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COMPARATIVE TOLERANCE AND SENSITIVE RESPONSE OF INDIAN MUSTARD (*Brassica juncea* L. Czern and Coss) GENOTYPES TO HIGH TEMPERATURE STRESS

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SUMMARY

Indian mustard (*Brassica juncea* L. Czern & Coss) is an important source of edible oil in India. This crop experiences substantial yield loss due to high temperature, especially at the seedling stage. Therefore, improving seedling stage heat tolerance in Indian mustard is an important breeding objective. In this study, 53 genotypes, including two checks (BPR-543-2 and RH-30), were evaluated for heat tolerance under heat stress (maximum temperature 40.1 °C at 0-10 cm depths on seeding date on September 26, 2012) and normal (maximum temperature 31.0 °C at 0-10 cm depth on seeding date on October 23, 2012) conditions at the research farm of ICAR- Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, India. Results indicate that most Indian mustard genotypes respond differently for eight physiological traits under normal and high temperature stress conditions. Among the 53 genotypes used for the study, BPR-349-9, Urvashi, BPR-541-2, BPR-605-40, Pusa Tarak (EJ9912-13), RGN-48, BPR-549-2, DRMR-729 and DRMR-1918 were identified as heat-tolerant at seedling stage with heat susceptibility index (S) and yield stability ratio (YS), ≤ 0.5 and > 80%, respectively. The relative water content showed significant negative correlation with excised-leaf water loss (r = -0.385**) under heat stress condition. The higher membrane stability index and high population survival might also have provided high temperature stress tolerance in genotypes DRMR-541-44 and RGN-48. The heat tolerant genotypes identified in this study could be included in future breeding programmes.

Key words: Genotype, *Brassica juncea*, excised-leaf water loss, relative water content, membrane stability index, heat susceptibility index

Key findings: Based on heat stability index, yield stability ratio and higher RWC values, genotypes BPR-349-9, Urvashi, BPR-541-2, RGN-48, BPR-549-2, DRMR-729 and DRMR-1918 were identified as heat tolerant at seedling stage.

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INTRODUCTION

Indian mustard (*Brassica juncea* L. Czern and Coss) is an important edible oil yielding crop in India. It accounts for nearly 30% of the total oilseed production and 31.4% to edible oil pool of the country (Singh *et al*., 2014). Comparing three species of oilseed brassica for variation in critical temperature and the most sensitive crop growth stage for high temperature stress, (Angadi *et al*., 2000) identified Indian mustard to have greater tolerance to heat and water stress than the canola quality Indian mustard. The cultivation of Indian mustard is largely carried out under the rainfed farming systems where sowing commences after south-west monsoon rains (Venkateswarlu and Prasad, 2012). Early rains may cause the farmers to sow the crop early in the season to take advantage of the conserved moisture in the soil. But high temperature prevailing at the time of sowing reduces seed germination and causes seedling mortality, resulting in poor crop stand and reduced seed yield (Azharudheen *et al*., 2013). Wide variations in diurnal soil temperatures ranging from 28 °C to 56 °C at the surface and from 33 °C to 37 °C at 300 mm depth were observed in Rajasthan (Gupta, 1986). The average surface soil temperature may reach as high as 45 °C during second fortnight of September and first fortnight of October in Rajasthan. This may lead to situation where the crop needs to be re-sown before a final successful crop is established. This causes a lot of economic loss to the farmers (Salisbury and Gurung, 2011). More frequent weather aberrations and high temperature events are expected due to climate change scenario resulting in greater impact of heat on mustard production. Efforts to strengthen resilience are required by utilizing diverse heat tolerant genotypes in brassica crop improvement programmes. This will help to stabilize the productivity and to meet the growing demands of edible oil of the country. Therefore, this study was designed to assess the genetic variability for seedling heat tolerance in the Indian mustard germplasm and to characterize the selected heat tolerant and susceptible genotypes. Thermo-tolerant genotypes identified in the study would have important bearing on brassica breeding programmes aimed at improving tolerance to heat stress at seedling stage.

MATERIALS AND METHODS

Fifty-three Indian mustard genotypes were procured from the ICAR-Directorate of Rapeseed-Mustard Research (ICAR-DRMR), Bharatpur. Two hundred seeds of each genotype including two checks (BPR-543-2 and RH-30), were sown under heat stress (maximum temperature 40.1 °C at 0-10 cm depth on seeding date on September 26, 2012) and normal conditions (maximum temperature 31.0 °C at 0-10 cm depth on seeding date on October 23, 2012) in complete randomized block design with three replications at the ICAR-DRMR research farm, Bharatpur (77.27º E longitude; 27.12º N latitude and 178.37 m above mean sea level), Rajasthan, India. The soil of the experimental site was sandy loam with EC 1.5 dSm^{-1}, organic carbon (0.25 - 0.30%), available N (125-135 kg/ha), P (20-22 kg/ha), K of 240-260 kg/ha, and pH of 8.1. The crop was raised strictly under conserved moisture conditions. All genotypes were grown in two rows of 5 m length; with row to row and plant to plant spacing of 30 cm and 10 cm, respectively. Growth and physiological characters, including, population survival (PS) at 10 and 25 days after sowing (DAS), membrane stability index (MSI), excised-leaf water loss (ELWL), relative water content (RWC), water retention capacity of leaves (WRCL), oil content, seed yield per plant (g), heat susceptibility index (S) and yield stability ratio (YS) were recorded from five randomly selected plants of each genotype.

Determination of growth and physiological parameters

Leaf membrane stability index (MSI) was determined following the method of Premachandra *et al*. (1990) as modified by Sairam (1994). Leaf stripes (0.2 g) of uniform size were placed in test tubes containing 10 ml of double distilled water in two sets. Test tubes in one set were kept at 40 °C in a water bath for 30 min and electrical conductivity of the water containing the sample was measured (C_1) using a conductivity bridge. Test tubes in the other set were incubated at 100 °C in boiling water in water bath for 15 min and electrical conductivity was measured as above (C_2). The MSI was calculated using the following formula:

$$MSI= [1- C_1/ C_2] \times 100$$

For determining excised- leaf water loss (ELWL), the leaves were weighed at three stages viz. immediately after sampling (fresh weight); after drying in an incubator at 28 °C and 50% R.H. for 6 h; and after oven drying for 24 h at 70 °C as suggested by Clarke, (1987). The ELWL was calculated using the following formula:

$$ELWL = [\text{fresh weight} - \text{weight after 6 h}) / (\text{fresh weight} - \text{dry weight}] \times 100$$

The samples for relative water content (RWC) were also weighed immediately to obtain fresh weight (FW); 2 cm leaf sections were floated in distilled water for 4 h, blot-dried and weighed to obtain turgid weight (TW); The 2.0 cm leaf sections were oven dried at 60 °C for 24 h and weighed to obtain dry weight (DW). The RWC was calculated using the formula of Barrs (1968):

$$RWC (\%) = [FW - DW) / (TW - DW] \times 100$$

The water retention capacity of leaves (WRCL) was estimated by the method proposed by Ashraf and Ahmad (1998).

The heat susceptibility index (S) was calculated using the formula of Fisher and Maurer (1978):

$$S = (1 - Y/Yp) / (1 - X/Xp)$$

where Y= mean seed yield of a genotype in a stress environment, Yp= mean seed yield of a genotype in a stress free environment (normal), X= mean Y (seed yield) of all genotypes in stress environment, Xp= mean Yp (seed yield) of all genotypes in normal. S is the relative heat stress tolerant ($S \leq 0.5$ stress tolerant, $S > 0.5 - 1.0$ moderately stress tolerant and $S > 1.0$ susceptible).

Yield stability ratio (YS) was using the formula of Lewis (1954):

$$YS = (\text{seed yield under stress} / \text{seed yield under normal condition}) \times 100$$

All mature siliquae from five randomly selected plants were threshed and average seed weight per plant was calculated. The oil content (%) was estimated using nuclear magnetic resonance (NMR), according to the protocol of the AOCS (1980).

Statistical analysis

Analysis of variance (ANOVA) was performed on the data following Panse and Sukhatme (1978), and critical difference (CD) calculated at 5% probability level. Correlation analysis between seed yield per plant and physiological parameters were determined using Windostat version 8.5 software.

RESULTS AND DISCUSSION

Temperature is an important factor which affects growth and development of plants. All plants require a certain amount of heat units during growth periods and the duration to achieve heat units depends upon the climatic conditions. For each set of experiments, high temperature stress was created by sowing in the last week of September under conserve moisture conditions. Results indicate that most genotypes respond differently under normal and high temperature stress conditions. The population survival at 10 and 25 DAS decreased with increasing heat stress in all genotypes. However, the decrease was lesser in BPR-349-4, RGN-48, DRMR-541-44, DRMR-1321 and Narendra Rai. Under non-stress condition, the decrease in population survival in 10 and 25 DAS was lesser in CS-54 and Pusa Tarak (Table 1).

Heat shock increases cell membrane permeability, thereby inhibiting cellular function, as a result of the denaturation of proteins and increments of unsaturated fatty acids that disrupt water, ion, and organic solute movement across membranes. Thylakoid membranes typically show swelling, increased leakiness, physical separation of the chlorophyll light harvesting complex II from the PSII core complex, and disruption of PSII-mediated electron transfer (Ristic *et al*., 2008).

Membranes are main loci affected under heat stress conditions. In this investigation, membrane stability index (MSI) increased under heat stress in most genotypes.

Table 1. Mean performance of genotypes under high temperature stress and normal regime.

Genotype	PS at 10 DAS		PS at 25 DAS		MSI	
	Normal	Stress	Normal	Stress	Normal	Stress
NPJ-93	69.1	48.1	62.6	42.2	12.4	14.5 (17.1)
NPJ-113	66.4	47.0	57.5	37.4	6.5	8.4 (28.6)
NPJ-124	70.8	52.5	60.4	41.0	6.2	7.0 (11.7)
NPJ-112	58.5	37.2	45.3	25.3	21.7	24.6 (13.6)
Pusa Agrani	58.7	39.4	46.5	26.5	8.1	11.2 (37.5)
EJ-17	54.3	35.4	48.6	28.6	16.5	20.0 (21.4)
NRCDR-02	62.6	41.4	48.9	29.6	37.5	39.4 (5.1)
BPR-543-2 (Check)	68.5	49.8	58.7	42.1	15.3	16.2 (6.2)
BPR-349-9	64.2	46.3	54.5	36.5	27.5	28.5 (3.7)
BPR-540-6	75.3	54.5	62.3	42.4	16.1	17.3 (7.4)
RGN-13	62.4	41.2	49.5	29.4	34.4	35.2 (2.1)
RGN-12	67.5	46.3	33.5	33.4	36.3	38.8 (6.8)
NRCDR-601	58.4	21.7	40.7	17.0	14.3	15.3 (6.8)
NRCHB-101	54.4	15.3	35.4	13.2	31.3	32.5 (4.0)
RH-30 (Check)	54.5	18.3	36.2	16.0	20.6	22.2 (8.1)
Urvashi	56.4	32.9	40.5	20.5	25.3	28.7 (13.4)
BPR-549-9	63.0	29.3	37.6	23.3	20.6	22.4 (8.7)
BPR-541-2	85.4	65.5	71.2	51.2	7.7	9.2 (19.0)
JR-042	62.1	42.3	50.2	29.2	8.7	11.2 (29.4)
JN-032	64.8	61.2	72.1	52.3	36.4	38.3 (5.1)
JN-031	60.6	40.2	49.3	29.3	25.1	27.8 (10.8)
Varuna	53.5	34.5	41.1	21.5	21.8	24.3 (11.9)
GM-2	87.0	71.3	78.8	59.4	50.3	56.2 (11.7)
RB-50	87.6	69.7	78.0	58.2	6.6	10.4 (57.0)
DFS-45	56.8	46.1	54.3	34.2	26.4	28.6 (8.4)
BPR-659-49	68.1	54.9	61.6	42.4	28.6	31.2 (9.0)
BPR-605-40	74.5	55.3	64.1	43.5	20.6	22.2 (8.1)
DRMR-537-40	84.1	61.3	68.6	48.3	36.6	39.1 (6.8)
Pusa Tarak (EJ9912-13)	94.5	90.3	94.5	82.4	41.5	43.3 (4.4)
CS-54	90.5	79.4	97.7	68.5	35.5	39.2 (10.2)
Kanti	94.7	81.3	82.4	72.3	39.6	41.5 (4.8)
Pusa Jaikisan	93.3	83.5	84.7	74.6	49.5	51.1 (3.1)
GM-3	94.4	87.2	87.6	77.6	23.6	25.5 (8.0)
RGN-48	97.1	94.1	96.9	88.3	38.3	42.8 (11.7)
Rohini	92.4	81.5	80.3	70.3	16.5	18.3 (10.6)
Narendra Rai	95.9	90.4	90.9	81.2	25.1	29.1 (15.7)
BPR-549-2	91.8	82.7	83.8	73.7	50.0	52.5 (5.0)
BPR-541-4	95.2	91.2	90.0	80.5	32.7	34.3 (5.1)
DRMR-541-44	96.6	96.1	91.9	89.1	56.6	57.8 (2.1)
BPR-349-4	97.5	96.2	94.1	88.0	7.0	9.8 (39.8)
BPR-325-4	94.7	90.3	91.1	81.5	10.8	14.5 (34.9)
BPR-181-14	92.1	89.7	89.2	80.3	21.5	23.8 (10.5)
DRMR-1346	90.7	80.3	81.3	70.4	11.2	12.7 (13.2)
DRMR-1351	87.4	77.1	85.3	65.5	5.4	7.3 (25.0)
DRMR-1826	91.4	86.2	86.6	76.4	26.5	30.4 (14.5)
DRMR-1321	96.1	94.2	89.3	80.1	29.8	32.6 (9.5)
DRMR-1350	90.2	81.2	85.3	72.3	24.6	26.1 (6.2)
DRMR-324	90.9	88.1	88.2	78.0	45.6	49.2 (7.9)
DRMR-1780	81.0	62.2	70.9	50.3	14.5	16.6 (14.4)
DRMR-729	85.4	65.3	75.4	55.3	36.6	40.8 (11.3)
DRMR-1918	90.4	84.6	84.9	74.5	21.3	23.5 (9.9)
Maya (EC-98)	80.7	72.2	80.3	60.3	6.8	8.7 (28.3)

Genotype	PS at 10 DAS		PS at 25 DAS		MSI	
	Normal	Stress	Normal	Stress	Normal	Stress
DRMR-675	89.2	86.5	89.1	79.6	24.8	30.3 (22.2)
Mean	78.2	63.6	69.4	53.7	24.8	27.2 (9.7)
Range	53.5-97.5	15.3-96.2	33.5-97.7	13.2-89.1	5.4-56.6	7.0-57.8
CV (%)	4.2	2.8	5.9	7.3	9.6	18.3
CD at 5%	5.3	2.9	6.7	6.3	3.8	8.1

Genotype	ELWL		RWC		WRCL	
	Normal	Stress	Normal	Stress	Normal	Stress
NPJ-93	30.4	34.9	79.5	78.5 (1.3)	28.8	24.2 (15.9)
NPJ-113	24.6	29.8	81.5	62.6 (23.2)	27.6	22.4 (18.8)
NPJ-124	22.8	25.5	80.2	63.5 (20.8)	20.4	18.3 (10.3)
NPJ-112	20.1	23.4	73.2	65.4 (10.6)	24.5	21.7 (11.5)
Pusa Agrani	18.3	21.4	76.2	68.4 (10.2)	29.5	25.3 (14.2)
EJ-17	22.5	24.3	77.2	59.4 (23.0)	23.6	21.2 (10.1)
NRCDR-02	23.4	25.6	78.5	71.1 (9.4)	30.4	26.3 (13.6)
BPR-543-2 (Check)	25.3	27.0	80.9	60.9 (24.7)	30.8	28.3 (8.1)
BPR-349-9	14.3	16.6	76.3	65.3 (14.3)	28.3	25.2 (11.0)
BPR-540-6	20.4	22.1	81.2	67.6 (16.7)	37.3	24.6 (34.1)
RGN-13	19.3	21.6	79.3	70.1 (11.5)	45.0	40.4 (10.2)
RGN-12	20.6	22.8	79.3	68.9 (13.2)	41.5	37.3 (10.2)
NRCDR-601	25.4	27.3	85.7	64.3 (25.0)	29.6	27.5 (7.3)
NRCHB-101	19.3	21.6	78.9	70.7 (10.4)	39.5	35.7 (9.3)
RH-30 (Check)	31.1	33.4	72.2	65.6 (9.2)	38.3	34.3 (10.3)
Urvashi	26.3	28.4	83.4	68.3 (18.2)	35.2	32.4 (8.1)
BPR-549-9	22.5	24.1	83.5	70.4 (15.8)	27.0	25.5 (5.7)
BPR-541-2	19.5	21.7	76.4	65.9 (13.7)	38.4	36.4 (5.2)
JR-042	30.3	32.4	82.5	74.4 (9.9)	34.4	32.5 (5.6)
JN-032	16.4	18.5	78.7	68.9 (12.4)	38.0	34.4 (9.5)
JN-031	21.7	24.8	77.8	65.6 (15.8)	22.4	20.5 (8.7)
Varuna	20.1	22.4	78.3	66.7 (14.8)	44.6	40.6 (9.0)
GM-2	28.2	30.3	81.4	68.4 (16.0)	29.7	25.9 (12.9)
RB-50	22.6	26.5	85.3	75.6 (11.3)	38.0	35.9 (5.8)
DFS-45	21.0	24.2	81.5	54.7 (32.9)	32.0	28.4 (11.1)
BPR-659-49	22.7	25.2	82.3	59.3 (28.0)	27.4	24.5 (10.5)
BPR-605-40	34.4	36.2	56.5	51.0 (9.9)	20.3	15.5 (23.8)
DRMR-537-40	26.4	28.2	82.3	57.1 (30.6)	40.3	35.5 (12.1)
Pusa Tarak (EJ9912-13)	20.2	22.4	75.3	70.5 (6.3)	24.4	22.4 (8.3)
CS-54	21.6	23.0	84.9	70.2 (17.3)	30.3	24.4 (19.4)
Kanti	24.5	26.2	76.5	70.4 (8.0)	22.4	19.5 (12.9)
Pusa Jaikisan	24.3	26.5	83.5	60.8 (27.2)	20.5	18.6 (9.5)
GM-3	16.4	20.2	79.3	66.6 (16.0)	33.3	30.3 (9.0)
RGN-48	21.3	23.9	80.5	65.5 (18.6)	71.5	62.3 (12.8)
Rohini	23.6	25.5	80.9	64.5 (20.2)	30.5	24.5 (19.7)
Narendra Rai	21.3	23.5	77.1	67.9 (67.9)	18.2	15.7 (13.5)
BPR-549-2	23.4	24.8	78.6	74.5 (5.2)	24.4	22.7 (7.0)
BPR-541-4	21.1	23.2	80.9	60.5 (25.3)	33.4	29.4 (11.9)
DRMR-541-44	16.3	18.5	84.5	67.5 (20.2)	19.2	17.7 (7.9)
BPR-349-4	22.6	24.5	79.8	66. (17.33)	19.4	16.2 (16.6)
BPR-325-4	16.6	20.5	78.4	67.3 (14.2)	24.3	20.7 (14.9)
BPR-181-14	18.4	20.4	81.2	60.4 (25.7)	28.4	25.4 (10.7)
DRMR-1346	26.1	26.5	81.4	68.4 (16.0)	30.4	25.5 (16.2)
DRMR-1351	18.4	19.4	87.8	70.3 (19.9)	30.4	24.4 (19.8)

Genotype	ELWL		RWC		WRCL	
	Normal	Stress	Normal	Stress	Normal	Stress
DRMR-1826	23.7	26.4	92.2	69.9 (24.2)	35.3	29.4 (16.8)
DRMR-1321	18.2	21.3	94.6	67.8 (28.3)	39.4	36.5 (7.3)
DRMR-1350	18.7	21.9	90.5	71.2 (21.4)	27.3	25.1 (8.0)
DRMR-324	26.3	30.8	77.6	65.4 (15.6)	38.3	35.4 (7.6)
DRMR-1780	18.2	20.3	95.4	57.4 (39.8)	32.3	30.5 (5.7)
DRMR-729	20.2	22.2	85.5	80.3 (6.1)	49.2	42.4 (13.8)
DRMR-1918	17.5	19.5	87.5	86.4 (1.3)	32.4	28.5 (12.2)
Maya (EC-98)	22.2	23.3	75.5	47.5 (37.1)	29.3	26.6 (9.0)
DRMR-675	34.2	36.8	45.7	42.5 (6.9)	21.2	20.2 (4.5)
Mean	22.4	24.8	80.1	66.2	31.5	27.8
Range	14.3-34.2	16.7-36.8	45.7-95.4	42.5 -86.4	18.2-71.5	15.5 -62.3
CV (%)	8.7	11.2	4.3	5.6	6.0	7.3
CD at 5%	3.1	4.5	5.5	6.0	3.1	3.3

Genotype	Oil content (%)		Seed yield per plant (g)		Heat susceptibility index (S)	Yield stability ratio (YS)
	Normal	Stress	Normal	Stress		
NPJ-93	40.6	38.8	25.7	13.0 (49.3)	1.7	50.7
NPJ-113	42.6	41.9	24.3	21.2 (12.7)	0.4	87.3
NPJ-124	42.5	41.8	41.3	20.3 (50.8)	0.2	49.2
NPJ-112	43.6	42.9	97.4	57.8 (40.6)	1.4	59.4
Pusa Agrani	42.3	41.5	23.5	15.6 (33.5)	1.2	66.4
EJ-17	42.5	42.0	29.0	11.4 (60.8)	2.1	39.2
NRCDR-02	41.8	41.4	27.6	16.5(11.1)	1.4	59.8
BPR-543-2 (Check)	41.6	42.2	25.5	14.0 (45.0)	1.6	54.9
BPR-349-9	42.6	42.4	18.3	17.3 (5.6)	0.2	94.4
BPR-540-6	42.2	41.5	21.6	18.0 (16.6)	0.6	83.3
RGN-13	41.1	40.0	28.4	17.3 (11.0)	1.3	61.1
RGN-12	41.7	40.6	32.6	27.2 (16.4)	0.6	83.5
NRCDR-601	42.3	40.6	39.7	32.6 (17.8)	0.6	82.2
NRCHB-101	42.3	41.9	34.6	20.5 (40.7)	1.4	59.3
RH-30 (C)	42.4	41.4	21.5	18.1 (15.8)	0.6	84.2
Urvashi	42.6	41.5	24.5	22.6 (8.0)	0.3	92.0
BPR-549-9	43.2	41.8	24.6	20.4 (20.0)	0.6	82.7
BPR-541-2	41.6	42.0	28.6	26.5 (7.5)	0.3	92.4
JR-042	41.8	41.6	19.5	17.4 (10.7)	0.4	89.2
JN-032	42.6	41.9	22.6	20.2 (10.7)	0.4	89.3
JN-031	41.8	40.7	29.5	20.4 (30.7)	1.1	69.3
Varuna	41.6	41.4	29.4	13.4 (54.4)	1.9	45.6
GM-2	41.7	40.9	26.4	23.1 (12.2)	0.4	87.8
RB-50	41.2	40.8	23.7	21.4 (9.7)	0.3	90.3
DFS-45	42.1	40.7	21.7	17.0 (21.6)	0.8	78.3
BPR-659-49	42.6	42.5	26.0	21.5 (17.2)	0.6	82.8
BPR-605-40	42.4	41.2	21.0	19.4 (7.4)	0.3	92.5
DRMR-537-40	41.7	41.4	23.6	20.5 (13.0)	0.5	87.0
Pusa Tarak (EJ9912-13)	41.4	41.0	23.0	21.1 (8.1)	0.3	92.0
CS-54	42.4	41.8	24.0	20.2 (16.0)	0.6	84.0
Kanti	42.6	42.4	25.8	21.5 (16.6)	0.6	83.3
Pusa Jaikisan	42.6	41.5	26.0	23.5 (9.6)	0.3	90.4
GM-3	42.0	41.4	26.6	16.5 (37.8)	1.3	62.1
RGN-48	42.7	41.4	24.7	22.6 (8.4)	0.3	91.5
Rohini	42.9	41.5	24.9	20.5 (17.5)	0.6	82.5

Genotype	Oil content (%)		Seed yield per plant (g)		Heat susceptibility index (S)	Yield stability ratio (YS)
	Normal	Stress	Normal	Stress		
Narendra Rai	42.6	40.4	25.6	19.5 (6.1)	0.8	76.2
BPR-549-2	41.4	39.9	25.0	23.2 (7.2)	0.3	92.8
BPR-541-4	41.2	39.7	25.0	18.4 (28.4)	0.9	73.6
DRMR-541-44	41.0	40.9	29.0	23.1 (20.3)	0.7	79.7
BPR-349-4	41.7	41.3	24.0	18.2 (24.1)	0.9	75.8
BPR-325-4	42.3	40.3	26.5	22.0 (17.0)	0.6	82.9
BPR-181-14	41.5	42.1	30.0	28.2 (5.9)	0.2	94.1
DRMR-1346	41.3	41.9	24.6	21.3 (17.2)	0.5	86.7
DRMR-1351	41.9	40.7	16.5	11.0 (33.1)	1.2	66.8
DRMR-1826	42.5	40.0	13.5	7.6 (43.8)	1.5	56.2
DRMR-1321	41.4	41.4	19.2	12.0 (37.5)	1.3	62.4
DRMR-1350	41.1	40.8	35.0	12.6 (64.0)	2.2	36.0
DRMR-324	41.5	40.9	23.0	17.6 (23.6)	0.8	76.4
DRMR-1780	41.6	39.5	25.0	22.0 (12.0)	0.4	88.0
DRMR-729	41.7	40.7	18.0	16.8 (6.6)	0.2	93.3
DRMR-1918	40.5	40.1	22.6	20.6 (8.9)	0.3	91.0
Maya (EC-98)	41.8	40.4	24.7	16.6 (32.6)	1.1	67.3
DRMR-675	42.5	40.2	30.2	21.4 (29.2)	1.0	70.8
Mean	42.0	41.2	26.9	19.3		
Range	40.5-43.6	38.8-42.9	18.0-97.4	7.6-32.6		
CV (%)	1.5	2.0	8.0	11.4		
CD at 5%	0.4	0.4	3.5	3.6		

Among the genotypes MSI was high in DRMR-541-44, GM-2, BPR-549-2, Pusa Jaikisan and DRMR-324 under normal and stress conditions, while it was low in NPJ-124, DRMR-1351, NPJ-113, Maya (EC-98) and BPR-541-2 at high temperature stress relative to the normal (Table 1). Maximum increase in MSI under stress condition was experienced in RB-50 (57.0%), BPR-349-4 (39.6%) and Pusa Agrani (37.5%). Since membrane damage increased with increase in stress level, MSI can be considered a very significant tool for evaluating the heat tolerance potential in Indian mustard genotypes. Similar increase in cell membrane stability under high temperature stress has also been reported in Indian mustard (Ram *et al.*, 2012) and in cowpea (Ismail and Hall, 1999).

Thermo-tolerant genotypes have less excised-leaf water loss (ELWL) compared to thermo-susceptible genotypes. Mean values of all genotypes were significantly lower for ELWL and higher for RWC than overall means value (Table 1). Genotypes JR-042, BPR-605-40 and NPJ -93 were identified as heat sensitive and BPR-349-9, JN-032, GM-3, DRMR-541-44 and BPR-325-4 as heat tolerant for ELWL, irrespective of environmental condition. This finding is in good agreement with sorghum genotypes at seedling and post-anthesis stages (Ali *et al.*, 2009). The results revealed a significant difference in relative water content in Indian mustard genotypes (Table 1). In general, heat stress adversely affected relative water content of mustard genotypes. The significantly lower RWC values under heat stress conditions (1.3% - 39.8%) indicated that in general, the loss of water from leaves at reproductive stage was higher with poor retention (Table 1). Under the heat-stress conditions, the RWC values of genotypes DRMR-1918 (86.4%; 1.3), DRMR-729 (80.3%; 6.1), NPJ-93 (78.5%; 1.3) and RB-50 (75.6%; 11.3) were higher compared to genotypes DRMR-675 (42.5%; 6.9), Maya (47.5%; 37.1), BPR-605-40 (51.0%; 9.9) and DFS-45 (54.7%; 32.9) (Table 1). Higher RWC values in leaves are good indicators of heat resistance. Our present findings are in agreement with the earlier studies on wheat (Dhanda and Sethi, 1998) and Indian mustard (Ram *et al.*, 2012, 2014; Sudhir *et al.*, 2013).

Irrespective of the environmental conditions, the water retention capacity of leaves

(WRCL) values of genotypes RGN-48, DRMR-729, RGN-13 and Varuna were high. Under heat-stress conditions, the significantly lower WRCL values ranged from 4.5 to 34.1%; under normal conditions, the higher WRCL values were observed in genotypes RGN-48, DRMR-729, RGN-13 and Varuna (Table 1). The water retention capacity of leaves (WRCL) appears to be a good characteristic of heat/drought resistance in wheat cultivars (Kirkham *et al*., 1980; Salim *et al*., 1969); Pelissier with an extensive root system has long been recognized as a heat/drought hardy cultivar (Hurd, 1964; Aamodt and Johnston, 1936). The leaf water retention trait can be screened relatively easily and may be used in breeding heat tolerant Indian mustard.

Under normal conditions oil content was high in cv. NPJ-112, PBR-549-9, Rohini, RGN-48 and Narendra rai compared to DRMR-1918, DRMR-541-44 and NPJ-93 genotypes; genotypes NPJ-112, BPR-349-9 and BPR-659-49 recorded high oil content compared to check varieties BPR-543-2 and RH-30 under heat stress condition. The findings that oil content decreased considerably under high temperature stress at seedling stage are in agreement with several earlier studies on brassica (Zada *et al*., 2013; Heenam and Armstrong, 1993; Mendham *et al*., 1981, 1990).

Heat-stress significantly reduced seed yield per plant. The reduction in seed yield per plant ranged from 64.0% in DRMR-2350 to 50.8% in NPJ-124; genotype BPR-349-9 recorded the lowest (5.6%) reduction in seed yield per plant.

The genotypes NPJ-112, NPJ-124, NRCDR-601 and DRMR-1350 recorded the maximum seed yield per plant under normal condition, while genotypes NRCDR-601, BPR-181-14 and RGN-12 the maximum seed yield per plant under stress conditions. The reduction in the total plant biomass due to heat stress in the early sown crops may have also resulted in the reduction of seed yield per plant. Similar reduction in seed yield and genotypic differences in early sown brassica species (Chauhan *et al*., 2009; Singh *et al*., 2010; Lallu *et al*., 2010; Ram *et al*., 2012) have also been reported. Based on the heat stability index (≤ 0.5) and yield stability ratio (>80%) genotypes, BPR-349-9, Urvashi, BPR-541-2, BPR-605-40, Pusa Tarak (EJ9912-13), RGN-48, BPR-549-2, DRMR-729 and DRMR-1918 were identified as heat tolerant at seedling stage (Table 1). This finding is also in agreement with earlier study on *B. juncea* genotypes at seedling and post-anthesis stages (Lallu *et al*., 2010).

The significant correlation between seed-yield and other physiological traits ranged from 0.295*- 0.993** (Table 2). The seed yield per plant had significant positive correlation with oil content (r = 0.295*) (Table 2) under normal stress condition while the oil content was significantly negatively correlated with population survival (r = -0.267*) (Table 2) under heat stress condition. The relative water content had a highly significant negative correlation with the excised-leaf water loss (r= -0.385**) under heat stress conditions, and the population survival at 25 DAS had significant positive correlation with the population survival at 10 DAS (r = -0.993**). Similar correlation between seed yield per plant and physiological traits had also been reported in Indian mustard by Ram *et al*., (2012); Sharma and Sardana (2013).

Holland (2006) observed that genetic correlations between traits are due to linkage and/or pleiotropy indicating the magnitude and direction of correlated response to selection. He also emphasized the relative efficiency of correlations facilitating indirect selection. The present findings indicate that since the traits are highly correlated, selection based on correlations may be a useful breeding strategy for indirect selection for higher seed yield potential (Ojaghi and Akhundova, 2010).

Thus, because of these results it can be inferred that the stress tolerance mechanism exists at seedling stage of Indian mustard genotypes. Based on heat stability index, yield stability ratio and higher RWC values, genotypes BPR-349-9, Urvashi, BPR-541-2, BPR-605-40, Pusa Tarak (EJ9912-13), RGN-48, BPR-549-2, DRMR-729 and DRMR-1918 were identified as heat tolerant at seedling stage. The genotypes DRMR-1918 and DRMR-729 are also comparatively tolerant to high temperature stress owing to higher in RWC.

Table 2. Correlation coefficient among seed yield per plant and stress tolerance parameters**.**

Character	Environment	Population survival 10 DAS	Population survival 25 DAS	Membrane stability index	Excised-leaf water loss	Relative water content	Water retention capacity of leaves	Oil content (%)	Seed yield/ plant (g)
Population survival 10 DAS	Normal	1.000	0.947**	0.180	-0.123	0.117	-0.091	-0.284*	-0.249
	Stress	1.000	0.993**	0.221	-0.183	0.091	-0.089	-0.260	0.011
Population survival 25 DAS	Normal		1.000	0.151	-0.147	0.063	-0.114	-0.263	-0.266*
	Stress		1.000	0.228	-0.166	0.066	-0.093	-0.267*	0.023
Membrane stability index	Normal			1.000	-0.047	0.110	-0.063	-0.111	0.002
	Stress			1.000	-0.041	-0.023	-0.062	-0.094	0.138
Excised-leaf water loss	Normal				1.000	0.049	-0.009	0.101	-0.073
	Stress				1.000	-0.385**	0.006	-0.118	0.016
Relative water content	Normal					1.000	0.098	-0.287*	-0.103
	Stress					1.000	0.056	-0.099	-0.140
Water retention capacity of leaves	Normal						1.000	-0.137	-0.155
	Stress						1.000	-0.055	-0.052
Oil content (%)	Normal							1.000	0.295*
	Stress							1.000	0.105
Seed yield per plant (g)	Normal								1.000
	Stress								1.000

* and ** Significant at 5 and 1 per cent level of significance, respectively

The higher membrane stability index and high population survival might have also imparted high temperature stress tolerance in genotypes DRMR-541-44 and RGN-48. This study identified several high temperature tolerant Indian mustard genotypes in north-western India, which could now be used in breeding programmes.

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