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RESEARCH ARTICLE

Physiological and biochemical responses of garden pea genotypes under reproductive stage heat stress

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Abstract High temperature causes several morphological, physiological, and biochemical changes in crop plants, and garden pea is highly sensitive to a higher temperature than other legume crops. This study assessed garden pea genotypes' physiological and biochemical responses during a reproductive stage in regular and heat stress season at the Division of Vegetable Science, Indian Agricultural Research Institute, New Delhi (India). Forty-five garden pea genotypes, including 15 tolerant, 15 moderately tolerant, and 15 susceptible genotypes, were analyzed for three physiological, six biochemical, and 11 quantitative morphological traits under regular and heat stress seasons. Our results showed a considerable decrease in leaf water content, greenness index, and membrane stability index in heat stress season and a substantial increase in malondialdehyde, hydrogen peroxide, and antioxidant enzymes in heat stress season compared to the regular season. The 15 heat-tolerant genotypes showed a significant increase in antioxidant enzymes compared to the 15 heat-susceptible genotypes, which impart thermotolerance by scavenging reactive oxygen species generated in high-temperature stress conditions. Further, correlation and biplot analysis of morpho-physiological and biochemical traits indicated that physiological and biochemical traits were important in determining yield and related traits under heat stress conditions in garden pea genotypes. Thus, estimating critical physiological and biochemical traits could facilitate in differentiating

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thermotolerant genotypes from susceptible genotypes in garden peas and aid in heat-tolerant breeding programs of similar cool-season legume crops.

Keywords Heat · Tolerance · Pisum sativum · Terminal Heat · Pea

Abbreviations

RWC	Relative water content
CT	Canopy temperature
GI	Greenness index
MSI	Membrane stability index
MDA	Malondialdehyde
H_2O_2	Hydrogen peroxide
SOD	Superoxide dismutase
CAT	Catalase
TS	Total sugar
PH	Plant height
IL	Internode length
DFF	Days to 50% flowering
RSL	Reproductive stem length
RGD	Reproductive growth days
NPP	Number of pods per plant
PL	Pod length
NSP	Number of seeds per pod
APW	Average pod weight
DM	Days to maturity
YPP	Yield per plant

Introduction

Garden pea (*Pisum sativum* L., 2n = 2x = 14) is commonly grown in India as a cool-season leguminous vegetable crop. Peas belong to the third largest family of flowering plants, Fabaceae having more than 450 genera and 1200 species. Garden pea is an excellent source of protein, fiber, minerals, and vitamins source. Wrinkle-seeded pea cultivars have 26–33% protein, while smooth-seeded cultivars have 23–31% protein (Cousin 1997). It is a source of vitamins A, B, and C, contains 35–40% starch and 4–7% fiber, and has relatively high lysine levels. These features make peas an appropriate dietary complement to cereals. The crop is usually grown in temperate, subtropical, and mild tropical climates. The optimum temperature requirement is 13–18 °C, and growth stops at 29–30 °C. Temperature is a major factor affecting legumes' yield and quality (Christophe et al. 2011). Higher temperature limits the economic opportunity of the crop only for a few months (November-February) in the North Indian plains. Delayed sowing had to be practiced to extend the crop duration for early summer cultivation (end of March- mid-April). However, this causes terminal heat stress in garden peas, disrupting cellular processes at physiological and biochemical levels (Aleem et al. 2020) and ultimately affecting their survivability and yield.

Therefore, developing thermotolerant genotypes is of prime importance to sustain its productivity in the early summer. High temperature causes several morphological, physiological, and biochemical changes in the crop. Early exposure to high temperatures causes untimely flowering in plants, while pre-flowering heat stress causes a low number of flowering buds, and post-flowering heat stress cause flower drying (Venugopalan et al. 2021). Heat stress also causes visual symptoms in cool-season legumes, like sun scorching, leaf discoloration, leaf burn, and senescence (Ismail and Hall. 1999; Vollenweider et al. 2005). Kumar et al. (2016) reported that a rise in temperature above 25 °C in cool season pulses like field peas, chickpeas, lentils, and fava beans leads to flower drop, pod abortion, and yield reduction of up to 20–70%. All the morphological changes at vegetative and reproductive growth stages due to heat stress are results of disturbance in cellular processes at physiological and biochemical levels (Aleem et al. 2020).

In high temperatures, physiological processes like photosynthesis, respiration, and membrane stability are primarily disturbed due to oxidative stress created by the excessive generation of reactive oxygen species. Other notable heat stress effects include structural changes in tissues and cell organelles, disorganization of cell membranes, disturbance of leaf water relations, and impedance of photosynthesis. Lipid peroxidation via the production of ROS and changes in antioxidant enzymes, and altered patterns of synthesis of primary and secondary metabolites are also of considerable importance (Wahid et al. 2007).

Adaption to heat stress in crops involves the activation of many heat-responsive genes due to altered membrane stability (Mittler et al. 2012). Genes involved in the synthesis of various osmoprotectants, antioxidants, heat shock proteins, and regulating transcriptional controls and signal transduction molecules are thought to be up-regulated in thermotolerant genotypes, and an increase in the proportion of respective

gene products had been seen in tolerant genotypes (Galsurker et al. 2018; Thakur et al. 2018). Osmoprotectants and antioxidants are ROS scavengers to minimize oxidative stress while signaling molecules can enhance this antioxidant activity, leading to heat tolerance in heat-resistant genotypes. Thus, the key physiological and biochemical adaptations had to be studied between heat tolerant and heat susceptible genotypes to understand the basis of heat tolerance.

Cool season legumes were found to be more sensitive during the reproductive stage, resulting in considerable loss of flowers and pods (Leport et al. 2006; Shrestha et al. 2006; Wang et al. 2006; Krishnamurthy et al. 2011; Devasirvatham et al. 2012; Hamidou et al. 2013; Farooq et al. 2017). Exposure of food legumes during the reproductive stage to higher day temperatures causes reduced pod set and seed yield. For example, exposing a pea to a higher day temperature of 30 °C affects its pod set and yield (McDonald and Paulsen 1997). Hence reproductive stage heat stress had to be investigated in garden peas as very limited research has been conducted. Therefore, the present study aims to understand the heat stress response of tolerant, moderately tolerant, and susceptible garden pea genotypes regarding physiological and biochemical basis during the reproductive stage. The major objective of the study was to identify the importance of physiological and biochemical parameters in the heat stress tolerance of garden peas during the reproductive stage and to determine their association with growth and yield parameters with the help of statistical analysis.

Materials and methods

Plant materials and field experiment

In the current study, 45 garden pea genotypes (Table S1), including fifteen tolerant, fifteen moderately tolerant, and fifteen susceptible genotypes, were selected from screening 86 genotypes for heat tolerance in the heat stress season of 2021 (January to April 2021). All the genotypes were subjected to physiological and biochemical characterization under normal and heat stress conditions. The genotypes consist of improved cultivars and exotic germplasm lines of garden peas. These genotypes were characterized from November 2021 to February 2022 in the field for the regular season (22.27/8.46 °C) (max/min) and from January to April 2022 for heat stress season (28.21/12.47 °C) (max/min) in randomized block design with three replications. The sowing was delayed by three months for the heat stress study (18th January 2022) than the regular season (5th November 2021) such that the reproductive phase coincides with high temperature (37.7/19 °C) (max/min) during March–April. Line sowing was done with 30 cm spacing between rows and 10 cm between plants. All the recommended cultural practices were followed (Chadha 2019). The mean maximum and minimum temperatures during normal and heat stress seasons were presented in Fig. 1A and B.

Physiological characterization

After morphological analysis, the genotypes were taken for physiological and biochemical characterization under heat stress and normal condition.

The relative water content of the leaf was estimated according to the method of Barrs and Weatherley (1962). Fully expanded whole leaves were collected from each genotype, and their fresh weight was measured. Then the leaves were placed in distilled water and kept in a petri dish for 4 h, and their turgid weight was measured. The leaves were then oven dried at 65 °C for 48 h, and their dry weight was measured. The RWC of the leaf can be calculated using the formula,

$$\mathrm{RWC}(\%) = \left[(\text{Fresh weight} - \text{Dry weight})/(\text{Turgid Weight} - \text{Dry Weight})\right] \times 100.$$

All the genotypes' canopy temperature (°C) was measured using IR thermography during heat stress and regular season.

The leaf greenness (SPAD) index was measured from fresh, fully opened leaves using SPAD-502 m, giving rapid and non-destructive measurements of leaf chlorophyll concentration.

Biochemical characterization

The membrane stability of the cell was determined by measuring the amount of electrolyte leakage in leaves. 0.1 g of leaves were cut into uniformly sized pieces and taken in a test tube having 10 ml distilled

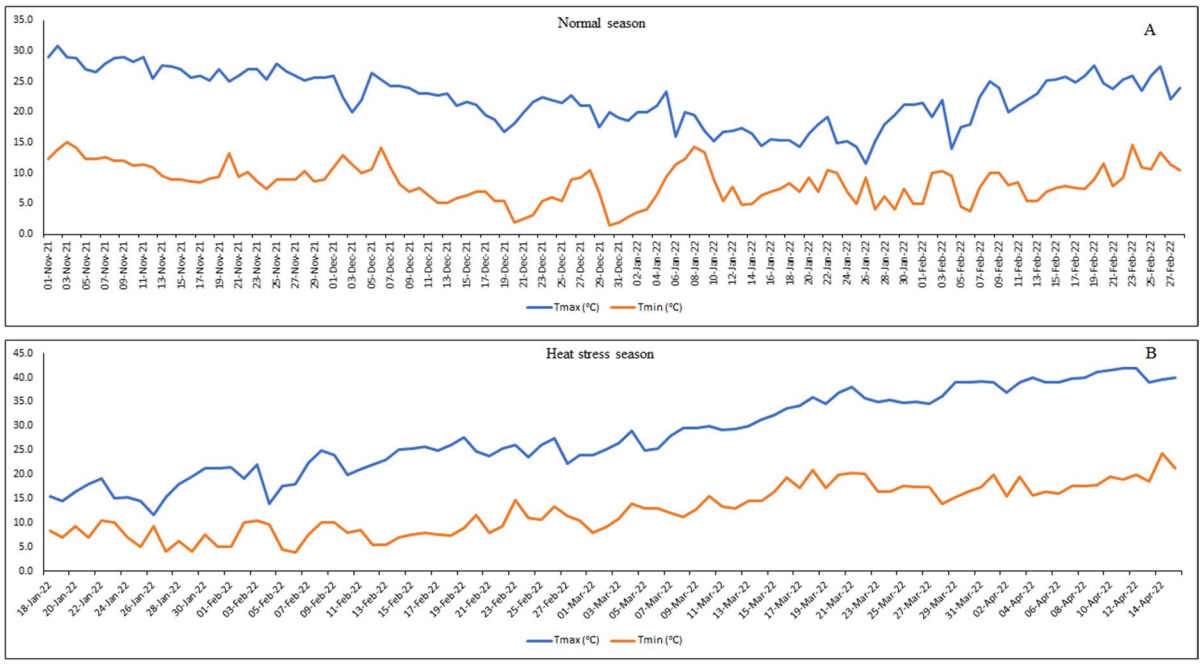

Fig. 1 Graph depicting minimum and maximum temperature. **A** Normal season. **B** Heat stress season

water in two sets. 1 set was kept in a water bath at 40 °C for 30 min, and another set was kept at 100 ◦C in a boiling water bath for 15 min. Their electrical conductivities, C1 and C2, were measured by a conductivity meter.

$$\text{Membrane stability index MSI}(\%) = \left[1 - (C1/C2)\right] \times 100$$

Malondialdehyde content (MDA), the final product of membrane lipid peroxidation, was measured using TBARS assay according to Health and Packer (1968). 0.2 g of leaves were ground with 4 ml of 0.1% TCA and centrifuged at 12,000 rpm at 4 °C for 20 min. 0.1 ml of supernatant was taken, and 4 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95° C for 30 min in an electric oven, then by cooling in an ice bath, and then, the aliquot was centrifuged at 10,000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm and 600 nm. The TBARS content was calculated according to its extinction coefficient $\in = 155\ \text{mM}^{-1}\ \text{cm}^{-1}$ and expressed as nmol of MDA g^{-1} fresh weight.

H_2O_2 was assayed according to the method described by Alexieva et al. (2001). 0.2 g of leaf sample was homogenized with 4 ml of trichloroacetic acid and centrifuged at 12,000 rpm at 4 °C for 20 min. 0.5 ml of trichloroacetic acid supernatant, 2 ml of 1 mM KI, and 0.5 ml of 100 mM of phosphate buffer were mixed. The reaction mixture was kept for 1 h in darkness, and absorbance was taken at 390 nm against TCA as blank. Hydrogen peroxide concentration was calculated by comparing it with a standard curve made using a known concentration of H_2O_2 and expressed as μmol g^{-1} fresh weight.

Antioxidant enzyme activity

The enzyme extract for antioxidant activity was prepared by grinding 0.1 g of leaf sample with 10 ml extraction buffer containing 0.1 M phosphate buffer (pH 7.5) and 0.5 mM EDTA. Then it was centrifuged at 15,000 rpm for 20 min. The resultant enzyme extract was used for enzyme activity determination.

The superoxide dismutase (SOD) activity was estimated using the reaction mixture containing 0.2 ml methionine, 0.1 ml nitro blue tetrazolium chloride, 0.1 ml EDTA, 1.5 ml phosphate buffer, and 0.1 ml sodium carbonate. To this reaction mixture, 0.1 ml of enzyme extract was added, and the final volume

was made up to 3 ml using double distilled water. The reaction was initiated by adding two mM riboflavin, and the tubes were incubated under 15w fluorescent lamps for 15 min. Reaction mixture without enzyme with maximal color served as control. A non-irradiated reaction mixture that does not develop color acts as blank. The absorbance was recorded at 560 nm using a spectrophotometer. One unit enzyme activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction per min. The enzyme activity was expressed as U g-1 FW min-1.

Catalase (CAT) activity was measured by monitoring the decrease of absorbance at 240 nm after 1 min caused by the decomposition of H_2O_2. The reaction mixture contained potassium phosphate buffer of pH 7.0 (1.5 ml), hydrogen peroxide (0.5 ml), and 0.05 ml of enzyme extract, and the volume was made up to 3 ml using double distilled water. The reaction starts by adding hydrogen peroxide, and the absorbance is measured in a spectrophotometer for one minute. The difference between the initial and final H_2O_2 concentration provides catalase activity and is expressed as µmol H_2O_2 reduced/min/g FW.

With some modifications, total sugar (TS) was estimated using the anthrone reagent method as per Roe (1955). 0.5 g of the sample was crushed in 80% ethanol, and the filtrate was taken. The volume was made up to 10 ml and centrifuged, and the supernatant was collected. The supernatant was diluted in a 1:10 ratio with ethanol. In a test tube, 20 µl of diluted supernatant was dried in a boiling water bath. Once the contents were dried, 1 ml of distilled water was added and vortexed. 4 ml of ice-cold anthrone reagent was added in each tube and heated for 8 min in a boiling water bath and read the absorbance at 630 nm after cooling. The amount of carbohydrates in the sample was calculated from a standard curve made from a known glucose concentration.

Morphological data

The various morphological quantitative traits of garden peas recorded in normal and heat stress conditions correlated with their physiological and biochemical traits. The recorded morphological quantitative traits include growth parameters like plant height (PH), internode length (IL), days for 50% flowering (DFF), reproductive stem length (RSL), reproductive growth days (flowering to maturity) (RGD), days to maturity (DM) and yield parameters like number of pods per plant (NPP), pod length (PL), number of seeds per pod (NSP), average pod weight (APW) and yield per plant (YPP). All the data were recorded by randomly selecting five plants from each replication in normal and heat stress conditions.

Statistical analysis

The data obtained from normal and heat stress conditions were subjected to a one-factor analysis of variance in XLSTAT. Significance was established at 1% (highly significant) and 5% (significant). Correlation coefficients among different parameters were calculated using XLSTAT. K-mean clustering using the elbow method and principal component analysis was performed using OPSTAT. Heat map, cluster dendrogram, and biplot analysis were performed using the software RStudio version 2022.07.2 + 576.

Results

Forty-five genotypes, including 15 tolerant, 15 moderately tolerant, and 15 susceptible genotypes identified from heat tolerant screening, were subjected to physiological and biochemical characterization in heat stress and normal condition. Tolerant, moderately tolerant, and susceptible genotypes during heat stress and regular season were presented in Fig. 2. Further, their growth and yield parameters were also recorded and correlated with physiological and biochemical traits to understand the significance of physiological and biochemical basis in heat tolerance.

Physiological characterization

The mean value of physiological and biochemical traits of fifteen tolerant, moderately tolerant, and susceptible genotypes under regular season and heat stress season was presented in Tables 1 and 2, respectively. The mean RWC of 45 genotypes in heat stress conditions was 57.47% which was 27.58% less than in normal conditions (79.36%). The tolerant genotypes showed an 11.76% decrease in RWC content, while the moderately tolerant and susceptible genotypes showed a 22.84 and 49.01 percent reduction than in normal conditions. The tolerant pea genotypes viz. EC-598649 (80.86%), followed by GP-915-II

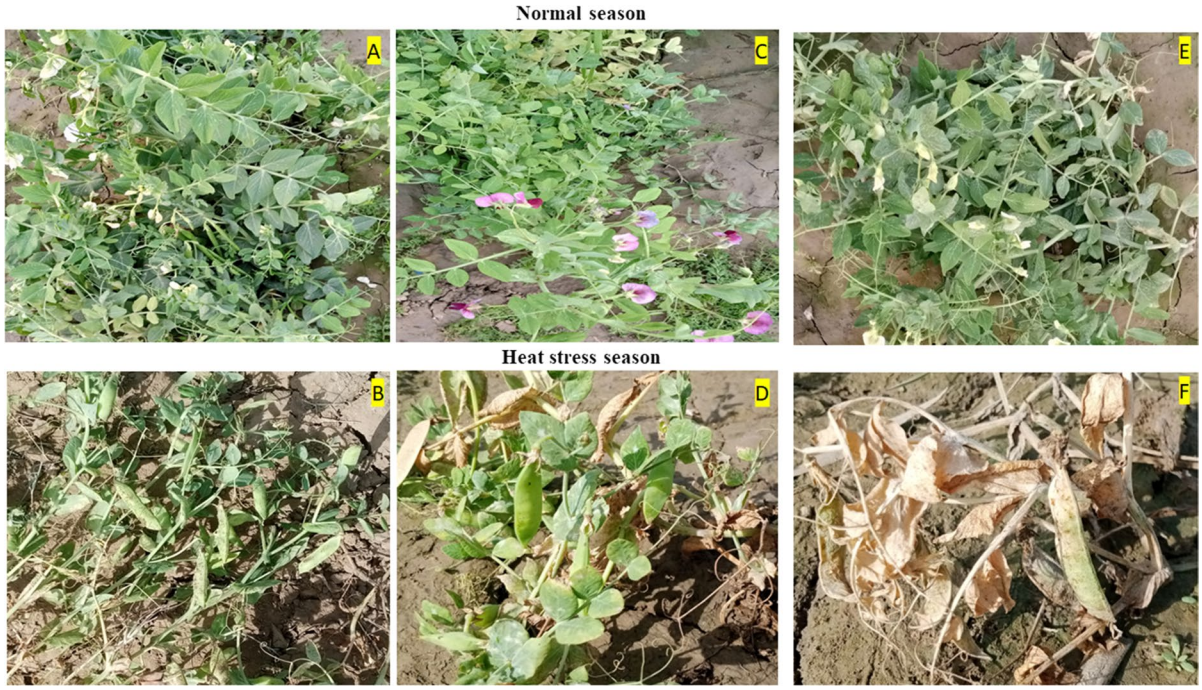

Fig. 2 Tolerant, moderately tolerant, and susceptible genotype during normal and heat stress season **A**, **B**: GP-61 (Tolerant) **C**, **D**: EC-598892–1 (Moderately tolerant) **E**, **F**: GP-1708 (Susceptible)

(77.03%) and EC-677211 (76.33%), showed the highest leaf water content, while the susceptible genotype AP-3 showed the least water content of 30.12%. However, in heat stress conditions, RWC showed a broad range from 30.12 to 80.86% than in normal conditions (Fig. 3A; Table 3).

On observing the canopy temperature of the genotypes, it was found that there was no significant difference between tolerant, moderately tolerant, and susceptible genotypes in normal conditions. However, in heat stress conditions, the susceptible genotypes showed 4.61% and 6.32% higher canopy temperatures than moderately tolerant and tolerant genotypes (Fig. 3B). The average canopy temperature in heat stress conditions was 56.32% higher than in normal conditions. The genotypes that maintained lower canopy temperature were EC-598638(31.52 °C), EC-598892–1 (32.25 °C), Golden pod (32.3 °C) and EC-598593 (32.3 °C), while the susceptible genotype Apoorva (40.6 °C) recorded maximum canopy temperature (Table 2).

Under heat stress conditions, the genotypes showed a 26.01% reduction in the average greenness index than in normal conditions. The rate of reduction in average greenness index intolerant, moderately tolerant, and susceptible genotypes during heat stress season was 8.04, 26.58, and 46.87% than in their respective normal condition (Fig. 3C). Tolerant genotype, 2019/PMPM-4 showed the highest greenness index of 46.90 in heat stress season followed by GP-1104 (44.75) and GP-912-II (44.54). The lowest greenness index of 10.96 was recorded in the susceptible genotype GP-917. Garden pea genotypes viz. 2019/PMPM-4, EC-677211, MEGH-2, GP-1104, and GP-912-II showed a non-significant increase in the greenness index during heat stress conditions compared to the regular season (Table 2).

Biochemical characterization

High-temperature stress causes severe cellular injury and cell death, which cause changes in cellular organization, protein denaturation, and increased membrane fluidity. These effects, in turn, lead to the production of toxic compounds and reactive oxygen species, which in turn cause the peroxidation of lipids and pigments. Subsequently, tolerant plants will produce antioxidant enzymes like superoxide dismutase

Table 1 The mean value of physiological, biochemical traits of 45 garden pea genotypes under normal condition

Genotypes	Physiological			Biochemical					
	RWC	CT	GI	MSI	MDA	H_2O_2	SOD	CAT	TS
Tolerant									
EC-598646	78.4 ± 1.16	20.92 ± 1.13	38.87 ± 0.63	77.25 ± 2.03	12.49 ± 0.62	16.52 ± 0.42	640.77 ± 24.19	2.33 ± 0.22	7.08 ± 0.07
EC-598638	78.58 ± 1.67	22.85 ± 1.65	37.71 ± 1.62	68.9 ± 2.47	14.99 ± 0.62	14.69 ± 0.33	703 ± 18.06	2.94 ± 0.19	7.19 ± 0.17
2019/PMPM-4	80.98 ± 1.91	22.72 ± 1.24	45.92 ± 0.96	77.91 ± 0.93	27.48 ± 0.95	11.99 ± 0.13	625.59 ± 31.85	3.22 ± 0.18	7.27 ± 0.15
GP-61	79.95 ± 1.97	20.83 ± 1.16	37.7 ± 0.93	74.24 ± 1.41	25.6 ± 0.36	15.59 ± 0.34	672.3 ± 16.56	2.81 ± 0.19	11.3 ± 0.38
GP-902	84.45 ± 1.88	25.1 ± 0.29	40.07 ± 0.75	71.22 ± 1.43	23.1 ± 0.72	15.42 ± 0.21	655.28 ± 29.69	3.36 ± 0.19	10.89 ± 0.71
GP-912-II	82.82 ± 1.78	24.77 ± 1.45	44.12 ± 0.62	80.08 ± 1.06	14.36 ± 0.95	17.61 ± 0.14	662.12 ± 38.47	4.89 ± 0.32	9.71 ± 0.36
EC-598649	86.21 ± 0.27	21.46 ± 0.7	40.74 ± 0.8	65.24 ± 1.08	13.74 ± 0.62	14.37 ± 0.31	554.02 ± 23.07	3.22 ± 0.18	7.56 ± 0.1
GP-48	80.7 ± 1.42	22.9 ± 0.56	48.95 ± 1.3	71.3 ± 0.41	16.24 ± 0.95	12.11 ± 0.13	770.23 ± 30.54	2.88 ± 0.14	8.06 ± 0.24
EC-677211	81.23 ± 1.39	23 ± 0.65	34.58 ± 1.4	72.45 ± 1.34	28.1 ± 0.62	14.79 ± 0.26	598.48 ± 26.83	3.45 ± 0.24	7.12 ± 0.12
EC-598654	77.38 ± 0.73	24.07 ± 0.72	45.12 ± 0.81	76.3 ± 0.52	24.98 ± 0.62	15.65 ± 0.27	554.02 ± 43.54	2.5 ± 0.09	7.55 ± 0.1
GP-1104	85.85 ± 3.2	20.52 ± 1.32	44.24 ± 0.63	71.23 ± 0.71	22.44 ± 1.53	15.48 ± 0.14	647.61 ± 12.1	4.22 ± 0.18	7.43 ± 0.32
EC-677214	79.37 ± 0.49	21.5 ± 0.62	36.36 ± 0.37	76.03 ± 1.03	19.36 ± 0.36	18.54 ± 0.22	630.6 ± 48.24	3.14 ± 0.14	7.11 ± 0.17
GP-57	79.77 ± 1.25	23.08 ± 1.85	39.14 ± 0.99	68.16 ± 1.29	25.6 ± 0.72	13.86 ± 0.18	729.86 ± 24.08	4.15 ± 0.13	7.17 ± 0.22
GP-915-II	83.82 ± 1.4	24.75 ± 0.49	40.26 ± 1	74.25 ± 1.16	26.85 ± 1.3	16.19 ± 0.17	620.25 ± 38.31	2.97 ± 0.25	6.35 ± 0.1
EC-598602	79.11 ± 0.42	23.35 ± 1.2	36.23 ± 1.44	67.64 ± 0.89	23.11 ± 1.91	17.55 ± 0.25	700.49 ± 27.06	3.25 ± 0.19	7.16 ± 0.15
Moderately tolerant									
GP-1707	88.67 ± 2.59	24.92 ± 1.45	38.06 ± 1.84	75.82 ± 1.9	13.11 ± 0.96	17.32 ± 0.21	642.44 ± 51.41	4.78 ± 0.11	7.46 ± 0.07
MEGH-2	83.76 ± 0.81	21.28 ± 1.61	39.72 ± 0.93	66.74 ± 1.81	25.58 ± 0.95	13.55 ± 0.34	675.8 ± 40.24	6.67 ± 0.28	7.96 ± 0.18
EC-598593	87.68 ± 1.75	24.59 ± 1.42	39.3 ± 0.89	70.56 ± 1.11	12.8 ± 1.1	16.47 ± 0.41	647.28 ± 21.28	2.33 ± 0.29	7.36 ± 0.14
GP-801	84.65 ± 1.35	23.8 ± 0.55	37.28 ± 1.51	66.3 ± 0.46	18.73 ± 1.91	13.61 ± 0.11	713.84 ± 16.41	3.36 ± 0.3	14.75 ± 1.35
Purple pod sel-1	78.37 ± 0.39	25.37 ± 0.31	37.92 ± 1.71	65.85 ± 0.97	14.9 ± 0.98	19.7 ± 0.24	695.32 ± 22.62	2.14 ± 0.02	6.96 ± 0.09
VP-1436	75.36 ± 0.38	23.41 ± 1.29	36.66 ± 1.6	72.54 ± 1.81	21.23 ± 1.65	20.74 ± 0.3	765.3 ± 30.86	2.85 ± 0.14	6.89 ± 0.06
Golden pod	82.99 ± 2.07	20.94 ± 2.19	34.31 ± 0.75	67.55 ± 0.65	15.05 ± 1.23	17.83 ± 0.11	723.85 ± 18.59	4.39 ± 0.39	7.22 ± 0.24
VP-625–1	76.21 ± 1.32	23.82 ± 0.93	34.79 ± 2.13	70.41 ± 0.51	25.6 ± 0.72	16.75 ± 0.21	734.03 ± 15.79	4.15 ± 0.18	6.82 ± 0.11
PMR-38-II	72.14 ± 0.5	23.02 ± 0.95	38.5 ± 1.65	72.35 ± 0.6	22.08 ± 1.53	14.03 ± 0.13	621.42 ± 12.22	3.26 ± 0.22	7.13 ± 0.24
EC-328758	76.15 ± 0.93	24.07 ± 1.18	37.55 ± 1.43	71.26 ± 2.01	12.49 ± 0.72	18.28 ± 0.38	556.78 ± 18.53	3.75 ± 0.06	7.85 ± 0.2
EC-318760	77.69 ± 1.93	22.88 ± 1.39	34 ± 1.19	65.55 ± 1.76	18.73 ± 0.96	18.45 ± 0.47	702.5 ± 26.88	2.66 ± 0.22	7.16 ± 0.21
GP-55	74.45 ± 0.56	21.84 ± 0.14	37.52 ± 2.8	72.45 ± 2.68	28.72 ± 1.3	14.11 ± 0.13	450.43 ± 18.18	3.87 ± 0.61	6.82 ± 0.1
EC-334160	75.55 ± 0.57	23.9 ± 2.57	36.42 ± 1.77	70.22 ± 2.35	14.15 ± 0.55	15.81 ± 0.1	646.44 ± 38.3	4.16 ± 0.24	6.76 ± 0.19
JP-625	78.22 ± 0.46	23.36 ± 0.82	36.65 ± 1.08	69.15 ± 2	16.82 ± 1.26	17.94 ± 0.1	542.18 ± 31.85	4.12 ± 0.13	7.13 ± 0.12
EC-598892–1	78.12 ± 1.02	21.73 ± 1.72	38.81 ± 0.75	78.41 ± 1.44	10.95 ± 1.02	13.35 ± 0.22	690.49 ± 12.26	4.12 ± 0.23	7.73 ± 0.21
Susceptible									
GP-1706	75.12 ± 0.59	23.43 ± 0.37	32.69 ± 2.74	69.12 ± 1.25	14.36 ± 0.36	13.18 ± 0.1	694.41 ± 38.02	2.54 ± 0.38	6.99 ± 0.08
MA-7	76.25 ± 0.52	23.33 ± 2.06	33.44 ± 0.97	72.53 ± 0.78	11.49 ± 1.82	9.44 ± 0.26	483.79 ± 35.96	2.68 ± 0.32	7.2 ± 0.2
VP-233	78.12 ± 0.81	24.7 ± 2.3	35.66 ± 1.98	73.15 ± 2.13	25.39 ± 0.55	10.46 ± 0.21	556.78 ± 37.44	3.47 ± 0.16	7.15 ± 0.14
2014/PEV-2	76.66 ± 1.39	21.02 ± 1.36	37.08 ± 1.01	76.75 ± 1.02	10.43 ± 1.12	11.43 ± 0.09	598.48 ± 25.74	4.17 ± 0.22	6.76 ± 0.12
IP-3	75.12 ± 0.31	22.32 ± 1.37	34.37 ± 0.77	77.15 ± 0.17	24.14 ± 0.91	10.11 ± 0.37	652.7 ± 38.02	3.98 ± 0.24	7.36 ± 0.31
GP-6	80.12 ± 0.95	22.58 ± 0.66	26.57 ± 0.44	67.52 ± 1.54	21.86 ± 0.72	8.59 ± 0.15	767.39 ± 39.42	4.26 ± 0.21	7.07 ± 0.16
GP-1805	79.54 ± 1.55	23.96 ± 0.57	36.36 ± 1.91	70.14 ± 0.97	13.74 ± 0.95	11.39 ± 0.13	719.43 ± 28.49	3.67 ± 0.06	7.05 ± 0.12
VRP-6	78.26 ± 2.37	23.1 ± 0.71	32.29 ± 2.03	76.48 ± 0.86	23.52 ± 1.16	12.07 ± 0.23	711.09 ± 46.45	2.89 ± 0.37	7 ± 0.07
EC-552779	69.45 ± 1.83	23.71 ± 1.63	35.3 ± 0.85	77.41 ± 0.48	22.48 ± 1.57	11.31 ± 0.18	661.04 ± 37.44	2.66 ± 0.24	7.22 ± 0.18
GP-1705	82.14 ± 0.59	21.6 ± 0.34	34.74 ± 1.95	78.16 ± 1.14	18.11 ± 1.91	10.8 ± 0.09	679.81 ± 35.47	3.47 ± 0.29	7.1 ± 0.08
VP-457	78.54 ± 1.2	22.89 ± 1.25	34.86 ± 2.23	65.84 ± 0.91	11.24 ± 0.36	18.28 ± 0.35	556.78 ± 37.6	4.51 ± 0.25	7.27 ± 0.14
GP-1708	80.15 ± 1.78	20.95 ± 1.84	32.02 ± 0.97	67.69 ± 1.31	16.24 ± 1.65	10.03 ± 0.43	759.05 ± 21.44	3.67 ± 0.52	7.81 ± 0.1
AP-3	76.45 ± 1.41	22 ± 2.03	35.37 ± 0.6	78.45 ± 2.69	12.32 ± 0.57	14.28 ± 0.22	623.51 ± 35.56	3.84 ± 0.15	7.14 ± 0.15
GP-917	77.32 ± 1.37	23.82 ± 2.42	34.79 ± 1	72.56 ± 1.86	20.61 ± 1.08	14.74 ± 0.21	548.43 ± 39.16	2.64 ± 0.27	7.24 ± 0.1
Apoorva	79.14 ± 1.42	23.06 ± 1.65	35.19 ± 0.33	71.25 ± 1.83	13.11 ± 1.3	17.94 ± 0.18	723.6 ± 17.97	3.87 ± 0.32	6.89 ± 0.1

Table 1 (continued)

Genotypes	Physiological			Biochemical					
	RWC	CT	GI	MSI	MDA	H_2O_2	SOD	CAT	TS
Mean ± SE	79.36 ± 1.24	22.96 ± 1.20	37.29 ± 1.25	72.03 ± 1.31	18.85 ± 1.02	14.72 ± 0.23	651.31 ± 29.38	3.52 ± 0.23	7.60 ± 0.20
CD 5%	2.61	2.68	3.29	2.17	2.82	0.33	52.40	0.45	0.67
CV %	2.02	7.19	5.43	1.85	9.21	1.36	4.95	7.91	5.43

RWC Relative water content, *CT* Canopy temperature, *GI* Greenness index, *MSI* Membrane stability index, *MDA* Malondialdehyde, *H_2O_2* Hydrogen peroxide, *SOD* Superoxide dismutase, *CAT* Catalase, *TS* Total sugar, *SE* Standard error, *CD* Critical difference, *CV* Co-efficient of variation

and catalase to combat reactive oxygen species. Thus, estimating important biochemical traits like membrane stability index, malondialdehyde content, hydrogen peroxide content, superoxide dismutase, catalase activity, and total sugar may suggest their importance in heat tolerance.

The membrane stability index was estimated to determine the amount of solute leakage. It was observed from the study that the tolerant genotypes maintained high membrane stability than moderately tolerant and susceptible genotypes under heat stress conditions. The mean MSI% intolerant, moderately tolerant, and susceptible genotypes were 24.75%, 43.74%, and 64.96% decrease in heat stress conditions compared to normal conditions (Table 3). The overall decrease in MSI of all the genotypes during heat stress conditions was 44.51% (Fig. 3D). The range of MSI during normal conditions was 65.24–80.08%, and during heat stress conditions was 12.28–76.31%. The highest MSI during heat stress conditions was found in the tolerant genotype EC-598654 (76.30%), followed by 2019/PMPM-4 (65.91%) and EC-598649 (57.91%) (Table 2).

Malondialdehyde, the premium product of lipid peroxidation, increased more during heat stress than under normal conditions. Also, the increase in MDA content was higher in susceptible genotypes than in intolerant and moderately tolerant genotypes. The mean MDA intolerant, moderately tolerant, and susceptible genotypes under normal conditions were 21.23, 18.06, and 17.27 nmol/g FW, and under heat stress conditions, were 83.94, 73.87, and 164.89 nmol/g FW respectively. In heat stress conditions, MDA was increased 2.95 times intolerant, 3.09 times in moderately tolerant, and 8.55 times in susceptible genotypes (Fig. 3E; Table 3). The tolerant genotypes with the lowest MDA contents were EC-328758 (53.08 nmol/g FW), GP-912-II (57.19 nmol/g FW), and GP-902 (57.74 nmol/g FW), while the susceptible genotype 2014/PEV-2 showed highest MDA content of 196.08 nmol/g FW (Table 2).

Similarly, H_2O_2 contents were also increased during heat stress conditions, and the highest increase was found in susceptible genotypes. The comprehensive range of hydrogen peroxide contents in normal conditions was 8.59–20.74 µmol/g FW, while in heat stress conditions, it ranged from 19.71 to 52.96 µmol/g FW. The mean hydrogen peroxide content in heat stress conditions showed a 74.09, 74.53, and 262.92% increase in tolerant, moderately tolerant, and susceptible genotypes than in normal conditions (Fig. 3F; Table 3). The tolerant and susceptible genotypes with the highest and lowest H_2O_2 contents were EC-598646 (19.71 µmol/g FW) and Apoorva (52.96 µmol/g FW), respectively (Table 2).

Antioxidants like superoxide dismutase and catalase was found to be increased during heat stress condition to counteract ROS and the highest increase was found in tolerant genotypes. In heat stress season, SOD showed a broad range from 719.99 to 1376.30 U/g FW with a mean value of 1041.75 U/g FW, while in normal conditions, it ranged from 450.43 to 770.23 U/g FW with a mean value of 651.31 U/g FW (Table 2). The tolerant genotype with the highest SOD activity in heat stress conditions was 2019/PMPM-4 (1376.30 U/g FW), followed by EC-677214 (1352.94 U/g FW) and EC-598892-1 (1337.93 U/g FW) while the susceptible genotype GP-1805 (719.99 U/g FW) had lowest SOD activity. The tolerant, moderately tolerant, and susceptible genotypes showed 69.61, 76.38, and 33.70% increases in SOD content in the heat stress season compared to the regular season (Fig. 3G; Table 2). Similarly, the catalase activity was also increased in heat stress season. The highest activity was found in the tolerant genotype GP-57 with 42.89 µmol H_2O_2 reduced/min/g FW followed

Table 2 The mean value of physiological, biochemical traits of 45 garden pea genotypes under heat stress condition

Genotypes	Physiological			Biochemical					
	RWC	CT	GI	MSI	MDA	H_2O_2	SOD	CAT	TS
Tolerant									
EC-598646	67.35 ± 1	36.41 ± 0.68	36.22 ± 1.97	51.25 ± 2.29	72.44 ± 6.88	19.71 ± 0.17	1272.03 ± 36.12	29.75 ± 1.61	7.08 ± 0.15
EC-598638	70.24 ± 2.8	31.52 ± 2.49	36.97 ± 1.03	48.65 ± 1.05	94.92 ± 5.96	26.6 ± 0.64	1097.7 ± 25.23	29.54 ± 1.4	6.94 ± 0.04
2019/PMPM-4	69.64 ± 0.49	36.42 ± 0.46	46.9 ± 0.74	65.91 ± 2.65	66.19 ± 2.37	28.92 ± 2.02	1376.3 ± 33.34	34.17 ± 2.37	6.88 ± 0.05
GP-61	69.99 ± 2.92	33.97 ± 0.17	29.4 ± 2.77	54.72 ± 2.34	103.66 ± 7.67	26.54 ± 1.09	1131.07 ± 20.67	30.5 ± 1.89	7.16 ± 0.26
GP-902	73.98 ± 3.77	35.23 ± 1.02	39.56 ± 1.31	48.67 ± 7.28	57.74 ± 2.6	28.75 ± 2.42	1037.65 ± 20.98	33.39 ± 1.38	11.32 ± 0.58
GP-912-II	67.76 ± 2.16	32.89 ± 0.46	44.54 ± 0.35	52.29 ± 2.82	57.19 ± 3.22	26.31 ± 2.52	1151.09 ± 32.48	30.12 ± 2.24	10.3 ± 0.25
EC-598649	80.86 ± 1.52	36.82 ± 1.68	30.6 ± 0.91	57.91 ± 1.32	72.44 ± 5.01	27.33 ± 1.81	1111.05 ± 47.55	36.33 ± 3.56	7.64 ± 0.33
GP-48	70.41 ± 3.98	34.96 ± 1.61	44.5 ± 3.19	53.36 ± 4.04	65.57 ± 3.82	28.69 ± 2.78	877.5 ± 32.18	37.83 ± 2.17	7.73 ± 0.3
EC-677211	76.33 ± 2.84	35.15 ± 1.18	35.5 ± 0.78	54.84 ± 4.76	65.15 ± 2.89	28.99 ± 3.16	859.15 ± 30.1	30.1 ± 2.22	6.65 ± 0.21
EC-598654	74.49 ± 7.36	33.7 ± 0.88	44.12 ± 1.76	76.3 ± 7.6	104.28 ± 5.44	23.93 ± 1.46	1117.72 ± 17.35	32 ± 0.6	7.05 ± 0.13
GP-1104	75.83 ± 4.14	33.18 ± 1.04	44.75 ± 0.55	50.33 ± 2.49	71.19 ± 4.69	31.72 ± 4.32	1050.99 ± 40.54	29.89 ± 1.79	6.89 ± 0.11
EC-677214	68.22 ± 2.44	35.17 ± 1.47	30.3 ± 0.91	52.55 ± 5.99	76.6 ± 3.61	22.4 ± 2.77	1352.94 ± 42.29	33.33 ± 2.09	7.04 ± 0.03
GP-57	59.77 ± 1.49	38.1 ± 0.9	27.98 ± 1.7	56.76 ± 4.91	123.64 ± 2.87	31.58 ± 2.11	1151.09 ± 46.51	42.89 ± 2.83	6.78 ± 0.05
GP-915-II	77.03 ± 3.91	34.88 ± 2.01	33.77 ± 1.58	49.86 ± 1.21	105.53 ± 6.52	21.5 ± 3.55	1034.31 ± 40.53	29.05 ± 0.54	6.12 ± 0.02
EC-598602	73.39 ± 5.19	36.07 ± 1.39	35.95 ± 1.57	48.48 ± 2.06	122.6 ± 3.56	28.13 ± 2.05	940.89 ± 27.68	29 ± 0.85	6.54 ± 0.08
Moderately tolerant									
GP-1707	53.97 ± 1.99	34.08 ± 0.7	22.78 ± 0.49	34.48 ± 3.24	68.07 ± 5.45	25.86 ± 0.6	1207.81 ± 35.16	26.67 ± 2.31	6.83 ± 0.1
MEGH-2	56.51 ± 2.47	33.3 ± 1.89	40.6 ± 1.26	39.71 ± 1.94	65.19 ± 4.34	27.05 ± 0.84	1191.12 ± 49.6	19.72 ± 2.3	7.17 ± 0.1
EC-598593	62.32 ± 3.03	32.3 ± 2.29	30.65 ± 1	44.77 ± 3.03	64.52 ± 3.07	27.45 ± 0.99	1104.38 ± 38.65	34.89 ± 2.2	6.87 ± 0.05
GP-801	74.47 ± 5.14	38.77 ± 0.83	13.05 ± 0.83	34.39 ± 3.08	72.64 ± 3.61	30.79 ± 2.8	1211.14 ± 35.16	35.33 ± 3.63	6.87 ± 0.17
Purple pod sel-1	66.47 ± 5.51	37.38 ± 1.2	34.83 ± 1.48	44.94 ± 1.62	73.06 ± 2.82	31.58 ± 0.64	1101.04 ± 39.64	32.22 ± 3.32	7.27 ± 0.21
VP-1436	50.14 ± 0.73	37.6 ± 0.74	24.63 ± 2.08	41.16 ± 0.55	66.82 ± 3.82	35.02 ± 1.2	980.09 ± 49.44	26.45 ± 1.63	6.46 ± 0.08
Golden pod	69.26 ± 4.54	32.3 ± 0.22	15.63 ± 0.85	41.47 ± 2.52	68.9 ± 3.25	27.56 ± 1.56	1337.09 ± 14.26	21.39 ± 1.07	7.1 ± 0.09
VP-625–1	54.26 ± 1.8	34.1 ± 0.7	24.66 ± 0.98	38.74 ± 1.12	58.91 ± 2.8	30.26 ± 0.35	1021.8 ± 53.18	25.58 ± 5	6.32 ± 0.04
PMR-38-II	54.12 ± 3.65	37.26 ± 0.7	30.86 ± 1.22	42.14 ± 1.05	63.28 ± 4.65	29.5 ± 3.19	1015.54 ± 42.41	26.65 ± 3.11	6.58 ± 0.06
EC-328758	56.45 ± 3.05	35.57 ± 0.95	32.59 ± 1.36	40.46 ± 0.97	53.08 ± 1.08	34.17 ± 1.92	1084.36 ± 48	24.74 ± 1.56	6.65 ± 0.04
EC-318760	66.09 ± 3.49	36.57 ± 1.18	31.3 ± 0.2	37.76 ± 2.48	89.3 ± 0.95	29.54 ± 2.48	1194.46 ± 22.66	26.45 ± 2.53	7.11 ± 0.16
GP-55	52.65 ± 0.62	35.63 ± 1.55	32.88 ± 0.99	36.28 ± 1.61	66.21 ± 4.17	27.63 ± 2.47	896.68 ± 31.86	27.64 ± 3.22	7.79 ± 0.28
EC-334160	64.2 ± 3.12	37.25 ± 1.34	27.27 ± 1.5	36.27 ± 2.9	69.5 ± 3.03	29.54 ± 1.58	1331.26 ± 46.23	28.39 ± 2.41	6.36 ± 0.05
JP-625	68.92 ± 3.8	38.56 ± 0.61	26 ± 1	42.28 ± 2.44	127.39 ± 0.96	21.21 ± 2.4	1284.54 ± 55.38	23.31 ± 1.82	6.87 ± 0.05
EC-598892–1	68.3 ± 4.67	32.25 ± 1.74	21.54 ± 1.23	38.74 ± 1.02	101.16 ± 5.01	25.63 ± 3.48	1337.93 ± 39.34	13.72 ± 2.25	6.89 ± 0.12
Susceptible									
GP-1706	46.23 ± 2.51	38.15 ± 1.07	11.63 ± 0.91	29.45 ± 3.2	150.49 ± 3.08	41.4 ± 1.61	854.97 ± 43.86	13.39 ± 0.81	6.13 ± 0.02
MA-7	50.58 ± 2.72	37.65 ± 0.53	19.59 ± 0.42	32.21 ± 4.68	166.1 ± 6.85	40.12 ± 1.66	967.58 ± 38.18	11.5 ± 0.45	6.45 ± 0.04
VP-233	34.61 ± 2.4	38.53 ± 1.15	20.89 ± 0.42	24.39 ± 2.01	140.5 ± 3.97	38.68 ± 1.87	854.97 ± 32.41	10.17 ± 0.18	6.33 ± 0.02
2014/PEV-2	42.75 ± 3.74	38.15 ± 0.22	16.87 ± 1.64	32.45 ± 2.25	196.08 ± 4.73	41.4 ± 4.73	922.54 ± 53.66	10.5 ± 0.6	6.24 ± 0.06
IP-3	35.15 ± 2.48	39.41 ± 1.51	17 ± 1.41	30.66 ± 1.98	186.08 ± 6.06	52.02 ± 1.57	884.16 ± 35.66	8.39 ± 0.59	6.65 ± 0.06
GP-6	31.77 ± 3.57	37.25 ± 0.83	23.95 ± 1.82	14.15 ± 1.45	202.32 ± 6.83	41.48 ± 1.05	942.56 ± 47.49	11.17 ± 0.75	6.42 ± 0.05
GP-1805	38.74 ± 1.93	32.55 ± 1.24	11.9 ± 0.98	15.67 ± 2.49	138 ± 1.91	48.28 ± 0.84	719.99 ± 2.9	13.22 ± 1.3	6.16 ± 0.04
VRP-6	42.21 ± 3.23	36.45 ± 1.46	13.86 ± 1.49	27.92 ± 1.95	132.38 ± 5.11	40.29 ± 3.05	877.49 ± 9.07	14.79 ± 1.52	6.33 ± 0.02
EC-552779	41.25 ± 1.42	39.63 ± 1.57	22.87 ± 1.07	21.89 ± 1.28	179.84 ± 5.44	44.97 ± 4.22	742.37 ± 26.46	12.69 ± 1.12	6.27 ± 0.02
GP-1705	36.75 ± 2.84	35.78 ± 1.52	17.84 ± 1.8	21.25 ± 0.97	171.72 ± 2.95	44.63 ± 3	786.16 ± 22.53	14.01 ± 0.35	6.45 ± 0.05
VP-457	45.66 ± 3.76	36.69 ± 0.67	20.59 ± 0.89	30.17 ± 3.2	197.32 ± 5.66	44.54 ± 2.66	842.46 ± 43.3	11.89 ± 1.08	6.33 ± 0.04
GP-1708	42.34 ± 2.88	37.96 ± 1.28	25 ± 2.33	26.22 ± 2.48	185.46 ± 4.6	43.44 ± 1.02	871.65 ± 41.8	13.33 ± 2.03	6.13 ± 0.03
AP-3	30.12 ± 1.66	34.69 ± 1.46	23.47 ± 0.87	12.28 ± 0.9	152.16 ± 4.61	46.5 ± 5.14	942.55 ± 41	16.89 ± 1	6.45 ± 0.06
GP-917	36.78 ± 2.73	34.1 ± 0.7	10.96 ± 1.34	30.76 ± 2.13	142.48 ± 5.13	47.18 ± 1.13	888.34 ± 36.18	14.05 ± 0.47	6.24 ± 0.02
Apoorva	37.74 ± 3.04	40.6 ± 0.88	14.95 ± 0.41	33.89 ± 2.83	132.39 ± 3.82	52.96 ± 2.59	920.04 ± 49.11	15.22 ± 1.09	6.12 ± 0.02

Table 2 (continued)

Genotypes	Physiological			Biochemical					
	RWC	CT	GI	MSI	MDA	H_2O_2	SOD	CAT	TS
Mean ± SE	57.47 ± 2.99	35.89 ± 1.11	27.59 ± 1.23	39.97 ± 2.58	107.57 ± 4.15	33.37 ± 2.12	1041.75 ± 35.96	23.83 ± 1.76	6.89 ± 0.11
CD 5%	6.37	2.75	3.34	7.63	12.52	5.19	92.08	4.79	0.39
CV %	6.82	4.71	7.45	11.74	7.16	9.57	5.44	12.36	3.51

RWC Relative water content, *CT* Canopy temperature, *GI* Greenness index, *MSI* Membrane stability index, *MDA* Malondialdehyde, H_2O_2 Hydrogen peroxide, *SOD* Superoxide dismutase, *CAT* Catalase, *TS* Total sugar, *SE* Standard error, *CD* Critical difference, *CV* Co-efficient of variation

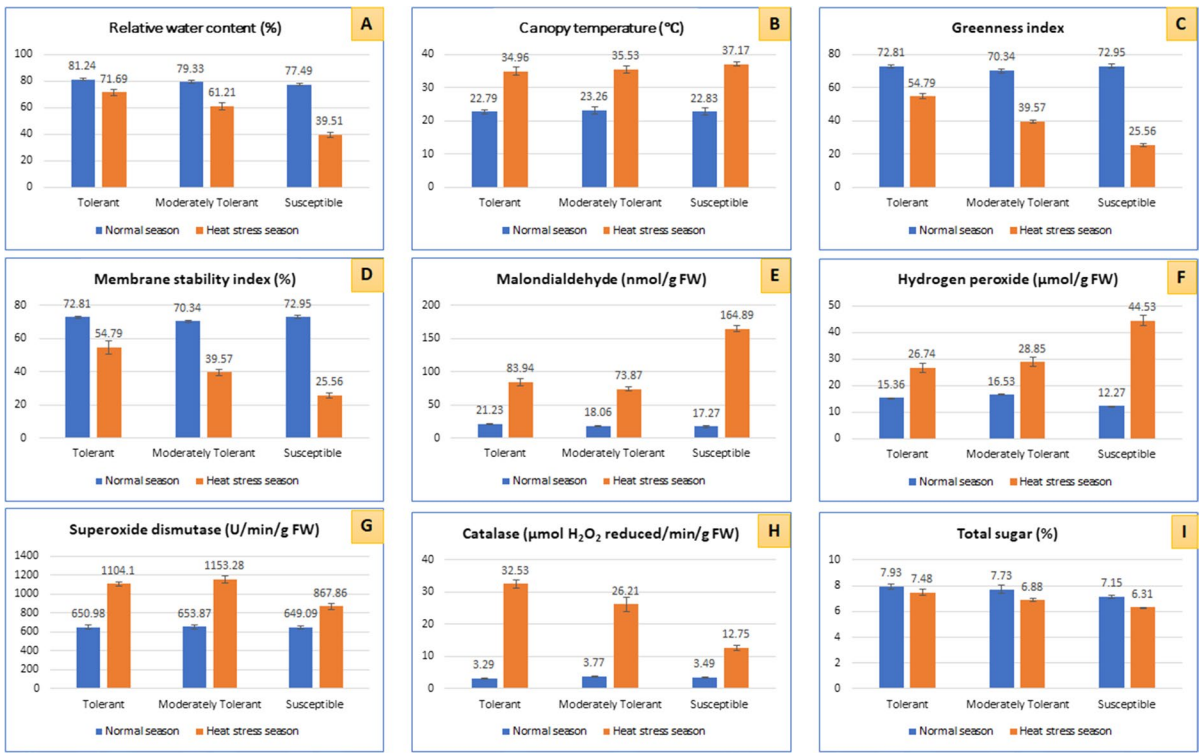

Fig. 3 Mean value of physiological and biochemical traits of tolerant, moderately tolerant, and susceptible genotypes under normal and heat stress season

by GP-48 (37.83 µmol H_2O_2 reduced/min/g FW) and EC-598649 (36.33 µmol H_2O_2 reduced/min/g FW) while lowest activity was observed. In the susceptible genotype IP-3 (8.39 µmol H_2O_2 reduced/min/g FW). The tolerant genotypes had an average of 8.89 times higher catalase activity than in the regular season, while the moderately tolerant and susceptible genotypes showed 5.95 times and 2.65 times higher catalase activity (Fig. 3H; Table 3).

On estimating total sugar %, it was found that the tolerant genotypes showed only a 5.67% decrease in heat stress season as compared to the regular season, while moderately tolerant and susceptible genotypes showed 11% and 11.75% reduction (Fig. 3I; Table 3). The highest total sugar % was found in the moderately tolerant genotype GP-801 (14.75%); however, in heat stress conditions, the highest total sugar was found in the tolerant genotypes GP-902 (11.32%), GP-912-II (10.30%) and GP-55 (7.79%). Meanwhile, purple pod sel-1 and GP-55 genotypes showed a significant increase in total sugar content

Table 3 Mean values of physiological and biochemical traits in garden peas under normal and heat stress conditions

	Normal condition				Heat stress condition			
	Mean	SE	CV%	Range	Mean	SE	CV%	Range
Relative water content (%)								
Tolerant	81.24	0.95	2.02	77.38–86.21	71.69	2.30	5.56	59.77–80.86
Mod. Tolerant	79.33	0.99	2.16	72.14–88.67	61.21	2.57	7.27	50.14–74.47
Susceptible	77.49	0.76	1.69	69.45–82.14	39.51	1.96	8.61	30.12–50.58
Total	79.36	0.93	2.02	69.45–88.67	57.47	2.26	6.82	30.12–80.86
Canopy temperature (°C)								
Tolerant	22.79	0.67	5.12	20.52–25.10	34.96	1.16	5.72	31.52–38.10
Mod. Tolerant	23.26	1.01	7.53	20.94–25.37	35.53	1.07	5.20	32.25–38.77
Susceptible	22.83	1.14	8.67	20.95–24.70	37.17	0.64	3.00	32.55–40.60
Total	22.96	0.95	7.19	20.52–25.37	35.89	0.98	4.71	31.52–40.60
Greenness index								
Tolerant	40.67	0.90	3.82	34.58–48.95	37.40	1.53	7.09	27.98–46.90
Mod. Tolerant	37.17	1.24	5.79	34.00–39.72	27.29	0.91	5.74	13.05–40.60
Susceptible	34.05	1.32	6.71	26.57–37.08	18.09	1.07	10.25	10.96–25.00
Total	37.29	1.17	5.43	26.57–48.95	27.59	1.19	7.45	10.96–46.90
Membrane stability index (%)								
Tolerant	72.81	0.79	1.87	65.24–80.08	54.79	3.93	12.43	48.48–76.31
Mod. Tolerant	70.34	0.80	1.97	65.55–78.41	39.57	1.79	7.84	34.39–44.94
Susceptible	72.95	0.72	1.71	65.84–78.45	25.56	1.66	11.26	12.28–33.89
Total	72.03	0.77	1.85	65.24–80.08	39.97	2.71	11.74	12.28–76.31
Malondialdehyde (nmol/g FW)								
Tolerant	21.23	0.70	5.72	12.49–28.10	83.94	4.86	10.02	57.19–123.64
Mod. Tolerant	18.06	1.18	11.35	10.95–28.72	73.87	3.46	8.12	53.08–127.39
Susceptible	17.27	1.05	10.49	10.43–25.39	164.89	4.92	5.17	132.38–202.32
Total	18.85	1.00	9.21	10.43–28.72	107.57	4.45	7.16	53.08–202.32
Hydrogen peroxide (µmol/g FW)								
Tolerant	15.36	0.10	1.12	11.99–18.54	26.74	1.66	10.72	19.71–31.72
Mod. Tolerant	16.53	0.14	1.43	13.35–20.74	28.85	1.67	10.00	21.21–35.02
Susceptible	12.27	0.12	1.62	8.59–18.28	44.53	2.00	7.77	38.68–52.96
Total	14.72	0.12	1.36	8.59–20.74	33.37	1.84	9.57	19.71–52.96
Superoxide dismutase (U/min/g FW)								
Tolerant	650.98	18.37	4.89	554.02–770.23	1104.10	24.65	3.87	859.15–1376.30
Mod. Tolerant	653.87	18.82	4.99	450.43–765.30	1153.28	37.95	5.70	896.68–1337.93
Susceptible	649.09	15.46	4.12	483.79–767.39	867.86	30.05	6.00	719.99–967.58
Total	651.31	18.61	4.95	450.43–770.23	1041.75	32.71	5.44	719.99–1376.30
Catalase (µmol H_2O_2 reduced/min/g FW)								
Tolerant	3.29	0.11	5.81	2.33–4.89	32.53	1.41	7.51	29.00–42.89
Mod. Tolerant	3.77	0.18	8.03	2.14–6.67	26.21	2.24	14.81	13.72–35.33
Susceptible	3.49	0.19	9.26	2.54–4.51	12.75	0.86	11.65	8.39–16.89
Total	3.52	0.16	7.91	2.14–6.67	23.83	1.70	12.36	8.39–42.89
Total sugar (%)								
Tolerant	7.93	0.21	4.51	6.35–11.30	7.48	0.22	4.99	6.12–11.32
Mod. Tolerant	7.73	0.33	7.38	6.76–14.75	6.88	0.10	2.46	6.32–7.79
Susceptible	7.15	0.13	3.03	6.76–7.81	6.31	0.03	0.83	6.12–6.65

in heat stress season compared to normal conditions (Table 2).

Dendrogram and heat map for physiological and biochemical traits

Highly significant differences were found among treatments for all physiological and biochemical traits studied in normal and heat stress conditions (Tables 4 and 5, respectively). A cluster dendrogram was generated based on physiological and biochemical traits of normal and heat stress conditions (Fig. 4A and B, respectively). The genotypes were classified into two major clusters, with 22, 6 genotypes in cluster I and 23, 39 in cluster II during heat stress and normal condition, respectively. In heat stress season, cluster I was found to be closely related and consisted of 15 tolerant and seven moderately tolerant genotypes, whereas cluster II was found to be further subdivided into two sub-clusters. Subcluster IIa consists of 14 susceptible genotypes, and subcluster IIb consists of 8 moderately tolerant and one susceptible genotype. It was observed that during heat stress conditions, the tolerant and susceptible genotypes were grouped into distinct clusters (Fig. 4B). However, in the control season, such distinct clusters were not formed among the genotypes, as cluster I consisted of 3 tolerant and three moderately tolerant genotypes and cluster II consists of 12 tolerant, 12 moderately tolerant and 15 susceptible genotypes (Fig. 4A).

A heat map was generated for normal and heat stress conditions to visualize the closely related genotypes concerning physiological and biochemical traits. It was perceived from Fig. 5 that three distinct groups of genotypes were formed in the regular season, with the genotype EC-552779 as an outlier as it has the least relative water content (69.45%) in this season. RWC was found to be maximum, and CT was found to be minimum in the regular season. No specific pattern was observed in the heat map among the genotypes in the regular season (Fig. 5). However, in heat stress season, the genotypes were grouped into three distinct clusters with specific patterns concerning physiological and biochemical data (Fig. 6). The traits like RWC, CT were placed together. In contrast, the traits like MDA, SOD, CAT, and TS were placed under one sub-cluster with H_2O_2, GI, and MSI in another sub-cluster. The susceptible genotypes

Table 3 (continued)

	Normal condition				Heat stress condition			
	Mean	SE	CV%	Range	Mean	SE	CV%	Range
Total	7.60	0.24	5.43	6.35–14.75	6.89	0.14	3.51	6.12–11.32

Table 4 Analysis of variance for physiological and biochemical traits in normal condition

Source of variation	df	Mean sum of squares								
		RWC	CT	GI	MSI	MDA	H_2O_2	SOD	CAT	TS
Replications	2	153.06**	126.03**	77.12**	204.70**	32.87**	6.50**	84,895.27**	5.22**	3.44**
Treatments	44	46.82**	5.07**	47.69**	52.13**	93.67**	26.64**	16,988.38**	2.16**	6.21**
Error	88	2.57	2.72	4.11	1.78	3.01	0.04	1039.27**	0.08	0.17

Table 5 Analysis of variance for physiological and biochemical traits in heat stress condition

Source of variation	df	Mean sum of squares								
		RWC	CT	GI	MSI	MDA	H_2O_2	SOD	CAT	TS
Replications	2	777.67**	79.22**	69.61**	258.97**	57.01	341.78**	52,277.24**	177.56**	0.50**
Treatments	44	660.84**	15.17**	301.20**	548.80**	6537.31**	233.54**	92,679.43**	257.85**	2.79**
Error	88	15.34	2.85	4.22	22.03	59.36	10.19	3209.36	8.68	0.06

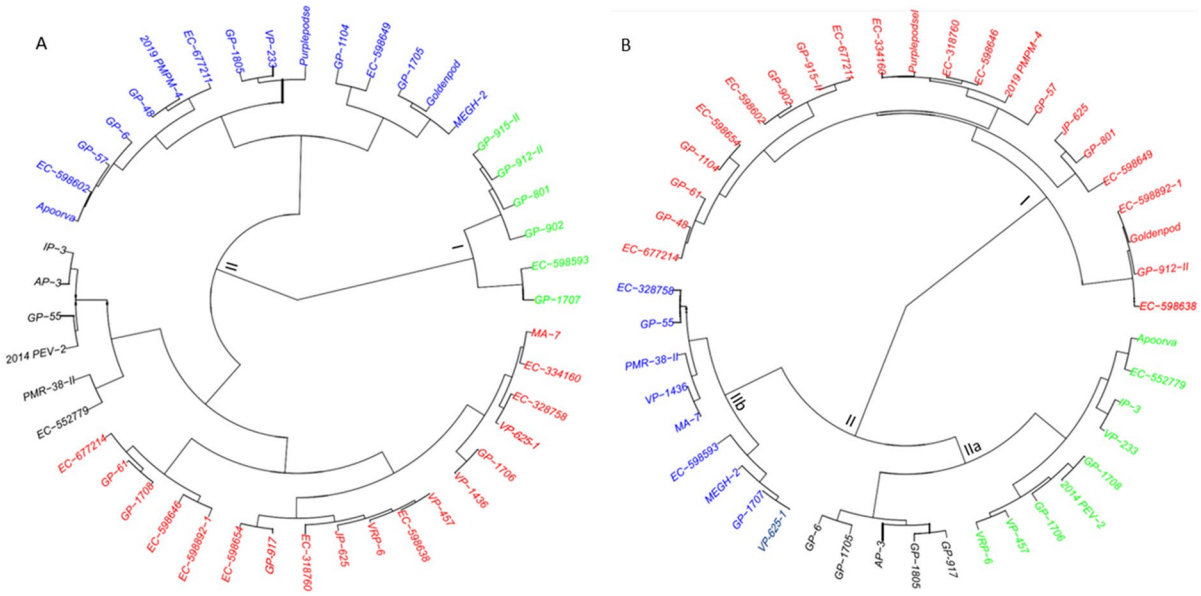

Fig. 4 Cluster dendrogram of 45 garden pea genotypes based on physiological and biochemical traits. **A** Normal condition. **B** Heat stress condition

viz., Apoorva, AP-3, GP-6, IP-3, and VP-233 were found to be grouped. This group had lower RWC, GI, and MSI with higher CT and MDA. The genotypes GP-1705, GP-917-II, EC-552779, VRP-6, GP-1708, 2014-PEV-2, VP-457, GP-1805, GP-1706, MA-7, and VP-1436 were placed together under a group which was found to have better RWC, GI, and MSI than group 1 with comparatively less CT and MDA. The remaining tolerant and moderately tolerant genotypes were grouped in one sub-cluster and were characterized by higher RWC, MSI, GI, CAT, and lower CT and MDA.

Correlation between morphological, physiological, and biochemical traits under heat stress and normal condition

The mean value of quantitative morphological traits of 45 garden pea genotypes under normal and heat stress conditions was presented in supplementary tables S2 and S3, respectively. Significant correlations were found between morphological, physiological, and biochemical traits under heat stress conditions. In contrast, normal conditions showed very few significant correlations among these traits (Fig. 7). It was observed that RWC is significantly correlated with only GI and TS, while GI is significantly correlated with only NPP under normal conditions. Thus, it was clear that there was no correlation between yield parameters and physiological and biochemical traits in the regular season.

However, growth parameters like PH showed a significant negative correlation with MSI and a significant positive correlation with H_2O_2. Also, MDA and H_2O_2 showed a significant positive correlation with DFF and IL, respectively, and CAT showed a highly significant positive correlation with RSL under normal conditions. The growth and yield parameters were found to be correlated within themselves in normal conditions, as shown in Fig. 7.

Physiological traits- RWC, CT, GI; biochemical traits- MSI, MDA, H_2O_2, SOD, CAT, TS; growth parameters- PH, IL, DFF, RSL, RGD, DM; yield parameters- NPP, PL, NSP, APW, YPP

In heat stress conditions, important yield parameters like NPP and YPP showed significant positive correlations with physiological traits like RWC and GI and biochemical traits like MSI, CAT, and TS. In contrast, they showed a significant negative correlation with CT, MDA, and H_2O_2. However, NPP showed a significant positive correlation with SOD also. Likewise,

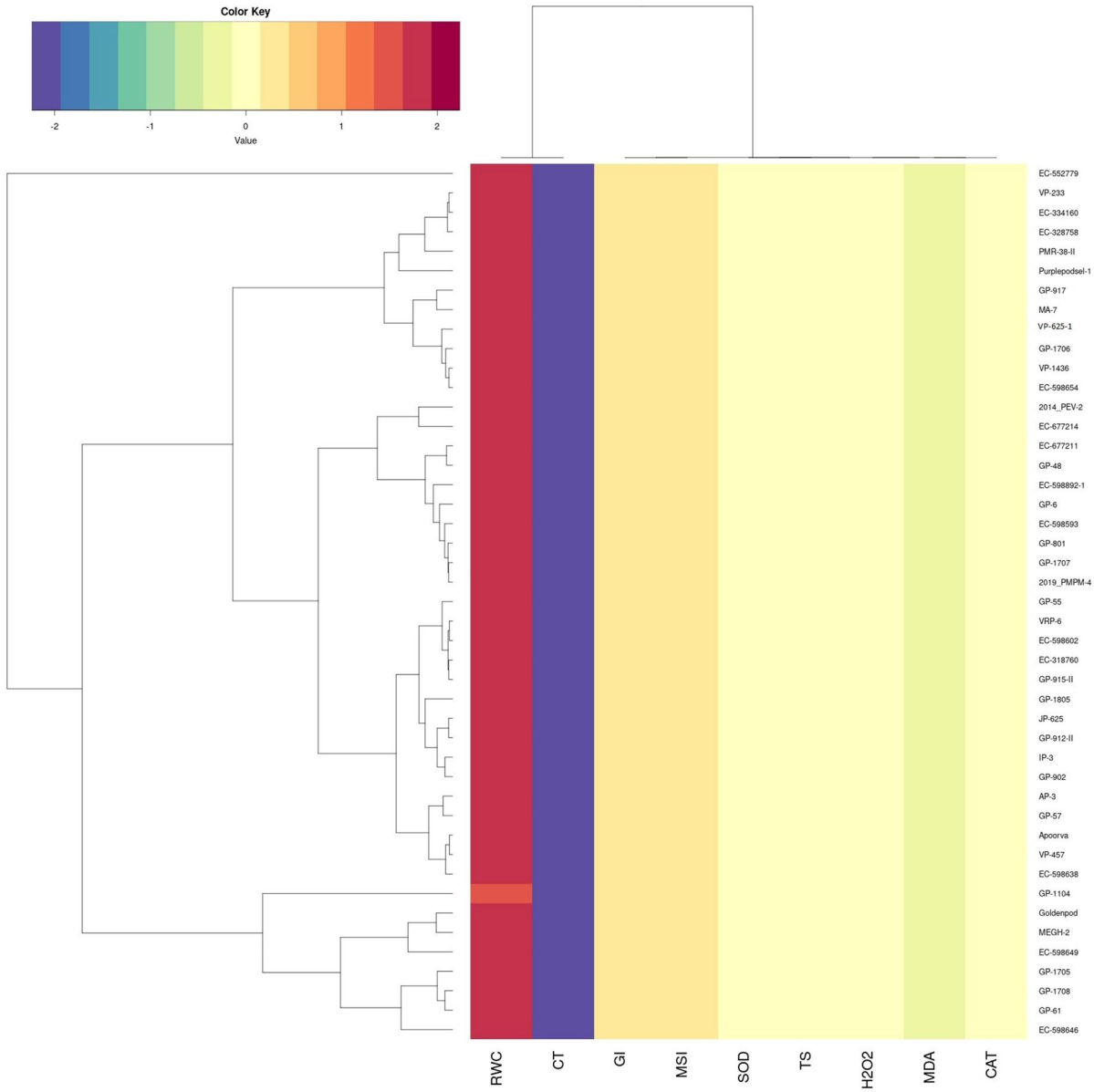

Fig. 5 Heat map of 45 garden pea genotypes for physiological and biochemical traits during normal season

other yield indices like PL, NSP, and APW also showed a significant positive correlation with RWC, GI, MSI, and SOD and a significant negative correlation with MDA and H_2O_2. Growth indices like PH, DFF, RSL, RGD, and DM showed a significant positive correlation with RWC, GI, MSI, SOD, and CAT and a significant negative correlation with MDA and H_2O_2. These findings imply that MDA and H_2O_2 negatively affect most yield and growth parameters except CT in heat stress conditions.

Furthermore, most growth parameters were significantly associated with yield parameters except DFF and IL. Similarly, all physiological and biochemical traits were positively correlated except CT, MDA, and H_2O_2. The figure suggested that the physiological and biochemical traits are more

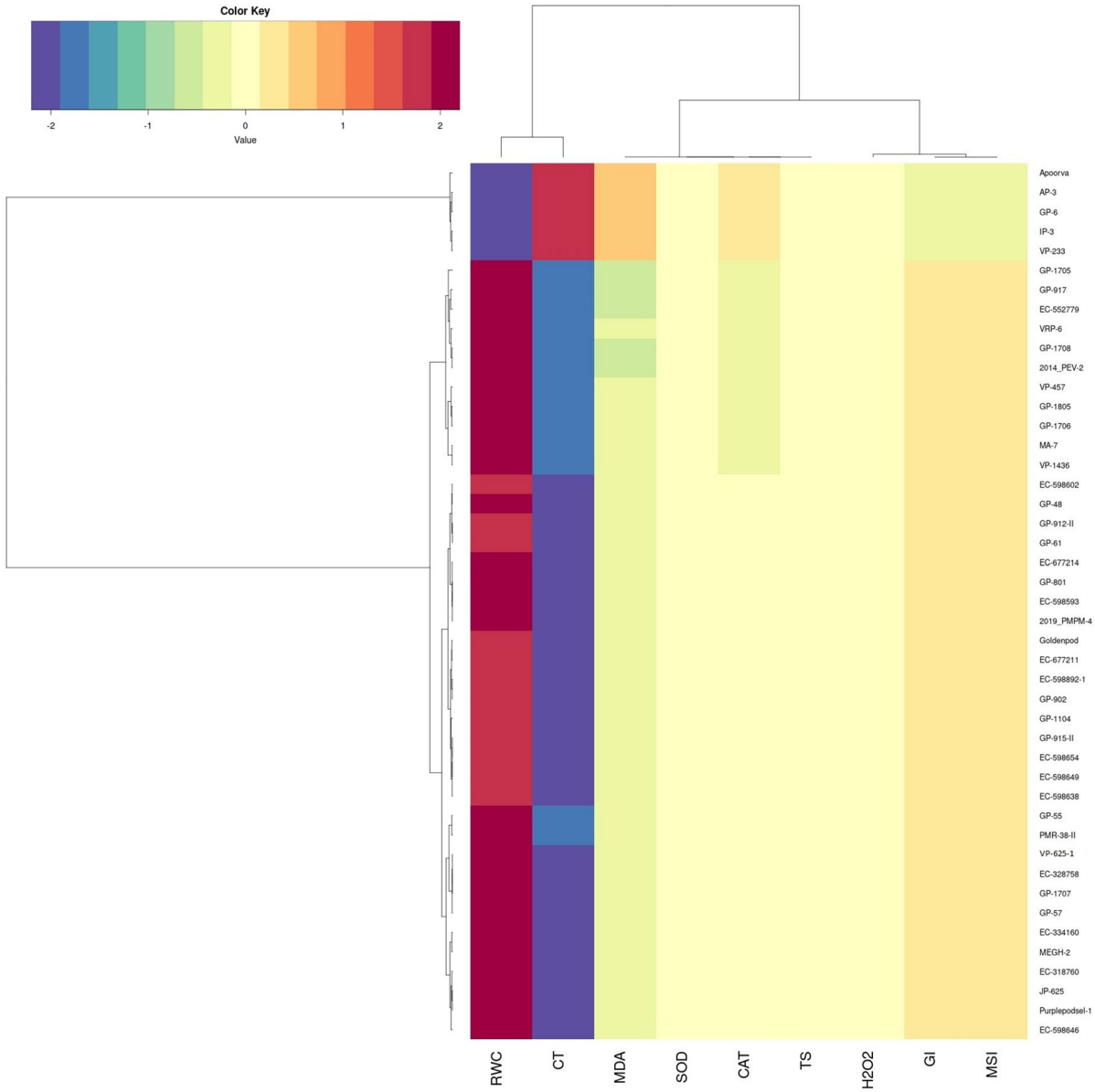

Fig. 6 Heat map of 45 garden pea genotypes for physiological and biochemical traits during heat stress season

important in determining yield parameters in heat stress than in normal conditions.

PCA analysis for morpho-physiological and biochemical traits

The mean data of 20 variables (morphological, physiological, and biochemical) were subjected to principal component analysis. It was clear from the scree plot (Fig. 8A and B, respectively) that the first two principal components contributed the highest variance than other factors under normal and heat stress conditions. The maximum variability was observed in PC1 (22.1% and 50.8%), followed by PC2 (12.5% and 11.7%) under normal and heat stress conditions, respectively. In normal conditions, seven principal components contributed 72% of the total variance with an eigenvalue more than 1, while in heat stress

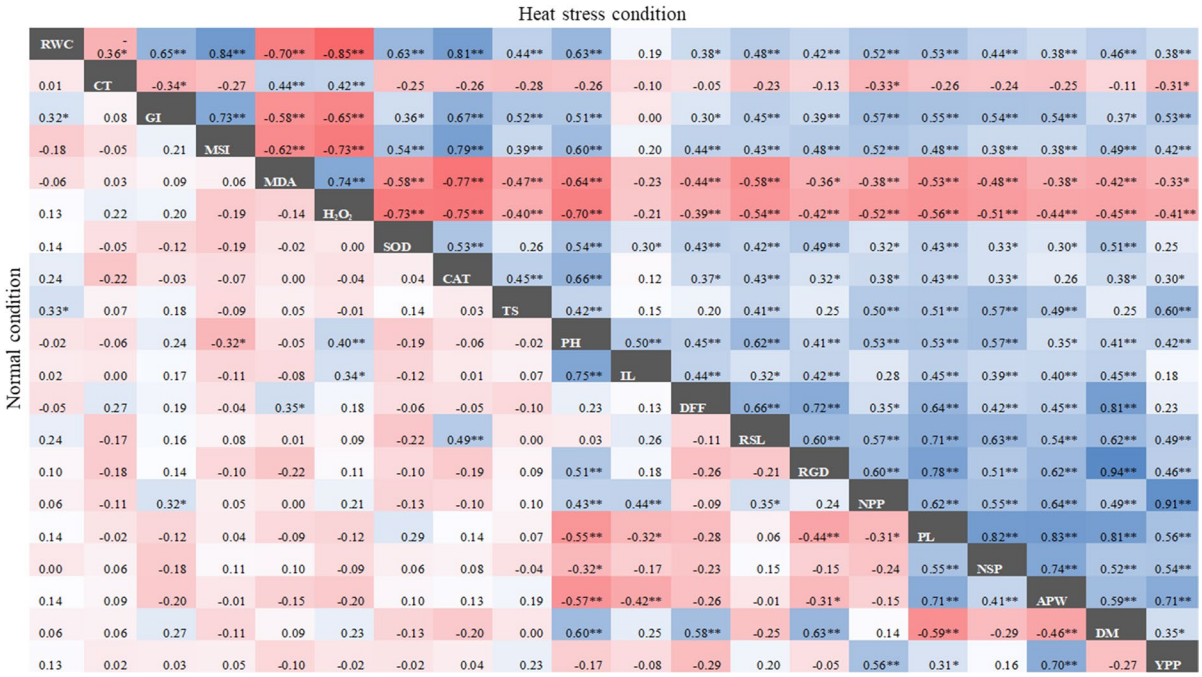

Fig. 7 Pearson correlation analysis between morphological, physiological, and biochemical traits of 45 garden pea genotypes

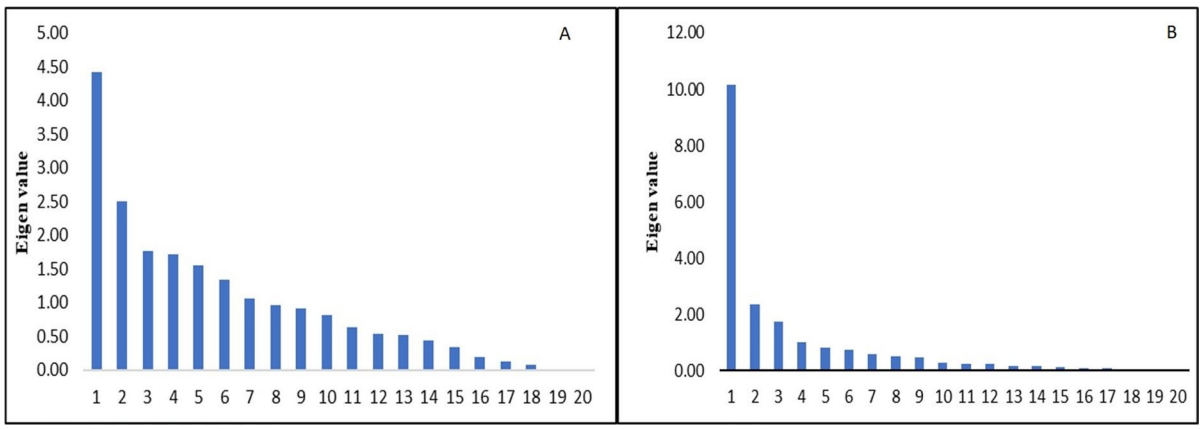

Fig. 8 Scree plot based on morphological, physiological, and biochemical traits. **A** Normal season. **B** heat stress season

conditions, three principal components contributed to 71.3% of the total variance with an eigenvalue greater than 1 (Table 6).

In normal conditions, the characters like PH, IL, RGD, and DM showed considerable positive contributions, while PL, NSP, and APW showed considerable negative contributions in PC1. In PC2, the diversity among genotypes was due to the negative contribution of RWC, GI, TS, IL, RSL, NPP, and YPP and the positive contribution of DFF under normal conditions. The PC3 showed variation among genotypes due to MSI, MDA, CAT, DFF, and RSL with positive and RGD with negative denominations. The variation in PC4 was due to the positive contribution of MSI and the negative contribution of RWC, CT, H_2O_2, SOD, TS, and DFF. In PC5, the traits that

Table 6 Eigenvectors and eigenvalues of first seven principal components (normal) and first three principal components (heat stress) of 20 traits of garden pea genotypes

Variable	Normal condition							Heat stress condition		
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC1	PC2	PC3
Eigenvalues	4.43	2.51	1.77	1.71	1.56	1.35	1.07	10.16	2.35	1.76
Variance %	22.10	12.50	8.80	8.60	7.80	6.80	5.30	50.80	11.70	8.80
Cumulative %	22.10	34.70	43.50	52.10	59.90	66.70	72.00	50.80	62.50	71.30
RWC	−0.014	**−0.303**	−0.003	**−0.365**	0.222	**0.336**	**−0.235**	**−0.25**	**−0.29**	0.09
CT	0.01	0.107	0.064	**−0.441**	**−0.249**	**−0.363**	−0.188	0.12	**0.18**	0.20
GI	0.16	**−0.232**	0.206	−0.214	**−0.237**	**0.306**	**−0.315**	**−0.23**	**−0.20**	−0.20
MSI	−0.072	0.034	**0.234**	**0.25**	**−0.446**	**0.217**	**−0.332**	**−0.24**	**−0.24**	0.09
MDA	0.021	0.145	**0.384**	−0.139	−0.143	**0.216**	**0.449**	**0.24**	**0.24**	−0.10
H_2O_2	0.187	−0.156	0.014	**−0.262**	0.101	**−0.408**	**−0.267**	**0.26**	**0.26**	−0.09
SOD	−0.112	0.048	−0.208	**−0.262**	**0.291**	0.101	**0.264**	**−0.20**	−0.14	**0.29**
CAT	−0.098	−0.2	**0.307**	0.067	**0.457**	0.151	−0.003	**−0.23**	**−0.35**	0.13
TS	−0.025	**−0.233**	−0.141	**−0.345**	−0.049	**0.315**	0.183	**−0.19**	−0.06	**−0.35**
PH	**0.406**	−0.141	−0.064	0.026	0.13	−0.175	0.072	**−0.24**	−0.11	0.10
IL	**0.288**	**−0.238**	0.095	0.055	0.136	**−0.29**	0.073	−0.13	**0.27**	0.21
DFF	0.195	**0.232**	**0.37**	**−0.355**	−0.063	−0.053	0.137	**−0.20**	**0.24**	**0.34**
RSL	−0.03	**−0.391**	**0.429**	0.188	0.161	−0.032	−0.073	**−0.24**	0.14	0.05
RGD	**0.262**	−0.093	**−0.458**	0.12	−0.026	**0.235**	−0.204	**−0.23**	**0.31**	0.17
NPP	0.184	**−0.433**	−0.001	0.125	**−0.293**	−0.083	**0.306**	**−0.24**	0.10	**−0.31**
PL	**−0.385**	−0.087	−0.014	−0.147	0.076	−0.099	−0.124	**−0.27**	**0.25**	−0.03
NSP	**−0.255**	−0.03	0.053	−0.019	0.005	−0.167	**−0.243**	**−0.24**	0.17	−0.18
APW	**−0.374**	−0.162	−0.143	−0.174	−0.146	−0.099	0.052	**−0.23**	**0.27**	**−0.25**
DM	**0.371**	0.103	−0.091	−0.186	−0.075	0.157	−0.07	**−0.23**	**0.29**	**0.26**
YPP	−0.175	**−0.418**	−0.135	−0.046	**−0.351**	−0.129	**0.268**	**−0.21**	0.10	**−0.45**

RWC Relative water content, *CT* Canopy temperature, *GI* Greenness index, *MSI* Membrane stability index, *MDA* Malondialdehyde, *H_2O_2* Hydrogen peroxide, *SOD* Superoxide dismutase, *CAT* Catalase, *TS* Total sugar, *PH* Plant height, *IL* Internode length, *DFF* Days to 50% flowering, *RSL* Reproductive stem length, *RGD* Reproductive growth days, *NPP* Number of pods per plant, *PL* Pod length, *NSP* Number of seeds per pod, *APW* Average pod weight, *DM* Days to maturity, *YPP* Yield per plant, Bold numeric digits significant values

contributed significantly to the variations were SOD and CAT, while traits with significant negative contributions were CT, GI, MSI, NPP, and YPP. The traits with significant positive contributions in PC6 and PC7 were RWC, GI, MSI, MDA, TS, RGD, MDA, SOD, NPP, and YPP, respectively. In contrast, the traits CT, H_2O_2, IL and RWC, GI, MSI, H_2O_2, and NSP showed considerable negative contributions to variation among genotypes in PC6 and PC7, respectively.

However, in heat stress conditions, three principal components had eigenvalue greater than 1, and the characters with the substantial positive contribution in PC1 were MDA and H_2O_2. In contrast, the traits with substantial negative contributions were RWC, GI, MSI, SOD, CAT, TS, PH, DFF, RSL, RGD, NPP, PL, NSP, APW, DM, and YPP. The diversity among genotypes in PC2 was due to CT, MDA, H_2O_2, IL, DFF, RGD, PL, APW, DM in the positive denomination and RWC, GI, MSI, and CAT in the negative denomination. The PC3 explained that variation among genotypes was due to the appreciable positive contribution of SOD, DFF, and DM traits and the negative contribution of TS, NPP, APW, and YPP (Table 6).

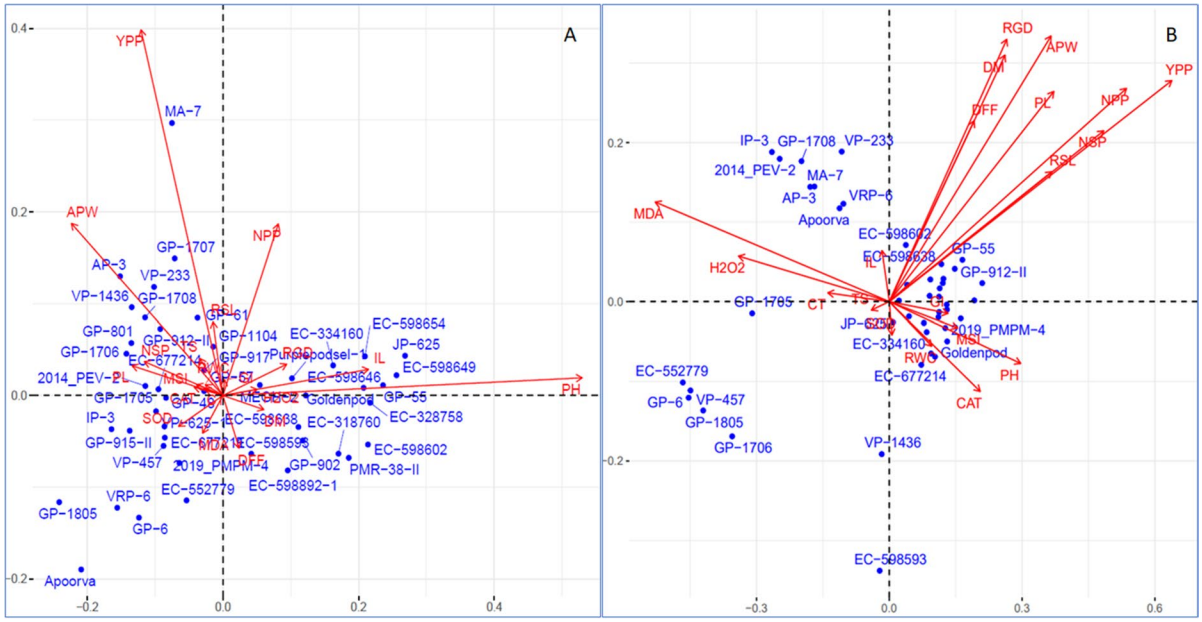

Fig. 9 Principal component analysis biplot of 45 garden pea genotypes for morpho-physiological and biochemical traits. **A** Biplot analysis for normal season. **B** Biplot analysis for heat stress season

Biplot analysis for morpho-physiological and biochemical traits

The mean data of 20 traits (morphological, physiological, and biochemical) were used to construct the biplot separately for normal and heat stress conditions to understand better the relationship between genotypes and variables (Fig. 9A and B, respectively). The biplot revealed three distinct clusters of genotypes for heat stress season. Almost all tolerant and moderately tolerant genotypes were positioned near each other in the first quadrant of PC1 in heat stress season and were aligned with most of the morphological and yield-related traits, indicating their positive association (Fig. 9B). Likewise, the physiological and biochemical traits like GI, CAT, MSI, RWC were positioned in the second quadrant of PC1. They showed an association with morphological traits in heat stress season. However, there was no such clustering of genotypes in the regular season.

It was evident from the figure that MDA, H_2O_2, and CT were negatively associated with most of the traits and showed maximum variability in the negative direction during heat stress season. Almost all susceptible genotypes were positioned on the negative side of PC1, along with MDA, H_2O_2, and CT. The susceptible genotypes EC-552779, GP-6, VP-457, GP-1805, and GP-1706 were positioned at an obtuse angle with yield-related traits. In contrast, most physiological and biochemical traits showed the least contribution in the regular season (Fig. 9A).

Discussion

The current study deals with forty-five genotypes: fifteen tolerant, fifteen moderately tolerant, and fifteen susceptible. The study was carried out to perceive the importance of physiological and biochemical traits in heat stress tolerance. High temperature during the reproductive stage could be achieved by delayed sowing, which restricts vegetative growth, and hastens crop maturity leading to poor yield (Reddy 2009). Plants undergoing high or low-temperature stress exhibit various adaptive mechanisms morphologically, physiologically, and biochemically. When

plants undergo stress, physiological change occurs first, followed by morphological change. Therefore, key physiological and biochemical traits imparting heat tolerance were analyzed between tolerant, moderately tolerant, and susceptible genotypes. The key physiological traits recorded in the study were relative water content, canopy temperature, and greenness index.

Relative water content was found to represent the water status of plant leaves and is affected by physiological characteristics (Kramer and Boyer 1995). An increase in leaf temperature in cool season legumes causes a decrease in relative water content, ultimately reducing crop photosynthetic rate (Farooq et al. 2009). Similarly, changes in photosynthetic parameters are good indicators of plant stress as photosynthesis is interconnected with plant growth and yield (Kocal et al. 2008; Tomaz et al. 2010). Of the numerous photosynthetic parameter, chlorophyll content is one of the parameters used to evaluate the degree of plant stress and is measured as greenness index by SPAD meter. In the current study, the average RWC and GI were found to be 27.58% and 26.01% lower in heat stress conditions than in normal conditions. The reduction was highest in susceptible genotypes (49.01%, 46.87%) than in moderately tolerant (22.84%, 26.58%) and tolerant genotypes (11.76, 8.04%). This reduction in photosynthetic parameters during heat stress conditions could be due to the irreversible damage of thylakoid membranes, chloroplast ultrastructure, and PSII reaction centers (Havaux 1993; Allakhverdiev et al. 2003; Wang et al. 2009; Chen et al. 2012). A similar reduction in photosynthesis pigment and chlorophyll was reported in chili heat stress research by Ghai et al. (2016), Kaur (2014). In our study, a significant decrease in RWC and GI was found in heat stress conditions, which suggests that these physiological traits could be potentially used to differentiate tolerant and susceptible genotypes. However, some tolerant genotypes (2019/PMPM-4, EC-677211, MEGH-2, GP-1104, and GP-912-II) showed a non-significant increase in greenness index during heat stress conditions. Weng et al. (2021) also reported that tolerant melon genotypes produced more chlorophyll content in the early stages of stress to resist humidity and heat stress. It was also observed that relative water content and greenness index was significantly correlated under normal (0.32*) and heat stress condition (0.65**). Additionally, these two traits showed a significant positive correlation with all the yield parameters and growth parameters except internode length. These findings imply that higher leaf water content and greenness index in heat stress conditions would enable tolerant genotypes to produce higher yields.

Cooler leaf or high leaf temperature depression was used as a criterion to improve heat tolerance in plants (Lawlor et al. 2002; Oshino et al. 2011; Zhang et al. 2004; Hedden and Thomas 2012). Leaf/canopy temperature depression, *i.e.*, the ability of plants to maintain low leaf/canopy temperature, is an important heat avoidance strategy in plants that helps maintain better assimilation rates by enhancing stomatal conductance and protecting chloroplasts (Oshino et al. 2011). In our study, canopy temperature was used to identify cooler canopy, which is used to differentiate tolerant and susceptible genotypes. Compared to the regular season, the tolerant and susceptible genotypes showed 53.40 and 62.81% increased canopy temperature in the heat stress season. The tolerant genotypes show a lower increase in CT than susceptible genotypes. The ability of tolerant genotypes to maintain lower canopy temperatures may be due to transpirational cooling (Singh et al. 2007). It was also reported that genotypes with minimum canopy temperature use more available soil moisture to cool the canopy by transpiration (Lepekhov 2022). It was observed that canopy temperature is negatively correlated with GI, NPP, and YPP and positively correlated with MDA and H_2O_2. Therefore, genotypes with lower canopy temperatures had to be selected for heat tolerance study.

It is a fact that various abiotic stresses like high temperature, low temperature, salinity, and drought lead to the accumulation of reactive oxygen species (Mittler 2002; Rivero et al. 2004, 2007; Gill and Tuteja 2010). The reactive oxygen species, namely hydroxyl radical, singlet oxygen, superoxide anion, and hydrogen peroxide, are produced faster during high temperatures, affecting cellular membranes and cellular activities (Reddy et al. 2004). Such damage to cell membranes enhances their permeability and decreases their membrane stability. Hence, it has been used to measure heat tolerance in many plants, including potatoes and tomatoes (Chen et al. 1982) and cowpea (Ismail and Hall 1999). Our study used the membrane stability index (MSI) to determine cell membrane thermostability. It was previously used to

evaluate heat tolerance in crops like brassica (Ram et al. 2014) and cowpea (Ismail and Hall 1999). MSI gives an idea about the amount of electrolyte leakage from plant cells where lower electrolyte leakage indicates higher membrane stability and vice versa. In the current study, MSI has found to be decreased by 44.51% during heat stress conditions, and the reduction was found to be highest in susceptible genotypes (64.96%) while it was lowest in tolerant genotypes (24.75%). Similar reports of low electrolyte leakage in tolerant genotypes had been reported by Pastori and Trippi (1992) and Kraus et al. (1995), and Cheng et al. (2009) in tomatoes. The tolerant genotypes maintain high membrane stability in this study, even under heat stress. Thus, it was suggested to be used as a selection criterion for thermotolerance as the genetic makeup of thermotolerant genotypes tends to increase the stability of membranes (Sikder et al. 2001; Ashraf and Foolad 2007; Dhanda and Munjal 2006). These findings enable them to produce good plant growth and yield since yield parameters like NPP (0.52**), PL (0.48**), NSP (0.38**), APW (0.38**), and YPP (0.42**) were found to be positively correlated with MSI. A similar significant relationship was observed between cell membrane stability and yield in sorghum (Sullivan and Ross 1979).

The damage to the cellular membrane leads to the accumulation of malondialdehyde, the product of lipid peroxidation (Wahid et al. 2007). The study shows that higher temperatures increase MDA content by 4.71 times than normal conditions. Weng et al. (2021) also reported increased MDA content in melon under high temperature and humidity stress. Further, the highest increase was found in susceptible genotypes (8.55 times) than in tolerant genotypes (2.95) during heat stress conditions which imply that higher MDA causes greater oxidative damage in susceptible genotypes, and tolerant genotypes tend to show less MDA production, thus undergoing less oxidative damage. Liu et al. (2013, 2017) also confirmed that thermotolerant rice cultivars with high cell membrane stability and low malondialdehyde content showed less cell membrane damage.

Hydrogen peroxide generation from the electron transport chain is a normal physiological process (Gill and Tuteja 2010). However, high-temperature stress may induce oxidative stress by overproduction of activated oxygen species like singlet oxygen ($^1O_2$), superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-), causing cellular injury (Liu and Huang 2000). Similarly, in the present study, heat stress season showed increased H_2O_2 content than a normal season, and susceptible genotypes showed higher H_2O_2 content than tolerant and moderately tolerant genotypes. A similar accumulation of hydrogen peroxide content was reported in mustard seedlings (Dat et al. 1998) and fava beans (Siddiqui et al. 2015) after experiencing high-temperature stress. Further, malondialdehyde and hydrogen peroxide were positively correlated with each other (0.74**) and canopy temperature. In contrast, they were negatively correlated with other physiological, biochemical, and other morphological quantitative traits except for internode length. Thus, it was confirmed from the study that increased oxidative stress by reactive oxygen species tends to affect yield parameters.

Generally, plants have internal defense mechanisms manifested with antioxidant enzymes like superoxide dismutase and catalase to scavenge activated oxygen species. Superoxide dismutase (SOD) scavenges the superoxide radical (O^{2-}), resulting in the production of hydrogen peroxide (H_2O_2), which in turn is removed by the enzyme catalase. The current study showed increased activity of SOD in both tolerant (69.61%) and susceptible genotypes (33.70%) during heat stress conditions compared to the regular season. Similar responses of increase in SOD were observed in tomato and watermelon plants by Rivero et al. (2001). However, the increase was lower in susceptible genotypes than intolerant and moderately tolerant ones. This response of increased SOD activity in tolerant plants could be understood by the fact that higher SOD enzymes could help them to scavenge more superoxide radicals than susceptible genotypes and make them more tolerant (Siddiqui et al. 2015).

As mentioned earlier, ROS like H_2O_2 were found to accumulate during high-temperature stress and are scavenged by the enzyme catalase. The enhanced catalase activity indicates fewer toxic effects of H_2O_2 (Sudhakar et al. 2001). In this study, the catalase activity was increased 8.89, 5.95, and 2.65- times during heat stress conditions intolerant, moderately tolerant, and susceptible genotypes, respectively. A similar increase in catalase activity in heat stress conditions was reported by Fu and Huang (2001). The highest catalase activity in tolerant plants is associated with accelerated H_2O_2 scavenging, thus providing higher tolerance to heat stress, as suggested by

Liu and Huang (2000), Sairam et al. (2000), and Tian et al. (2012). It was also reported that the upregulation of CAT in cucumber and SOD in potato turn on antioxidant defense in thermotolerant genotypes (Galsurker et al. 2018; Kim et al. 2010; Tang et al. 2006; Wang et al. 2006). It was reported in wheat genotypes that antioxidants like SOD and CAT activities were correlated with their thermotolerance capacity and less oxidative damage (Sairam and Tyagi 2004). Likewise, decreased antioxidant activity in susceptible genotypes leads to higher levels of activated oxygen species which in turn causes more injury to plants (Fadzillah et al. 1996). From correlation research, it was found that antioxidant activities (SOD, CAT) were positively correlated with NPP (0.32*, 0.38*), PL (0.43**, 0.43**), and NSP (0.33*, 0.33*). However, SOD was positively correlated with APW (0.30*), while CAT was positively correlated with YPP (0.30*). Since most of the yield-related parameters were positively correlated with antioxidant activities and negatively correlated with MDA and H_2O_2, it is evident that increased antioxidant activity decreases oxidative stress and increases plant growth and yield.

Total sugar content was also estimated, and it was found that the average total sugar percent exhibited a 9.34% reduction in heat stress season compared to normal conditions, and the reduction was lowest in tolerant genotypes (5.67%) than in moderately tolerant (11%) and susceptible genotypes (11.75%). A similar report of decreased sugar content has been found in French beans due to moderate osmotic stress (Sassi-Aydi et al. 2014). This decrease could be due to osmotic stress-induced photosynthesis reduction leading to photo assimilates shortage (Hussin et al. 2013; Tejera et al. 2006). However, three tolerant genotypes showed a non-significant increase in total sugar content in heat stress conditions. In comparison, two tolerant genotypes (Purple pod sel-1 and GP-55) showed a significant increase compared to normal conditions, which is in line with the findings of Arunkumar et al. (2012) in chickpeas, where he reported that the total sugar content reduced was less in tolerant genotypes than susceptible genotypes, and under high-temperature stress tolerant genotype showed increased total sugar (16.2%) content while in others it declined. Also, total sugar was positively correlated with all the yield parameters.

The analysis of variance (Tables 4 and 5) showed that treatments have significant differences among them. In heat stress season, cluster analysis classified the susceptible and tolerant genotypes into distinct clusters according to their physiological and biochemical basis of tolerance which was further confirmed by biplot analysis, where the susceptible and tolerant genotypes were placed on the negative and positive sides of PC1, respectively (Fig. 9B). Biplot and correlation analysis confirmed that MDA, H_2O_2, and CT were negatively associated with most of the yield contributing traits and genotypes with higher MDA, H_2O_2, and CT found to be susceptible to heat stress. It also validated that yield parameters were more highly affected by physiological and biochemical changes under heat stress than in normal conditions.

Conclusion

The comprehensive analysis of garden pea genotypes' physiological and biochemical response under heat stress showed that tolerant genotypes tend to undergo better physiological and biochemical adaptations in response to heat stress. All the fifteen tolerant genotypes that survived high temperatures of > 38 °C were able to protect against chlorophyll degradation (GI) and maintain cooler canopy temperature (CT) and high leaf water content (RWC). Further, they maintained good cell membrane stability causing low electrolyte leakage in these genotypes and lower accumulation of MDA and H_2O_2. The enhanced antioxidant enzyme activity (SOD and CAT) in tolerant genotypes reduced oxidative stress by scavenging reactive oxygen species generated during high-temperature conditions. Estimating the correlation coefficient showed that physiological and biochemical traits were very important in determining the yield parameters of plants under heat-stress conditions, which was further confirmed by principal component analysis and biplot analysis. Our results demonstrated that heat tolerance in garden pea genotypes was closely associated with key physiological and biochemical traits like relative water content, greenness index, membrane stability index, malondialdehyde, hydrogen peroxide, superoxide dismutase, and catalase.

Further, these traits could be used as an identification index for heat tolerance in other cool-season legumes and aid in heat-tolerant breeding programs.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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