



RESEARCH ARTICLE

Optimization of sowing dates in Indian mustard (*Brassica juncea* L.) to combat yield losses caused by high temperature at reproductive stage

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Abstract

A field experiment was undertaken during the Rabi seasons of 2019-20 and 2021-22 at organic farm of Amity Institute of Organic Agriculture, Amity University, Noida, Uttar Pradesh, India, for the optimization of the sowing time period to reduce the effect of heat stress on Indian mustard (*Brassica juncea* L.) for increasing productivity. 32 Indian mustard germplasms were sown at three different time periods viz., the last week of September, the last fortnight of October, and the second week of November. Our results revealed that the mustard crop showed a substantial increase in yield supporting characters such as the number of siliqua and seeds per siliqua when sown at last week of September as compared to the second week of October, whereas the late sown crop resulted in less yield due to the heat stress faced by crop during the anthesis stage as compared to early and optimum sowing. The mean seed yield per plant showed a positive correlation with mean test weight. We established the positive effect of early sowing over optimal and late sowing in Indian mustard; further, we found a few mustard accessions were able to resist different climatic conditions. The mustard germplasm accessions IC296688, IC296703, IC296732, IC305130, IC401575, IC426385, and IC589669 were high yielding among all the accessions and can be considered as heat stress tolerant.

Keywords

Rabi; late sown; *Brassica*; genetic variability; terminal heat stress; agromorphological traits.

Introduction

Mustard (*Brassica juncea* L. Czern) is a major Rabi season oilseed crop belonging to the Cruciferae family and the genus *Brassica*, a natural amphidiploid with chromosomal number (2n=36) (1). After soybeans, rapeseed-mustard is presently the world's second-most-produced edible oilseed crop (2). Mustard is often grown in rain-fed environments and is moderately acid-tolerant. It necessitates well-drained soil with a pH close to neutral. Mustard utilizes the C3 carbon absorption pathway, resulting in an effective photosynthetic response at temperatures of 15-20°C. Due to the presence of glucosinolate and its hydrolysis products, such as allyl isothiocyanate (0.30-0.35 %), the seed and oil of mustard have a noticeable pungency, making them suitable for use as condiment in pickles, flavouring curries and vegetables (3). India's most important oilseed crop is Indian mustard

(*Brassica juncea* L. Czern), which accounts for more than 80% of the entire area under rapeseed-mustard cultivation (4). Rapeseed-mustard production, area, and productivity in India were 6.69 million ha, 10.11 million tonnes, and 1511 kg/ha, respectively, in the year 2020-21 (5). It is a self-pollinated plant; however, insects have some role in cross pollination (2-15 %). Mustard is planted as a winter crop in our country, but due to globally changing climatic conditions, it is exposed to heat stress conditions, especially during reproductive stages. Heat stress in plants is defined as a temperature increase of even a single degree above the threshold level for a period sufficient to cause irreversible damage to plant growth and development (6, 7). Climate change has caused heat stress to worsen, and increasing temperatures are causing heat stress in many parts of the world, including India (8). It is predicted that the average worldwide air temperature will increase by 1.8 to 4.0°C in the mid-20th century (9). The temperature is likely to rise more in the Rabi season than in the Kharif season. According to previous studies (10-12), high temperature stress causes a 10-40 % decrease in crop productivity in India.

Plant responses to heat stress depend on their development stage. Indian mustard is vulnerable to heat stress at the reproductive stage since it is a thermo-sensitive and photosensitive crop. Different sowing time period give different environmental conditions for crop growth and development, as well as yield stability, within the same location (13). That is why sowing date is an essential determinant of crop yield, and non-monetary inputs influence the productivity of seed and oil to a great extent (14). Flowering parts and developing grains are highly susceptible to heat stress, affecting anthesis, fertilization, and seed set, resulting in poorer crop production (15). Inadequate grain filling and flower abortion were caused by high temperatures, resulting in a significant decrease in seed output. Late sowing reduces seed yield by synchronizing the silique filling period with high temperatures, lowering assimilate production, causing drought stress, shortening the silique filling period, and hastening plant maturity. Choosing an appropriate sowing time period is crucial for coordinating plant growth stages

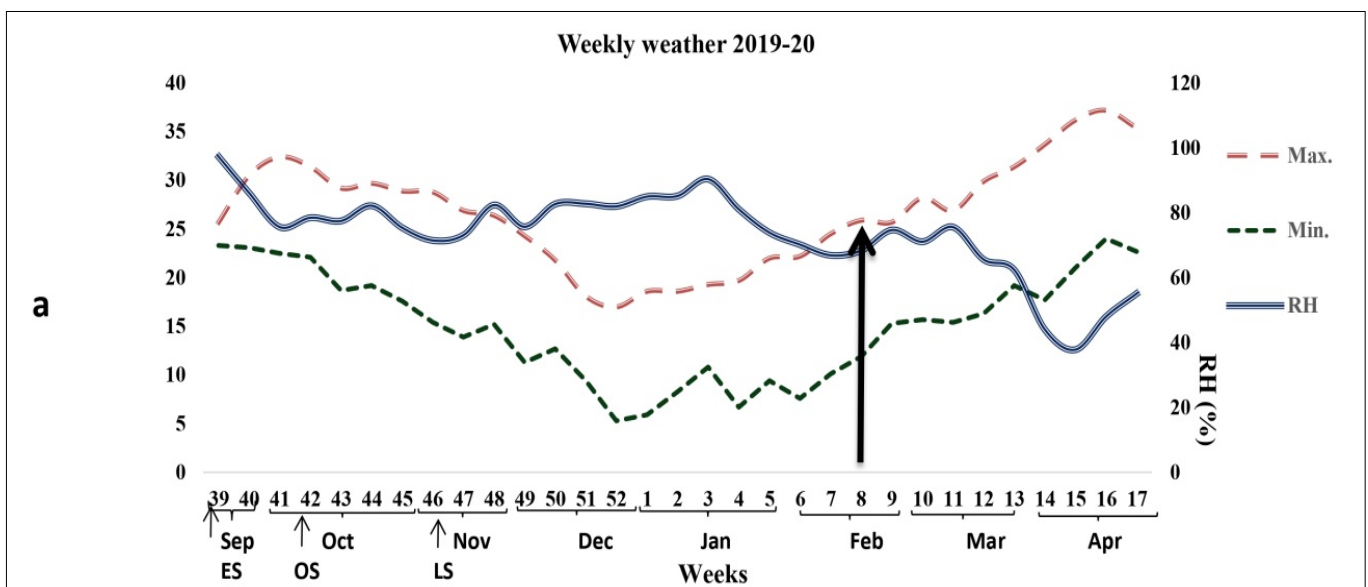
with the ideal environmental conditions that provide the highest output and the plant's endurance to high temperatures.

Here, we have taken three different time points for mustard sowing under field conditions to optimise the time of sowing. This experiment would provide the idea for timely sowing of mustard under field conditions to escape heat stress for a higher seed yield. Further, the accessions observed to be tolerant to heat stress at the post-anthesis stage may be taken up by breeders or researchers to develop climate resilient mustard or related plant species to acclimatise under globally changing climatic conditions.

Materials and Methods

Plant material and sowing condition

Thirty-two Indian mustard (*Brassica juncea*) germplasm procured from the National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India, were taken for study at the organic farm of the Amity Institute of Organic Agriculture, Amity University, Noida, Uttar Pradesh, India during the two years 2019-20 and 2021-22. The study area is located at 28° 54' N latitude and 77° 33' E longitude at an elevation of 201 m, with a major soil type of sandy loamy, with 68.6 % sand, 12.8 % silt, and 18.6 % clay, with EC ranging from 0.40 to 0.79 dS^m⁻¹, low organic carbon (0.06–0.76 %), low available N (0.045 %), available P (1.54–18.47 kg/acre), and available K of 46.46–116.36 kg/acre, as well as a pH ranging from 7.9 to 8.5. The healthy seeds, free from any infection, were selected further and sown under field conditions at three different time periods *i.e.*, early sown (ES) in the last week of September, optimum sown (OS) in the third week of October, and late sown (LS) in the second week of November. Meteorological data such as minimum temperature, maximum temperature, and relative humidity were recorded throughout the experiment (Fig. 1a and Fig. 1b). Data of sunrise, sunset and day length for both the years is presented in Supplementary Table 4. Recommended agronomic practices were followed for growing the crop. Weeding, insect and pest management, and other intercultural operations were performed as needed in the study.



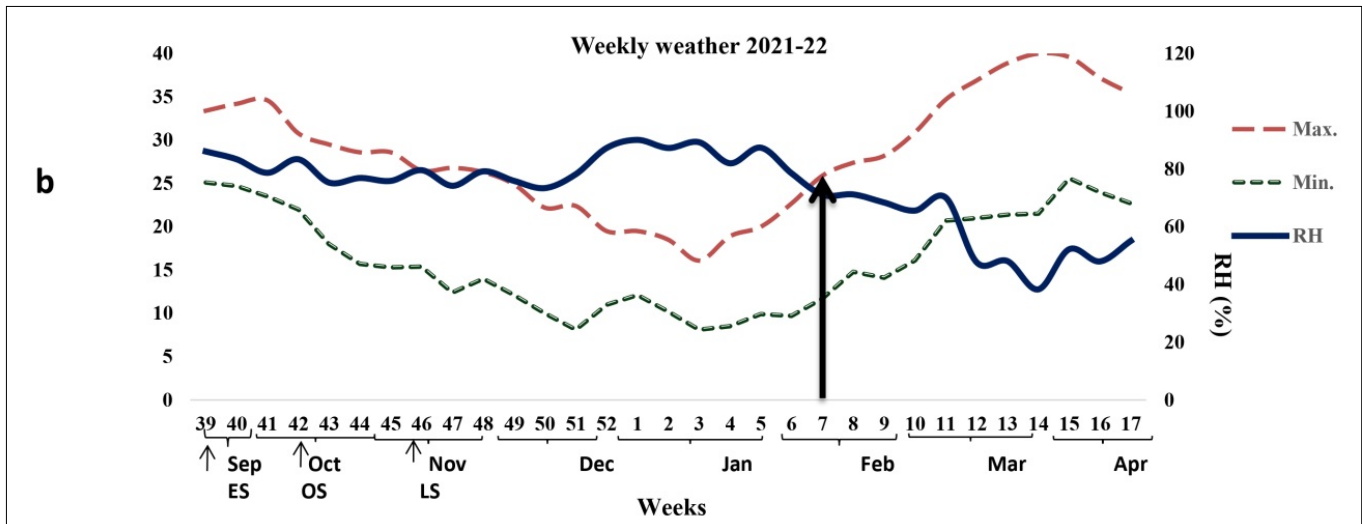


Fig. 1. Weekly different meteorological data recorded under field conditions. a) Meteorological data for the year 2019-20; b) Meteorological data for the year 2021-22.

Experimental design, data recording for agro-morphological traits

Three sets of 32 mustard germplasm accessions were sown in paired rows (2 m in length) in a factorial randomized block design with plant spacing of 45 × 15 cm. The agro-morphological traits viz., number of siliqua per plant, siliqua length (cm), number of seeds per siliqua, 1000 seed weight or test weight (g) and seed yield per plant (g) were recorded under study. The percent decrease/increase was computed under early and late sown over optimum sown condition. Five plants from each accession were chosen (tagged as P1, P2, P3, P4 and P5) for biometric measures. The data was recorded as per minimal descriptors for *Brassica sp* (16).

Statistical analysis

The method of analysis of variance (ANOVA) as described by Panse and Sukhatme (17) was used to statistically examine all experimental data. The heat stress effect was calculated as a percent change in a character's mean under late sown compared to early and optimum sown condition. The "F" (variance ratio) test was used to determine whether treatment effects were significant. For distinguishing treatment effects from those of change effects, appropriate standard errors and critical differences (C.D. at 5%) were calculated. To investigate the genotype, environment, and genotype × environment interaction, OPSTAT software (<http://14.139.232.166/opstat/>) was used to conduct an analysis of variance utilizing replication mean values. Coefficient of variation,

Heritability and Genetic Advance was calculated using R software R package version 0.1.0. (<https://CRAN.R-project.org/package=variability>), PCA (Principal component analysis) was carried out using Statskingdom software (<https://www.statskingdom.com/pca-calculator.html>). Hierarchical clustering was carried out using Minitab software (<https://www.minitab.com/en-us/>).

Results

Temperature during sowing time is a key component that influences plant growth and development. During growth periods, all plants require a particular amount of heat units, and the time it takes to attain these units is dependent on the climatic circumstances. An analysis of variance (ANOVA) was carried out to test the significance of variance among 32 diversified germplasm of Indian mustard for all the 5 yield attributing traits (Table 1). The mean squares because of treatments were highly significant for all the five characters in both years *i.e.*, 2019-20 and 2021-22. The variance resulting from replications was found to be non-significant for all the characters in both the years. The ANOVA result revealed that genotype was responsible for a significant amount of variability. Significant differences were observed in most of the agro-morphological traits and yields under different sowing conditions. The plant height and number of branches were significantly higher when the crop was sown in the last week of September (ES) as compared to the 2nd week of November (LS). This could be attributed to a longer vegetative growth period as

Table 1. Analysis of variance (ANOVA) for 5 characters in Indian mustard in 2019-20 and 2021-22.

Sources	Degree of freedom	Years	Number of siliqua	Siliqua length	Seeds per siliqua	Test weight	Seed yield per plant
Replications	2	2019-20	9,831,330	0.07	6.53	0.69	816.56
		2021-22	8,015,447	0.07	20.99	3.98	269.57
Treatment	31	2019-20	57,606*	0.10	6.26*	1.35*	111.02*
		2021-22	63,099*	0.16*	0.73*	1.87*	102.27*
Error	62	2019-20	16,908	0.08	2.69	0.16	16.91
		2021-22	16,792	0.07	0.84	0.24	9.70

*significance at 5% level

a result of favourable environmental conditions, especially temperature, resulting in improved growth characteristics under early sowing.

Effect of heat stress on agro-morphological traits in mustard

Traits included in the study were siliqua per plant, siliqua length, number of seeds per siliqua, test weight, and seed yield per plant, as these are important traits that positively

the ES condition ranged from 435.3-1466.7 & 592-1322.3 and 36.7-401.3 & 83.7-386 under the LS condition during both years, respectively (Supplementary Table 1). IC426385, IC305130, and IC296703 were found to have significantly higher number of siliqua for all the sowing dates for both the years of experiments. Minimum, maximum, range, and other statistical parameters associated with different traits observed for 2 years are given in Table 2. When the percentage increase in siliqua counts was

Table 2. Range, mean, standard error and standard deviation of morpho-physiological characters in Indian mustard under different sowing time.

Trait	Year	Mean	SEM	Standard deviation	Coefficient of variance	Minimum	Maximum	Range
Number of siliqua	2019-20	839.6	24.5	138.6	16.5	457.8	1078.4	620.6
	2021-22	782.0	25.6	145.0	18.6	439.1	1010.9	571.8
	Mean	810.8	22.8	129.0	15.9	464.1	1044.7	580.6
Siliqua length	2019-20	4.4	0.03	0.181	4.1	4.0	4.8	0.8
	2021-22	4.6	0.04	0.229	5.0	4.2	5.1	0.9
	Mean	4.5	0.03	0.153	3.4	4.2	4.8	0.6
Seeds/ siliqua	2019-20	14.7	0.25	1.439	10.0	12.8	17.7	4.9
	2021-22	13.7	0.09	0.495	3.6	12.4	14.8	2.4
	Mean	14.1	0.12	0.689	4.9	13.2	16.0	2.8
Test weight	2019-20	3.5	0.12	0.670	19.0	2.3	5.6	3.3
	2021-22	3.7	0.14	0.795	21.5	2.2	5.2	3
	Mean	3.6	0.12	0.654	18.1	2.2	5.4	3.2
Seed yield per plant	2019-20	19.8	0.99	5.638	28.5	7.6	29.4	21.8
	2021-22	16.3	0.58	3.270	20.1	8.6	21.4	12.8
	Mean	18.0	0.67	3.776	20.9	8.1	24.4	16.3

impact yield. Heat stress during critical crop growth stages, such as post-anthesis or flowering stages, significantly reduces mustard yield. In this study, we observed a rise in temperature during the reproductive stages of Brassica under LS as compared to OS. Under LS conditions, productivity declines primarily due to the shortening of the vegetative and reproductive phases which forces early plant maturity, reducing yield and productivity. Here, we observed heat stress in Brassica accessions in the month of February, when the crop reached the post-anthesis or flowering stage (Fig. 1a and Fig. 1b) which ultimately affected flowering and pod formation.

Siliqua per plant under ES, OS and LS

The number of siliqua per plant in 32 accessions of Brassica germplasm under different sowing time periods over the years showed a higher number of siliqua in the early and optimum sown plants compared to the late sown and the percent decrease in siliqua count of the early and late sown conditions is shown in Supplementary Table 1. The germplasm accessions showed high genetic variability for the number of siliqua under different sowing times. A higher number of siliqua per plant was recorded at the optimal sown (OS) condition i.e., the last fortnight of October for both years and ranged from 758.7 (IC362912) to 1496 (IC426385) in 2019-20 and from 542.3 (IC570279) to 1453.7 (IC491128) during 2021-22. Whereas, siliqua counts under

compared under ES and LS over OS conditions, it was observed that out of 32 accessions of Indian mustard germplasm, IC267695, IC296702, IC353575, IC401575, IC426388, IC447833, IC491263, IC491429, IC570279, IC570301, and IC571686 showed the percent increase in number of siliqua at the ES condition over the years as compared to OS. The seed counts under LS conditions drastically decreased when compared to optimum sowing conditions and ranged from 64.19 % to 92.4 %. The data is represented in the Fig. 2a and 3a.

Siliqua length (cm) under ES, OS and LS

The siliqua length was also observed to be higher in early and optimum sown plants compared to late sown. There was high genetic variation observed among the accessions. The longest siliqua was seen in the optimum sown condition for both years. For the year 2019-20, the germplasm IC491044 recorded the longest siliqua (5.2 cm), and the germplasm IC491509 (5.7 cm) showed the longest siliqua for the year 2021-22 (Supplementary Table 1). Whereas in the early sown condition the siliqua length ranged from 4-5 cm and 4.2-5.2 cm and in the late sown condition, the siliqua length ranged from 3.6-4.9 cm and 4-5.2 cm for both years, respectively. The percentage change for siliqua length was computed for both early sown and late sown and was compared to the optimum sown condition. Out of the 32 accessions, IC267695, IC267699,

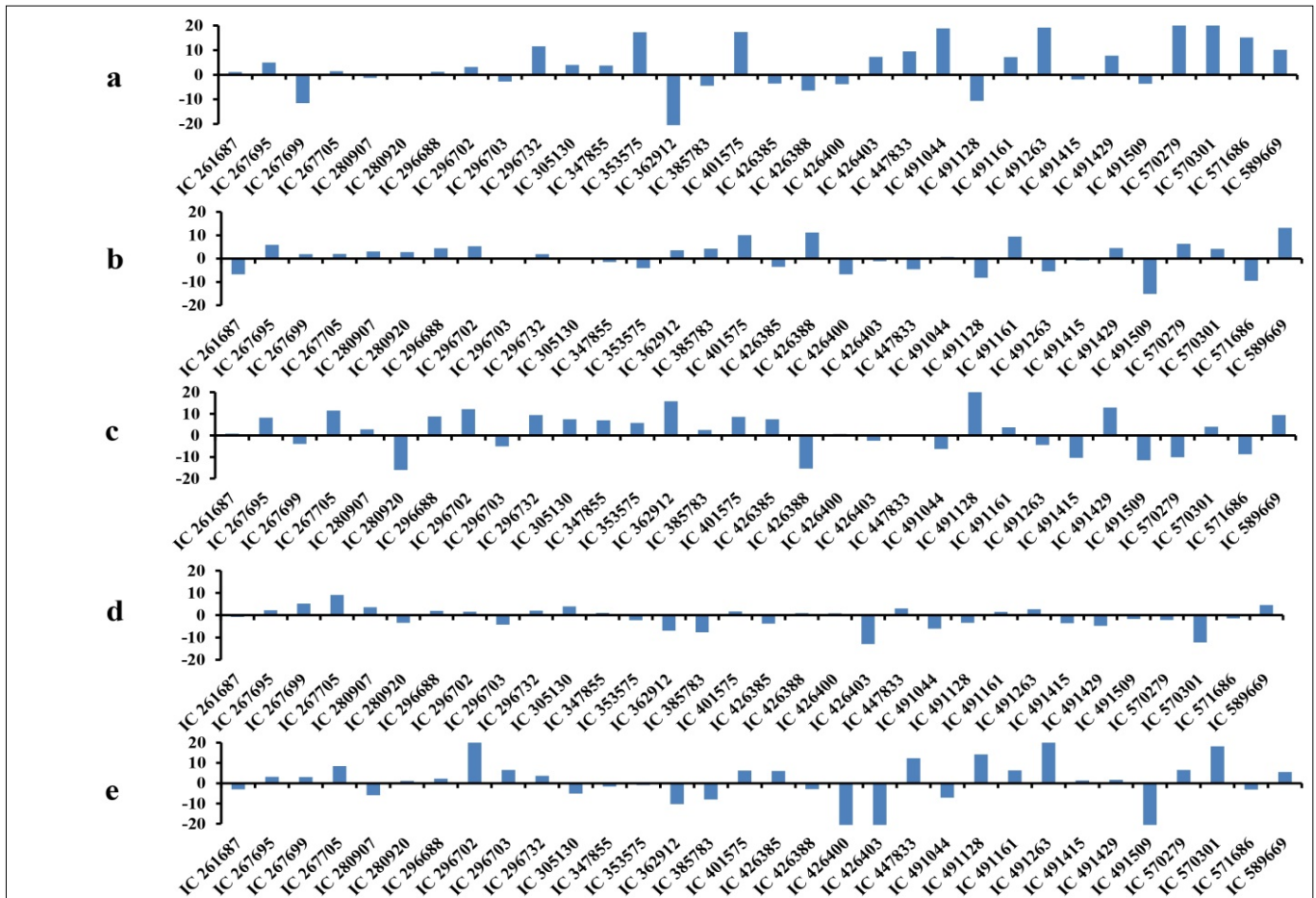


Fig. 2. Percent change in early sown condition compared to optimum sown for 32 accessions of Indian mustard over the years. **a)** Number of siliquas; **b)** Siliqua length (cm); **c)** Number of seeds per siliqua; **d)** Test weight (g); **e)** Seed yield per plant (g).

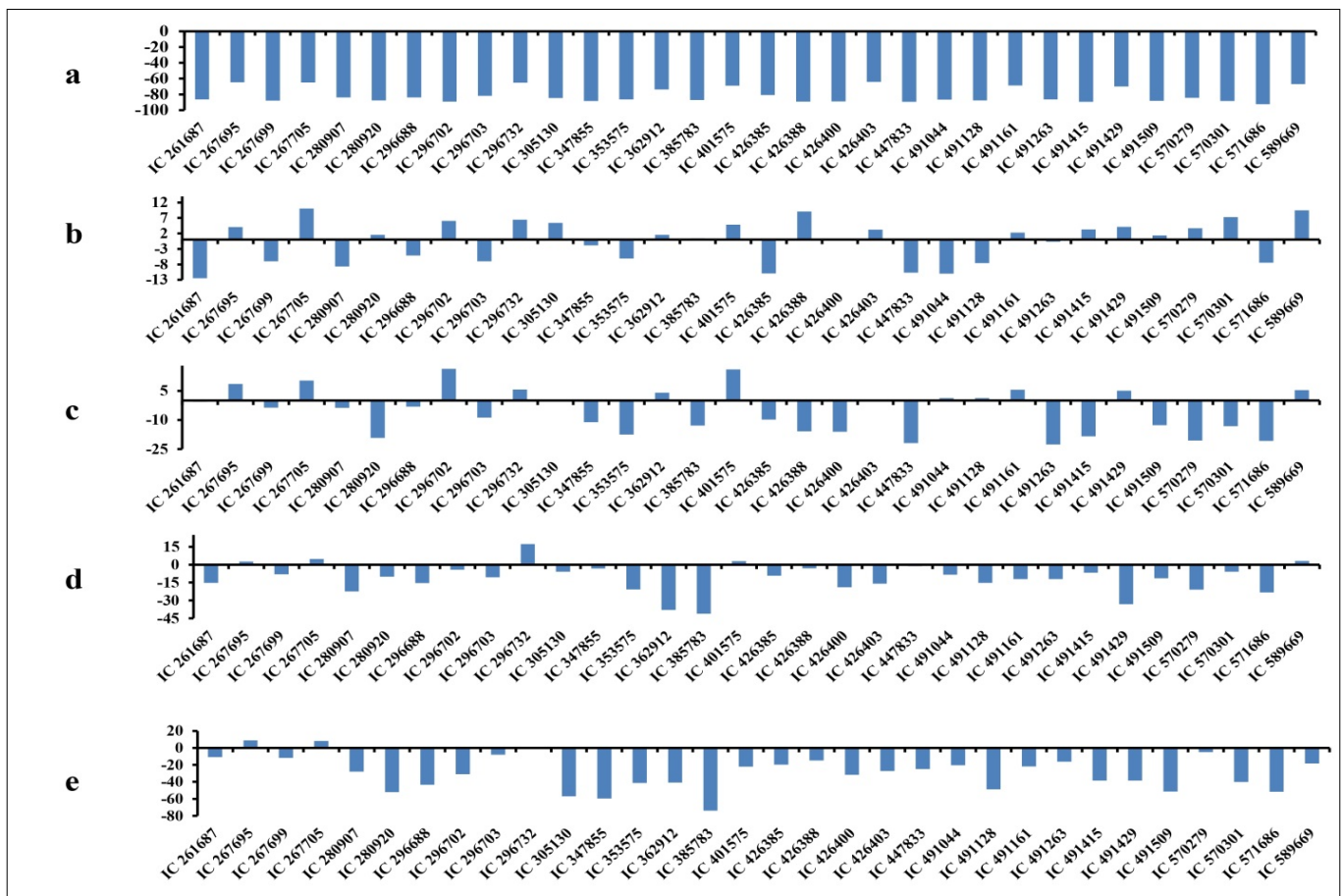


Fig. 3. Percent change in late sown condition compared to optimum sown for 32 accessions of Indian mustard over the years. **a)** Number of siliquas; **b)** Siliqua length (cm); **c)** Number of seeds per siliqua; **d)** Test weight (g); **e)** Seed yield per plant (g).

IC267705,

IC296688, IC296703, IC296732, IC385783, IC401575, IC426388, IC491161, IC491429, IC570279, IC570301, and IC589669 showed a positive increase in siliqua length for the early sown condition for both years as compared to OS (Fig. 2b). Not much variation was seen in the siliqua length of late sown crops when compared to optimum sown ones (Fig. 3b).

No. of seeds per siliqua under ES, OS and LS

The quantity of seeds per siliqua showed a declining tendency with delayed sowing during both years of the experiment. Accessions during optimum and early sown conditions showed a higher number of seeds per siliqua as compared to late sown conditions. The findings showed that sowing in the last week of September and the second fortnight of October were at par. The optimum sown condition showed the highest number of seeds per siliqua was 12.7 (IC 491128) to 20.0 (IC 447833) in 2019-20 and the range was 12.1 (IC 362912) to 16.3 (IC 491509) in 2021-22. Whereas seeds per siliqua under early sown conditions varied from 11.0 to 19.3 and 12.4 to 15.4, and under late sown conditions from 10.7 to 17.7 and 10.1 to 14.1, respectively, across both years (Supplementary Table 1). The percent reduction was seen more in late sown compared to early ones. Of the 32 accessions of Indian mustard germplasm, IC267695, IC296705, IC296732, IC491161, IC491429, and IC589669 demonstrated the percent increase in the number of siliqua at both early and late sown condition over the years as compared to OS. (Fig. 2c and Fig. 3c).

Test weight (g) under ES, OS and LS

Information on test weight (1000 seed weight), and percent change in early and late sown condition compared to optimum sowing conditions for both years is shown in Supplementary Table 1. In comparison to the year 2019-20, test weight was higher during the years 2021-22. Under late sown condition in both years, the test weight dropped significantly, based on our analysis (Fig. 2d and 3d, respectively). The minimum test weight was recorded in IC362912 (1.5 g) under late sowing conditions and the maximum test weight was recorded in IC426388 under all the sowing condition for both years of the experiment. There was signifi-

cant variation in test weight at the optimum date of sowing, which declined significantly under late sown. During both study years, IC426388 had the greatest test weight. It was also significantly better than the other accessions. The lowest test weight was recorded in genotypes IC296703, IC280920, and IC362912, they recorded the fewest siliqua per plant. The reduction in test weight in late sown is due to very short grain filling period of the growing mustard crop.

Seed yield per plant (g) under ES, OS and LS

At the normal and early date of sowing, there was notable genotypic variability in seed yield per plant, which considerably decreased under late seeded conditions. For the year 2019-20, seed yield (g/plant) for optimum sown crops ranged from 10.5 g (IC362912) to 35.4 g (IC296688), whereas values for early-sown and late-sown crops ranged from 9.8 g (IC 362912) to 37.2 g (IC296703) and 6.1 g (IC385783) to 32.0 g (IC296732), respectively. For the year 2021-22, seed yield per plant ranged from 11.5 g (IC362912) to 32.4 g (IC305130) in the normal sown condition, with a mean of 21.5 g and for late sown condition the seed yield ranged from 6.2 g (IC362912) to 30.4 g (IC296703) (Supplementary Table 1). There was a decrease in seed yield per plant in early sowing condition, the loss was upto 27.3 % (Fig. 2e), whereas in late sown condition it ranged from 8.03 % to 73.92 % (Fig. 3e) for both the years. The decrease in seed yield per plant could be due to a decrease in the plant's overall biomass as well as a negative effect on yield parameters because of heat stress faced under late sown conditions. However, only three germplasm IC267695, IC267705, and IC296732 showed a positive increase in yield for all the sowing conditions.

Coefficient of variation, Heritability and Genetic Advance

The genotypic (GCV) and phenotypic (PCV) coefficients of variation are simple measures of variability that are commonly used to evaluate variability. The relative values of the coefficients provide information about the degree of genetic population diversity. The population's genetic diversity offers many opportunities to select genotypes with desirable characteristics. As a result, the genotype pool was evaluated for the study of variability. Table 3 shows the phenotypic and genotypic coefficients of heritability,

Table 3. Estimates of phenotypic (PCV) and genotypic (GCV) coefficient of variation, heritability and genetic advance in percent of mean among 5 characters in Indian mustard

Parameter	Year	Coefficient of variation (%)		Heritability (broad sense) %	Genetic advance (GA)	GA as % mean
		GCV (%)	PCV (%)			
Number of siliqua	2019-20	13.9	20.8	44.52	160.1	19.1
	2021-22	15.9	23.0	47.90	177.1	22.7
Siliqua length	2019-20	1.91	6.6	8.36	0.05	1.14
	2021-22	3.5	7.0	25.24	0.17	3.65
Seeds per siliqua	2019-20	7.40	13.4	30.53	1.24	8.42
	2021-22	-1.3	6.6	-4.09	-0.08	-0.6
Test weight	2019-20	17.8	21.0	71.43	1.09	30.92
	2021-22	19.9	23.9	69.22	1.27	34.1
Seed yield per plant	2019-20	25.7	31.9	64.98	9.3	42.76
	2021-22	25.7	31.9	64.98	9.3	42.7

genetic advance, and variability in 2019-20 and 2021-22 for each of the five traits. The magnitude of PCV was marginally higher than GCV for all the characters, indicating that the visible variation in the expression of characters was not only due to the varying influence of environmental factors. Data in the table showed maximum GCV and PCV were recorded for seed yield per plant, number of siliqua, and test weight for both years. High heritability, in conjunction with high genetic advance was observed for test weight and seed yield per plant for both years of the study.

PCA and hierarchical clustering

PCA is a useful tool for reducing data in genetic diversity investigations. PCA eliminates interrelationships between components and identifies variables that contribute the most to genetic variability, which can then be used to characterize genotypes further (18). The PCA gave us eigenvalues and factor scores, which we used to figure out

how well the axes and their associated features could be used to tell them apart (Fig. 4). PCA (19) was used to figure out the degree of divergence, the direction of their evolutionary patterns, and how much different parts of the data contributed to the overall divergence. Positive effects are being produced by each of PC's characteristics. The existence of both positive and negative loading in a single component indicates that the components and variables have positive and negative correlation trends. PC₁ (42.8%) and PC₂ (23.4%) contributed to the total 66.2 % variability. The results of the principal component analysis explained the genetic variation among the genotypes for all characters studied. Table 4 shows the results of the PCA utilizing 5 yield attributing traits of Indian mustard for both years combined. PC₁ and PC₂ had eigen root values greater than 1, but PC₃ had a value almost close to 1. These 3 principal components of characters viz., number of siliqua, seed yield per plant, and siliqua length cumulatively added

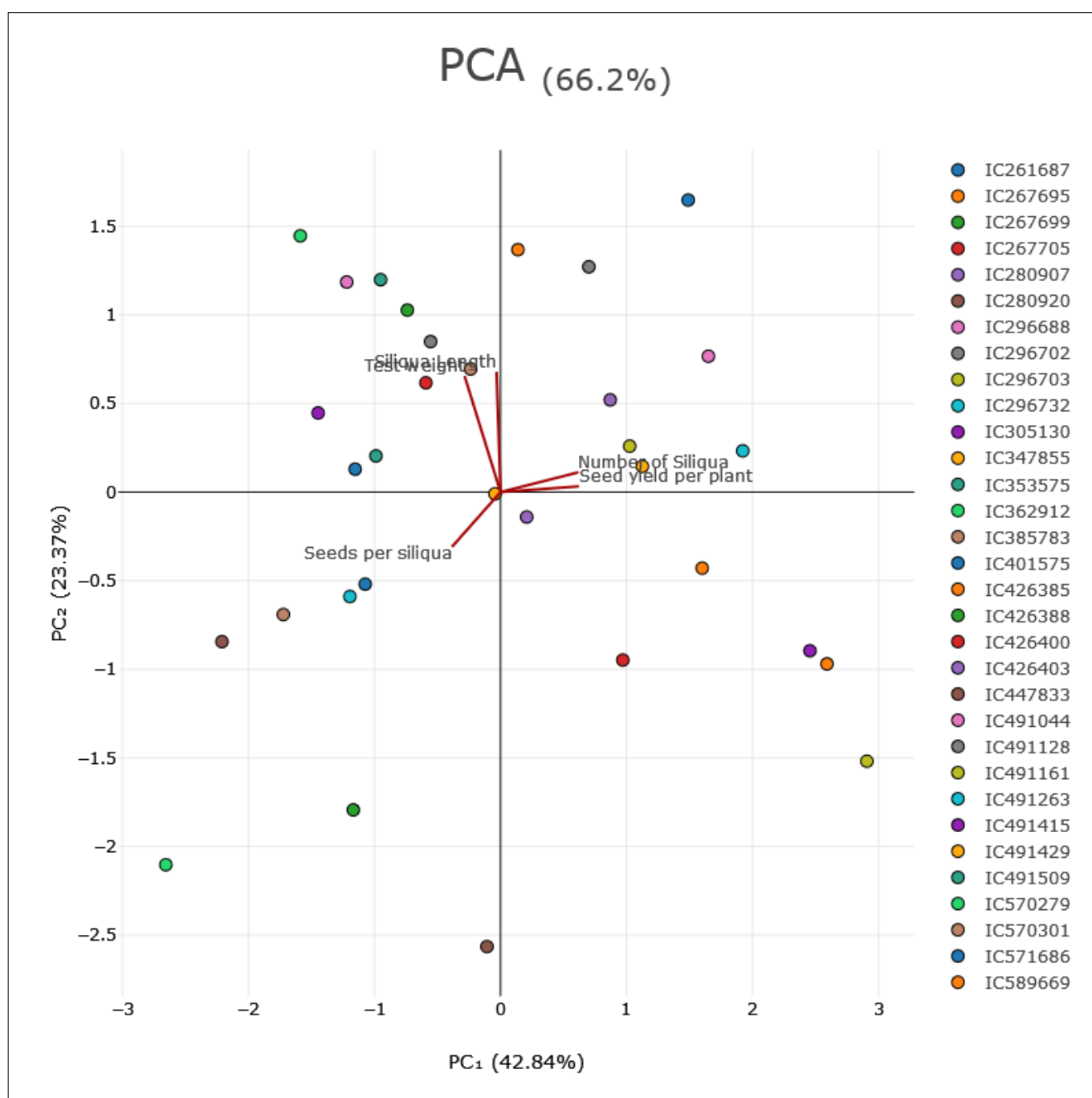


Fig. 4. Principal Component Analysis (PCA) plots of variables under study.

Table 4. Eigen values and percentage of variation explained by first five principal components for agronomic traits of Indian mustard.

Parameter	PC ₁	PC ₂	PC ₃	PC ₄	PC ₅
Eigenvalue	2.14	1.17	0.95	0.53	0.21
% of Variance	42.84	23.37	19.03	10.61	4.15
Cumulative (%)	42.84	66.20	85.24	95.85	100.00

85.24% to the total variation. The following PCs gradually accounted for a decreasing amount of variation while the first one absorbed and accounted for the greatest share of the overall variability in the collection of all PCs. IC267695, IC491161, IC267699, IC267699, IC426400, IC426403, IC296688, IC296732, IC401475, IC426403, IC491128, IC491161, and IC491429 showed maximum variability among the germplasms for both years combined.

PCA was analysed separately for all three sowing dates of both years and data with the PCA plot is presented in Supplementary Table 3. For early sown condition PC₁ and PC₂ characters viz., number of siliqua and siliqua length, was the major distinct variability contributing characters which accounted for nearly 64.28 % of the total

IC491263, IC280920, IC353575, IC426388, IC347855, IC571686, IC491044, IC570301, IC491415 and IC491509 were grouped in the first cluster. The accessions IC267695, IC491161, IC267699, IC267699, IC426400, IC426403, IC280907, IC296702 and IC385783 were grouped in the second cluster. The accessions IC296688, IC305130, IC296703, IC426385, IC296732, IC401575, IC589669, IC491128 and IC491429 grouped in the third cluster. In this study, the highest correlation was observed between yield per plant and number of siliqua followed by test weight and siliqua length (Fig. 5b).

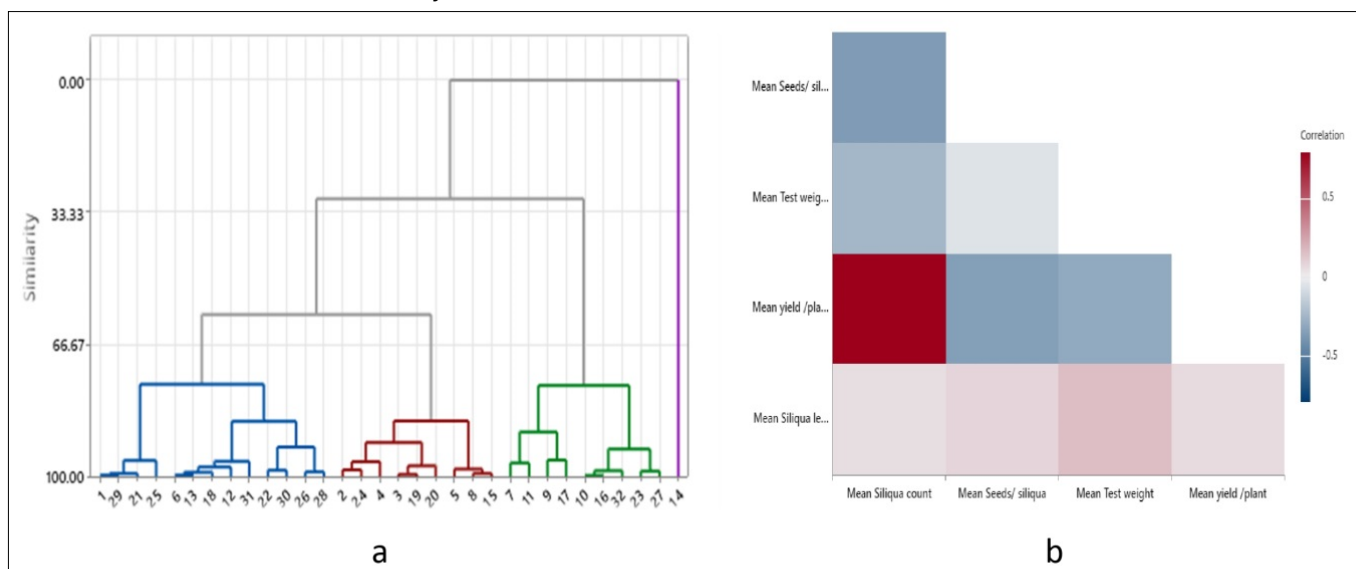


Fig. 5. a) Dendrogram showing different clustering pattern among 32 Indian mustard accessions on the basis of agro-morphological traits **b)** Collelogram showing correlation among 32 Indian mustard accessions on the basis of agro morphological traits.

variation and germplasms IC296702, IC296688, IC401575, IC491161 and IC491429 contributed for maximum variability. Similarly, for PCA of optimum sown condition here, the first two PCs number of siliqua and seed yield per plant each have eigenvalues greater than one and together account for 42.27%, and of the overall variation. IC296703, IC296688, IC426385, IC426403, and IC491128 showed higher variability. For late sown condition in sum, the first two components account for 64.13% of the variance, and germplasm IC267695, IC296702, IC296732, IC401575 and IC426403 showed variability along with the characters seeds per siliqua and siliqua length, this is a reasonable approximation of the initial variation among the germplasm under study (Supplementary Table 3).

The hierarchical cluster shows the grouping of accessions in four clusters, represented by blue, red, green, and purple. The first cluster comprising thirteen accessions was the biggest, while the fourth cluster comprising one accession (IC362912) was the smallest (Fig. 5a). The accessions viz., IC261687, IC570279, IC447833,

Discussion

Abiotic stresses especially heat stress adversely affects crop plants due to globally changing climatic condition. Oilseed Brassica is a major source of vegetable oil throughout the world, but it is highly susceptible to heat stress especially at the post-anthesis stage. Therefore, to meet the increasing demand of vegetable oil, necessary steps should be taken for sustainable productivity of Brassica (20). Over time, the crop Brassica has lost its natural ability to withstand heat stress due to domestication. The time of sowing is of utmost importance to combat heat stress; generally, high temperatures at seedling as well as the anthesis stage led to poor crop growth and lower seed yields along with the search for new Brassica genotypes to survive under heat stress conditions. In present study, screening of 32 Brassica accessions showed significant variability when planted under different environmental condition (ES, OS and LS) for two years and an overall reduction was observed for important agro-morphological traits viz., siliquas per plant, siliqua length, number of

seeds per siliqua, 1000 seed weight or test weight and seed yield per plant under heat stressed late sown condition. But few accessions IC267695, IC267705, IC296688, IC296703, IC296732, IC305130, IC401575, IC426385, IC491128, IC570301 and IC589669 found as good performers even under heat stress, their performance was better on par with ES and LS condition. Therefore, the genetic makeup of accessions and climatic conditions are major factors determining seed yield. Here, under LS condition, the duration of reproductive phase was shortened and the crop reached physiological maturity at early stage. The number of siliqua per plant is one of the most important components of seed yield in mustard (21). Here, we observed a greater number of siliqua per plant under ES and OS as compared to LS. A high genetic variability was also observed within the treatment as well as across the treatment. This can be due to the genetic makeup of distinct genotypes and the effect of environment on genotypes in the mustard crop (22, 23). Our study was in concurrence with the earlier report in terms of less number of siliqua per plant reported under heat stress conditions as compared to the control (24). The ES crop had a favourable temperature for a longer period of time as compared to the LS, ES crop showed improved growth and yield characteristics, and increased production. Similar findings were observed in earlier studies carried out on mustard (25).

The time of sowing in the Brassica crop had a significant impact on the number of seeds per siliqua, and as sowing was delayed, the number of seeds per siliqua gradually decreased. It was primarily caused by a hospitable environment, especially the temperature that prevailed during sowing and during the vegetative and reproductive stages. High temperature throughout the reproductive phases of late planted crops forced the crop to mature resulting in the decreased number of seeds/siliqua (26). Large variation has been observed in previous studies on Brassica in terms of siliqua per plant, seeds per siliqua and siliqua length in various genotypes on varied sowing dates (23, 27 and 28). Similarly, in our study high genetic variability was also observed in agro-morphological traits.

Here we observed that the LS crop has the highest yield reduction of ~35%, as compared to ES and OS. Transient heat stress during flowering or the post-anthesis stage is a threat to yield in *Brassica napus* (29). Late sown Brassica crops showing reductions in seed yield and genotypic variations have been reported in earlier findings by Shargi *et al.* (30), Rafiei *et al.* (31) and (32–35). Several studies indicated the disastrous effects of heat stress alone or along with other abiotic stresses such as drought on plant metabolism, physiological changes, reproduction and ultimately crop production. Rahaman *et al.* (36) found genes associated with pollen sterility, embryo abortion etc. in Brassica under heat stress conditions. They observed increased pollen sterility (26.1 to 84.4 times) under heat stress condition as compared to control, which led to 13.8% reduction in the number of pods in heat-stressed Brassica plants.

The results were also in agreement with those of other crop plants such as wheat, where terminal heat stress significantly affected grain yield due to the shorter grain-filling duration, resulting in fewer reproductive spikes (37). Singh *et al.* (38) noticed a similar pattern, late-sown crops had an unfavourable temperature regime that slowed their growth in comparison to crops that were sown on normal or mid-dates at earlier dates. Heat stress at the flowering or post-anthesis stage causes a decline in seed reduction by aborting pollen grain production and improper development of other reproductive flower parts (39). Forced maturation resulting from a quick rise in temperature affected post-fertilization development. Here, germplasm IC296688, IC296703, IC305130, and IC426385 showed early maturity as compared to other accessions. This may be because of their genetic capacity to perform better in a short duration of time with available nutrients and moisture. These accessions can be bred with other high yielding varieties to develop short duration and high yielding mustard crop. Test weight is an important agro-morphological trait, which was shown to be considerably affected by different dates of sowing. It was significantly highest for crops planted on early and optimum time, and gradually reduced under late sown condition. Dinda *et al.* (40) noted a decrease in the quantity of siliqua per plant, seeds per siliqua, and the weight of 1,000 seeds. Average reduction in the seed yield when sown on third and first date in comparison to second sowing were in the range 2 to 90 % in all the genotypes as shown in table (Supplementary Table 1). Lodhi *et al.* (41) also conducted an analysis of variance and discovered extremely significant differences among all genotypes for all the characters under study. High heritability coupled with high genetic advance indicated that most likely the heritability is due to additive gene effect and selection directly based on these traits may be effective. In earlier studies, high genetic advance in percent of mean conjugated with high heritability have also been reported for seed yield per plant by Tripathi *et al.* (42), Pal (43) also observed resilient PCV and GCV for secondary branches per plant, seeds per siliqua, siliqua per plant, seed yield per plant, siliqua length, and biological yield per plant. These greater GCV and PCV values suggested increased potential for phenotypic selection using these traits for further improvement. Under late sown conditions, there was up to 90% yield loss in most of the genotypes during both years. The reason for this loss is the rising temperature during the reproductive phase of the late sown crop which resulted in stunted vegetative growth. Also, the numbers of siliqua were reduced which ultimately was responsible for the shrivelled seeds and low yield. Due to the Indian mustard's late seeding, Dinda *et al.* (40) also noted a decrease in the quantity of siliquae per plant, seeds per siliqua, and the weight of 1,000 seeds.

A multitude of processes are used to carry out PCA, many of which require subjective judgements. The number of variables being studied and the number of extracted components are equal. Principle component analysis was used by Pankaj *et al.* (44) to identify nine principal

components (PCs) that explained about 77% variability. Neeru *et al.* (19) likewise discovered 11 principal components (PCs) that explained about 75% variability in Indian mustard. It is clearly showed that the variation in this component is contributed by the combination of yield as well as quality characters. The results of the principal component analysis corroborated with results obtained by Gupta *et al.* (45). Jolliffe and Cadima (46) found that relevant components for explaining variation were those with eigenvalues greater than one and accounting for at least 10% of the variation. A few recent studies have also investigated the use of wild relatives for the improvement of abiotic stress tolerance. The use of Brassica wild relatives can also be used to improve abiotic stress tolerance in Indian mustard, as they store economically important traits in them but still remain uninvestigated (47, 48).

The identified potential accessions here would be used as potential donors for breeders or researchers in the genetic improvement program of mustard, especially under heat stress in the scenario of globally changing climatic conditions. Additionally, omics-based studies emphasizing the transcriptome, proteome, and metabolome are required to gain insights into how heat stress alters yield determining traits in mustard.

Conclusion

Here, the results effectively established the effect of different sowing dates on Indian mustard germplasm. The highest plant growth attributes were recorded early (last week of September), while the lowest attributes were recorded for the late sown condition (2nd week of November). So, this study laid the foundation for farmers or researchers to carry out mustard sowing at the optimum time to escape heat stress. However, IC296703, IC296732, IC296688, IC305130, IC401575, IC426385, and IC589669 showed the highest yield per plant and yield attributes under all the three sowing time period for both the years. Their performance was under heat stress condition (LS) when compared to ES and OS. The germplasm IC267695, IC296688, IC296732, IC401575, IC426403, IC491128, IC491161, and IC491429 showed variability in their performance for all the characters for each year as well as both the years combined. With the help of the principal component analysis utilised in the current study, we were able to identify germplasm with the best yield attributing characters as well as germplasm promising for different combinations of characters. These results will be useful in understanding the genetic diversity within a group of germplasm. These accessions would be considered potential donors for heat stress tolerance in mustard breeding programmes or related plant species for climate resilient agriculture.

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Authors contributions

RY and NR conceptualized and planned the experiment. BP performed the field experiment, data collection and prepared manuscript draft. BP and HV did analysis of data and interpretation of statistical data. RY, SP and HV revised and improved the manuscript. The final manuscript was read and approved by all authors.

Compliance with ethical standards

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

Ethical issues: None.

Supplementary data

Table 1. Impact of various planting dates on the number of siliqua per plant, siliqua length (cm), seeds per siliqua, test weight (g) and yield per plant (g) of mustard.

Table 2. Pooled data of the year 2019-20 and 2021-22 for number of siliqua per plant, siliqua length (cm), seeds per siliqua, test weight (g) and yield per plant (g) of Indian mustard.

Table 3. PCA analysis data of each sowing time of the year 019-20 and 2021-22 combined together.

Table 4. Data on sunshine hours of the crop season during both the years.

Figure 1. Boxplot, histogram and 3D scatterplot of year 2019-20 and 2021-22 for number of siliqua per plant, siliqua length (cm), seeds per siliqua, test weight (g) and yield per plant (g) of Indian mustard.

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