Journal of Oilseed Brassica, 16 (2) : 158-164, July 2025

Research article

Morpho-physiological variability and stability of early maturing thermo-tolerant Indian mustard (*Brassica juncea* L. Czern&Coss) genotypes for seed yield under heat stress condition in semi-arid region of India

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https://doi.org/10.56093/job.v16i2.2

Abstract

Impact of climate change like global warming, drought, flooding, and other extreme events are posing severe challenges to global crop production. Heat stress at seedling stage is one of the major factors responsible for the low productivity of crop plants and has become an increasing threat for agriculture. Indian mustard grown under rain-fed conditions is usually affected by heat stress at seedling stages resulting in negative effect on yield. In this context, the selection of heat tolerant genotypes may be one of the remedies. Therefore, a study was carried out at the research farm of ICAR-Indian Institute of Rapeseed-Mustard Research, Bharatpur, Rajasthan during Rabi 2018-19, 2019-20 and 2020-21 to estimate stability and morpho-physiological variability of early maturing thermo-tolerant genotypes in order to identify some of the promising early maturing heat stress tolerant advanced breeding lines. The crop was raised strictly under conserved moisture early sown conditions. G x E interaction was significant for all the characters *viz*., days to flowering, days to maturity, seed yield (kg/ha), membrane stability index (%), excised leaf- water loss (%), relative water content (RWC %), and water retention capacity of leaves (%) except 1000-seed weight. G x E interaction (linear) was also significant for all these eight characters, indicating substantial amount of predictable G x E interaction. All ten genotypes were tested for three stability parameters, namely mean, b_i and S^2di. Genotype DRMRHT-13-13-5-4 attained minimum days to maturity (122.88) alongwith regression coefficient near to unity and S^2di near to zero considered as stable. While the genotype DRMRHT-13-13-5-5 attained minimum days to maturity (124.44) alongwith regression coefficient near to unity exhibit average stability.

Keyword: *Variability, Stability, Thermo-tolerant, Indian mustard*

Introduction

Indian mustard (*Brassica juncea* L.) is an amphidiploids plant (2n=36) belongs to mustard family called (*Brassicaceae*). *Brassicas* are most important vegetable oil seed crop followed by soybean. In the 2023-24 season, rapeseed-mustard production in India reached 131.61 lakh tonnes, surpassing soybean production, which stood at 130.54 lakh tonnes, to become the country's leading oilseed crop. Rapeseed-mustard now accounts for 33.24% of India's total oilseed production, which amounts to 395.94 lakh tonnes (AICRP-RM 2024 www.drmr.res.in). The recent surge in rapeseed-mustard production can be attributed to improved high-yielding varieties, agronomic practices, and favorable climatic conditions. Government initiatives supporting oilseed cultivation and increased awareness of the crop's economic benefits have further driven its growth in productivity and acreage.

Adaptation of Indian mustard (*Brassica juncea* L.) and canola (*B. napus* L.) to low rainfall, short duration, Mediterranean-type environment in south Western Australia was studied by investigating the effects of genotype, environment and their interaction on crop growth and seed yield. Seed yield of Indian mustard and canola in low rainfall environments (Merredin, Mullewa and Newdegate) were higher when sown early in the season (May). Mustards were generally more adapted than canola to stressful environments associated with low rainfall, high temperature and late sowing as inferred from principal component and Finlay Wilkinson analyses (Gunasekera *et al*., 2006).

Rajasthan is among the largest producers, accounting for about 50% of the total rapeseed-mustard production in India. The growing of rapeseed-mustard in Rajasthan is mostly carried out under conserve soil moisture conditions where sowing commences after south west monsoon rains. Early rains may lead the farmers to sow the crop early in the season to take advantage of conserved moisture in the soil (Venkateswarluand Prasad, 2012). However, at the time of early sowing (second fortnight of September to first fortnight of October), the mean surface soil temperature may reach as high as 45°C. High soil temperature often results in seedling mortality upon initial germination which may eventually require re-sowing (Salisbury and Gurung, 2011).

Identification of gene or genes responsible for the desired characteristics of heat tolerance at distinct stages of plant growth and development is of great importance. Consequently, the present investigation was come up with to develop, evaluate and identify some of the promising early maturing heat stress tolerant genotypes.

Genotypic differences play a major role in adaptation of crop plant to specific stress environments. The differential response off genotypes to environmental changes is a genotype x environment interaction (G x E) (Vargas *et al.,* 2001). Understanding the biological significance and causes for G x E interactions could potentially lead to improved adaptation and yield in specific stress environments by betterexploitation of appropriately adapted genotypes (Turner *et al.,* 2001).Stability analysis helps in understanding the genotypic-adaptation under variable environmental changes.

Material and Methods

Indian mustard is sensitive to elevated temperature, especially at the seedling stage, which causes yield losses**.** To estimate stability and genetic variability of early maturing thermo-tolerant genotypes of Indian mustard, ten advanced breeding lines including two checks, were grown in the field under heat stress conditions **(**maximum temperature 40**.**5^{0}C at 0 to 10 cm depths on seeding date on September 28, 2018; maximum temperature 41**.**02^{0}C at 0 to 10 cm depths on seeding date on September 28, 2019; maximum temperature 43**.**2^{0}C at 0 to 10 cm depths on seeding date on September 25, 2020**)** in RBD with 3 replications at the research farm of ICAR-Indian Institute of Rapeseed-Mustard Research, Bharatpur Rajasthan, India.Geographically, the experimental farm of ICAR-IIRMR, Bharatpur is situated at the altitude of178.37 m above mean sea level (77.27^{0} E longitude; 27.12^{0} N latitude). The area has a semi-arid, sub-tropical climate having mean precipitation of about 664mm most of which is received in rainy season spreading from July to September.The soil of the experimental site was sandy loamwith 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.

Morpho-physiological characters, including, Days to 50 percent flowering (DTF), Days to maturity (DTM), 1000-seed weight (1000 SW in gm), Membrane Stability Index % (PMSI), Excised-leafwater loss % (PELWL), Relative water content % (PRWC), Water retention capacity of leaves %(PWRCL) and Seed yield (SY in kg/ha) were recorded from five randomly selected plants of each genotype. Various physiological characters including (PMSI), (PELWL), (PRWC), and (PWRCL) were determined by the procedures described by Ram *et al.,* (2015).

Analysis of variance (ANOVA) was calculated according to the formula described by PanseandSukhatme(1978) and critical differences (CD) were determined at 5 and 1% probability level. Estimation of phenotypic and genotypic coefficient of variation, heritability in broad sense, genetic gain, and stability based on Eberhert and Russel Model (1966) were determined using Windostat version 8.5 software.

Results and Discussion

The analysis of variance for all the traits showed highly significant difference among the genotypes indicating sufficient amount of variability in the material. The highest genotypic and phenotypic coefficient of variation was observed for membrane stability index (%) followed by seed yield (kg/ha) in allthe environments (Table 1 & 2). High genotypic and phenotypic coefficients of variation for seed yield and membrane stability index were also reported by Ram *et al.*(2021); Yadav *et al.* (2021); Sagolsem *et al.* (2023) in Indian mustard. High heritability (in broad sense) estimates were focused for water retention capacity of leaves (99.98% E_1), membrane stability index (99.96 E_3), seed yield (99.78 E_3), relative water content (99.07 % E_1), water retention capacity of leaves (99.05% E_3), relative water content (99.41% E_3), excised leaf water loss 97.40% E_1) and 1000 seed weight (99.03% E_3) indicating that these characters were less influenced by the environmental factor and direct selection for these characters would be effective for further improvement. The heritability (in broad sense) of membrane stability index (93.60% E_1) with maximum genetic advance (66.79% E_1) was observed which might be due to heritability with additive gene effect therefore selection may be effective (Table 2).

For each environment analysis of variance was carried out individually as well as pooled over the years on all the selected characters. Analysis of variance revealed significant differences amongst genotypes for the observed characters in each of the three environments was also carried out in order to verify presence of G x E interactions. G x E interactions variance was significant for all the observed characters. Variance due to genotype was also significant for all the observed characters. Variance due to environment was also significant for all the observed

Table 1. Range, critical difference, genotypic coefficient of variation (GCV), phenotypic coefficient of variation, heritability (b.s.) and genetic advance in Indian mustard in 3 environments.

	Days to flowering			Days to maturity			1000 seed weight (g)			Seed yield (kg/ha)		
	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3
Range	37.66	38.50	39.00	116.66	121.50	130.00	3.95	3.56	3.66	1209.16	1084.65	1344.44
	-48.00	-47.50	-48.00	-132.33	-144.00	-149.33	-5.85	-5.22	-5.61	-3109.29	-2899.18	-2422.22
CD (P=0.05)	2.90	1.38	1.21	1.31	1.64	2.46	0.57	0.40	0.07	403.99	9.38	21.02
CD (P=0.01)	4.33	2.06	1.81	1.96	2.45	3.67	0.84	0.60	0.10	602.24	13.99	31.33
GCV	9.26	6.65	5.83	3.77	5.30	3.72	11.68	10.40	14.17	20.20	27.47	21.44
PCV	10.86	7.19	6.30	3.89	5.42	4.04	15.81	13.33	14.24	24.80	27.48	21.46
h^2 (b.s.)	72.72	85.50	85.80	94.11	95.68	84.83	54.61	60.86	99.03	66.34	130.28	99.78
GA as % of mean	16.28	12.67	11.14	7.55	10.69	7.06	17.79	16.72	29.06	33.90	56.58	44.12

E_1: Year 2018-19, E_2: Year 2019-20 and E_3: Year 2020-21

Table 2. Range, critical difference, genotypic coefficient of variation (GCV), phenotypic coefficient of variation, heritability (b.s.) and genetic advance in Indian mustard in 3 environments.

Genetic variability	Membrane Stability Index (%)			Excised leaf- water loss (%)			Relative water content (RWC %)			Water retention capacity of leaves (%)		
parameters	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3
Range	8.42	27.06	25.06	20.80	14.63	23.02	76.54	79.62	81.07	54.71	21.59	23.02
	-25.34	-46.43	-44.15	-36.49	-22.48	-31.90	-85.50	-88.69	-88.20	-69.09	-29.62	-31.90
CD (P=0.05)	1.67	12.10	0.12	0.98	6.58	2.63	0.27	2.33	0.24	0.15	2.09	0.30
CD (P=0.01)	2.50	18.03	0.17	1.47	9.81	3.93	0.40	3.48	0.36	0.10	3.12	0.44
GCV	33.51	5.98	14.26	19.96	8.39	9.56	3.01	3.48	3.30	9.63	9.88	10.02
PCV	34.63	25.62	14.27	20.22	31.67	12.81	3.03	4.24	3.31	9.64	12.20	10.07
h^2 (b.s.)	93.60	05.46	99.96	97.40	07.03	55.65	99.07	67.51	99.41	99.98	65.63	99.05
GA as % of mean	66.79	2.88	29.38	40.58	4.58	14.69	6.18	5.90	6.78	19.85	16.49	20.56

E_1: Year 2018-19, E_2: Year 2019-20 and E_3: Year 2020-21

Table 3. Stability parameters of different genotypes for morpho-physiological traits in Indian mustard.

Genotype	Days to flowering			Days to maturity			1000 seed weight (g)			Seed yield (kg/ha)		
	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i
DRMRHT-13-13-6-5	41.16	-0.40	1.13	125.11	1.21	2.33*	4.15	0.57	-0.026	2045.40	0.365	-6837.08
DRMRHT-13-13-5-6	41.72	-1.21	5.09**	126.61	0.85	19.89**	4.34	-0.69	0.60	2008.52	1.072	2887.54**
DRMRHT-13-13-5-4	39.22	-4.02	0.54	122.88	0.87	-0.88	4.30	1.77	-0.03	2201.44	0.940	21100.63**
DRMRHT-13-13-5-5	42.27	9.83**	2.67*	124.44	1.19	4.07**	4.45	-0.10	0.18	2375.40	0.162	13669.39**
DRMRHT-13-22-9	46.38	2.20*	-0.98	125.94	1.07	16.90**	4.25	0.28	-0.035	1934.67	0.804	244831.25**
DRMRHT-13-22-10	47.05	-1.40	0.19	127.38	0.63	1.64	5.03	3.07**	0.050	2810.23	0.982	33168.00**
DRMRHT-13-22-8	46.61	0.004	-0.08	129.55	1.08	5.03**	5.07	4.77**	0.003	2264.60	2.084*	386665.50**
DRMRHT-13-28-16	46.50	-0.593	3.41**	128.11	0.93	-0.77	5.00	-0.72	-0.019	2010.10	2.615**	26731.50**
NPJ-112 (Check)	44.02	1.368*	-0.66	128.55	1.05	0.79	3.91	1.29	-0.041	1227.56	-0.184	25999.28**
BPR-543-2	48.50	4.22**	4.98**	141.88	1.08	14.05**	4.06	-0.25	-0.032	1945.46	1.159	71985.99**
Population mean	44.35			128.05			4.46			2082.34		

Table 4. Stability parameters of different genotypes for morpho-physiological traits in Indian mustard.

Genotype	Membrane Stability Index (%)			Excised leaf- water loss (%)			Relative water content (RWC %)			Water retention capacity of leaves (%)		
	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i
DRMRHT-13-13-6-5	31.75	1.23	-11.68	25.35	0.85	0.34	81.11	-0.77	1.16	35.10	0.86	1.00
DRMRHT-13-13-5-6	34.46	1.29	-8.81	22.63	1.43	-4.15	82.41	0.34	-0.15	35.27	0.87	2.93**
DRMRHT-13-13-5-4	38.35	0.88	-11.67	23.39	-0.55	-1.11	87.46	1.06	-0.027	39.67	1.24	18.58**
DRMRHT-13-13-5-5	33.16	1.20	1.09	23.40	1.25	17.88**	85.71	2.50**	-0.28	36.10	0.93	8.16**
DRMRHT-13-22-9	28.00	1.32	-12.32	27.53	1.75	40.14**	80.55	0.06	0.009	34.87	0.87	12.86**
DRMRHT-13-22-10	33.42	0.68	-3.20	20.07	0.81	-2.06	85.63	1.39	-0.136	40.03	1.21	14.31**
DRMRHT-13-22-8	30.28	0.45	107.96**	20.64	1.17	1.58	84.58	0.70	0.443	41.46	1.20	13.70**
DRMRHT-13-28-16	24.74	0.78	29.40**	27.10	1.59	-4.21	82.01	1.17	0.96	39.11	0.86	8.99**
NPJ-112 (Check)	30.10	0.96	-10.86	23.70	0.26	9.64**	81.28	2.65**	-0.44	35.89	1.01	8.74**
BPR-543-2	30.72	1.18	-6.84	25.71	1.40	-3.49	81.17	0.85	-0.118	37.19	0.88	4.23**
Population mean				23.96			83.20			37.47		

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

characters. These results indicated presence of substantial amount of genotype x environment interaction.

Stability analysis was carried out as per Eberhart and Russell (1966) model for all the observed characters in order to verify the presence of variance due to component of G x E interaction. Samuel *et al.* (1970) and Paroda and Hays (1971) emphasized that linear regression (bi) could simply be regarded as a measures of response of a particular genotype, where the deviation around regression of a particular genotype, where the deviation around regression line (S^2di) is the most appropriate measure of stability. Genotype with b_i=01 and lowest deviation around regression line could be termed most stable and vice-versa. Accordingly, it was possible to judge the stability of genotypes with due consideration to their mean performance and linear response.

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.

The genotype x environment interaction was present and it was highly significant for all the characters studiedby Singh and Bhajan (2020). As the environments selected in the present study were diverse (three consecutive year), the presence of significant G x E for observed characters indicates the relevance of the stability analysis. Genotype DRMRHT-13-13-5-5, DRMRHT-13-13-5-4, DRMRHT-13-22-10 and DRMRHT-13-13-5-6 possess relative stable and high performance for relative water content (%), membrane stability index (%), days to maturity, water retention capacity of leaves (%) and seed yield (kg/ha) over its respective population mean. The result indicated that genotype DRMRHT-13-13-5-5, DRMRHT-13-13-5-4, DRMRHT-13-22-10 and DRMRHT-13-13-5-6 were expressed relatively wider range for seed yield (kg/ha), excised leaf-water loss (%) and water retention capacity of leaves (%). Analysis of variance for stability indicated significant differences were observed, indicating the diversity in the selected genotypes. Significant differences were also observed among the environments two, indicating the significant effect of environment in the expression of the traits. Genotype x environment interaction was significant for seed yield (kg/ha), membrane stability index, relative water content and water retention capacity of leaves indicating that genotypes are varying over the environment due to G x E. The significant G x E interaction has been earlier reported by Gunasekera *et al.*(2006); Sagolsem Diana *et al.* (2013); Singh and Bhajan, (2016); Ram *et al.*(2023); Prathamesh *et al.* (2025).

Results are in accordance with reports by Ram *et. al.*(2013); Iqbal *et al.* (2014); Singh and Bhajan (2016); Koundinya *et al.*(2021).

The genotype DRMRHT-13-13-5-5 attained more membrane stability index (%) alongwith regression coefficient equilant to unity and S^2di near to zero considered as stable. While, the genotype DRMRHT-13-13-5-4 had attained maximum membrane stability index alongwith regression coefficient equilant near to unity, exhibit average stability.The stability parametersfor water retention capacity of leaves (%) revealed that genotype DRMRHT-13-22-10 had higher mean water retention capacity of leaves to general mean alongwith regression coefficient near to unity considered as average stable and desirable genotype. The genotype DRMRHT-13-22-10 recorded maximum seed yield (kg/ha) alongwith regression coefficient near to unity exhibiting average stability, where as genotype DRMRHT-13-13-5-4 had recorded high seed yield to general mean along with regression coefficient near to unity considered as average stable and desirable.

Considering pooled mean performance of membrane stability index (%), it was found that five genotypes viz., DRMRHT-13-13-5-4 (38.35 %), DRMRHT-13-13-5-6 (34.46%), DRMRHT-13-13-5-5 (33.16 %) and DRMRHT-13-22-10 (33.42 %) had significantly higher membrane stability index than check variety NPJ-112 (30.10 %) (Table 4).

Out of 10 genotypes, three genotypes viz., DRMRHT-13-13-5-5, DRMRHT-13-13-5-4 and DRMRHT-13-22-10 possessed significantly higher mean than general mean, regression coefficient equivalent to zero, exhibiting average stability and adaptability, hence most suitable and desirable. The genotypes viz., DRMRHT-13-13-5-5, DRMRHT-13-13-5-6 and DRMRHT-13-13-6-5 had mean significantly higher than general mean, regression coefficient more than unity and S^2di equivalent to zero, exhibiting below average stability. Hence, this genotype was suited for better environmental condition. Results are in accordance with reports of Ram *et al.* (2013); Iqbal *et al.*,(2014); Singh and Bhajan (2016); Prathamesh *et al.* (2025).

The mean performance of excised leaf water loss (%) of genotypes ranged from 20.80% to 36.49 % (E_1), 14.63% to 22.48% (E_2)and 23.02 to 31.90 % (E_3).Four genotypes viz., DRMRHT-13-22-10, DRMRHT-13-22-8, DRMRHT-13-13-5-6 and DRMRHT-13-13-5-4 had minimum excised leaf water loss than check variety BPR-543-2 (25.71%). The deviation from regression was significantly greater than zero in one genotype DRMRHT-13-13-6-5. Genotype DRMRHT-13-13-5-6 and DRMRHT-13-22-10 showed significantly higher mean, with regression coefficient unity and non-significant deviation from regression coefficient indicating average stability suitable for all environmental conditions.Results are in accordance with reports of Ram *et al.*(2013); Iqbal *et al.*(2014); Singh and Bhajan (2016); Koundinya *et al.*(2021); Supratim *et al.* (2024).

Means of relative water content (RWC%) varied from 80.55 % in DRMRHT-13-22-10 to 87.46% in DRMRHT-13-13-5-4. Five genotype viz., DRMRHT-13-13-5-4, DRMRHT-13-13-5-5, DRMRHT-13-22-10, DRMRHT-13-22-8 and DRMRHT-13-13-5-6 recorded higher relative water content than check variety NPJ-112 (81.17%). Considering all stability parameters *i.e.* high mean RWC, b_i near to one and S^2di close to zero, two genotypes viz., DRMRHT-13-13-5-4 and DRMRHT-13-22-10 were found superior and stable across environments.Genotype DRMRHT-13-13-5-6 recorded more RWC (%) and b_i value less than one with non-significant S^2di, explaining its suitability in poor environments (unfavorable) showed above average stability. The results are concomitant with the results reported by Ram *et al.*(2013); Singh and Bhajan, (2016); Koundinya *et al.*(2021); Singh *et al.*(2023).

With respect to water retention capacity of leaves (%), the mean value varied from 34.87% in DRMRHT-13-22-9 to 41.46% in DRMRHT-13-22-8 with general mean of 37.47%. Five genotypes i.e. DRMRHT-13-13-5-4, DRMRHT-13-22-10, DRMRHT-13-22-8 and DRMRHT-13-28-16 registered higher water retention capacity of leaves (%) than check variety NPJ-112 (35.89%) and general mean (37.47%) (Table 4).Out of 10 genotypes, two genotypes and S^2di close to zero indicating that these genotypes were stable across the environments. Considering mean above average, b_i near to unity and S^2di close to zero, the genotype, DRMRHT-13-13-6-5, DRMRHT-13-13-5-4, DRMRHT-13-13-5-5 and DRMRHT-13-22-10 were found stable. However, the genotypes DRMRHT-13-13-6-5 were considered specially adapted to rich environments as b_i is significant >1. While, genotype DRMRHT-13-28-16 recorded above average stability due to bi valueless than one and non-significant deviation from regression line. The results obtained are in agreement with the finding of earlier worker Ram *et al.* (2013); Singh and Bhajan, (2016); Koundinya *et al.* (2021).

Based on the stability analysis over three environments, four genotypes viz., DRMRHT-13-13-5-4, DRMRHT-13-13-5-5, DRMRHT-13-22-10 and DRMRHT-13-13-6-5 were identified as promising for seed yield (kg/ha), membrane stability index, minimum excised leaf water loss, relative water content and water retention capacity of leaves were considered as desirable and stable over the environments. These genotypes can be utilized in pedigree crop improvement and can be released for commercial cultivation. The selected germplasm can be used as parental source for the development of superior heat tolerant/drought tolerant stable Indian mustard varieties for commercial cultivation.

Key findings : The present results provide useful information to aid the choice of heat tolerant and early maturing genotypes. DRMRHT-13-13-5-4, DRMRHT-13-13-5-5 and DRMRHT-13-22-10 could be included in breeding programme where objective is to develop high yielding and heat tolerant stable genotypes over the environments.

Author's Contribution

Conceptualization of research (BR, V V Singh, K H Singh); Designing of the experiments (BR, Priyamedha); Contribution of experimental materials (BR); Execution of field/lab experiments and data collection (Execution); Analysis of data and interpretation (BR, Reema Rani, M S Sjith K, V V Singh, Arun Kumar BL Meena, LK Meena); Preparation of the manuscript (BR, V V Singh).

Acknowledgements

We sincerely acknowledge Director, Indian Council of Agricultural Research-Indian Institute of Rapeseed-Mustard Research, Bharatpur-321303 (Rajasthan) India, for providing financial support and the facilities to carry out this research work.

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