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RESEARCH ARTICLE

Indexing heat stress-induced changes in Indian mustard germplasm using biochemical traits, stress tolerance indices and seed morphological features

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Abstract

Heat stress in *Brassica* is a great threat to its productivity and it is a major abiotic challenge in the current scenario of changing global climatic conditions. Oil production from *Brassica* is the second largest production after soybean, globally. In this study, 32 Indian mustard accessions were evaluated (post-anthesis stage) under heat stress in field conditions during the *rabi* season of 2019-20, by being exposed to 3 different growing conditions i.e., early, optimum and late sowing. Biochemical assays were performed at the post-anthesis stage to analyze the best-performing accessions under heat stress during the *rabi* season of 2021-22. Seed morphological parameters and stress indices (MDA, proline content) were used to find high-performing accessions. The results showed a significant correlation between yield under stress and STI (stress tolerance index), YI (yield index), SSPI (stress susceptibility percent index) and MP (mean productivity), indicating the utility of these indices in the selection of heat-tolerant and high-yielding lines. Based on the morphological, seed yield and quality parameters, accessions IC280920, IC401575, IC426400, IC491509 and IC570301 were found tolerant to heat stress as compared to other accessions. Therefore, the selected accessions can be utilized to improve crop *Brassica,* especially under heat stress.

Keywords

biochemical assays; *Brassica*; climate change; heat stress; stress indices

Introduction

Plants are exposed to a variety of environmental challenges throughout their lifespan, which limit their growth and development and pose serious risks to global food security (1, 2). The constantly rising temperature due to global warming has a significant impact on plant growth, development and productivity, among other dynamic abiotic elements of the environment (3). The average temperature is predicted to increase by 2 °C to 4 °C by 2050 (4). Temperature variation has an impact on the grain and seed production of annual crops. Indian mustard (*Brassica juncea* L.) is the most essential edible oilseed crop in the world. India, which produces ~11 % of the world's mustard, is the third-largest producer after China and Canada (5). It is predominantly grown in tropical and sub-tropical areas during the *rabi* season under rainfed and irrigated, early and late conditions (6). Mustard oil contains a balanced ratio of monounsaturated and polyunsaturated fatty acids. *Brassica* is vulnerable to many abiotic stresses, such as heat and drought, due to globally changing climatic conditions, which ultimately affect yield (7). Mild fluctuations in temperature and humidity during the seedling stage may significantly impact all growth phases (8). Among *Brassica*, Indian mustard covers more than 80 % of the land in India, because it is slightly more resilient to biotic and abiotic stresses than other *Brassica* species. High temperature and low moisture levels during the reproductive and maturity period, result in significant yield and quality loss (9, 10). High temperature (about 40 ° C) harms embryonic cells. The effects of temperature stress on plant morphology include stunted root and shoot growth, leaf scorching/abscission, stem burning and fruit discoloration, which eventually reduce crop output (11).

Heat stress has the potential to decouple metabolic and enzyme processes that build up undesirable and detrimental reactive oxygen species (ROS), which are the source of oxidative stress (12). ROS often causes cellular damage by oxidizing different biomolecules (13, 14). Heat stress changes in cells can be measured using different biochemical assays. Malondialdehyde (MDA) is used as a potential biomarker for lipid peroxidation because it results from the peroxidation of unsaturated fatty acids in phospholipids, which damages cell membranes. Cell membranes contain unsaturated lipids, the ROS produced during high-temperature stress reacts with them and causes lipid peroxidation, which results in the accumulation of MDA (15). *B. juncea*, tolerant genotypes show reduced MDA content under high temperature stress conditions, then sensitive genotypes (16). Proline (another biochemical marker for assessing stress) is an osmoprotectant, that protects the strength of cellular membranes, helps the cytoplasm's osmotic balance, protects protein structures and enzyme activities against denaturation, and scavenges hydroxyl free radicals (17). Plants accumulate proline to boost their tolerance against abiotic stress and to maintain high relative water contents and osmotic potential (18).

The present research study was aimed at exploring the effects of heat stress on Indian mustard germplasm. The accessions were screened for high yield under heat stress conditions during the *rabi* 2019-20 (19) and heat stress tolerant accessions were validated under heat stress in fields during the *rabi* 2021-22 at the flowering stage using oxidative stress indicators and seed morphological features. This information is important to identify suitable germplasm accessions as donors for the improvement of *Brassica* through a breeding program.

Materials and Methods

Plant material and experimental site

Thirty-two accessions of Indian mustard germplasm used in the study, procured from the ICAR-National Bureau of Plant Genetic Resources, New Delhi, India, are shown in Table 1. They were planted at 3 different sowing dates (year 2019-20, *rabi* season), i.e., early (last week of September, S1), optimum (third week of October, S2) and late sown (second week of November, S3), to study the effect of terminal heat stress on the *Brassica* crop (19). The experiment was laid out in a factorial randomized block design with 3 replications at the research farm of the Amity Institute of Organic Agriculture, Amity University, Noida, India. The recommended package of practices was followed to raise a good crop of mustard. Based on yield attributing traits (number of siliquas per plant, siliqua length (cm), number of seeds per siliqua, 1000 seed weight or test weight (g) and seed yield per plant (g)), high-yielding tolerant accessions from the previous study was validated for heat stress under field conditions during the *rabi* 2021-22 using biochemical assays (MDA and Proline content) and seed morphological features. From each plot, 5 healthy plants were picked at random and tagged during the vegetative stage to record all morphological data and collected samples for biochemical analysis at the reproductive stage.

Table 1. List of Indian mustard germplasm used in this study.

Sr. No	Accessions	Sr. No	Accessions
1	IC261687	17	IC426385
2	IC267695	18	IC426388
3	IC267699	19	IC426400
4	IC267705	20	IC426403
5	IC280907	21	IC447833
6	IC280920	22	IC491044
7	IC296688	23	IC491128
8	IC296702	24	IC491161
9	IC296703	25	IC491263
10	IC296732	26	IC491415
11	IC305130	27	IC491429
12	IC347855	28	IC491509
13	IC353575	29	IC570279
14	IC362912	30	IC570301
15	IC385783	31	IC571686
16	IC401575	32	IC589669

Weather conditions and heat stress treatment

Weather information on temperature (°C, maximum and minimum) and relative humidity (%) during crop season was recorded for the years 2019-20 and 2021-22. The average weather data was considered for further studies. The weather was typical for the environment in North India. Only changes in aerial temperature were taken into consideration because trials were carried out in well-irrigated conditions. There was a gradual drop in relative humidity from February to April due to a sudden rise in maximum temperature starting from 2nd week of February (Fig. 1). Thus, heat stress occurred in late-sown accessions from anthesis to the grain filling stage.

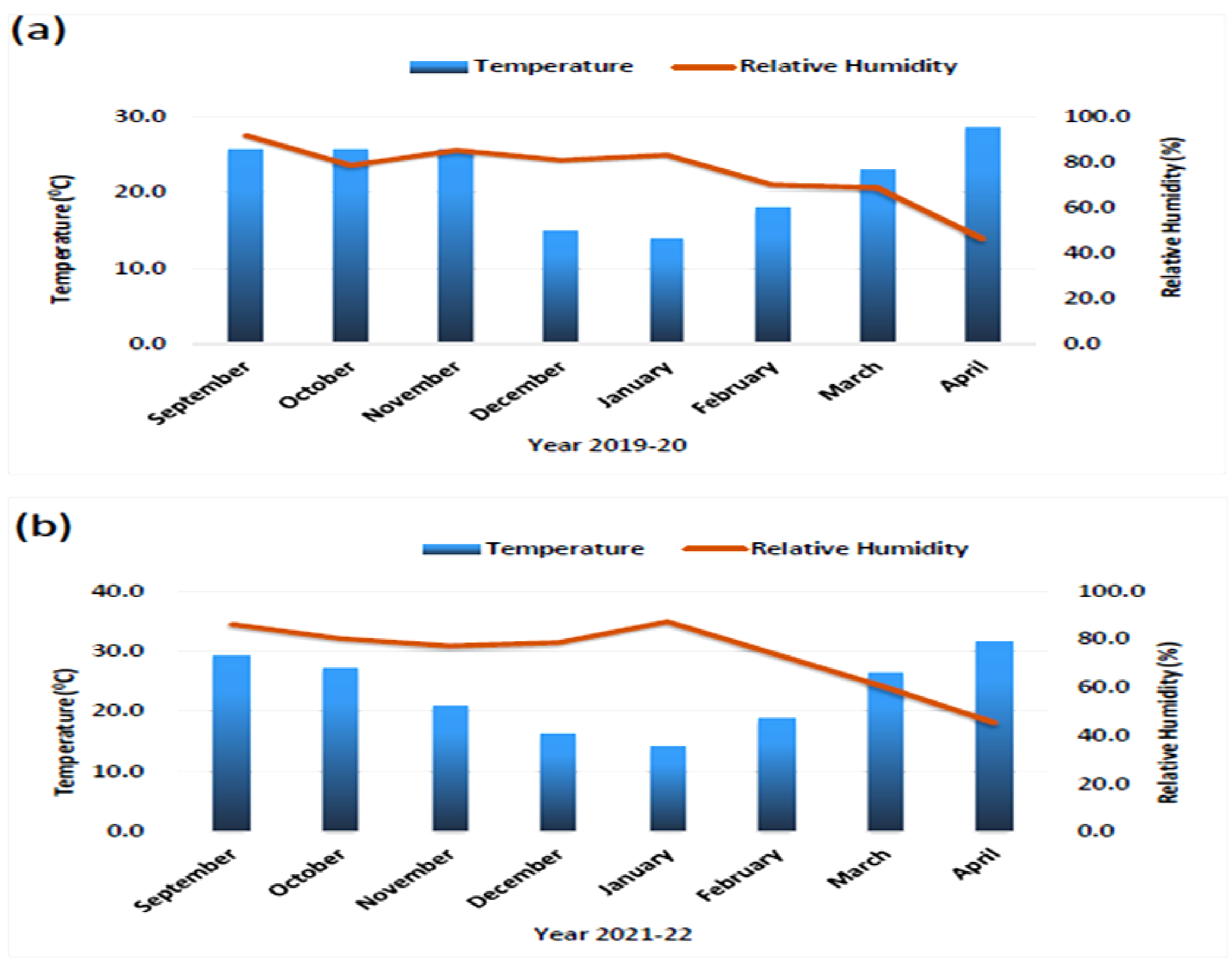


Fig. 1. Weather data for the crop growing season of Indian mustard germplasm during *rabi* season (**a**) 2019-20 and (**b**) 2021-22.

Biochemical assays and seed morphological parameters for measuring heat stress tolerance in Brassica

Malondialdehyde (MDA) concentration was measured in *Brassica* plants at the 50 % flowering stage according to Heath and Packer's method (1968) (20). Proline content was evaluated in leaf samples from control and treated plants to evaluate the levels of accumulated proline under heat stress conditions (21). *Brassica* seeds per germplasm were taken to determine the 1000-seed weight (TSW, in grams). *Brassica* seeds were scanned in an Epson scanner to examine various seed features, such as seed area, seed length, seed breadth, seed perimeter and seed roundness. Further, grain analysis software was used to retrieve the seed-related parameters. A stereo zoom microscope ACUSZM-745T (Acucal, India), was used to capture good-quality seed images.

Statistical data calculation and analysis

The grain yield (g) of each *Brassica* accession was measured by harvesting the mature crop individually from each plot. All stress tolerance indices were computed using the grain yield (Yp and Ys) of each accession and the mean yield (Xp and Xs) of all accessions under normal and late sowing conditions, respectively, using the following mentioned in 1 to 9 equations:

Stress tolerance (TOL) = $Yp - Ys$ (22)........................(Eqn. 1)

Stress tolerance index (STI) = $(Yp \times Ys)/Xp^2$ (23)......(Eqn. 2)

Stress susceptibility % index (SSPI) = $Yp - Ys/\ 2(Xp) \times 100$ (24) ....(Eqn. 3)

Yield index (YI) = Ys/Xs (25) ....(Eqn. 4)

Yield stability index (YSI) = Ys/Yp (26) ....(Eqn. 5)

Relative stress index (RSI) = $(Yp/Ys)/(Xs/Xp)$ (27) .....(Eqn. 6)

Mean productivity (MP) = $(Yp + Ys)/2$ (22) ....(Eqn. 7)

Percent yield Reduction (PYR) = $(Yp{-}Ys)/Yp \times 100$ (28)...(Eqn. 8)

Stress Susceptibility Index (SSI) = $1- (Ys/Yp)/1- (Xs/Xp)$ (29) ....(Eqn. 9)

where, Yp and Ys represent the yield performance of the various accessions and Xp and Xs represent the average yield of all the accessions under normal and heat stress conditions respectively.

The stress indices were calculated using Microsoft Excel. We used Minitab (https://www.minitab.com/en-us/) software for hierarchical clustering and correlograms. The data was subjected to various statistical analyses, such as descriptive statistics analysis and analysis of variance

(ANOVA two-way) using OPSTAT software (http://14.139.232.166/opstat/).

Results and Discussion

To understand the heat stress impact on *Brassica*, it is necessary to establish appropriate sowing times under field conditions that can be precisely controlled and repeated. Indian mustard is one of the most significant oilseed crops, which accounts for a considerable portion of the *Brassica* family's total acreage. In our previous study, we screened the *Brassica* accessions based on yield-attributing traits under heat stress in field conditions (19). In this study, the filtered high-yielding accessions were validated for heat stress tolerance using biochemical assays and seed morphological features. Here, at 3 different sowing dates, S1 (early sown), S2 (optimum sown) and S3 (late sown), the effect of the heat stress and their interaction with germplasm at the flowering stage showed high variability in mustard accessions for all the traits based on biochemical assays and seed morphological characteristics. The anthesis and post-anthesis stages of late-sown accessions were negatively impacted by a rise in temperatures above 25 °C in mid-February.

The constantly increasing ambient temperature is the most harmful for *Brassica*. High temperatures (> 28–30 °C) can significantly reduce the weight of ripe mustard seeds during the anthesis and post-anthesis stages, hence reducing yields. The most temperature-sensitive stages of *Brassica* crops are seedling and flowering (30).

MDA and Proline content

MDA levels in the controlled environment ranged from 0.20 nmole/g FW (IC296688) to 0.49 nmole/g FW (IC362912). According to Fig. 2a, the range under S2 and S3 stressed conditions was 0.31 nmole/g FW (IC447833) to 0.65 nmole/g FW (IC362912) and 0.53 nmole/g FW (IC296702) to 1.04 nmole/g FW (IC362912) respectively. The membrane damage due to heat stress, as measured in terms of MDA content, was observed in all the accessions. The MDA content was higher in the case of accessions, viz., IC362912, IC570301, IC426385, IC267705, IC296688 and IC385783 and lowest in the case of IC296702, IC280920, IC261687, IC401575, IC296732, IC491263 and IC491415 under high-temperature stress of 36 °C. Lipid peroxidation, assessed by malondialdehyde (MDA) in stressed conditions, was 4.66 (MDA g (-1) f. wt. of tissue) in tolerant genotypes, 7.44 (MDA g (-1) f. wt. of tissue) in susceptible genotypes and correlated significantly (r = 0.563) with electrolyte leakage (16). We observed more MDA content in *Brassica* accessions under heat stress, but the content was significantly less in heat-stress-tolerant accessions. The MDA level was 1.58 (TPM1) to 8.47 (JM-2) fold greater than the corresponding controls (31). According to reports, *B. juncea* tolerant genotypes accumulated less MDA under high temperatures, indicating less oxidative damage, whereas sensitive genotypes showed higher levels of damage (16). The range of MDA in our study for the heat stress condition is 0.53 to 1.04 μmol g^{-1} FW. MDA buildup caused by heat stress was found and measured in various crops during the seedling stage wheat (32) and *Brassica* (16). Under heat

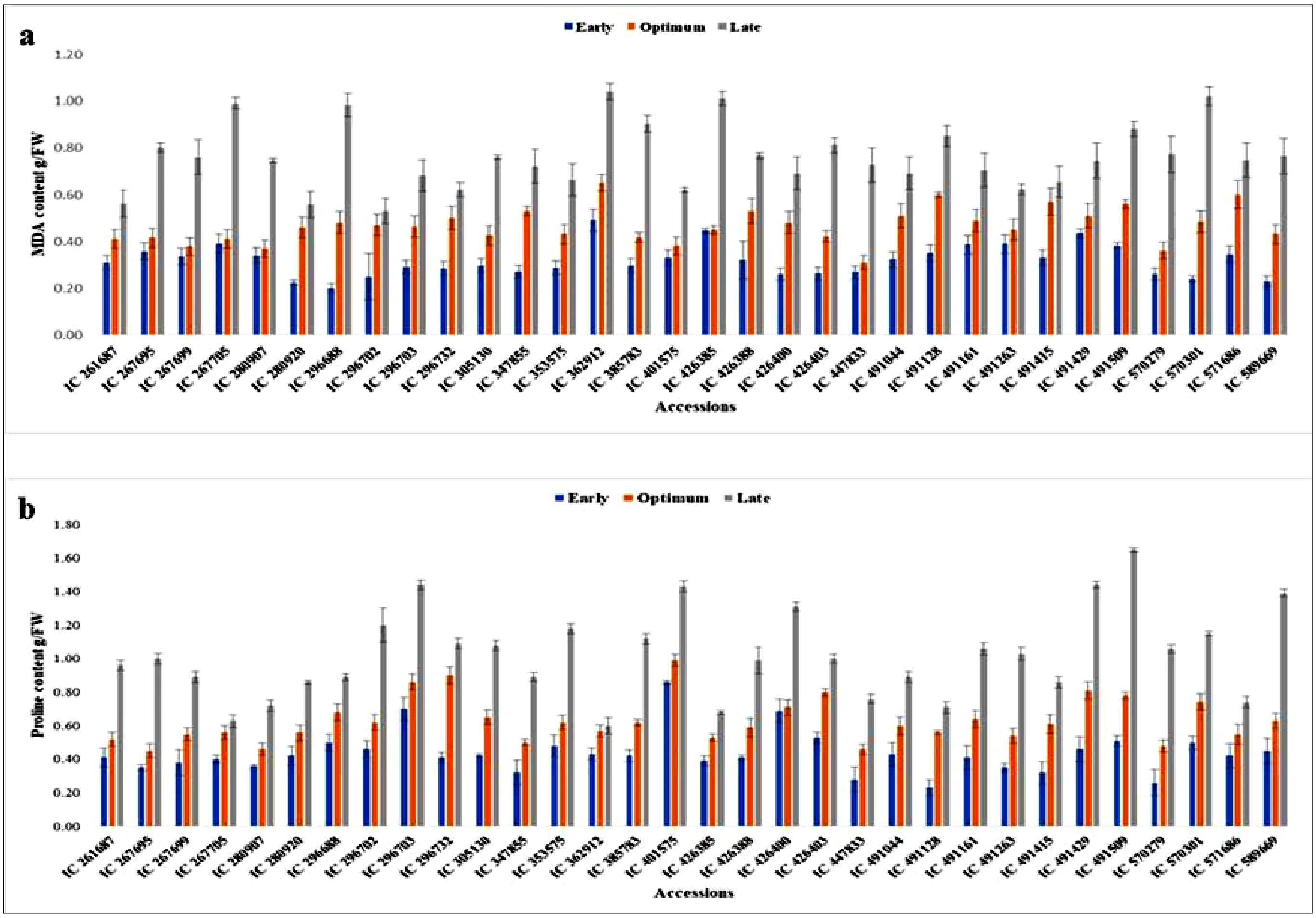


Fig. 2. Evaluation of Indian mustard germplasm under heat stress conditions based on biochemical assays. (**a**) MDA content and (**b**) Proline content .

stress, the cell metabolism is severely affected, resulting in membrane damage and the accumulation of ROS. Using antioxidant enzymes like ascorbate peroxidase (APX), peroxidase (POX), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), polyphenol oxidase (PPO) and metabolites like glutathione (GSH), ascorbic acid (AsA), tocopherol and carotenoids, plants protect their cells and sub-cellar systems from the damaging effects of ROS (31). Proline content showed a significant increase in the plants under high-temperature stress conditions. Proline content under early sown conditions in this experiment ranged from 0.23 µg/g FW (IC491128) to 0.86 µg/g FW (IC401575) and in timely sown it ranged from 0.45 µg/g FW (IC267695) to 0.99 (IC401575) µg/g FW. According to Fig. 2b, the range for the temperature-stressed plants was 0.60 µg/g FW (IC362912) to 1.65 µg/g FW (IC491509). There was no significant change in proline content in accessions.

In the present study, accessions IC491509, IC296703, IC491429, IC401575, IC589669, IC426400, IC296702, IC353575, IC570301 and IC385783 showed a high increase in proline content under heat stress conditions, while accessions IC362912, IC267705, IC426385, IC491128 and IC280907 showed the lowest value of proline content. The increase in proline content indicates that these *Brassica* accessions are capable of adapting to heat stress. Accumulation of osmoprotectants such as proline in the cell cytoplasm is also an adaptation against heat stress conditions (33). Here, we observed significantly higher proline content in thermotolerant accessions as compared to thermosensitive accessions. So, our study is in agreement with the previous reports (34). Therefore, proline can be used as a reliable indicator of heat stress imposed on *Brassica* plants.

The estimation of proline aids in the selection of genotypes that are heat tolerant (7). Proline accumulates more in genotypes that are thermotolerant than in genotypes that are thermosensitive, with a significant difference of 36.8 % to 28.1 % in RGN 368 and RH-0749 (35). We observed high proline content in accessions IC296702, IC296703, IC353575, IC385783, IC401575, IC426400, IC491429, IC491509, IC570301 and IC589669 under heat stress conditions. A recent study also classified mustard accessions based on proline content and found that high proline content was related to a group of thermotolerant cultivars (PM-21, PM-22 and PM-30) (34). So, our study was in concurrence with this report. Further, deep understanding can be achieved concerning *Brassica*-heat stress interaction by combining multiple 'omics' strategies.

Effect of heat stress on seed morphological traits in Indian mustard germplasm

The descriptive statistics of seed yield and seed morphological traits with analysis of variance (ANOVA) for different sowing dates are presented in Tables 2 and 3. For seed morphological features and 100-seed weight, the ANOVA findings revealed statistically significant genotypic variation in mustard accessions (p ≤0.05) for seed parameters such as seed area, length, breadth, perimeter, diameter

Table 2. The mean, standard error (± SE), minimum, maximum and variance components observed for Indian mustard germplasm based on seed yield and seed morphological features.

S1 (Early Sown)						
Parameters	**Seed Yield (kg/ha)**	**Area (mm^2)**	**Length (mm)**	**Breadth (mm)**	**Perimeter (mm)**	**Roundness**
Min.	364	1.3	1.4	1.2	4.3	0.6
Max.	2201	4.4	2.6	2.3	7.9	0.9
Mean	1453	3.0	2.1	1.8	6.5	0.8
SEm (±)	95.8	0.14	0.06	0.05	0.16	0.01
SD	542	0.81	0.33	0.26	0.93	0.07
CV	37.3	27.1	15.8	14.1	14.3	8.1
S2 (Timely Sown)						
Min.	294	2.1	1.7	1.5	5.4	0.7
Max.	2201	4.2	2.5	2.2	7.8	0.9
Mean	1477	3.1	2.2	1.9	6.6	0.8
SEm (±)	101	0.11	0.04	0.04	0.12	0.01
SD	572	0.65	0.21	0.20	0.68	0.05
CV	38.8	20.6	9.9	10.9	10.2	6.1
S3 (Late Sown)						
Min.	177	1.8	1.7	1.4	5.0	0.7
Max.	837	4.0	2.6	2.1	7.7	0.9
Mean	527	2.9	2.1	1.8	6.4	0.8
SEm (±)	30.5	0.11	0.04	0.04	0.12	0.01
SD	172	0.63	0.23	0.20	0.70	0.04
CV	32.7	22.0	10.9	11.3	11.1	4.7

Min. =Minimum; **Max**. =Maximum; **SEm**= Standard error of mean; **SD**= Standard deviation; **CV**= Coefficient of Variance.

Table 3. ANOVA shows the effects of different sowing dates on seed yield and seed morphological features in Indian mustard germplasm.

Source of Variation	df	Seed Yield (kg/ha)	Area (mm²)	Length (mm)	Breadth (mm)	Perimeter (mm)	Roundness
Sowing Dates (S)	2	28120733.3	1.74	0.11	0.24	1.64	0.016
Accessions (A)	31	1549066.23	2.71	0.35	0.28	3.39	0.011
Accessions X Sowing dates	62	201412.05	0.85	0.14	0.09	1.02	0.007
Error	190	3291.19	0.17	0.03	0.02	0.22	0.004

df= degree of freedom Mean square values presented in the table and means significant at the 0.05.

and roundness. The analysis of variance revealed significant mean squares of accessions for area, length, breadth, perimeter and roundness, demonstrating substantial differences among the germplasms in all the sowing conditions. Significant mean squares for all studied characteristics revealed variations in heat stress levels and their effect on these characters. The interaction effects of sowing dates and germplasm accessions were significant for all variables, revealing a distinct pattern of variances among the germplasms at various sowing times.

We phenotype seed morphological features from all the accessions at 3 sowing dates. Values for area, length, breadth, perimeter and roundness were averaged and compared for each sowing time (Fig. 3a to Fig. 3e). The individual seed area was recorded for all the sowing dates. For early sowing, the area range was 1.3 to 4.4 mm^2. The accessions IC426388 and IC267695 (4.4 mm^2) showed maximum area and the minimum value was found in accession IC570279 (1.3 mm^2). For optimum sowing conditions, the range of the area was between 2.1 to 4.2 mm^2, with a maximum for IC491128 and IC296688 (4.2 mm^2) and the least for IC570279 (2.1 mm^2). In high temperature stressed conditions, the accessions IC296732 and IC571686 showed the highest area of 4.0 mm^2, the accession IC589669 showed the lowest seed area value of 1.8 mm^2 (Fig. 3a). The average seed was measured between 1.2 and 2.3 mm in breadth and 1.4 to 2.6 mm in length in early sowing. In optimum sowing, the range of length and breadth was from 1.7 to 2.5 mm and 1.5 to 2.2 mm, respectively. In the heat-stressed condition, the average size of the mustard seed ranged from 1.7 to 2.6 mm in length and 1.4 to 2.1 mm in breadth. In early sowing conditions, accession IC426388 and IC267695 showed the highest length and breadth of 2.6 and 2.3 mm respectively. In late sowing conditions, the length (2.6 mm) and breadth (2.1 mm) were highest in accession IC296732 (Fig. 3b and Fig. 3c). In early sowing, accessions IC267695 and IC426388 had the largest perimeter (7.9 mm), whereas IC570279 seeds had the

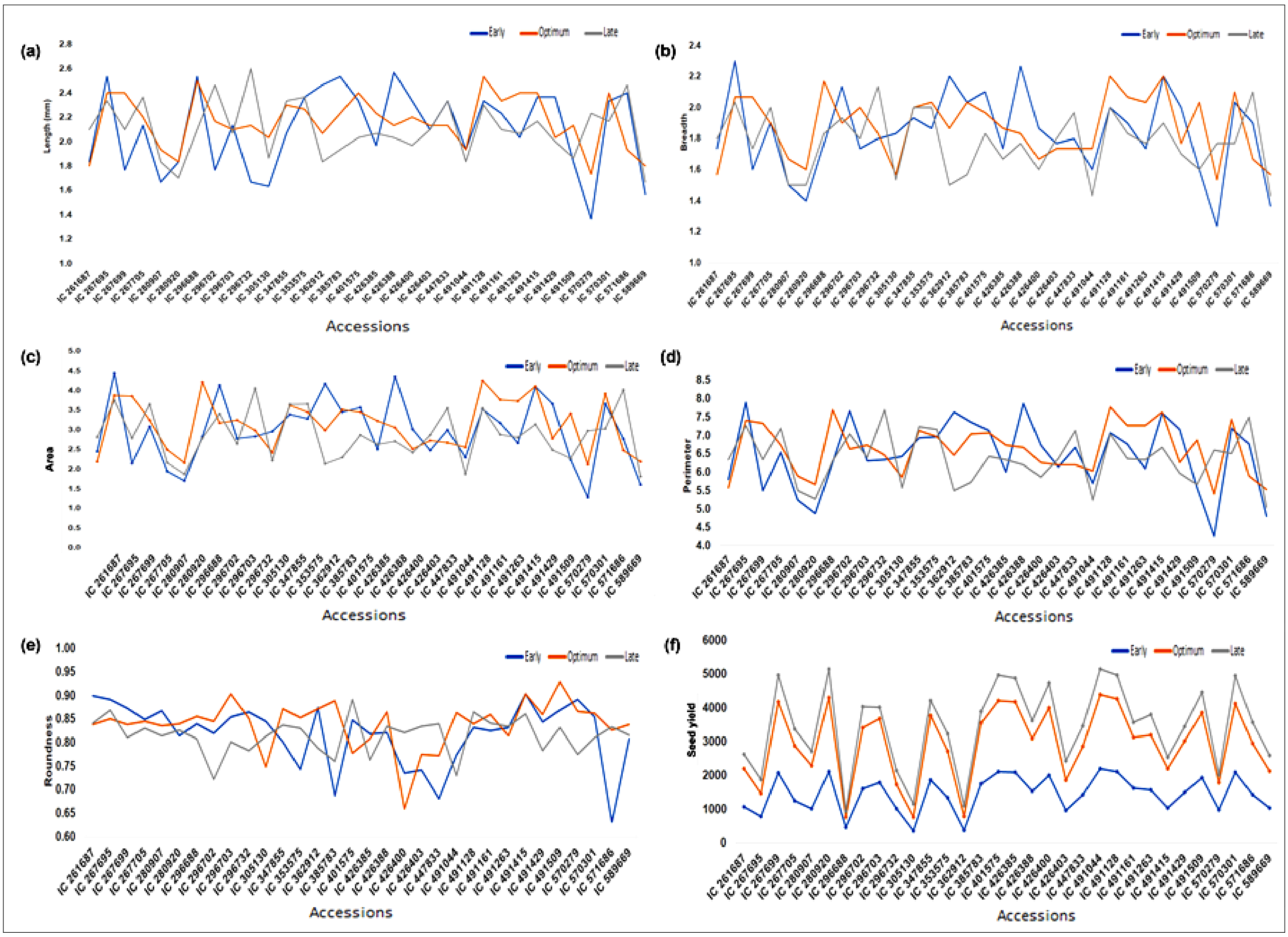


Fig. 3. Effect of heat stress on Indian mustard germplasm under three different sowing conditions. (**a**) Seed length (mm), (**b**) Seed breadth (mm), (**c**) Seed area (mm²), (**d**) Seed perimeter (mm), (**e**) Seed roundness and (**f**) Seed yield (kg/ha).

smallest (4.3 mm). For optimum sowing, the value of this perimeter ranged from 5.4 to 7.8 mm; the highest value was found to be IC491128 (7.8 mm) and the lowest in accession IC570279 (5.4 mm). The range of values for the perimeter for late sowing was 5 to 7.7 mm; the highest perimeter was observed in IC296732 (7.7 mm) and the lowest in IC589669 (5.0 mm). The roundness of the seeds was the geometric trait that most significantly differentiated the samples under study. For all the sowing, the average values of seed roundness varied from 0.63 to 0.93. At early sowing, accession IC491415 showed the highest value (0.90) and IC571686 showed the lowest value (0.63). The accession IC491509 recorded the highest value of 0.93 and IC426400 recorded the lowest value (0.66) for roundness in optimum sowing. For late sowing, the maximum value was observed in IC401575 (0.89) and the minimum value was observed in IC296702 (0.72). Relatively higher seed size and shape were observed under optimal conditions than late. This might be due to optimum climatic conditions during the flowering, maturity and harvesting stages of the *Brassica* life cycle. Late-sown crops matured early with less pod filling and improper seed development. Seeds obtained from these sowings had relatively low moisture content and these seeds are small seed size and shape. High temperatures from February to March forced the plant to mature fast, resulting in a small seed size (36).

Effect of heat stress on yield in Indian mustard germplasm

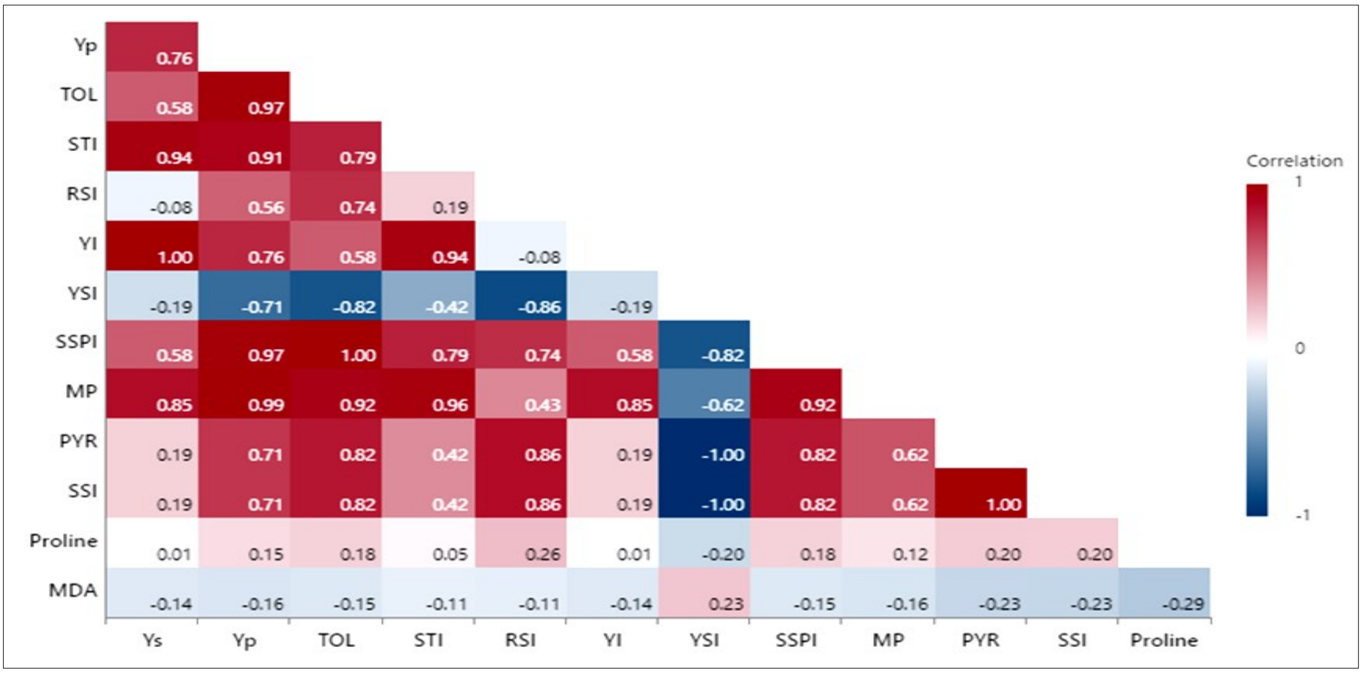
The culmination of several yield components established under a certain set of environmental conditions is expressed in the final seed yield. The seed yield from the corresponding net plot was used to compute the seed yield/ha. In this study, seed yield was significantly affected by the date of sowing. The last sowing's lower seed yield was eventually caused by a decrease in the number of pods and seeds due to an increase in temperature at the flowering stage, which eventually caused a reduction in flowers and vegetative growth too. The seed yield ranged from 2201 to 364 kg/ha and the average for the accessions under study was 1453 kg/ha in early sowing conditions. In optimum conditions, the seed yield was in the range of 2201 to 294 kg/ha, with an average of 1477 kg /ha. The range of seed yield in the late sowing condition was found to be between 837 to 177, with an average of 527 kg/ha. The results indicated that for normal and early sowing dates, seed yield was at par in different mustard accessions, which significantly decreased under late sowing conditions. Under normal and heat stress conditions, the maximum seed yield was recorded in IC280920 (2201 and 837 kg/ha respectively). Whereas, the minimum seed yield under normal and heat stress conditions was noted in IC296688 (294 and 177 kg/ha respectively). The mean seed production decreases by 59.81 % under heat-stressed conditions. The findings of an author, in late sown conditions, there was an overall 33.92 % decrease in *Brassica juncea* seed yield (37). The results were also in agreement with the wheat cultivars, where fewer grain numbers were because of fewer reproductive spikes and shorter grain-filling times, which resulted in lower grain weight (38). The seed yield of IC491044 was the highest in the early sown, with a value of 2201 kg/ha while the lowest value (364) was found in IC301530. We also reported that a few accessions, viz., IC491044, IC280920, IC491128, IC426385 and IC267699, were sensitive to heat stress, but they showed an overall high yield on all three sowing dates. A study reported similar conclusions that reduced seed yield from delayed sowing could be caused by temperature changes in the late-sown crop (39).

Stress Tolerance Indices in Indian Mustard Germplasm

Several stress indices, including STI, TOL, SSPI, YSI, YI, RSI, MP and PYR were computed in this study based on yield in both normal and heat-stressed conditions. Accession IC296703 (1541.8) and IC385783 (1483) have higher TOL values. Under stressful conditions, these accessions produced fewer yields. As a result, these accessions were regarded as susceptible to heat. The lowest TOL value was related to IC305130 (10.3) and IC362912 (64.0). IC296703 was found to be the accession most susceptible to heat stress; it also had the highest values for TOL, SSPI, YSI, RSI and PYR. It showed a high grain yield under normal, non-stress conditions and a low grain yield under late-sown, heat-stress conditions. This accession is best suited for normal sown conditions. Ys, Yp, YI, STI and SSI (low) were used to determine the top accessions that performed the best (IC280920, IC570301, IC267695, IC401575, IC491044, IC426400 and IC426385). Based on SSI, the top 5 heat stress tolerant accessions were IC305130, IC362912, IC426403, IC296688 and IC267695, but they showed poor yields in all the sowing conditions. It was found similar results in rice, indicating that SSI and YSI might be utilized to find accessions with higher yields when under stress as compared to normal conditions. Such tolerant accession might breed high-yielding varieties for *Brassica* crop improvement (40).

Correlation study of stress and yield indices in Indian mustard germplasm

The yield under stress conditions (Ys) exhibited significant positive correlations with the potential yield (Yp), indicating that a higher yield under stress conditions is not necessarily the consequence of a high potential yield under optimal conditions (Fig. 4). Further, Ys is also positively correlated with TOL, STI, YI, SSPI and MP while it is negatively correlated with RSI, YSI and MDA. In rapeseed, similar relationships between Ys and YI, MP, STI, GMP, TOL and SSI were noted (41). Therefore, it is possible to determine appropriate accessions for heat stress resistance using these parameters. RSI has a positive correlation with Yp (0.56) but a negative correlation with Ys (-0.08). TOL, STI, YI, SSPI, MP, SSI, Proline and PYR had a high positive relationship with seed yield (Ys and Yp). However, in the current study, the accession with the highest YSI showed a yield drop of only 2.52 when planted under late conditions as compared to optimal. YI had a strong positive relationship with Ys, Yp, TOL, STI, SSPI, MP, PYR, SSI and proline, whereas it had a negative correlation with RSI (-0.08), YSI (-0.19) and MDA (-0.14), indicating that these indices can be used to distinguish the accessions that are stable and heat



	Ys	Yp	TOL	STI	RSI	YI	YSI	SSPI	MP	PYR	SSI	Proline
Yp	0.76											
TOL	0.58	0.97										
STI	0.94	0.91	0.79									
RSI	-0.08	0.56	0.74	0.19								
YI	1.00	0.76	0.58	0.94	-0.08							
YSI	-0.19	-0.71	-0.82	-0.42	-0.86	-0.19						
SSPI	0.58	0.97	1.00	0.79	0.74	0.58	-0.82					
MP	0.85	0.99	0.92	0.96	0.43	0.85	-0.62	0.92				
PYR	0.19	0.71	0.82	0.42	0.86	0.19	-1.00	0.82	0.62			
SSI	0.19	0.71	0.82	0.42	0.86	0.19	-1.00	0.82	0.62	1.00		
Proline	0.01	0.15	0.18	0.05	0.26	0.01	-0.20	0.18	0.12	0.20	0.20	
MDA	-0.14	-0.16	-0.15	-0.11	-0.11	-0.14	0.23	-0.15	-0.16	-0.23	-0.23	-0.29

Fig. 4. Correlation coefficients between seed yield (**Ys** and **Yp**), biochemical parameters (Malondialdehyde (**MDA**) and **Proline**) and different indices of stress tolerance (Stress tolerance (**TOL**), Stress tolerance index (**STI**), Relative stress index (**RSI**), Yield index (**YI**), Yield stability index (**YSI**), Stress susceptibility % index (**SSPI**), Mean productivity (**MP**), % yield Reduction (**PYR**) and Stress Susceptibility Index (**SSI**) in Indian mustard germplasm. The color key on the right-hand side denotes the level of correlation.

tolerant. These indices showed that accession IC280920 showed the highest yield under both circumstances, followed by IC570301, IC401575 and IC267699. The most appropriate stress indices for wheat, according to past research, are MP, GMP and the Stress Tolerance Index (STI) (42, 43). According to a recent study, an appropriate selection index must strongly relate to seed yield in both stressed and non-stressed conditions (44).

Hierarchical cluster analysis of Indian mustard germplasm

All studied mustard accessions were grouped into five clusters based on biochemical parameters (proline and MDA) and stress indices such as TOL, STI, RSI, YI, YSI, SSPI, MP, SSI and PYR. Cluster III had the highest number of accessions (13), while Cluster V had the lowest number of accessions (3). The accessions within the same cluster exhibited greater similarity, whereas the accessions between other clusters displayed diversity in the stress indices and biochemical parameter values. The accessions belonging to cluster III showed the highest value for STI, YI, and MP, while the minimum was observed in accessions belonging to cluster V (Fig. 5). Based on hierarchical clustering, genotypic variability could be exploited in the crossing program for crop improvement.

To accelerate the breeding program, the selection of heat stress tolerant *Brassica* accessions is an essential step. This selection is a time-saving process and would also reduce the workload. We have efficiently screened *Brassica* accessions based on biochemical assays and seed

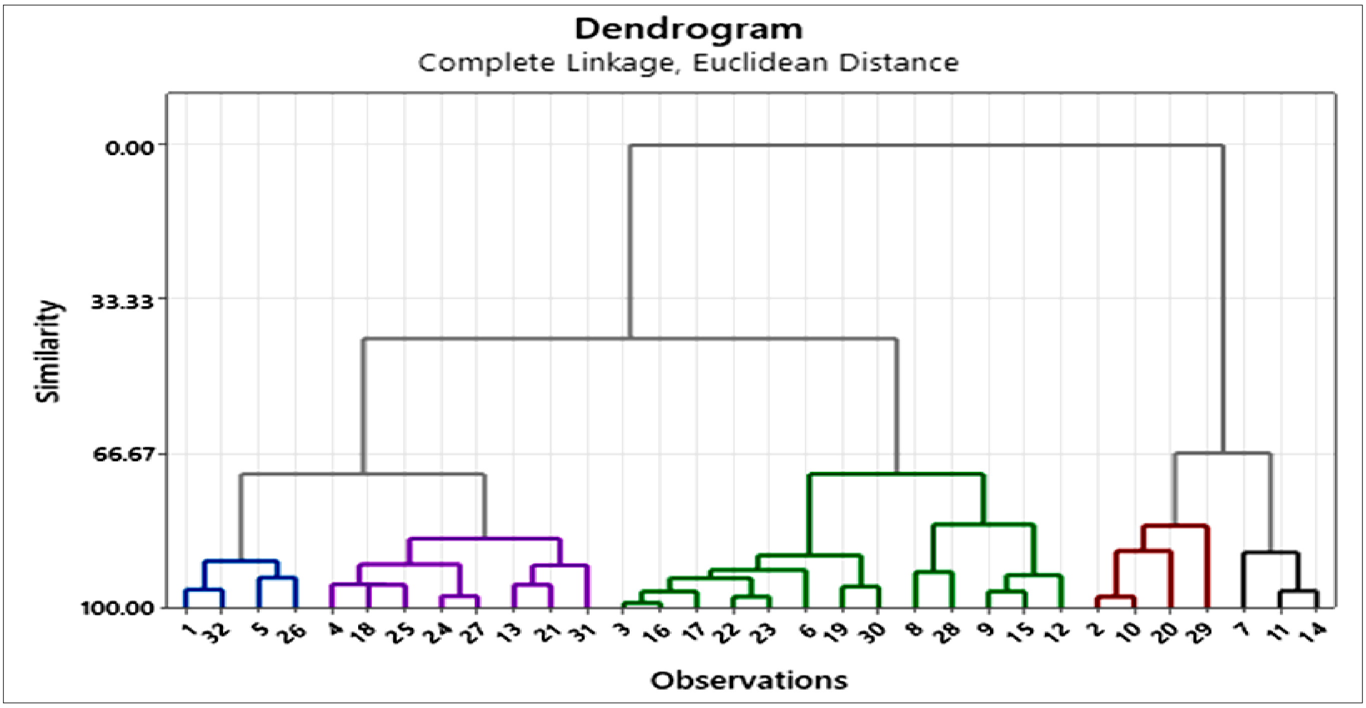


Fig. 5. Dendrogram depicting the clustering pattern of Indian mustard germplasm based on biochemical parameters and stress indices.

parameters under heat stress conditions. Under high temperatures, only tolerant accessions could perform better, which is the outcome of physiological and biochemical changes. So, heat stress tolerance is directly linked to the natural capacity of *Brassica* accessions to bring about changes in cell metabolism and physiological functions. Therefore, heat stress tolerant *Brassica* accessions such as IC280920, IC401575, IC426400, IC491509 and IC570301 can be used soon to develop thermotolerant *Brassica* and related crops for future sustainability. The accession IC401475 showed the highest yield under heat stress conditions in our previous study (19) and showed a high level of heat stress tolerance as indicated through biochemical assays. So, further studies can be concentrated on these accessions for studying *Brassica*-heat stress interaction.

Conclusion

In the current study, there were very significant differences for all the studied traits, as indicated by the analysis of variance. Correlation coefficient analysis results showed that STI, YI, SSPI and MP had a strong positive connection with Yp and Ys, while YSI and RSI had a negative correlation with Ys. The same stress indices (STI, YI, SSPI and MP) were utilized in the cluster analysis to differentiate between heat stress tolerant and susceptible accessions. As a result, it is determined that these indices are appropriate for the selection of high-yielding and tolerant accessions. We observed that accessions IC280920, IC401575, IC426400, IC491509 and IC570301 were heat stress tolerant as well as showed high yields based on seed morphological traits, biochemical assays, and stress indices. These accessions can be further utilized by breeders for breeding programs for agricultural improvement initiatives to develop heat-stress tolerant *Brassica* crops.

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Authors' contributions

BP and RY conceptualized the research work. RY and SP participated in the designing of the experiments. RY contributed the experimental material. BP, HV, SK, AK, KS and Ka executed the field and lab experiments. BP, HV and SA carried out an analysis of the data and their interpretation. BP, HV, RB, RY and SP participated in the preparation of the manuscript. After reading the paper, each author gave their approval.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

Supplementary data

Table. 1. Origin and Pedigree of list of accessions used in the study

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