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RESEARCH ARTICLE

Genetic analysis of adaptive and physiological traits in response to high temperature stress in rapeseed (*Brassica napus* L.)

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Abstract

A study was conducted to find out the genetic variation for physiological traits in response to high temperature in diverse crosses between yellow sarson (B9, YSH401 and NRCYS05-03) and toria (Jeuti and TS06) of *Brassica napus* L. screened under temperature gradient tunnel. Significant variation was observed for all traits in high temperature stress environment. Parents, Jeuti and TS46 along with the crosses B9 x Jeuti, YSH401 x TS46, NRCYS05-03 x Jeuti and NRCYS05-03 x TS46 were found promising based on mean performance for yield attributing characters under stress conditions. YSH401 was a good general combiner for heat susceptibility index and B9 x YSH401, B9 x TS46 and YSH401 x Jeuti were observed as heat tolerant. Main shoot length, number of flowers on main shoot, number of siliquae on the main shoot, siliqua density, per cent flower drop, number of siliquae on a terminal shoot, biological yield per plant and seed yield per plant showed high genetic variation with high heritability and genetic advance. The number of flowers on main shoot was positively correlated with siliquae on the main shoot and seed yield per plant.

Keywords: Genetic variation, heat susceptibility index, heritability, genetic advance

Introduction

The increasing threat of climate change has a profound impact on agricultural production as heat waves cause significant yield losses with great risks for future global food security (Christensen and Christensen, 2007). Rapeseed-mustard (*Brassica napus* ssp. *napus* L.), a cool season crop, constitutes an important source of world edible oil. The area planted and area harvested for crops differ, which accounted for crop loss due to several environmental stresses. Besides biotic and abiotic stresses, cultivation under rainfed or with conserved/limited irrigation in marginal areas causes differences in the overall productivity of mustard growing regions of India. Further, due to intensive cropping patterns but gaining genetic increment under stress conditions is always challenging (Snowdon et al. 2021). In Assam, rapeseed is the main oilseed crop. Farmers preferably grow in the field more than mustard for its short life cycle. Normally it is sown from mid-October to mid-November. However, due to various reasons sowing of rapeseed is delayed, in which case, the sensitive reproductive stage may encounter moderately high-temperature stress. Stress indices have been widely used in different crops for screening tolerant genotypes based on yield reduction under stress conditions (Banerjee et al. 2020). Several evidences reported response of stress indices based on yield under drought stress (Meena et al. 2013; Nowosad et al. 2017; Sandhu et al. 2019). However, limited information is available for these responses in Indian mustard under heat stress. The reproductive stage is the most susceptible stage for temperature stress in most crops in which temperature response has been studied (Hall, 1992; Paulsen, 1994). The development of heat-stress tolerant cultivars would be an ideal option for sustainable production. In this context, the present investigation was undertaken to study the response of rapeseed cultivars to high temperature under temperature gradient tunnel (TGT), [Genesis Technologies, Maharashtra, India] established in the Department of Crop Physiology, Assam Agricultural

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University (AAU), Jorhat. This forms the basis of the selection of the plant materials in the situation that prevailed in Assam.

Materials and methods

Plant material and the experimentation

Three yellow sarson (B9, YSH401 and NRCYS05-03) and two toria lines (Jeuti and TS46), which are popular in the state of Assam and mostly grown by the farmers, were used for this study. They were crossed in a diallel fashion without reciprocal.

During *rabi* 2017-18 the 15 entries comprising five parents and ten F_1s were sown in randomized block design with two replications. The genotypes were grown in (high-temperature stress condition as well as in non-stress field condition, ambient CO2 and ambient temperature to record the performance of the genotypes. The temperature gradient tunnel (TGT) is a structure consisting of a rectangular block, fabricated by metallic sheet pipe, covered with polycarbonate sheet (100 µ gauge). The polycarbonate sheets showed >85% transmission of light. The tunnel was fitted with 4 RTD (remote terminal unit) or data loggers**,** temperature sensors, humidity transmitters and infrared heaters. A temperature sensor and a humidity transmitter were placed outside the chamber to record ambient data. The computer was used for software run and to store daily data. The tunnel was filled with fine tilth soil for growing the plants, and all the agronomic practices were followed as per package. The flowering period started in the 2nd week of December and the temperature inside the TGT was recorded to be nearly 30°C (+4°C) throughout the flowering period and outside the TGT was nearly 25°C. The elevated temperature was maintained through Infra-Red Heater regulated by SCADA software. The following morpho-physiological traits were recorded.

Observations recorded

Pollen viability was studied to know the temperature effect on pollen in stress and non-stress conditions. This was done by staining the pollen grains with 1% IKI solution. Anthers were collected from six randomly chosen flower buds. The fertile (fully stained) and sterile pollen grains (unstained) were counted in five microscopic fields under a compound microscope (40X). Pollen viability was calculated as follows:

$$\text{Pollen viability\%} = \frac{\text{Number of p\textbackslash viable pollens}}{\text{Total number of pollens}} \times 100$$

The date on which 50% of the plants in a plot came to flowering was noted and the number of days from sowing was counted. Agronomic parameter such as number of seeds per siliqua, 1000 grain weight (g), 1000 grain weight (g), Biological yield gm plant^{-1} (BYP), 1000 grain weight (g) (TSW), Seed yield gm plant^{-1} (SYP) were also recorded during harvest of the crop.

Leaf canopy temperature (LT)

Leaf canopy temperature was measured by an infrared thermometer (DHS135XEL/DHS215XEL, Wahl Instruments, Inc.) for high-temperature screening. The temperature was recorded at three levels of the plant i.e., at the ground level, mid-level of canopy and at the top of the plant canopy.

Chlorophyll stability index (CSI)

The chlorophyll stability index was estimated using a spectrophotometer following the method of Koleyoreas (1958). It was calculated as the difference in light transmission in percentage between stressed and non-stressed leaf samples. CSI was done to screen the genotypes for temperature stress.

Stressed sample = 5 g of leaf sample in a test tube at 55˚C for 1hr

Non-stressed = 5 g of leaf sample kept in a test tube in room temperature

$$\text{CSI} = \frac{\text{OD at 652 nm of treated sample}}{\text{OD at 652 nm of control}} \times 100$$

More physiological data viz. Days to maturity (DM) was measured by the date on which about 75% of plants of a plot turned brownish color was taken as attaining physiological maturity. The number of days from sowing to this date was counted. The length of the main shoot (MSL) of each sampled plant was measured in cm from the base of the inflorescence to the tip at crop maturing stage. The number of flowers on the main shoot (FMS) was measured in numbers on the main inflorescence at the flowering stage. The number of pods (siliquae) borne on the main shoot (SMS) of sampled plants was counted while measuring the main shoot length. The total number of siliquae borne per cm (SPS) of the main shoot was worked out. The total number of flowers borne on the terminal 15 cm of the main inflorescence and the number of siliquae borne on the terminal 15 cm of the main inflorescence were (STS) also recorded. Some other parameters like the difference between the number of flowers on the main shoot and siliquae developed on the main shoot, was considered as a number of flowers dropped. From this, the percent flower drop was worked out.

Heat susceptibility index (HSI)

Fisher and Maurer (1978) calculated the heat susceptibility index by the following formula.

$$\text{HSI} = \frac{(1\text{-}Y/Yp)}{(1\text{-}X/Xp)}$$

HSI ≤ 0.5 = Tolerant,
HSI > 0.5 –1.0 = Moderately tolerant,
HSI > 1.0 = Susceptible

Where,

Y= mean seed yield of a genotype in a stress environment, Yp= mean seed yield of a genotype in a stress-free environment, X = mean seed yield of all genotypes in stress environment, Xp = mean seed yield of all genotypes in a normal environment.

Statistical analysis

Analysis of the variance of each character was done following the standard statistical procedure. Combining ability analysis of the half diallel population was done following Model I, Method 2 of Griffing (1956).

Results

Analysis of variance of both stress and non-stress environments revealed that the genotypes differed significantly for all the characters studied. Mean values under TGT (stress condition) and non-stress condition are presented in Tables 1. A wide range for days to 50% flowering was recorded. The populations derived from the crosses, Jeuti x TS46, B9, YSH401 and YSH401 x TS46 were early flowering (40–43 days).

Pollen viability was affected by the increase in temperature and, therefore, the range for pollen leaf by was high under stress. Relatively high pollen viability was high under stress. Relatively high pollen viability was noticed in B9 x YSH401 (64.2%), YSH401 (63.5%), TS46 (60.9%) and B9 x TS46 (59.3%) crosses. On the other hand, in the open (non-stress), pollen viability showed narrow range (81.1 to 89.8%) as the temperature was always less than in the stress environment.

The cross, Jeuti x TS46 showed cooler leaf temperature (21.3°C) followed by TS46 (21.6°C), Jeuti (22.1°C) and NRCYS05-03 (22.6°C), while the cross B9 x TS46 recorded higher leaf canopy temperature (25.5°C). It was observed that leaf canopy temperature was lower outside the stress chamber. However, B9 x NRCYS05-03 and B9 x Jeuti showed 1 to 2°C higher temperatures in non-stress conditions. Again, in the non-stress environment, the chlorophyll stability index was found in the range of 55.01 to 81.66 per cent with a mean of 72%, whereas it was in the range of 45.69 to 76.09% with a mean of 64.3% in stress environment. In stress, the genotypes that exhibited good chlorophyll stability index were B9 (76.1%), B9 x TS46 (75.5%), YSH401 x TS46 (71%) and Jeuti (70.9%). Vegetative growth was more in stress than in non-stress conditions. In stress environment, the longest main shoot was produced in the cross NRCYS05-03 x Jeuti (84.8 cm) followed by Jeuti x TS46 (68.8 cm), B9 x YSH401 (67.8 cm), B9 x TS46 (66.5 cm) and YSH401 x NRCYS05-03 (62.7 cm). The mean value for main shoot length was 56.9 cm under stress and 48.7 cm under non-stress conditions. YSH401 x TS46, YSH401 x Jeuti, NRCYS05-03 x Jeuti and Jeuti x TS46 showed longer (>50 cm) main shoots than the others in a non-stress environment.

The parents and the hybrids produced 23 to 59 flowers on the main shoot under stress and 25 to 56 flowers in the non-stress environment (Table 1). The cross NRCYS05-03 x Jeuti showed the highest number of flowers, followed by B9 x Jeuti, Jeuti x TS46, TS46, YSH401 x Jeuti (>50) in stress. In non-stress YSH401 x TS46 exhibited the highest number of flowers followed by Jeuti x TS46 (>50). It was observed that high temperature affected siliqua development and as a result, in stress environment, only 26.2 siliquae were formed on main shoot on average while in non-stress, 33 siliquae were observed. In stress, Jeuti x TS46 exhibited the highest number of siliquae on the main shoot (38) followed by NRCYS05-03 x Jeuti (36.5), Jeuti (35.5), TS46 (33.5) and B9 x Jeuti (31.5). The average flower drop was 43.1 percent in stress and 21.3 percent in non-stress. Jeuti, Jeuti x TS46, TS46, B9 x NRCYS05-03, B9 x TS46 and NRCYS05-03 x Jeuti showed lesser flower drop in stress conditions. Siliqua density was also affected by temperature stress, with mean of 0.45 siliqua per cm of main shoot in stress and 0.66 siliqua per cm in non-stress. The toria parents Jeuti and TS46 showed higher siliqua density followed by NRCYS05-03 x TS46, Jeuti x TS46, B9 x NRCYS05-03 and B9 x Jeuti.

Due to flower drop the genotypes produced less siliquae in the terminal 15 cm of main shoot; on an average 7.5 siliquae were observed under stress and 9.4 siliquae under non-stress environment (Table 1). The cross YSH401 x TS46 showed the highest number of siliquae on terminal shoot (11.5) followed by B9 x TS46, YSH401 x NRCYS05-03, YSH401 x Jeuti, NRCYS05-03 x TS46, TS46 and B9 x Jeuti (8.5). In non-stress, B9 x NRCYS05-03 exhibited the highest number of siliquae on terminal shoot followed by B9 x TS46, YSH401 x TS46, YSH401 x Jeuti, YSH401 x NRCYS05-03, NRCYS05-03 x Jeuti, B9 x YSH401, B9 x Jeuti, NRCYS05-03, NRCYS05-03 x TS46 (>8).

A significant difference was recorded for the number of seeds per siliqua under the two environments. The mean value for this trait was 11.4 in stress and 16.2 seeds per siliqua in non-stress environment (Table 1). In stress environment, B9 x NRCYS05-03, Jeuti x TS46, TS46, YSH401 x NRCYS05-03, YSH401, Jeuti and B9 showed a higher number of seeds per siliqua. However, in non-stress environment, all the entries showed more than 10 seeds per siliqua. Seed size was adversely affected by high-temperature stress and as a result thousand seed weight was 2.87 g in stress as compared to 3.16 g in non-stress condition. YSH401 x NRCYS05-03, NRCYS05-03 x Jeuti, NRCYS05-03 x TS46, B9, B9 x Jeuti and B9 x TS46 exhibited heavier seeds under stress with more than 3 g per thousand seeds.

Biological yield was higher in stress condition with a mean value of 31.92 g per plant than in the non-stress condition (24.78 g) (Table 1). Under stress, NRCYS05-03 x Jeuti, NRCYS05-03 and YSH401 x Jeuti (>40 g) and under non-stress, NRCYS05-03, NRCYS05-03 x Jeuti, YSH401 x TS46 and B9 x NRCYS05-03 gave high biological yield per plant. Lesser seed yield (5.03 g per plant) was recorded in stress than in a non-stress environment (9.04 g/plant). In

Table 1. Mean values of diallel populations of rapeseed tested in high temperature stress (S) and non-stress (NS) environments

Population	Days to 50% flowering		Days to maturity		Pollen viability (%)		Leaf temperature (°C)		Chlorophyll stability index	
	S	NS	S	NS	S	NS	S	NS	S	NS
B9 x YSH401	45.5	42.5	116.5	102.5	64.16	87.72	23.83	23.42	66.21	75.66
B9 x NRCYS05-03	48.5	43.5	117.0	103.0	46.44	89.39	23.60	25.73	56.17	61.80
B9 x Jeuti	48.5	40.5	111.0	101.0	54.24	85.49	23.58	25.58	64.84	73.75
B9 x TS46	48.0	47.0	108.0	97.5	59.27	81.20	25.55	23.18	75.50	81.66
YSH401 x NRCYS05-03	46.0	48.5	124.0	103.5	46.45	85.82	25.20	21.95	49.81	58.24
YSH401 x Jeuti	44.0	43.5	114.5	98.0	44.81	85.84	25.16	24.03	45.69	55.04
YSH401 x TS46	43.5	50.0	108.5	97.0	43.53	89.05	24.20	23.20	71.00	79.90
NRCYS05-03 x Jeuti	45.5	41.5	113.5	101.5	43.40	83.78	23.95	22.51	55.94	67.78
NRCYS05-03 x TS46	47.5	36.5	114.0	102.0	37.84	87.74	23.12	22.74	66.83	77.87
Jeuti x TS46	41.5	38.5	98.5	93.5	51.80	89.75	21.28	20.94	66.25	78.83
B9	42.5	46.5	120.5	103.0	49.23	87.61	25.15	22.85	76.09	79.71
YSH401	43.5	44.5	120.5	102.5	63.54	88.89	23.90	21.77	67.89	71.94
NRCYS05-03	48.0	47.5	130.0	112.5	45.51	85.41	22.55	21.29	69.85	74.81
Jeuti	40.5	39.5	105.5	94.0	45.65	81.07	22.09	21.77	70.93	72.70
TS46	40.0	38.5	103.0	93.0	60.93	86.12	21.64	22.29	61.27	69.84
Mean	44.9	43.2	113.7	100.3	50.50	86.30	23.70	22.90	64.30	72.00
SE(m)	0.4	0.5	1.0	0.7	1.76	1.40	0.319	0.23	0.35	0.37
CD (P=0.05)	1.9	2.1	4.4	3.0	7.55	5.99	1.37	1.00	1.52	1.57
CD (P=0.01)	2.6	3.0	6.2	4.2	10.48	8.31	1.90	1.39	2.11	2.18

Table 1. Contd....

Population	Main shoot length (cm)		No. of flowers on main shoot		No. of siliquae on main shoot		Flower drop (%)		Siliqua density (No./cm)	
	S	NS	S	NS	S	NS	S	NS	S	NS
B9 x YSH401	67.85	46.90	46.7	42.3	28.0	37.0	40.00	12.45	0.41	0.79
B9 x NRCYS05-03	53.90	48.25	43.6	38.3	28.0	30.0	35.69	21.49	0.52	0.62
B9 x Jeuti	60.15	43.15	55.9	41.8	31.5	36.0	43.92	13.78	0.52	0.83
B9 x TS46	66.50	42.35	42.5	39.0	26.5	30.0	37.50	23.03	0.40	0.71
YSH401 x NRCYS05-03	62.70	48.30	37.1	45.3	20.5	37.0	44.74	18.06	0.33	0.77
YSH401 x Jeuti	56.85	63.95	50.2	44.1	27.5	39.5	45.16	18.83	0.48	0.62
YSH401 x TS46	58.70	70.75	41.5	56.5	24.5	56.5	40.83	9.73	0.42	0.80
NRCYS05-03 x Jeuti	84.83	63.25	59.5	49.8	36.5	47.0	38.69	15.65	0.43	0.74
NRCYS05-03 x TS46	47.80	43.75	45.8	37.5	26.5	34.0	42.08	15.58	0.55	0.78
Jeuti x TS46	68.85	55.50	54.7	51.3	38.0	51.5	30.39	11.40	0.55	0.87
B9	38.20	41.00	23.4	25.5	11.5	16.5	50.77	35.15	0.30	0.40
YSH401	43.00	33.80	40.5	26.0	14.5	12.8	64.10	49.70	0.34	0.39
NRCYS05-03	33.65	44.50	34.5	25.8	10.2	16.5	70.46	32.86	0.30	0.37
Jeuti	57.80	43.45	49.6	28.5	35.5	26.0	28.09	16.79	0.61	0.60
TS46	52.80	41.05	50.9	32.1	33.5	24.0	34.14	24.82	0.63	0.58
Mean	56.90	48.70	45.1	38.9	26.2	33.0	43.10	21.29	0.45	0.66
SE(m)	1.12	0.71	1.3	1.7	1.3	1.2	2.12	1.35	0.02	0.03
CD (P=0.05)	4.82	3.02	5.4	7.1	5.4	5.3	9.11	5.78	0.10	0.12
CD (P=0.01)	6.70	4.20	7.5	9.9	7.4	7.4	12.64	8.03	0.14	0.17

Table 1. Contd...

Population	No. of siliquae on terminal shoot		No. of seeds per siliqua		Thousand seed weight (g)		Biological yield per plant (g)		Seed yield per plant (g)		Heat susceptible index
	S	NS	S	NS	S	NS	S	NS	S	NS	
B9 x YSH401	6.5	9.5	10.5	12.5	3.020	3.105	25.05	21.10	5.50	5.70	0.383
B9 x NRCYS05-03	6.5	13.0	15.0	18.0	2.900	3.150	37.45	32.80	4.65	16.00	1.353
B9 x Jeuti	8.0	9.5	6.5	9.5	3.000	3.535	34.40	30.23	12.25	10.18	0.787
B9 x TS46	9.5	12.5	9.5	12.5	3.000	3.300	33.98	28.45	5.17	5.83	0.487
YSH401 x NRCYS05-03	8.5	10.5	12.5	16.5	3.210	3.405	27.90	19.48	3.02	8.15	1.208
YSH401 x Jeuti	8.5	11.0	10.5	11.5	2.695	2.920	29.95	22.00	5.88	6.74	0.250
YSH401 x TS46	11.5	11.5	8.5	11.5	2.640	2.960	46.25	33.56	9.49	11.43	0.572
NRCYS05-03 x Jeuti	6.5	10.5	11.5	14.5	3.100	3.400	46.85	37.70	7.77	13.40	0.805
NRCYS05-03 x TS46	8.5	8.5	11.0	13.5	3.100	3.200	35.45	26.70	7.54	16.13	1.050
Jeuti x TS46	5.5	7.0	14.0	16.0	2.300	2.960	27.35	20.80	4.05	14.60	1.411
B9	5.5	6.5	12.0	19.0	3.020	3.440	22.80	12.81	0.95	3.88	1.487
YSH401	5.5	6.0	12.5	18.5	2.900	3.050	22.39	13.30	1.68	4.30	1.199
NRCYS05-03	7.5	9.5	10.5	29.0	2.775	3.075	46.33	39.45	1.58	4.35	1.247
Jeuti	6.5	7.5	12.5	20.5	2.750	3.005	21.22	16.25	1.40	6.98	1.574
TS46	8.5	7.5	13.5	20.5	2.700	2.845	21.50	17.10	4.48	7.92	0.838
Mean	7.5	9.4	11.4	16.2	2.874	3.157	31.92	24.78	5.03	9.04	0.977
SE(m)	0.2	0.5	0.5	1.0	0.078	0.089	1.32	1.20	0.69	1.31	0.092
CD (P=0.05)	0.9	2.1	2.3	4.1	0.336	0.382	5.68	5.16	2.97	5.62	0.396
CD (P=0.01)	1.2	2.9	3.2	5.7	0.466	0.530	7.88	7.17	4.12	7.80	0.549

stress environment, B9 x Jeuti showed the highest seed yield per plant (12.25 g) followed by YSH401 x TS46 (9.49 g), NRCYS05-03 x Jeuti (7.77 g) and NRCYS05-03 x TS46 (7.54 g). These genotypes exhibited high seed yield also in non-stress conditions. The heat susceptibility index (HSI) was found to be lower in YSH401 x Jeuti (0.250), B9 x YSH401 (0.383) and B9 x TS46 (0.487).

Analysis of combining ability for morphological traits

Significant variation, in general, combining ability (GCA) effects was observed for all the characters except seeds per siliqua and seed yield per plant. Specific combining ability (SCA) effects showed significant variation for all the characters.GCA and SCA effects for yield adaptive characters are in stress conditions presented in Tables 2 and 3, respectively. YSH 401 showed good combining ability effect for heat tolerance as a parent (-0.150). The cross combinations YSH 401 x Jeuti (-0.654) and YSH 401 x TS46 (4.16) showed good combining ability effects for heat tolerance and seed yield per plant, respectively.

Evaluation of genetic parameters for morphological characters

To assess the magnitude and nature of genetic variation present in the materials, genotypic coefficient of variation (GCV), heritability in broad sense and expected genetic advance as a percentage of grand mean were worked out and presented in Table 4. The parents and crosses of the diallel responded to high temperature stress as reflected in highly significant changes in mean values for different characters. The high-temperature stress negatively affected the morpho-physiological yield attributes, resulting in drop of seed yield by 4.012 g per plant, which was 44.4 drop in seed yield.

Genotypic coefficient of variation was high for seed yield per plant (61.1%), number of siliquae on main shoot (32.6%), flower drop (25.7%), siliqua density (23.2%), main shoot length (22.7%), number of siliquae on terminal shoot (22.2%), biological yield per plant (22.1%) and number of flowers on main shoot (20.1%).

Table 2. General combining ability effects of the parental lines of rapeseed under high temperature stress condition

Parent	DM	PV	LT	CSI	MSL	STS	SPS	TSW	SYP	HSI
B9	1.64**	2.84**	0.71**	4.17**	-2.38**	-0.53**	-0.39	0.10*	-0.10	0.018
YSH401	3.21**	3.33**	0.61**	-2.46**	-1.33*	0.11	-0.17	0.02	-0.42	-0.150*
NRCYS05-03	6.64**	-5.37**	-0.13	-2.47**	-3.56**	-0.03	0.40	0.09*	-0.57	0.150*
Jeuti	-4.79**	-2.45**	-0.54**	-1.59**	6.41**	-0.53**	-0.10	-0.09*	0.37	0.077
TS46	-6.71**	1.65**	-0.64**	2.34**	0.86	0.97**	0.26	-0.11**	0.72*	-0.095
SE(gi)	0.49	0.84	0.15	0.17	0.54	0.09	0.26	0.04	0.33	0.044
SE(gi-gj)	0.78	1.33	0.24	0.27	0.85	0.15	0.41	0.06	0.52	0.070

* Significant at P=0.05 and ** Significant at P=0.01

Table 3. Specific combining ability effects of the diallel crosses in rapeseed under high temperature stress condition

Cross	DM	PV	LT	CSI	MSL	SMS	STS	SPS	TSW	SYP	HSI
B9 x YSH401	-2.02	7.54**	-1.14**	0.21	14.65**	8.63**	-0.62**	-0.31	0.03	0.99	-0.462**
B9 x NRCYS05-03	-4.95**	-1.48	-0.62	-9.82**	2.93*	8.29**	-0.48**	3.62**	-0.16*	0.30	0.209
B9 x Jeuti	0.48	3.40	-0.24	-2.02**	-0.79	1.41	1.52**	-4.38**	0.12	6.95**	-0.285
B9 x TS46	-0.60	4.33*	1.83**	4.70**	11.11**	-0.44	1.52**	-1.74	0.14	-0.48	-0.413*
YSH401 x NRCYS05-03	0.48	-1.97	1.07**	-9.55**	10.69**	1.86	0.88**	0.90	0.23**	-1.02	0.231
YSH401 x Jeuti	2.40	-6.52**	1.43**	-14.55**	-5.13**	-1.51	1.38**	-0.60	-0.10	0.90	-0.654**
YSH401 x TS46	-1.67	-11.91**	0.58	6.83**	2.27	-1.37	2.88**	-2.95**	-0.14	4.16**	-0.159
NRCYS05-03 x Jeuti	-2.02	0.76	0.97**	-4.29**	25.07**	7.14**	-0.48**	-0.17	0.23**	2.95**	-0.399*
NRCYS05-03 x TS46	0.40	-8.90**	0.24	2.67**	-6.41**	0.29	0.02	-1.02	0.25**	2.37**	0.018
Jeuti x TS46	-3.67**	2.15	-1.19**	1.21**	4.68**	1.41	-2.48**	2.48**	-0.37**	-2.06**	0.452**
SE(sij)	1.01	1.72	0.31	0.35	1.10	1.22	0.19	0.53	0.08	0.67	0.090
SE(sij-sik)	1.91	3.26	0.59	0.66	2.08	2.31	0.37	1.01	0.14	1.28	0.171
SE(sij-skl)	1.75	2.97	0.54	0.60	1.90	2.11	0.34	0.92	0.13	1.17	0.156

* Significant at P=0.05 and ** Significant at P=0.01

Heritability was found high for days to 50% flowering (91.3%), days to maturity (93.97%), pollen viability (82.98%), chlorophyll stability index (99.4%), main shoot length (97.06%), number of flowers on main shoot (92.76%), number of siliquae on main shoot (92.11%), siliqua density (83.85%), flower drop (87.19%), number of siliquae on terminal shoot (94.68%), biological yield per plant (91.98%) and seed yield per plant (83.12%). Genetic advance was high for main shoot length (46.1%), number of flowers on main shoot (39.8%), number of siliquae on main shoot (64.5%), siliqua density (43.7%), flower drop (49.5%), number of siliquae on terminal shoot (44.5%), number of seeds per siliqua (32.7%), biological yield per plant (55.5%) and seed yield per plant (114.7%). Moderate genetic advance was observed for pollen viability (28.91%) and chlorophyll stability index (28.43%).

Correlation analysis

Genetic correlation coefficients were estimated between all pairs of 13 quantitative characters in high-temperature

Table 4. Estimates of genetic parameters for different characters of diallel populations in Indian rapeseed under high temperature stress condition

Character	Mean		GCV (%)	Heritability$_{bs}$ (%)	Genetic advance (%)
	Stress	(S - NS)			
Days to 50% flowering	44.87	1.63**	6.35	91.28	12.51
Days to maturity	113.67	13.37**	7.18	93.97	14.35
Pollen viability (%)	50.45	-35.87**	15.41	82.98	28.91
Leaf temperature (°C)	23.65	0.77**	5.27	79.28	9.67
Chlorophyll stability index	64.28	-7.68**	13.85	99.37	28.43
Main shoot length (cm)	56.91	8.24**	22.72	97.06	46.11
No. of flowers on MS	45.08	6.63**	20.07	92.76	39.81
No. of siliquae on MS	26.18	-6.59**	32.64	92.11	64.53
Siliqua density (No./cm)	0.45	-0.20**	23.16	83.85	43.69
Flower drop (%)	43.10	21.62**	25.71	87.19	49.45
No. of siliquae on TS	7.53	-1.83**	22.19	94.68	44.48
No. of seeds per siliqua	11.37	-4.87**	17.97	77.80	32.65
1000 seed weight (g)	2.87	-0.28**	7.15	63.29	11.72
Biological yield per plant (g)	31.92	7.14**	28.08	91.98	55.49
Seed yield per plant (g)	5.03	-4.01**	61.06	83.12	114.68

Table 5. Genotypic correlation coefficients between different characters under high temperature stress

	DM	PV	LT	CSI	MSL	FMS	SMS	STS	SPS	TSW	BYP	SYP
DF	0.536*	-0.198	0.458	-0.099	0.004	-0.104	-0.276	0.212	-0.441	0.611*	0.604*	0.389
DM		-0.169	0.457	-0.136	-0.508	-0.653**	-0.835**	-0.135	-0.026	0.655**	0.238	-0.314
PV			-0.050	0.264	0.032	0.029	-0.028	-0.264	0.022	-0.051	-0.548*	-0.140
LT				-0.154	0.076	-0.434	-0.425	0.323	-0.415	0.695**	0.134	0.168
CSI					-0.327	-0.380	-0.269	-0.054	-0.247	-0.128	-0.064	-0.188
MSL						0.715**	0.783**	0.088	-0.066	0.067	0.174	0.492
FMS							0.906**	0.029	-0.078	-0.276	0.113	0.606*
SMS								0.022	0.111	-0.357	-0.055	0.449
STS									-0.605*	-0.001	0.456	0.545*
SPS										-0.231	-0.396	-0.716**
TSW											0.089	0.071
BYP												0.497

*Significant at P=0.05; ** Significant at P=0.01

stress conditions (Table 5). Days to 50% flowering positively correlated with DM, TSW and BYP. DM showed a positive correlation with TSW but a negative correlation with flower number on the main shoot and siliquae on the main shoot. PV is negatively correlated with biological yield per plant; leaf temperature positively correlated with TSW. On the other hand, STS showed a positive correlation with SYP seeds per siliqua showed a negative correlation with SYP.

Main shoot length showed a positive correlation with flowers as well as siliquae on main shoot. Number of flowers on main shoot was positively correlated with siliquae on main shoot and seed yield per plant.

Discussion

Variations in mean values for physiological and morphological characters were observed under stress and non-stress environments. In an open environment (non-stress), the genotypes matured 13 affected days earlier than in a stress environment. The vegetative growth was affected whereas the physiological traits and yield parameters were reduced under high-temperature environment. Annisa et al. (2013) observed that pollen viability during the high-temperature treatment decreased slightly as compared to control. Young et al. (2004) showed reduction of around 40% of pollen viability during the 5th day of high-temperature treatment in *B. napus*. Heat stress has adverse effect on crop yields if it occurs during the flowering and seed development stages (Saini et al., 1983; Jiang et al. 2015). High temperature beyond a certain threshold affects pollen development, pollination, fertilization and embryo development in cool-climate crops such as *Brassica* spp. (Prasad et al. 2017; Rieu et al. 2017). Chen et al. (2020) also reported such results in canola. In the present study, flower distortion and drying of flower buds were observed due to the high temperature effect. It was also observed that high temperature reduced flower and siliqua development, seed weight and siliqua density. Morrison and Steward (2002) reported that a temperature, more than 27˚C, induced floral sterility, reducing the number of siliquae on main shoot and seeds per siliqua. Nilsson (1994) and Pinnet et al. (2015) reported that temperature beyond a threshold level resulted in the formation of more number of flower buds but this higher flower induction was upset by reduced seed weight. Chugh and Sharma (2022) observed that temperatures more than 30˚C during seed filling influence stem reserve accumulation, mobilization and translocation in Indian mustard, leading to yield losses. Pokharel et al. (2020) reported that not only day temperature, high night temperature also cause a significant yield loss through alteration in the reproductive organ.

Heat susceptibility index is an important criterion for screening of temperature tolerant genotypes. In the present study, YSH401 x TS46, B9 x Jeuti, NRCYS04-03 x Jeuti and TS46 were found to be heat tolerant (HSI<1). Bhardwaj et al. (2017) reported in wheat that heat susceptibility index value less than unity is considered as tolerant to heat and taken as important criteria for breeding wheat genotypes suitable for heat stress. The results showed that high temperature mainly disturbed the siliqua formation without having adverse effects on plant growth and development. It was observed that flowers on main shoot and siliquae on terminal shoot were influential for seed yield under stress. Bahrami et al. (2021) reported that heat susceptibility index provides a measure of yield stability based on the minimization of yield loss under stressed conditions in *B. juncea* and, therefore, a stress tolerant genotype as identified by HSI need not necessarily have high yield potential, but should have minimum yield reduction. Jeuti and TS46 showed desirable adaptive yield attributes such as early maturity, low leaf temperature, high chlorophyll stability index, more siliquae on main shoot, less flower drop, high siliqua density, more seeds per siliqua. TS46 also exhibited heat tolerance.

The two toria parents (Jeuti and TS46) showed good combining ability for all yield-attributing characters. It is therefore, advocated that such genotypes may be useful in breeding for heat tolerance. However, the yellow sarson parent YSH401 was found as good combiner for heat tolerance. The crosses B9 x Jeuti, YSH401 x TS46, NRCYS05-03 x Jeuti and NRCYS05-03 x TS46 showed desirable characters, including heat tolerance. Both additive and non-additive gene actions were indicated as important for expression of characters in stress conditions. Singh et al. (2017) reported that the positive GCA effect for chlorophyll content and negative GCA effect for canopy temperature were considered good combiner for heat tolerance in Indian mustard. Kutlu and Sirel (2019) reported that negative GCA effects showed lower canopy temperatures in most tested materials and were desirable for higher grain yield in wheat.

A considerable level of genetic variation was present in the genotypes for main shoot length, number of flowers on main shoot, number of siliquae on main shoot, siliqua density, flower drop, number of siliquae on terminal shoot, biological yield per plant and seed yield per plant. Moreover, these characters showed high heritability with high genetic advance, indicating additive gene action for these traits. Therefore, simple selection methods such as mass selection or pedigree selection could effectively improve such characters under high temperature stress conditions. The chlorophyll stability index showed high heritability with moderate genetic advance, indicating the importance of non-additive gene action.

Correlation analysis showed that longer the main shoot higher would be the flower number, siliqua number per plant and ultimately increase the seed yield. Annisa et al. (2013) reported positive association between flower and siliquae number with seed number and yield under high-temperature stress in *B. rapa*. Islam et al. (2015) also reported positive association among yield per plant, number of siliquae per plant and seeds per siliqua in some inter-varietal crosses of *B. rapa* in a normal grown yield experiment. Stress indices have been widely used in different crops for screening tolerant genotypes based on yield reduction under stress conditions. The mathematical relationship between stress and optimum conditions based on seed yield has been anticipated by many scientists (Bahrami et al. 2014; Bahrami et al. 2021) to be helpful in selections. Chugh et al. (2022) identified promosing genotypes for heat tolerance based on higher values of yield index and yield stability index (YSI). However, YSI was significantly but negatively correlated with heat susceptibility index. It has been suggested that determination of promising genotypes based on heat stress indices may be a suitable option.

Chlorophyll stability index, leaf temperature, flowers on main shoot, siliquae on terminal shoot and thousand seed weight were found as the most important adaptive traits. Moreover, flowers on main shoot and siliquae on terminal shoot could be taken as sensitive indicator to heat susceptibility.

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Authors' contribution

Conceptualization of research (PKB, RNS, AP); Designing of the experiments (PKB, RNS, RD); Contribution of experimental materials (PKB, RD); Execution of field/ lab experiments and data collection (AP); Analysis of data and interpretation (PKB, AP); Preparation of manuscript (AP, RD, PKB).

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