDS protein profile. Journal of Wheat Research 8:51–53.

Shannon L, Kay E and Lew J (1966) Peroxidase isozymes from horseradish roots. I. Isolation and physical properties. Journal of Biological Chemistry 241:2166–2172. DOI: 10.1016/S0021-9258(18)96680-9.

Shariatinia F, Azari A, Rahimi A, et al. (2021) Germination, growth, and yield of rocket populations show strong ecotypic variation under NaCl stress. Scientia Horticulturae 278:109841. DOI: 10.1016/j.scienta.2020.109841.

Tang R, Niu S, Zhang G, et al. (2018) Physiological and growth responses of potato cultivars to heat stress. Botany 96:897–899. DOI: 10.1139/cjb-2018-0125.

Vuletic VM, Mihaljevic I, Tomas V, et al. (2022) Physiological response to short-term heat stress in the leaves of traditional and modern plum (Prunus domestica L.) cultivars. Horticulture 8:72. DOI: 10.3390/horticulturae8010072.

Weatherley PE (1950) Studies in water relation of cotton plant. The field measurement of water deficit in leaves. New Phytologist 49:81–87. DOI: 10.2307/2428690.

Wilson RA, Sangha MK, Banga SS, et al. (2013) Heat stress tolerance in relation to oxidative stress and antioxidative in Brassica juncea. Journal of Environmental Biology 35:383–385.

Wu W, Kang D, Kang X, et al. (2021) The diverse roles of cytokinins in regulating leaf development. Horticulture Research 8:118. DOI: 10.1038/s41438-021-00558-3.

Zhou R, Yu X, Ottosen C, et al. (2017) Drought stress had a predominant effect over heat stress on three tomato cultivars subjected to combined stress. BMC Plant Biology 17(24):1–13. DOI: 10.1186/s12870-017-0974-x.

Zou M, Yuan L, Zhu S, et al. (2017) Effects of heat stress on photosynthetic characteristics and chloroplast ultra structure of a heat-sensitive and heat-tolerant cultivar of wucai (Brassica campestris L.). Acta Physiologic Plantarum 39:30–34. DOI: 10.1007/s11738-016-2319-z.

Author Contributions

PS: Conceptualization of research, Designing and contribution of experimental materials, LB: Data collection; PS and LB: Analysis of data, interpretation and preparation of manuscript.

Acknowledgements

The provision of the Temperature Gradient Tunnel (TGT) facility built by the Department of Climate Change and Agricultural Meteorology and the germplasm provided for the study by the Oilseed Section, Department of Plant Breeding and Genetics at Punjab Agricultural University, Ludhiana, is fully acknowledged.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Competing interest

The authors declare no competing interests.

Ethics approval

Not applicable.

Citation: Sharma P and Brar L (2025) Evaluation of Advanced Breeding Lines of *Brassica juncea* (L.) in Temperature Gradient Tunnels: Physiological and Biochemical Traits Influencing Yield. Environmental Science Archives 4(2): 801-817.