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Assessment of Genetic Variability and Heritability of Morpho-Physiological Traits in Indian Mustard (*Brassica juncea*) Genotypes under Heat Stress

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ABSTRACT

To assess genetic variability, broad-sense heritability and genetic advance among Indian mustard genotypes, a study was conducted using 18 genotypes (including one check) under heat stress conditions. The experiment followed a Complete Randomized Block Design (CRBD) with three replications, carried out at the ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, during the *rabi* season of 2020-2021. In terms of mean performance, genotype DRMRHT-18-134 took the least number of days (33.5 days) to reach 50% flowering, while genotype DRMRHT-18-142 had the highest seed yield per plant (30.00 g). The phenotypic coefficient of variation (PCV) was greatest for the membrane stability index (44.42%), as was the genotypic coefficient of variation (GCV) (44.25%). Heritability estimates in the broad sense were notably high for the membrane stability index (99.48%). Additionally, high heritability combined with a significant genetic advance was observed for the membrane stability index, secondary branches per plant and seed yield per plant. For traits like days to 50% flowering and chlorophyll b content (mg/g FW), high heritability was associated with moderate genetic advance, while chlorophyll a content (mg/g FW) showed low heritability with moderate genetic advance.

Keywords Heritability, Phenotypic coefficient variation, Genotypic coefficient variation.

INTRODUCTION

The word “mustard” is thought to have originated from the early European tradition of blending the sweet “must” of old wine with crushed mustard seeds to create a hot paste, known as “mustum ardens” or “hot must,” which eventually evolved into the modern term (Hemingway 1976). Mustard belongs to the family *Crucifereae* (also known as *Brassicaceae*) and the genus *Brassica*. Indian mustard, or brown mustard, is an amphidiploid species with a chromosome number of 2n=36. Although it is predominantly self-pollinating, some level of cross-pollination (2-15%) can occur due to insect activity and other environmen-

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tal factors. Mustard is believed to have originated in China and was later introduced to India (Prain 1898, Bailey1922). Current Indian mustard varieties contain high levels of erucic acid, which, based on studies in birds and animals, has been suggested to have negative effects on heart health (Gopalan *et al.* 1974, Gurr 1992).

An increase of one degree Celsius in the average temperature during the growing season has been reported to reduce crop yields by 17% (Lobell and Asner 2003). Both temporary and prolonged exposure to high temperatures can lead to various morphological, physiological and biochemical changes in plants (Serraj *et al.* 1999, Moradshahi *et al*. 2004). Heat stress impacts plant growth at all stages of development, although the critical temperature threshold can vary depending on the growth stage. For example, during germination, elevated temperatures can either slow or completely inhibit the process, while at later stages, they can disrupt photosynthesis, respiration, water relations and membrane stability. The production of heat shock proteins and reactive oxygen species (ROS) are key plant responses to heat stress (Wahid and Close 2007, Camejo *et al.* 2006). Indian mustard demonstrates greater tolerance to heat and water stress compared to canola-quality Indian mustard. It is commonly cultivated under rainfed conditions, with sowing often beginning after the south-west monsoon rains (Venkateswarlu and Prasad 2012). Early rains may encourage farmers to plant the crop early in the season to utilize the conserved soil moisture. However, high temperatures during sowing can hinder seed germination and increase seedling mortality, leading to poor crop establishment and lower seed yields (Azharudheen *et al.* 2013).

MATERIALS AND METHODS

To assess the genetic variability among Indian mustard genotypes, a field experiment was conducted using 18 genotypes, including a check, under heat stress conditions. The trial was set up following the Complete Randomized Block Design (CRBD) with three replications at the ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, during the *rabi* season of 2020-2021. The research site is located at 77°30' E longitude, 27°15' N latitude and an elevation of 178.37 meters above sea level. Experimental site climate is classified as sub-tropical and semi-arid, with hot summers and an average maximum daily temperature ranging from 12°C to 19°C. The area receives approximately 700 mm of annual rainfall. The crop was grown under moisture-conserved conditions, with row and plant spacing set at 45 cm and 15 cm, respectively. Standard agronomic practices were followed to ensure proper crop growth.

Data were collected on various seed yield and yield-related traits, including days to 50% flowering, days to maturity, plant height (cm), number of primary and secondary branches per plant, main shoot length (cm), number of siliquae on the main shoot, total siliquae per plant, siliqua length (cm), seeds per siliqua, seed yield per plant (g), 1000-seed weight (g), membrane stability index (%), relative water content (%), excised leaf water loss (%), leaf water retention capacity (%), total phenol content (mg/g), and chlorophyll content (mg/g FW). Observations for all traits were made on five randomly selected plants from each genotype.

Membrane stability index (%)

Leaf strips (0.2g) of uniform size were placed in test tubes containing 10 ml of double-distilled water, divided into two sets. In the first set, the test tubes were kept in a water bath at 40°C for 30 minutes, after which the electrical conductivity of the solution was measured (C_1) using a conductivity meter. In the second set, the test tubes were placed in boiling water at 100°C for 15 minutes and the electrical conductivity was measured again (C_2) (Premachandra *et al.* 1990, Sairam 1994). The leaf membrane stability index (MSI) was then calculated using the following formula:

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

Relative water content (%)

For determining relative water content (RWC), the samples were first weighed to record their fresh weight (FW). Leaf sections, each 2 cm in length, were then floated in distilled water for 4 hours, after which they were blot-dried and weighed to measure their turgid weight (TW). These sections were subsequently oven-dried at 60°C for 24 hours to obtain the dry weight (DW). The RWC was calculated using the formula provided by Barrs (1968).

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

Excised leaf water loss (%)

To measure excised-leaf water loss (ELWL), leaves were weighed at three different stages: Immediately after sampling to record the fresh weight, after 6 hours of drying in an incubator at 28°C and 50% relative humidity, and finally after oven drying for 24 hours at 70°C, following the method described by Clarke (1987). ELWL was then calculated using the formula provided below:

ELWL= (Fresh weight – Weight after 6 h) / (Fresh weight- Dry weight) × 100

Water retension capacity of leaf (%)

Water retension capacity of leaf was estimated by the method proposed by Ashraf and Ahmad (1998).

WRCL =Wt of excised leaf each hours/Wt. of turgid excised leaf × 100

Total phenol (mg/g)

Total phenol of leaf was estimated by method proposed by Bray and Thorpe (2006).

Chlorophyll contains (mg/g FW)

Chlorophyll estimation was done in fresh leaf by a common method (Hiscox and Israelstam 1979) with the following formula for deriving Chlorophyll *a* (Chl. *a*), Chlorophyll *b* (Chl. *b*), Total chlorophyll (Chl. total) and Total carotenoids content.

$$\text{Chl. a (mg/g FW)} = [(12.7 \times A663) - (2.69 \times A645)] \times V/1000 \times W$$

$$\text{Chl. b (mg/g FW)} = [(22.9 \times A645) - (4.68 \times A663)] \times V/1000 \times W$$

$$\text{Carotenoids (mg/g FW)} = [(1000 \times A470) - (3.29 \times \text{Chl. a}) - (104 \times \text{Chl. b})]/198$$

Where,
V-volume of DMSO added W-weight of sample taken FW-fresh weight

Statistical analysis

The data collected for various genotypes across different parameters were analyzed using Analysis of Variance (ANOVA). The ANOVA was performed following the method outlined by Panse and Sukhatme (1957), and critical differences (CD) were calculated at the 5% and 1% probability levels. Phenotypic and genotypic coefficient of variation, heritability in broad sense, and genetic advance were estimated using Windostat Version 9.1 software. Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) values were classified as high if they exceeded 20%, low if below 10%, and intermediate if between 10% and 20%, according to Deshmukh *et al*. (1986).

RESULTS AND DISCUSSION

The analysis of genetic variability considered 21 traits across 18 genotypes in the study, focusing on genetic variance, heritability, and genetic advance. A broad range of variability within crops enhances the likelihood of selecting desirable types (Vavilov 1951). Table 1 presents the mean performance of various Indian mustard genotypes for morpho-physiological traits. Significant variation was observed among the genotypes. The number of days to 50% flowering ranged from 33.5 days for DRMRHT-18-134 to 46.5 days for DRMRHT-18-40, with an average of 40.5 days. Maturity varied by approximately 19 days among genotypes, with DRMRHT-18-142 being the earliest to harvest (109 days), and DRMRHT-17-23 taking the longest (128 days), with a mean of 119.58 days. The tallest plant height was recorded in DRMRHT-18-141, while the shortest was in DRMRHT-18-40, with an average height of 164.86 cm. The average number of primary branches per plant was 5.52, with a range from 4.7 to 6.7. DRMRHT-18-89 had the most primary branches, while DRMRHT-17-74 had the fewest. For secondary branches, DRMRHT-18-65 had the highest count (16.9), and DRMRHT-18-123 had the lowest (5.7), with an average of 12.11. The main shoot length averaged 71.11 cm, varying from 56.70 cm to 84.6 cm, with DRMRHT-17-83 showing

Table 1. Mean performance of different genotypes for morpho-physiological traits of Indian mustard.

Sl. No.	Genotype	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary branches	Secondary branches	Main shoot length (cm)	Siliqua on MSL
1	DRMRHT-18-40	46.5	123	171.8	4.7	9.1	66.85	39.5
2	DRMRHT-18-65	44.5	113	169.4	6.4	16.9	79.25	45.6
3	DRMRHT-18-88	39.5	116.5	164.3	6.1	15	75.75	46.8
4	DRMRHT-18-89	39.5	120	164.2	6.7	14.5	71.8	44
5	DRMRHT-18-91	42	120.5	185.5	6.6	14.7	75.65	43.8
6	DRMRHT-18-97	37	120	166.8	5.3	12.6	73.7	43.9
7	DRMRHT-18-123	40.5	121	169	4.8	5.7	63.95	35.7
8	DRMRHT-18-126	42	123.5	172.7	5	8.9	68.9	44.6
9	DRMRHT-18-134	33.5	115	152.7	4.9	14.8	71.25	36.4
10	DRMRHT-18-141	35	109.5	141.9	5.4	15.8	69.95	37.3
11	DRMRHT-18-142	37	109	152.3	5.6	14.9	75.8	39.3
12	DRMRHT-17-83	39.5	120.5	172.9	5.3	14.6	84.6	48.1
13	DRMRHT-17-74	41	126.5	153.7	4.7	14.7	75.05	47.4
14	DRMRHT-17-50	42.5	125.5	181.6	5.7	9.8	74.7	38.8
15	DRMRHT-17-23	41.5	128	167.2	5.6	6	56.7	41.2
16	DRMRHT-17-40	42.5	123.5	164.8	5.3	6.9	66.9	41.8
17	DRMRHT-17-21	43.5	121	157.5	5.7	8.9	57.85	35.1
18	CHECK NPJ-112	41.5	116.5	159.3	5.6	14.2	71.35	44.8
	Mean	40.5	119.58	164.86	5.52	12.11	71.11	41.89
	Range	33.5-46.5	109-128	141.9-185.5	4.7-6.7	5.7-16.9	56.70-84.6	35.1- 48.1
	SE(d)	0.24	0.979	3.8646	0.2248	0.4703	2.6286	1.8084
	CD	0.690	2.8143	11.107	0.6461	1.3515	7.5546	5.1974
	CV	1.027	1.4183	4.0601	7.0515	6.7253	6.4024	7.4766

Table 1. Continued.

Sl. No.	Genotype	Siliqua per plant	Siliqua length (cm)	Seeds per siliqua	Seeds yield siliqua (g)	1000 Seed weight (g)	Membrane stability index (%)	Relative water content (%)
1	DRMRHT-18-40	230.3	4.62	12.48	19	6.337	4.845	71.86
2	DRMRHT-18-65	353.1	5.52	13.96	25	5.644	14.47	75.69
3	DRMRHT-18-88	280.4	5.335	15.42	17.5	4.794	8.2767	74.95
4	DRMRHT-18-89	294.1	5.53	13.92	20.5	5.137	23.0567	75.09
5	DRMRHT-18-19	284.5	5.005	13.08	21.5	5.249	18.925	74.3
6	DRMRHT-18-97	254.6	4.81	14.6	19	4.513	20.7667	74.34
7	DRMRHT-18-123	152.1	5.105	14.4	15.5	6.433	18.51	78.395
8	DRMRHT-18-126	206.1	4.92	14.5	18.5	6.2115	13.1167	77.415
9	DRMRHT-18-134	296.6	5.605	15.4	25	5.671	29.6683	79.08
10	DRMRHT-18-141	341.4	4.965	14.98	17.5	4.0215	36.2467	78.53
11	DRMRHT-18-142	385.9	5.445	16.54	26	4.3055	29.8633	79.59
12	DRMRHT-17-83	334.2	5.32	16.22	30	5.643	17.2533	79.145
13	DRMRHT-17-74	324.1	4.87	16.56	23.5	4.685	19.7217	75.205
14	DRMRHT-17-50	270.1	5.18	14.04	18.5	4.7995	18.4583	79.87
15	DRMRHT-17-23	199.5	5.005	13.42	17.5	6.107	15.95	79.83
16	DRMRHT-17-40	208.4	5.295	14.06	14.5	5.0795	19.8617	75.89
17	DRMRHT-17-21	227.7	5.31	13.8	15.5	5.941	36.58	76.745
18	CHECK NPJ-112	295.4	5.075	15.54	25	5.931	10.4117	74.31
	Mean	274.36	5.16	14.60	20.52	5.36	19.7768	76.6797
	Range	152.1-385.9	4.62-5.60	12.48-16.56	14.5-30.00	4.02-6.43	4.84-36.58	71.86-79.87
	SE(d)	29.676	0.1291	0.4187	0.9555	0.2265	0.4479	0.8068
	CD.	85.290	0.3711	1.2033	2.746	0.651	1.2873	2.3187
	CV	18.734	4.3332	4.9645	8.0618	7.318	3.9227	1.8224

Table 1. Continued.

Sl. No.	Genotype	Excised leaf water loss (%)	Water retention capacity of leaf (%)	Total phenol (mg/g)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Carotenoids (mg/g FW)
1	DRMR HT-18-40	17.275	63.615	5.0433	4.09	0.78	4.87	13.85
2	DRMR HT-18-65	13.595	47.56	5.0267	4.8033	0.7	5.5033	13.08
3	DRMRHT-18-88	15.99	57.445	5.7867	3.53	0.54	4.07	10.3467
4	DRMRHT-18-89	18.945	64.695	5.5067	4.31	0.62	4.93	11.88
5	DRMRHT-18-91	18.77	59.625	5.8767	3.49	0.62	4.11	11.3567
6	DRMRHT-18-97	17.74	59.775	5.3433	4.39	0.74	5.13	13.7333
7	DRMRHT-18-123	24.05	67.63	5.1567	4.72	0.98	5.7	16.5867
8	DRMRHT-18-126	20.515	68.545	4.8033	3.75	0.57	4.32	11.9133
9	DRMRHT-18-134	15.915	46.16	5.5367	4.0333	0.76	4.7933	12.9
10	DRMRHT-18-141	17.515	55.835	4.6033	4.17	0.6	4.77	12.14
11	DRMRHT-18-142	16.68	56.935	4.8367	4.1167	0.72	4.8367	13.08
12	DRMRHT-17-83	18.86	58.05	5.1067	3.9433	0.62	4.5633	12.1567
13	DRMRHT-17-74	18.23	60.92	5.6233	3.5467	0.66	4.2067	12.3467
14	DRMRHT-17-50	23.105	62.83	5.4633	4.4867	0.71	5.1967	13.2067
15	DRMRHT-17-23	18.41	72.3	5.2167	3.7567	0.76	4.5167	12.79
16	DRMRHT-17-40	24.085	70.065	3.59	4.01	0.67	4.68	11.7267
17	DRMRHT-17-21	19.55	68.075	5.09	3.59	0.8	4.39	13.09
18	CHECK NPJ-112	20.87	53.32	5.4967	3.8733	0.66	4.5333	11.5867
	Mean	18.8944	60.7433	5.1726	4.0339	0.695	4.7289	12.6539
	Range	13.59-24.08	46.16-72.30	3.59-5.87	3.49-4.80	0.54-0.98	4.07-5.70	10.34-16.58
	SE(d)	0.9226	2.9877	0.2661	0.2358	0.0246	0.2379	0.4762
	CD	2.6515	8.5867	0.7649	0.6776	0.0707	0.6837	1.3687
	CV	8.4573	8.5192	8.912	10.1239	6.1272	8.7133	6.5184

the greatest length and DRMRHT-17-23 the least. The maximum number of siliquae on the main shoot was observed in DRMRHT-17-83 (48.1), while DRMRHT-17-21 had the fewest (35.1), with an average of 41.89. The highest number of siliquae per plant was found in DRMRHT-18-142 (385.9), and the lowest in DRMRHT-18-123 (152.1), averaging 274.36. The longest siliqua length was in DRMRHT-18-134 (5.60 cm) and the shortest in DRMRHT-18-40 (4.62 cm), with an average of 5.16 cm. The greatest number of seeds per siliqua was in DRMRHT-17-74 (16.56), and the least in DRMRHT-18-40 (12.48), with a mean of 14.60. For seed yield per plant, DRMRHT-18-142 yielded the highest (30.00 g), while DRMRHT-17-40 and DRMRHT-18-91 had the lowest yields (14.5 g and 18.5 g, respectively), with an average of 20.52 g. The 1000-seed weight ranged from 4.02 g in DRMRHT-18-140 to 6.43 g in DRMRHT-18-123, with an average of 5.36 g. The highest membrane stability index was found in DRMRHT-17-21 (36.58%), and the lowest in DRMRHT-18-40 (4.84%), with an average of 19.77%.

Relative water content averaged 76.67%, ranging from 71.86% to 79.87%, with DRMRHT-17-50 having the highest and DRMRHT-18-40 the lowest. The highest excised leaf water loss was in DRMRHT-17-40 (24.08%), and the lowest in DRMRHT-18-65 (13.59%), with an average of 18.89%. The greatest leaf water retention capacity was in DRMRHT-17-2 (72.30%), and the lowest in DRMRHT-18-134 (46.16%), with an average of 60.74%. The total phenol content averaged 5.17 mg/g, ranging from 3.59 to 5.87 mg/g, with DRMRHT-18-91 showing the highest and DRMRHT-17-40 the lowest. Chlorophyll a content was highest in DRMRHT-18-65 (4.80 mg/g) and lowest in DRMRHT-18-91 (3.49 mg/g), averaging 4.03 mg/g. Chlorophyll b averaged 0.695 mg/g, ranging from 0.54 to 0.98 mg/g, with DRMRHT-18-123 having the highest and DRMRHT-18-88 the lowest. The total chlorophyll content was highest in DRMRHT-18-123 (5.70 mg/g) and lowest in DRMRHT-18-88 (4.07 mg/g), with an average of 4.72 mg/g. The maximum carotenoid content was in DRMRHT-18-123 (16.58 mg/g), while

Table 2. Genetic variability for Morpho-physiological and quantitative traits of Indian mustard.

Characters	Genetic coefficient of variance (%)	Phenotypic coefficient of variance (%)	Heritability (broad sense) (%)	Genetic advance	Genetic advance value % mean
Days to 50% flowring	8.06	8.13	98.40	6.67	16.48
Days to maturity	4.45	4.67	90.80	10.46	8.74
Plant height (cm)	6.09	7.32	69.27	17.22	10.45
Primary branches	10.36	12.53	68.37	0.97	17.65
Secondary branches	30.15	30.89	95.26	7.34	60.62
Main shoot length (cm)	9.07	11.10	66.75	10.85	15.26
Siliqua on MSL	8.91	11.63	58.72	5.89	14.07
Siliqua per plant	19.72	27.20	52.57	80.81	29.45
Siliqua length (cm)	4.72	6.41	54.33	0.37	7.17
Seeds per siliqua	7.41	8.92	69.06	1.85	12.69
Seed yield per plant (g)	20.50	22.03	86.61	8.07	39.31
1000 seed wt (g)	13.02	14.94	76.01	1.25	23.39
Membrane stability index (%)	44.25	44.42	99.48	17.95	90.80
Relative water content (%)	2.92	3.44	72.04	3.92	5.11
Excised leaf water loss (%)	14.09	16.43	73.53	4.70	24.90
Water retension capacity of leaf (%)	11.00	13.91	62.52	10.88	17.92
Total phenol (mg/g)	8.72	12.47	48.95	0.65	12.57
Chlorophyll a (mg/g FW)	7.90	12.84	37.90	0.40	10.03
Chlorophyll *B* (mg/g FW)	14.35	15.60	84.59	0.18	27.19
Total chlorophyll (mg/g FW)	8.14	11.92	46.64	0.54	11.46
Carotenoids (mg/g FW)	9.75	11.73	69.13	2.11	16.70

the minimum was in DRMRHT-18-88 (10.34 mg/g), with an average of 12.65 mg/g.

Table 2 presents the phenotypic and genotypic coefficients of variation, heritability in the broad sense, and genetic advance for 21 traits across 18 Indian mustard genotypes. The highest phenotypic coefficient of variation (PCV) was observed for membrane stability index (44.42%), secondary branches per plant (30.89%), siliqua per plant (27.20%), seed yield per plant (22.03 g), excised leaf water loss (16.43%), and chlorophyll b (15.60 mg/g FW). Conversely, the lowest PCV was noted for days to maturity (4.67%) and relative water content (3.44%). The genotypic coefficient of variation (GCV) was highest for membrane stability index (44.25%), secondary branches per plant (30.15%), seed yield per plant (20.50 g), and siliqua per plant (19.72%). The lowest GCV was found for siliqua length (4.72 cm), days to maturity (4.45%), and relative water content (2.92%). GCV values were consistently lower than PCV values for all traits, suggesting substantial environmental influence. The significant gap between GCV and PCV across most traits indicates a strong environmental effect on the observed variations. Higher PCV compared to GCV in all traits reflects the substantial impact of environmental variance. These findings align with previous studies by Ram *et al.* (2017), Yadav and Pandey (2018), Gupta *et al.* (2019), Thapa *et al.* (2020), Ram *et al*. (2021), which similarly reported higher PCV compared to GCV, especially for traits like the membrane stability index. The highest GCV and PCV values for membrane stability index (%) suggest considerable variability for this trait (Ram *et al.* 2021).

Heritability estimates in the broad sense were highest for membrane stability index (99.48%), followed by days to 50% flowering (98.40%), secondary branches per plant (95.26%), days to maturity (90.80%), seed yield per plant (86.61%), and chlorophyll b (84.59%). In contrast, chlorophyll a had a lower heritability estimate of 37.90%. The highest expected genetic advance as a percentage of the mean was for membrane stability index (%) (90.80), secondary branches per plant (60.62%), and seed yield per plant (39.31%). Traits with high heritability and genetic advance included the membrane stability index, secondary branches per plant, and seed yield per plant. Traits with high heritability but medium genetic advance were days to 50% flowering and chlorophyll b. Chlorophyll a showed low heritability and medium genetic advance. Heritability estimates provide insights into the potential genetic gain in future generations. The value of heritability in genetic studies lies in its predictive ability for potential advances through selection, based on the relationship between phenotypic and genotypic values. High genetic advance is expected in traits with both high GCV and high heritability. Heritability, reflecting the proportion of genetic versus environmental variance, typically shows a negative correlation between broad sense and narrow sense heritability (Kempthorne 1957). If heritability is primarily due to additive gene effects, it is associated with high genetic gain, whereas non-additive effects lead to lower genetic gain (Panse and Sukhatme 1957).

CONCLUSION

High values of heritability in broad sense, genotypic coefficient of variation and expected genetic advance was recorded membrane stability index followed by days to 50% flowering, secondary branches per plant, days to maturity, seed yield per plant and chlorophyll. Direct selection by selecting these traits may be effective for yield improvement because traits are highly heritable and less affected by environment.

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