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SELECTION OF HEAT TOLERANT DONOR LINES IN INDIAN MUSTARD (*BRASSICA JUNCEA* L.)

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Abstract

Heat stress during the post anthesis (seed filling) stage negatively influences the movement of photosynthates to the developing sinks and, thus lowering the seed yield in Indian mustard [*Brassica juncea* (L.) Czern & Coss]. The present study was conducted with the objective to unravel the variability for terminal heat stress (THS) in a fixed diversity genetic stock of 500 lines of Indian mustard. Germplasm stock exhibited huge variability for seed yield and its contributing traits with variable levels of yield reduction under late sown conditions. The *per se* yield based computed heat stress susceptibility index (HSI) ranged from 32.3 (highly THS susceptible) to -1.15 (THS tolerant). Based on yield reduction due to THS, 500 test genotypes were grouped into 10 classes and genotypes from each class were randomly taken to constitute a diverse panel of 96 lines. This panel was evaluated under THS by raising crop under poly-tunnels during reproductive phase along with same set of 96 genotypes raised under normal field conditions (served as control). Based on agronomic advantage and HSI for economic traits, 23 heat tolerant donor lines were selected for utilization in commercial breeding programme.

Introduction

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is a major oilseed crop of Indian subcontinent, Northern China and East European countries. It is an important winter season oilseed crop in India and contributes nearly 28.6% of edible oil supplies (Shekhawat *et al.* 2012). With the development of canola quality, *B. juncea* further expanded to drier regions in Australia and Canada. Canola-quality *B. juncea* oil has developed as a complimentary oilseed crop to canola (*B. napus*) for cultivation in hot and low-rainfall areas, where canola did not perform well. Indian mustard is sensitive to climatic variable affecting its productivity significantly (Kovilpillai *et al.* 2010). In India, delayed sowing of mustard crop after harvesting rice and cotton exposes the crop to high temperature stress during reproductive stage (Chauhan *et al.* 2009). Delayed sowing also shortens the vegetative phase, advances flowering time and decrease the seed development period (Shrivastva and Balkrishna 2003 and Dhaliwal *et al.* 2007). High temperature causes multiple and undesirable changes in plant from germination till maturity (Hasanuzzaman *et al.* 2013) but its impact on plant varies with stage of plant, genotype and species. Flowering is the most sensitive stage for temperature stress damage probably due to vulnerability during pollen development, anthesis and fertilization leading to crop yield. Delayed sowing exposes the crop to terminal heat stress (THS) and result in considerable reduction in productivity due to impaired seed filling. Since the global temperature rising continuously, there is a prime need for the identification of novel genotypes/varieties with optimum yield potential under high temperature. The present study was conducted with objectives to characterize the available huge genetic stock under THS

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conditions, to differentiate genotypes into thermo-tolerant and susceptible groups based on the effect of THS on their productivity and its component traits, and to identify heat tolerant donors for commercial breeding programme.

Materials and Methods

The fixed diversity (through continuous selfing) set of 500 genotypes of Indian mustard was sown at Punjab Agricultural University (PAU) Regional Research Station, Abohar (representing arid zone of the Punjab state, India; located at 30.15° N, 74.19° E) during 2015-16 at two sowing dates – Mid Nov. (normal sown) and first week of December (late sown). Arid zone accompanied by delayed sowing allowed natural screening of germplasm for terminal heat tolerance. The data were recorded for yield contributing traits at crop maturity and was subjected to analysis of variance. The yield reduction under late sown condition in comparison to control (normal condition) was computed. A panel of 96 genotypes, representing a diverse range of yield reduction under THS, were selected from a stock of 500 genotypes and were planted under normal (field conditions) and heat stress conditions (poly-tunnels) in alpha lattice design during 2016-17 at PAU, Ludhiana; located at 30.90° N, 75.86°E. The heat stress conditions i.e., a set of germplasm raised under poly-tunnels, were created by placing iron rod frame in field and covering the crop by polythene sheets (3mm thickness) from 10.00 am to 3.00 pm every day from onset of siliquae initiation to onset of physiological maturity. Data were recorded at physiological maturity for yield and yield components traits viz., plant height (cm), main shoot length (cm), number of siliquae on main shoot, number of primary branches, number of secondary branches, seeds per siliquae, 1000 seed weight and seed yield per plot (gm) and were analysed statistically using SAS (Statistical Analysis System). Heat susceptibility index (HSI) was calculated using formula given by Fischer and Maurer (1978).

$$\text{Heat susceptibility index (HSI)} = \frac{1 - (Xs \;/\; Xn)}{1 - (\overline{Xs} \;/\; \overline{Xn})}$$

where X_n is trait value of n^{th} genotype under normal condition; X_s-the trait value of n^{th} genotype under stress conditions; $\bar{X}_n$ and $\bar{X}_s$ are the mean trait value of all genotypes under normal and stress conditions.

Results and Discussion

Temperature is an important factor which affects growth and development of plants. The sowing period of oilseed crops is also limited due to thermo and photo-sensitivity (Singh *et al.* 2017) and both early as well as late sown crops are affected by high temperature. High temperature stress significantly reduced yield in *Brasssica* cultivar by affecting yield and yield component traits (Gan *et al.* 2004). For the present study, THS was created during reproductive phase (Feb-March) by growing the crop under poly-tunnels. As depicted in Table 1, the temperature remained higher under poly-tunnels than that in open field conditions at every phase of crop growth from initiation of siliquae formation to onset of seed ripening. The harvest data of 96 genotypes were recorded at maturity. Significant variations were observed for all traits under NS (Table 2) and THS conditions (Table 3). The pooled analysis of variance across environments (NS and THS conditions considered as two environments) revealed significant interactions for seed yield and its component traits (Table 4). The mean data and coefficient of variation for all traits are given in Table 5. The yield contributing traits *viz.*, plant height, main shoot length, primary branches, secondary branches and number of siliquae on main shoot were affected with high temperature stress under poly-tunnels. The mean plant height of 96 genotypes under NS conditions was 148 cm and was reduced to137 cm under THS.

Table 1. Weekly maximum and minimum temperature during siliquae initiation stage and seed maturity of mustard crop during year 2017.

Week	Growth stage of crop	Normal sown conditions		Terminal heat stress conditions (under Poly -tunnels)	
		Temperature (°C)			
		Minimum	Maximum	Minimum	Maximum
II (8 to 14 Feb)	Siliquae initiation	6.4	21.6	17.9	30.6
III (15 to 21 Feb)	Siliquae development	12.9	25.8	22.5	34.5
IV (22 to 28 Feb)	Siliquae development	8.4	24.5	20.9	36.4
I (1 to 7 March)	Seed development & filling	9.7	25.2	21.5	37.7
II (8 to 14 March*)	Seed filling	9.4	19.4	21.2	33.5
III (15 to 21 March)	Seed filling & onset of physiological maturity	11.6	26.2	22.3	38.8

*32.2 mm rainfall observed during this week

Table 2. Mean sum of squares for various economic traits under natural field conditions.

Source	df	Traits†							
		PH	MSL	NPB	NSB	NSMS	SY	NS	TSW
Rep.	1	12.15	44.98	0.14	2.65	63.40	45.28	1.57	1.103
Block (rep)	14	23.45	11.90	0.10	4.32	7.07	32.01	0.89	0.07
Genotype	96	678.87**	95.28**	0.90**	11.03**	44.35**	164.46**	2.99**	1.20**
Error	81	43.71	17.37	0.26	2.34	9.34	18.06	0.70	0.83

** Significant at 0.001 level. †PH - Plant height (cm), MSL - Main shoot length (cm), NPB - Primary branches, NSB - Secondary branches, NSMS - Number of siliquae on main shoot, SY - Seed yield per plot, NS - Number of seeds/siliquae, TSW - 1000 seed weight (g)

Table 3. Mean sum of squares for various economic traits under terminal heat stress conditions.

Source	df	Traits†							
		PH	MSL	NPB	NSB	NSMS	SY	NS	TSW
Rep.	1	7.36	0.49	3.35	0.12	3.83	32.42	0.09	0.14
Block (rep)	14	35.81	66.87	0.78	2.14	19.61	40.48	0.77	0.05
Genotype	96	418.25**	110.94**	.03**	6.96**	45.48**	172.37**	2.92**	0.97**
Error	81	31.62	48.19	0.58	2.16	11.76	35.39	0.68	0.06

** Significant at 0.001 level. †PH - Plant height (cm), MSL-Main shoot length (cm), NPB- Primary branches, NSB- Secondary branches, NSMS- Number of siliquae on main shoot, SY- Seed yield per plot, NS-Number of seeds/ siliquae, TSW- 1000 seed weight (g)

Other traits were also affected due to heat stress and overall mean plot yield was reduced from 40.88 gm (NS) to 35.68 gm (THS). Crop grown under sub-optimal conditions would not be able to attain full genetic potential for growth and reproduction. Infertility and yield loss, due to heat stress, have been reported in *B. juncea* by Kalra *et al.* 2008. Boomiraj *et al.* (2010) confirmed that *Rabi* crops are vulnerable to high temperature during reproductive stage and exhibit yield reduction by effecting yield contributing traits. Lallu *et al.* (2010) reported significant reduction for plant height and intensity of siliquae born on different branches including main shoot. Singh *et al.* (2014) investigated reduction in plant height, seed yield under heat stress at two sowing dates. Reduction in growth and yield contributing traits *viz.*, main shoot length, siliquae on main shoot, seed yield had been reported by Sharma (2014). In present study, test genotypes showed variable response to THS in terms of mean values for economically important traits and hence, inferred the possibility of identification of heat tolerant donors.

Table 4. Pooled analysis of various for economic traits across environments.

Source	df	Traits†							
		PH	MSL	NPB	NSB	NSMS	SY	NS	TSW
Env	1	10960.03**	354.71**	0.40	0.01	516.15**	2320.91**	11.33**	11.42**
Rep (env)	2	9.75	22.73	1.74	1.38	33.61	38.85	0.83	0.12
Block (env*rep)	28	29.63	39.39	0.44	3.36	13.34	36.25	0.84	0.06
Genotype	96	707.31**	118.02**	1.02**	9.94**	56.52**	233.25**	3.83**	1.95**
Env* genotype	96	389.83**	88.20**	0.91**	8.06**	33.31**	103.59**	2.09**	0.21**
Error	162	37.67	32.79	0.42	2.26	10.56	26.73	0.69	0.07

** Significant at 0.001 level. Rep- replication, Env- environment, †PH - Plant height, MSL-Main shoot length, NPB- Primary branches, NSB- Secondary branches, NSMS- Number of siliquae on main shoot, SY-Seed yield per plot, NS- Number of seeds/ siliquae, TSW- 1000 seed weight.

Table 5. Genotype mean data and coefficient of variation (CV) for all traits under two environments.

Traits†	Natural sown conditions		Terminal heat stress conditions	
	Mean	CV	Mean	CV
PH (cm)	148.09	4.46	137.41	4.09
MSL (cm)	53.75	7.75	51.83	13.39
NPB	4.86	10.51	4.92	15.52
NSB	10.38	14.75	10.38	14.19
NSMS	35.34	8.66	33.02	10.39
SY (g)	40.89	10.47	35.68	16.67
NS	11.49	7.32	11.15	7.40
TSW(g)	4.26	6.24	3.92	6.34

`†PH - Plant height, MSL-Main shoot length, NPB- Number of primary branches, NSB- Number of secondary branches, NSMS- Number of siliquae on main shoot, SY- Seed yield per plot, NS- Number of seeds/ siliquae, TSW- 1000 seed weight.

As yield *per se* performance is important parameter to determine response to any stress, the yield reduction (YD) was calculated for each test genotype of the panel. It ranged from 56.218 (CRL-1359-60-75-4-3) to -40.566 (TM-106-1); given as supplementary information (TS 1). Five groups were made based on percent YD: -40 to -20, -20 to -0, 0.1 to 20, 20 to 40 and above 40, and genotypes were classified in each group as per respective per cent YD value. There were eleven genotypes which were having percent yield reduction value more than -30 (TM-106-1, PF-8, RK-06-1, CM-6-3, PTJ-3-79, RL-2106, Bio-209, NJHO-3-21, PCR-9404-2, NML-64 and Pusa-

Bold-DT-1). Genotypes TM-106-1, PF-8, RK-06-1 were having -40 per cent yield reduction value. These negative values for per cent YD recommended that there was no negative impact of high temperature on these genotypes; rather increment in yield was observed. On other hand, there were ten genotypes (CRL-1359-60-75-4-2, Bio-437, Bio-422-9, Bio-21-9-39, CM-21-8, Bio-2, CSR-47, PBR-357, Budh-3 and B-312) having YD more than 40% and/or HSI value more than 40 which reflects that these were the most susceptible genotypes to THS. Sharma (2014) mentioned that yield reduction less than 30 per cent can be used as a criterion for selection of heat tolerant genotypes. The wide range for per cent YD in present study and grouping of test genotypes in each constituted class recommended that this genetic stock may serve as good diverse panel for identification of heat tolerant donors.

All studied plant traits *viz.*, plant height, main shoot length, primary branches, number of siliquae on main shoot were found to be affected by THS under poly-tunnels during reproductive phase as indicated by difference in mean values. The mean values for each trait under each environment are presented in Table 5. HSI also one of the important indicators to determine the response of genotypes to heat stress, was computed on the basis of seed yield and its component traits for each genotype. The mean HSI for each class, constituted on bass of YD per cent and the respective number of genotypes grouped in each class, are presented in Table 6. According to Sio-Se *et al.* 2006, genotype which had the lowest HSI was categorized as most tolerant whereas genotype with the highest HSI was designated as most susceptible. HSI for seed yield ranged from 4.39 (CRL-1359-60-75-4-3) to -3.174 (TM-106-1). Negative HSI value indicates no reduction in trait value under heat stress conditions in comparison to that under normal. Genotypes PF-8, RK-06-1 and TM-106-1 had HSI value above -3 for SY, which showed that these genotypes were tolerant to heat stress condition as no yield reduction was observed in these genotypes. HSI values for different traits of tolerant genotypes and percent yield reduction are given in Table 7.

Table 6. Grouping of 500 mustard genotypes into five classes based on per cent yield reduction (YD) and heat susceptibility index (HSI) for seed yield.

Percent yield reduction	Mean HSI for SY	Number of genotypes
-40 to -20	-2.59	14
-20 to -0	-0.67	18
0.1 to 20	0.92	24
20 to 40	2.31	32
above 40	3.65	10

Table 7. HSI values of tolerant genotypes for different traits and percent yield reduction.

Genotype name	Traits†					% Yield reduction
	PH	NSMS	SY	TSW	NS	
JMG-951	0.4	0	-1.45	-0.19	-7.86	-18.58
RL-2106	0.78	-3.41	-2.59	-0.06	-6.03	-33.11
MSC-1	1.04	-2.01	-1.53	-0.64	-0.6	-19.53
PTJ-3-100	0.88	-3.93	-2.37	-0.05	-0.12	-48.41
RK-06-1	0.72	-0.14	-3.15	-0.23	-2.65	-40.28
MCP-12-211	0.64	-0.91	-0.67	-0.04	-3.34	-34.95
GRIRAJ	2.57	-12.8	-0.43	-0.01	-0.16	-38.55

†PH - Plant height, NSMS- Number of siliquae on main shoot, SY- Seed yield per plot, TSW- 1000 seed weight, NS- Number of seeds/ siliquae

Table 8. Mean values of different traits of heat tolerant donor lines of Indian mustard under normal sown and terminal heat stress conditions.

Genotype	Traits†							
	NSMS		SY (g)		OC (%)		OY (g)	
	NS	THS	NS	THS	NS	THS	NS	THS
Bio-169-95	38.83	22.67	41.00	52.75	37.76	40.84	15.48	21.54
Bio-209	29.83	32.33	25.25	33.50	40.09	38.84	10.32	12.84
CM-2	33.16	43.67	39.00	44.75	40.00	38.89	15.60	17.40
CM-6-3	42.50	36.33	25.00	34.00	39.13	38.95	9.78	13.24
CRL-1359-18-19-17	38.33	31.67	34.50	39.75	40.15	37.03	13.85	14.71
JMG-951	37.00	37.00	28.25	33.50	39.81	38.28	11.24	12.82
RL-2106	32.00	40.50	44.70	59.50	40.30	37.68	18.01	22.41
RRN-624	33.83	41.00	43.75	41.95	39.67	40.69	17.35	20.28
HYT-33	27.16	34.67	30.75	33.75	36.70	38.50	11.28	12.99
RKL-08-2	30.16	25.50	36.75	39.50	39.13	39.41	14.38	15.56
NML-64	30.67	40.83	50.25	65.75	40.53	39.38	20.36	25.89
RB-50	34.50	36.16	40.00	45.50	40.09	38.88	16.03	17.69
DRMR-08-293	34.00	31.83	41.25	49.50	41.24	38.26	17.01	18.93
MSC-1	35.17	40.67	32.00	38.25	39.54	38.19	12.65	14.60
PF-8	31.50	24.83	24.75	34.75	38.23	36.60	9.46	12.71
PTJ-3-79	36.00	34.50	28.50	38.25	41.06	38.36	11.70	14.67
PTJ-3-100	27.17	35.50	42.80	55.75	39.83	38.30	17.04	21.35
NJHO-3-2-1	44.67	43.17	50.50	66.75	41.79	40.61	21.10	27.10
PCR-9404-2	38.17	38.67	28.50	37.50	37.59	37.25	10.71	13.96
PUSA BOLD DT-1	31.17	28.50	35.00	45.50	40.18	40.49	14.06	18.42
RK-06-1	30.33	30.67	34.75	48.75	38.75	38.20	13.46	18.62
MCP-12-211	30.67	32.83	32.00	34.75	40.82	37.53	13.06	13.04
TM-106-1	41.17	31.33	26.50	37.25	38.85	38.87	10.29	14.47
Mean	34.26	34.55	35.47	43.96	39.62	38.69	14.10	17.18

NS - Normal sown, THS - Termnal heat stress, †NSMS - number of siliquae on main shoot, SY - seed yield, OC - oil content, OY- oil yield.

The HSI for heat tolerant checks viz., BPR-541-4 and Giriraj, were 0.431 and -0.435, respectively. There were 23 genotypes which exhibited lower HSI than Giriraj (the best check) and hence, were designated as heat tolerant genotypes. These selected genotypes may, serve as putative donors in breeding program for heat tolerance. THS donor lines were characterized for important economic traits *viz.*, NSMS, SY, OC and OY (multiple of seed yield and oil content) and mean trait values under normal as well as under heat stress conditions are presented in Table 8.

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