Heat stress effects on morpho-physiological characters of Indian mustard (*Brassica juncea* L.)

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ABSTRACT

Effects of heat (high temperature) stress during terminal stage were investigated on plant height, primary branches/plant, siliquae on main shoot, seeds/siliqua, 1000-seed weight, seed yield, oil and protein content, leaf area index (60 DAS), specific leaf weight (80 DAS), crop growth rate (40-60 DAS), chlorophyll stability index (60 DAS) and harvest index of 22 advanced breeding lines/varieties of Indian mustard (*Brassica juncea* L.) during 2008-09. The genotypes were grown in randomized complete block design with three replications in two environments, *viz.,* E_1 (9^{th} November sowing) and E_2 (25^{th} November sowing). Of the 45 days prior to physiological maturity, crop under E_2 was exposed to higher mean daily temperature differential of 0.9-5.6^{0}C for 28 days. Genotypic differences were significant for all the characters except protein content and chlorophyll stability index. Environment effects were highly significant for all the moropho-physiological characters investigated. Genotypes x environment interactions were significant only for 1000-seed weight, leaf area index and crop growth rate. Leaf area index, specific leaf weight, crop growth rate, chlorophyll stability index and harvest index were drastically reduced under E_2 by up to 49.4%, 52.1%, 76.2%, 54.2% and 28.9% respectively. The highest reduction in seed yield and its components ranged from 22.2% for seeds/siliqua to 69.2% for seed yield/plant. Four terminal high temperature tolerant genotypes as indicated by their low HIS for seed yield were BPR 538-10 (0.33), NRCDR 2 (0.44), RH 0216 (0.57) and NPJ 112 (0.58). Genotypes BPR 2, BPR 141-B-205-43 and BPR 540-6 having tolerance to high temperature for multiple characters were identified for utilization in the breeding programme.

Key words: High temperature**-** *Brassica juncea* L. - seed yield- physiological characters- heat susceptibility index

INTRODUCTION

Indian contribution to global rapeseed-mustard production (49.48 million tonnes), acreage (30.23 million hectares) and yield (1636 kg / ha) was 21.7%, 14.3% and 66.7%, respectively, during 2007 (FAO 2009). Rapeseed-mustard constitutes an important source of edible oil next to soybean and groundnut in India contributing 19.6% and 21.1% to the total oilseeds production and acreage 2007-08 (Anonymous 2009). Indian mustard [*Brassica juncea* L.) accounts for nearly 80% of the area under these crops in the country. This crop grows under diverse agro ecological situations such as timely / late sown, rainfed / irrigated, sole-& / or mixed crop with cereals (wheat, barley etc.) and *rabi (October-April)* pulses (chickpea, lentil etc.). The inter-/ mixed cropping with wheat as well as late sowing after rice and cotton exposes this crop to high temperature stress during reproductive stage. . Hall (1992) reported that flowering is the most sensitive stage for temperature stress damage probably due to vulnerability during pollen development, anthesis and fertilization leading to reduce crop yield. High temperature in *Brassica* enhanced plant development and caused flower abortion with appreciable loss in seed yield (Rao et al. (992). Flowering duration had a strong influence on seed yield and a rise of 3^{0}C in maximum daily temperature (21-24^{0}C) during flowering caused a decline of 430 kg / ha in canola seed yield (Nuttall et al. 1992. Therefore, improving seed yield of Indian mustard under late sown conditions by genetic up scaling of thermo tolerance at terminal stage would be vital for the sustainability in oilseed production. The present investigation is an attempt to analyze the effects of high temperature on seed yield, its components and growth parameters and also characterize genotypes for high temperature tolerance to identify suitable donors for utilization in the breeding program.

MATERIALS AND METHODS

The experimental materials for the present investigation comprised 18 advanced breeding lines and 4 varieties of Indian mustard grown in a randomized complete block design with three replications during *rabi* season (November-March) 2008-2009 after pre-sowing irrigation at the Experimental Farm, DRMR, Bharatpur (77.30^0 E longitude; 27.15^0 N latitude; 178.37 m above mean sea level), India. The experiment was conducted at 2 dates of sowing, *viz.,* November 09 (E_1) and November 25 (E_2) 2008. There were 5 rows of 5-m length for each genotype in a block. The row spacing was 30 cm and plant spacing with-in-a row was maintained at 10 cm by thinning. A fertilizer dose of 40: 40:40 kg/ha (N: P_2 O_5: K_2O) was applied at the time of sowing and 40 kg/ha N was top dressed 3-4 days after first irrigation. Standard agronomic practices were followed to raise a good crop. To study leaf area index, specific leaf weight, crop growth rate, the plant samples from 50 cm running row length were harvested above ground level at 40 and 60 and 80 days after sowing. The shoot and leaves were separated and leaf area was recorded using automatic leaf area meter (LICOR LI-3000A). Plant samples (shoot and leaves) were dried separately at 65^o ± 2^0 C till constant weight was achieved and dry matter of leaves, shoot as well as shoot + leaves was expressed in g/m^2. The total chlorophyll content was estimated following the method of Arnon (1949) and chlorophyll stability index (CSI) was computed as per the formula given by Kar et al. (2005). Observations were also recorded on 10 randomly taken plants/ replication/genotype on plant height, primary branches/plant, siliquae on main shoot, seeds / siliqua, 1000-seed weight (g) and seed yield /plant (g). Oil and protein content of dry seeds was determined by Dickey- John, Instalab 600 NIRS (Near Infrared Reflectance Spectrometer) as described by Kumar et al. (2003).

Analysis of variance using replication mean values for multiple randomized complete block design using indostat software was carried out to study the genotype, environment and genotype x environment interaction. Heat stress effect was computes as percent change in mean of a character under E_2 over that of under E_1. Heat susceptibility index (Fischer and Maurer 1978) was computed for all the characters /genotypes to characterize thermo tolerance.

RESULTS

Temperature regime

The temperature under two environments differed considerably during different phenological stages of the crop. The genotypes under E_1 were exposed to high temperature (29.2 ± 0.39^0 C) as compared to those under E_2 (26.2 ± 0.43^{0}C) during seedling stage. Thereafter, temperature sharply declined from 29.2^0 C to 20.9^0 C from seedling emergence to 100% flowering and again increased to 26.6^0 C till physiological maturity under E_1 (Table 1). However, under E_2 it declined from 26.2^{0}C to 23.1^{0}C until initiation of flowering and thereafter gradually increased to 29.3^0 C till physiological maturity. After initiation of flowering, the crop under E_2 experienced higher maximum temperature by 3-4^{0}C than that of under E_1.

The mean maximum temperature was about 2.7^{0}C higher under E_2 than E_1 during 100 % flowering and physiological maturity, the phase of active and rapid dry matter accumulation in seeds. Analysis of pattern of daily variation in minimum, maximum and mean temperature during 45 days prior to physiological maturity revealed that crop under E_2 was exposed to higher mean daily temperature differential of 0.9-5.6^{0}C for 28 days while the mean daily temperature was higher by 0.1-3.9^{0}C for 16 days under E_1 (Fig. 1a, b). The crop under E_1 was exposed to relatively cooler temperature until 30 days prior to physiological maturity.

Table 1. Trend of maximum and minimum temperature during different phenological stages of Indian mustard under contrasting environments, DRMR, Bharatpur, India

Growth stage	E_1/ E_2	Maximum temperature (^{0}C)		Minimum temperature (^{0}C)	
		Range	Mean ±SEM	Range	Mean ±SEM

Sowing-seedling emergence	E_1	27.7-30.2	29.2 ± □□0.39	11.5 -13.1	12.5 ± □□0.24
	E_2	23.2-27.4	26.2 ± 0.43	7.3 - 8.2	7.6 ± 0.01
Emergence-initiation of flowering	E_1	14.2-30.1	24.2 ± 0.50	2.2 - 17.4	8.5 ± 0.45
	E_2	14.2-28.5	23.1 ± 0.42	2.2 - 14.7	7.9 ± □□0.39
Initiation of flowering-50 % flowering	E_1	14.2-25.5	20.9 ± 0.60	2.2 - 12.5	6.2 ± 0.50
	E_2	19.8-27.4	23.8 ± □□0.41	4.2 - 14.0	7.7 ± □□□□0.56
50 %-100 % flowering	E_1	14.2-25.5	20.9□ ± □□0.70	2.2 - 13.0	6.7 ± □□0.50
	E_2	19.8-28.2	24.9 ± □□□□0.51	4.2 - 14.0	7.5 ± □□□□0.59
100 % flowering-physiological maturity	E_1	14.2-36.8	26.6 ± □□ 0.58	2.2 - 18.7	8.9 ± 0.37
	E_2	19.8-36.8	29.3 ± □□ 0.50	4.2 - 20.0	10.4 ±□□□□ 0.45

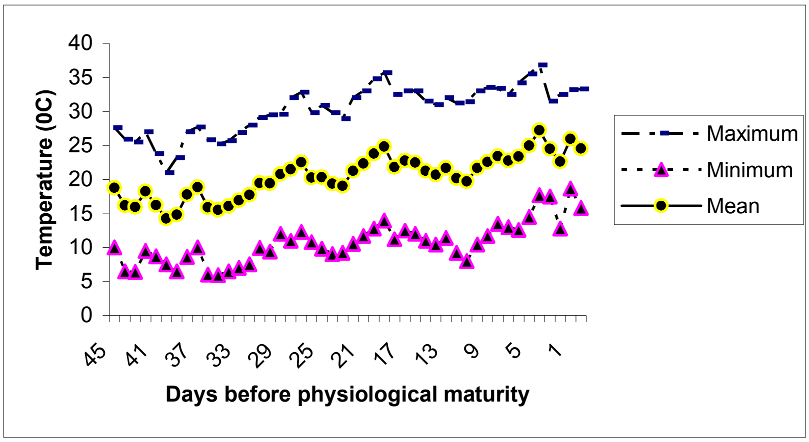

Fig 1a. Trend of daily minimum, maximum and mean temperature during 45 days prior to physiological maturity under E_1

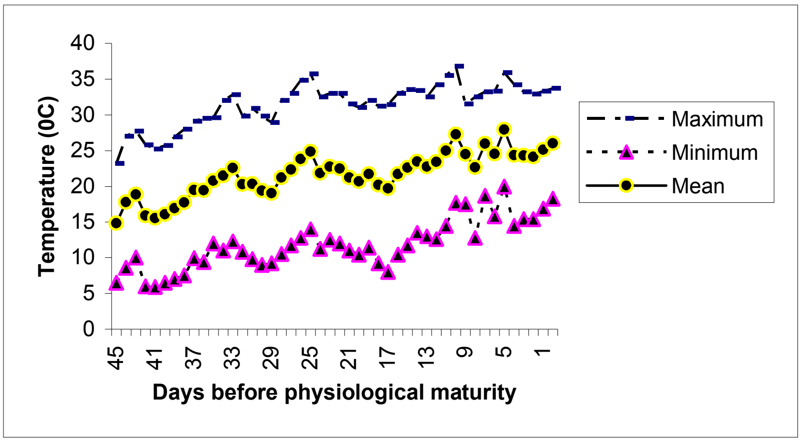

Fig 1b. Trend of daily minimum, maximum and mean temperature during 45 days prior to physiological maturity under E_2

Analysis of variance

Analysis of variance revealed significant genotypic differences for all the characters studied except protein content and chlorophyll stability index. Environment effects were highly significant for all the moropho-physiological characters. Genotypes x environment interactions were significant only for 1000-seed weight, LAI and CGR.

Effects

Heat (high temperature) stress during terminal stage had substantial effects on all the characters studied, varying with the genotypes and characters. Plant height was reduced considerably under E_2, ranging from 18.9 (SKM 531)-30.5% (Varuna) with a mean decline of 22.3%. Genotypes had fewer branches/plant except for the genotypes BPR 538-10, BPR 545-2 and BPR 548-3, which showed marginally higher primary branches/plant under heat stress (an increase of 0.7-8.5%). High temperature decreased siliquae on main shoot by up to 35.8% in the genotype NPJ 112 followed by RH 8814 (33.1%) while genotype BPR 549-3 having the minimum reduction (Table 2). The 1000-seed weight under heat stress showed considerable decrease. The mean decline in seed weight was 20.4% and genotype CS 3000-1-1-1 showed slightly improved seed weight under stress. Seeds/siliqua recorded relatively less reduction of 8.1% and genotype Varuna showing some increase in seeds. The genotypes with < 5% reduction in number of seeds/ siliqua were BPR-2, BPR 548-3, RH 0216 and BPR 560-6. The highest decrease due to heat stress was observed for seed yield/plant, ranging from 16.6-69.2%. The genotypes having the least reduction in seed yield under stress were BPR 538-10 (16.6%), NRCDR 2 (22.1%), RH 0216 (28.9%) and NPJ 112 (29.1%). Oil content was also slightly reduced by up to 6.7% whereas, protein content showed an increase of about 6.1%.

The genotypes grown under E_2 also showed very high reduction up to 49.4 % for the genotype RGN 193, 52.1 % for NPJ 112, 76.2 % for SKM 531 and 54.1% for NPJ 112, respectively for leaf area index, specific leaf weight, crop growth rate and chlorophyll stability index (Table 2). Harvest index also decreased by 14.4% under E_2 with the highest reduction of 28.9% for the genotype BPR 327-1-B. The genotypes showing less reduction in physiological characters were BPR 548-3 (7.5%), BPR 543-2 (12.5%) and SKM 531 (14.0%) for leaf area index, Bio 902 (19.6 %), BPR 327-1-3 (25.8%) and BPR 560-6-B (26.6%) for specific leaf weight, BPR 538-10 for crop growth rate and BPR 540-6 (1.3%), RGN 193 (5.5%) and BPR 868-3 (8.8%) for chlorophyll stability index.

Heat susceptibility index (HIS), a measure of tolerance of genotype/character to heat stress, also showed substantial variation. Large HSI values suggest greater sensitivity to the stress (Winter et al. 1988). The HIS differed among the genotypes as well characters. Although, mean HIS was close to unity for most of the characters except harvest index but varied widely. Higher HIS values were observed for harvest index, leaf area index, chlorophyll stability index, primary branches/plant and seeds/siliqua (Table 2) suggesting that these characters were more prone to high temperature. The characters showing HIS values close to unity or below were plant height, siliquae on main shoot, 1000-seed weight, oil and protein content, crop growth rate and seed yield indicating their relative tolerance to terminal high temperature. Genotypes showing low HSI for seed yield/plant, BPR-538-10 (0.33), NRCDR-2 (0.44) and RH-0216 (0.57) also showed low HIS values for plant height, primary branches/plant, 1000-seed weight, seeds/siliqua, siliquae on main shoot CGR and LAI. Genotypes BPR 141-B-205-43 (primary branches, siliquae on main shoot, seeds/siliqua, seed weight and oil content and specific leaf weight); BPR 540-6 (plant height, primary branches, siliquae on main shoot, seeds/siliqua, seed weight, and chlorophyll stability index) and BPR 2 (primary branches, seeds/siliqua, seed weight and crop growth rate) had tolerance to multiple characters.

Table 2. Heat stress effects on certain morpho-physiological characters in Indian mustard DRMR, Bharatpur, India

Character	Change in mean under E_2 over mean under E_1 (%)		Heat susceptibility index	
	Range	Mean ± SEM	Range	Mean ± SEM
Plant height (cm)	18.9- 30.5	22.3± 0.6	0.85-1.37	1.00 ± 0.03
Primary branches/ plant	- 8.5 -31.8	10.1 ± 2.1	-0.78-2.93	0.90 ± 0.19
Siliquae on main shoot	6.2 -35.8	18.9 ± 1.6	0.33-1.88	1.00 ± 0.08
Seeds / siliqua	- 2.4 -22.2	8.1 ± 1.0	-0.29-2.68	1.00 ± 0.12
1000-seed weight (g)	- 4.0 -31.3	20.2 ± 2.0	-0.20-1.54	1.00 ± 0.09
Seed yield /plant (g)	16.6 -69.2	49.5 ± 3.0	0.33-1.37	1.00 ± 0.06
Oil Content (%)	4.0-8.9	6.7 ± 1.0	-2.59-1.55	1.20 ± 0.18
Protein content (%)	- 8.0 – (-3.2)	- 5.8 ± 0.4	-0.09-1.37	1.10 ± 0.07
Leaf area index (60 DAS)	- 4.4 - 49.4	17.5 ± 4.1	-0.30-3.38	1.20 ± 0.28
Specific leaf weight (mg /cm^2) (60DAS)	- 4.0 - 52.1	37.4 ± 2.6	-0.10-1.30	1.00 ± 0.06
Crop growth rate (g / m^2/ day) (40-60 DAS)	5.9 - 76.2	54.8 ± 4.5	-0.21-1.33	1.00 ± 0.08
Chlorophyll stability index (60 DAS)	-9.0 - 54.1	18.1 ± 6.4	-4.73-3.07	0.90 ± 0.36
Harvest index (%)	- 2.0-28.9	14.4 ± 1.9	-1.05-15.23	7.60 ± 1.01

DISCUSSION

Terminal heat stress caused substantial reduction in all the characters except oil and protein content. Physiological characters like leaf area index (up to 49.4%), specific leaf weight (up to 52.1%), crop growth rate (up to 76.2%) and chlorophyll stability index (up to 54.2%) and harvest index (28.9%) were drastically reduced under E_2. The results revealed that a difference 3-4^{0}C in the mean high temperature after initiation of flowering (35-40 DAS) significantly reduced the growth of the Indian mustard. The highest reduction in seed yield and its components ranged from 22.2% for seeds/siliqua to 69.2% for seed yield. The findings of the present study corroborated earlier reports where reduction up to 76% in seed yield/plant, 20.9% in harvest index and 42.6% siliquae on main branch were reported (Lallu and Dixit 2008). The reduction in siliquae on main shoot and seeds / siliqua could be due to floral sterility as temperature > 27^0 C has been reported to induce floral sterility in canola (Morrison and Stewart 2002) as well as development of flowers in to seedless parthenocarpic fruits &/or flower abortion on the stem due to high temperature (Young et al. 2004). Considerable reduction in seed yield under E_2 in the present study could be due to less production of dry matter as a result of reduced LAI and CGR. Kumar and Srivastava (2003) reported positive correlation of seed yield with total chlorophyll content under late sown conditions, the reduced chlorophyll stability index (more destruction of chlorophyll under high temperature) and poor translocation of photosynthates from the sink to the source in the present study could be the other reasons for poor harvest index and consequently decreased seed yield in the present investigation. The results were in agreement with those of Subrahmanyam and Rathore (1994) who observed that high temperature during reproductive stage significantly inhibited the import of photosynthates by both upper and lower pods of terminal reaceme and thereby reduced sink strength. Heat stress also decreased oil content marginally but there was concomitant increase of the same magnitude in protein content as both are negatively correlated.

The present investigation also revealed that the top four terminal high temperature tolerant genotypes as indicated by their low HIS for seed yield were BPR 538-10 (0.33), NRCDR 2 (0.44), RH 0216 (0.57) and NPJ 112 (0.58). Their tolerance was coupled with tolerance to high temperature of plant height, primary branches/plant, 1000-seed weight, seeds/siliqua, siliquae on main shoot, CGR and LAI. Tall genotypes with more primary branches/plant, medium bold seeds and high chlorophyll content were suggested to be ideal for late sown conditions (Kumar and Srivastava 2003). In this context, genotypes having tolerance to high temperature (low HIS) for plant height, primary branches/plant, siliquae on main shoot, 1000-seed weight and chlorophyll stability index like BPR 2, BPR 141-B-205-43 and BPR 540-6 should be utilized in the breeding programme to improve seed yield under terminal heat stress.

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