Investigation of various physiological and yield characteristics for terminal heat stress in Indian Mustard (*Brassica juncea*)

Abstract

In present investigation we studied the influence of terminal heat stress on several agronomic, physiological and yield-related variables in 10 mustard genotypes such as; Advance-414, Anmol, Bond, Coral-432, JKMS 8532, MRR-8030, NMS-2018, Rajshree, RH-30 and Sonalika. The experiment conducted under control condition (Sown in October) and heat stressed environment (Sown in November) to assess the role of ambient and high temperatures, respectively. Different characteristics including relative water content (RWC), water saturation deficit (WSD), relative dry weight (RDW), membrane stability index (MSI), days to flowering, plant height and yield attributes were analysed in respect to heat stress. The results demonstrated strong relationship between genotype and treatment in responses to heat stress. In terms of RWC, RH-30 maintained high value in both normal (95.17 ± 0.991%) and late sown (72.78 ± 1.022%) circumstances, but Coral-432 exhibited susceptibility with decreased relative water content (51.30 ± 0.988%) under stress. Heat stress significantly raised water saturation deficit in all genotypes, with RH-30 retaining lower WSD (4.83 ± 0.083%; control) than Coral-432 (48.70 ± 0.179% stress) whereas membrane stability index ranged from 5.03 ± 0.033% to18.03 ± 0.176%. Yield attributes such as seed yield per plant, test weight drastically reduced under heat stress whereas, genotype RH-30 demonstrating the best resistance in seed yield (14.22 ± 0.215 g) and 1000-seed weight (test weight; 4.35 ± 0.066 g) under stress. Plant height and number of branches also showed similar reduction in respect to heat stress. Overall, genotypes RH-30 and Rajshree found superior to stress tolerance in all tested parameters and further used in the breeding programme to develop the heat resilience cultivar in mustard.

Keywords: Indian mustard, Terminal heat stress, Climate change, Membrane stability index, flowering stages.

Introduction

Indian mustard also known as Brown Mustard or Sarson (*Brassica juncea* L.) Czern & Coss (AABB, 2n = 36) is an important crop for Indian agriculture and account of 90% of overall brassica family [1]. In the global context the oleaginous brassica, rape and mustard are leading producers of edible oil after soybean and oil palm and second-largest producer of rapeseed mustard globally next largest producer to china and the third-largest producer next to Canada [2]. India possesses 19.8% of the global acreage and produces 9.8 % of the global output of rapeseed-mustard [3]. They grow the crop on 8.80 million hectares and produce 12.05 million metric tonnes annually with an average yield of 1,419 kg/ha. Rajasthan is the largest producer of rapeseed-mustard accounting for 46.63%, followed by Madhya Pradesh 14.36%, Haryana 11.63%, Uttar Pradesh 8.81% and West Bengal 6.5% [4]. Agricultural crops had ability to resist abiotic stresses; especially temperature influences an intricate point on the global food security [5]. Indian mustard *Brassica juncea* is an important oil seed crop adversely affected by climate change specifically heat stress during its reproductive phase [6]. The most significant impact on sustainable agriculture and crop enhancement is the information regarding the distinct responses of Indian mustard cultivars to normal and terminal high-temperature conditions [7]. Phenotypic and agronomic parameters are described and used as stress tolerance and performance predictors of a cultivar [8]. The flowering stage is most sensitive stage which affected by changing environmental conditions, such as high temperature [9, 10]. High temperature affected the pollen development, anthesis and fertilization during flowering stage and ultimately to the yield loss [11]. Heat stress profoundly affects the growth and development of the *Brassica juncea* and decreased the agronomic value [12]. Heat stress also drastically affects the number of primary and secondary branches, their number reduced under terminal high temperature [13] (Sakpal et al., 2023). The terminal high temperature damaged membrane and decreased the membrane stability index (MSI) and also reduce yield per plant, 1000 seed weight, seed yield/plant, and biological yield/plant [14, 15]). So that present study aimed to evaluate different morphological attributes in Indian mustard genotypes regarding their ability to tolerate high temperatures at terminal growth stage. Additionally, the study sought to identify specific characteristics that can be used as reliable indicators of thermotolerance under high temperature stress.

Materials and method

Rising of Crop and treatments- Genotypes, namely Advance-414, Anmol, Bond, Coral-432, JKMS 8532, MRR-8030, NMS-2018, Rajshree, RH-30, and Sonalika, were procured from the Oil Seed Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar, and grown in 1×4 m plots. Normal sowing was done in the second week of October (without heat treatment), while high temperature treatment was achieved by sowing a month later, in the second week of November. All recommended practices were followed timely, following standard protocols designed by the CCS HAU, Hisar.

Methodology

1. **Relative water content (RWC%):** Fresh leaf samples were obtained early in the morning and weighed immediately to determine fresh weight (FW). The leaves were then soaked in distilled water for 4–6 h at room temperature to soften them fully, following which the soft weight (TW) was measured. The leaves were then oven-dried at 70°C for 24 h to yield dry weight (DW). RWC was determined using the formula:
   RWC (%) $=(FW-DW/TW-DW)\times 100$
2. **Relative saturation deficit (RSD%):** RSD was determined using the soft weight (TW), fresh weight (FW) and dry weight (DW) of the leaves. The formula employed was:
   RSD (%) $=(TW-FW/TW-DW)\times 100$
3. **Water saturation deficit (WSD%):** WSD is another indicator of the water state of the plant. It was determined using the difference between turgid weight (TW) and fresh weight (FW), as follows:

WSD (%) =(TW−FW/TW) ×100

4. **Relative dry weight (RDW%):** RDW was obtained by comparing the dry weight (DW) of the sample to the fresh weight (FW). It was determined using the formula:
   RDW (%) =(DW/FW) ×100
5. **Membrane stability index (MSI%):** Leaf discs (1×3 cm) were soaked in distilled water, and conductivity was recorded after heating at 40°C for 30 min (C1) and 100°C for 10 min (C2). MSI was found as follows:
   MSI (%) =(1−C1/C2) ×100
6. **Days to flowering:** The interval of days from sowing to the emergence of the first flower was also noted for each plant.
7. **Days to 50% flowering:** It was determined when all plants in given plot had at least 50% of the flowers in their inflorescence opened and days to 50% flowering were estimated when 50% of the plants in each plot flowered.
8. **Plant height (root-shoot; cm):** The height of the plant from the root shoot junction to the tips of the tallest shoot at the time of physiological maturity measured in centimeters.
9. **Length of main shoot (cm):** The length of the main shoot was estimated from the base of primary shoot to the apex at the time of physiological maturity in centimetres.
10. **Number of primary branches:** The number of primary branches was counted which arise from the main stem of each plant.
11. **Number of secondary branches:** The maximum number of secondary branches that originated from primary ones for each plant was counted and the average calculated per plant.
12. **Biological yield per plant (g):** The biological yield was determined by collecting the above ground portion of the plant and includes stem, leaf, and pod. Fresh biomass was weighed on an electronic balance in grams.
13. **Seed yield per plant (g):** Once the plants were fully set, the seeds were harvested, washed and some of them were weighed in grams to estimate the seed yield per plant.

Results

The impact of sowing time on relative water content, relative saturation deviation, water saturation deficit, relative dry weight, membrane stability index, days to flowering, days to 50% flowering, plant height, length of main shoot, number of primary branches, number of secondary branches, biological yield per plant and seed yield per plant among various genotypes was evaluated under both normal and late sown conditions. The data reveal significant genotype-specific responses, with a remarked change in tested parameters under late sowing conditions.

The Relative Water Content (RWC): Higher RWC values reflect improved hydration, whereas lower values indicate water stress and decreasing water content. Under Normal Sown circumstances, most genotypes have high RWC values, indicating sufficient hydration. RH-30 and Rajshree display the greatest RWC values (95.17 ± 0.991 and 94.47 ± 1.133, respectively), demonstrating optimum water retention under typical conditions. Corel-432, however, exhibits a comparatively lower RWC (80.91 ± 0.085), indicating it may be slightly more prone to dehydration even under favourable settings. In the Late Sown condition, all genotypes show a considerable drop in RWC, showing greater water stress due to environmental variables. RH-30 again stands out with the greatest RWC (72.78 ± 1.022), demonstrating excellent resilience in retaining hydration under stress. In comparison, Corel-432 and MRR-8030 had lower RWC values (51.30 ± 0.988 and 53.23 ± 0.140), suggesting more vulnerability to water loss. These changes in RWC under varied circumstances give crucial insights for finding genotypes with greater water retention and drought resilience, relevant for breeding efforts focusing on stress tolerance.

Table 1: Impact of heat stress on relative water content (%) and relative saturation deficit of different Indian mustard genotypes

Genotypes	**Relative Water Content**		**Relative Saturation Deficit**	
	Control	**Heat Stress**	**Control**	**Heat Stress**
Advance-414	92.96±0.814	60.49±0.276	5.80±0.130	32.76±0.357
Anmol	83.88±1.398	55.74±0.408	13.51±0.071	38.36±0.179
BOND	91.29±1.900	60.01±0.904	7.34±0.161	33.70±0.158
Corel-432	80.91±0.085	51.30±0.988	16.14±0.203	42.12±0.220
JKMS 8532	87.11±0.815	57.37±1.017	10.83±0.158	36.20±0.472
MRR-8030	82.76±0.431	53.23±0.140	14.52±0.363	39.93±0.084
NMS-2018	84.99±0.266	56.58±1.150	12.69±0.054	37.33±0.351
Rajshree	94.47±1.133	62.96±1.409	4.64±0.042	31.71±0.019
RH-30	95.17±0.991	72.78±1.022	3.91±0.068	22.71±0.425
Sonalika	93.24±1.552	62.83±0.491	5.58±0.078	31.27±0.488
Factors	**C.D.**	**SE(m)**	**C.D.**	**SE(m)**
Treatments (T)	0.896	0.312	0.228	0.080
Genotypes (G)	2.004	0.698	0.510	0.178
Intraction (TxG)	2.834	0.988	0.722	0.252

Water Saturation deficit (WSD): Water Saturation deficit (WSD) is a measure of plant water stress that measures the deficiency of water content relative to full turgor. It represents the amount to which a plant is hydrated, with higher WSD values implying more water stress and lower hydration levels. Under Normal Sown circumstances, genotypes like Corel-432 and MRR-8030 show high WSD values (19.08 ± 0.467 and 17.23 ± 0.259, respectively), suggesting a bigger water deficit and perhaps higher sensitivity to water stress. In contrast, genotypes such as RH-30 and Rajshree display lower WSD values (4.83 ± 0.083 and 5.54 ± 0.009), indicating greater water retention and reduced stress levels. When exposed to Late Sown circumstances, most genotypes display elevated WSD values, indicating higher water stress owing to environmental variables such as warmth and lower moisture availability. Corel-432 and MRR-8030 display some of the highest WSD values (48.70 ± 0.179 and 46.77 ± 0.172), indicating considerable water stress, whereas RH-30 and Rajshree retain somewhat lower WSD values, showing more resilience. This data on WSD can assist in finding genotypes that are more drought-tolerant or resistive to water stress, which is useful for breeding efforts focused at increasing agricultural performance in water-limited areas.

Relative Saturation deficit (RSD): The Relative Saturation deficit (RSD) values for various genotypes under heat stress: RSD is a measure of variability that provides insights into the consistency and stability of each genotype's response to different conditions. In the Normal Sown condition, genotypes such as Corel-432, MRR-8030, and Anmol exhibit higher RSD values (16.14 ± 0.203, 14.52 ± 0.363, and 13.51 ± 0.071, respectively), indicating greater variability. In contrast, RH-30 and Rajshree have lower RSD values (3.91 ± 0.068 and 4.64 ± 0.042), suggesting more stable performance. Under the Late Sown condition, the RSD values generally increase, with Corel-432 and MRR-8030 showing the highest values (42.12 ± 0.220 and 39.93 ± 0.084, respectively). This pattern reflects the increased variability in genotype performance under late sowing conditions, likely due to stress factors such as temperature or moisture variations. The variation in RSD across genotypes and conditions underscores the differential responses of these genotypes to environmental changes, which could be valuable for selecting stable varieties in varying sowing conditions.

Table 2: Impact of heat stress on water saturation deficit and relative dry weight of different Indian mustard genotypes

Genotypes	**Water Saturation Deficit**		**Relative Dry Weight**	
	Control	**Heat Stress**	**Control**	**Heat Stress**
Advance-414	7.04±0.018	39.51±1.008	0.21±0.00	0.21±0.001
Anmol	16.12±0.134	44.26±0.369	0.19±0.00	0.15±0.001
BOND	8.71±0.214	39.99±0.166	0.19±0.00	0.19±0.003
Corel-432	19.08±0.467	48.70±0.179	0.18±0.00	0.16±0.004
JKMS 8532	12.88±0.241	42.62±0.399	0.19±0.00	0.18±0.004
MRR-8030	17.23±0.259	46.77±0.172	0.19±0.00	0.17±0.001
NMS-2018	15.01±0.023	43.42±0.046	0.18±0.00	0.16±0.004
Rajshree	5.54±0.009	37.04±0.770	0.20±0.00	0.17±0.002
RH-30	4.83±0.083	27.22±0.595	0.23±0.01	0.20±0.004
Sonalika	6.76±0.068	37.17±0.232	0.21±0.00	0.19±0.002
Factors	**C.D.**	**SE(m)**	**C.D.**	**SE(m)**
Treatments (T)	0.341	0.119	0.003	0.001
Genotypes (G)	0.762	0.266	0.006	0.002
Intraction (TxG)	1.078	0.376	0.008	0.003

Water Saturation deficit (WSD): Water Saturation deficit (WSD) is a measure of plant water stress that measures the deficiency of water content relative to full turgor. It represents the amount to which a plant is hydrated, with higher WSD values implying more water stress and lower hydration levels. Under Normal Sown circumstances, genotypes like Corel-432 and MRR-8030 show high WSD values (19.08 ± 0.467 and 17.23 ± 0.259, respectively), suggesting a bigger water deficit and perhaps higher sensitivity to water stress. In contrast, genotypes such as RH-30 and Rajshree display lower WSD values (4.83 ± 0.083 and 5.54 ± 0.009), indicating greater water retention and reduced stress levels. When exposed to Late Sown circumstances, most genotypes display elevated WSD values, indicating higher water stress owing to environmental variables such as warmth and lower moisture availability. Corel-432 and MRR-8030 display some of the highest WSD values (48.70 ± 0.179 and 46.77 ± 0.172), indicating considerable water stress, whereas RH-30 and Rajshree retain somewhat lower WSD values, showing more resilience. This data on WSD can assist in finding genotypes that are more drought-tolerant or resistive to water stress, which is useful for breeding efforts focused at increasing agricultural performance in water-limited areas.

Relative Dry Weight (RDW): The Relative Dry Weight (RDW) values for different genotypes under two conditions: Normal Sown and Late Sown. RDW measures the dry weight of the plant compared to its fresh weight, which might reflect the plant's water content and response to stress. Genotypes exhibit RDW values around 0.18 to 0.23, with RH-30 having the highest RDW (0.23 ± 0.01), indicating a somewhat greater dry matter content compared to others. This might signal higher structural along with drought tolerance under normal conditions. In the Late Sown condition, RDW values vary slightly, with a general tendency toward a modest reduction for most genotypes, possibly because to increasing water stress. Anmol and Corel-432 have among of the lowest RDW values (0.15 ± 0.001 and 0.16 ± 0.004, respectively), presumably indicating increased susceptibility to water stress. However, Advance-414 and RH-30 retain considerably higher RDW values (0.21 ± 0.001 and 0.20 ± 0.004), suggesting they could exhibit stronger resilience in preserving dry weight under water-limited situations. This information on RDW can aid in selecting genotypes that preserve structural integrity and adaptability under varied sowing conditions, useful for breeding stress-tolerant crops.

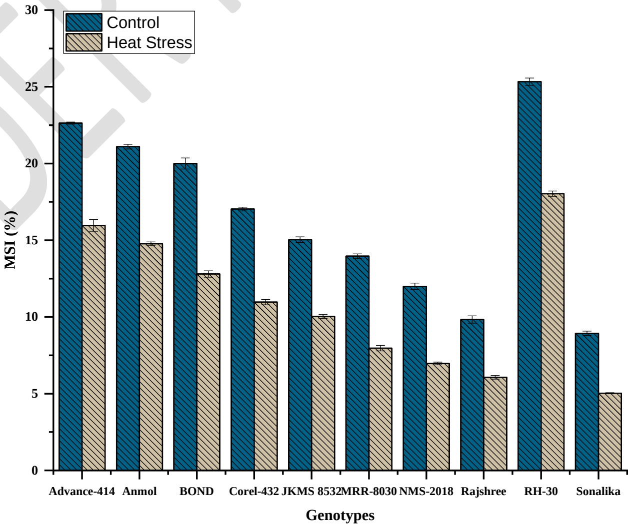

Figure 1: Impact of heat stress on membrane stability index (MSI; %) of different Indian mustard genotypes

Membrane stability index (MSI; %): The membrane stability index (MSI), reflecting plant resilience under stress, demonstrated significant variability across genotypes and conditions.

Significant differences were observed with respect to terminal heat stress (T), genotype (G) and T×G (Fig. 1). Under control conditions, RH-30 showed the highest MSI at 25.33±0.240%, while SONALIKA had the lowest at 8.93±0.145%. Heat stress generally led to a decline in MSI for all genotypes, with SONALIKA experiencing the greatest reduction, from 8.93±0.145% to just 5.03±0.033%. In contrast, RH-30, though decreasing, maintained the highest MSI under heat stress at 18.03±0.176%.

Day to Flower initiation: Heat stress significantly influenced the days to flower initiation across various genotypes. Under control conditions, RH-30 had the longest time to flower initiation at 42.33±0.504 days, while SONALIKA initiated flowering the earliest at 27.23±0.689 days. Heat stress accelerated flower initiation in most genotypes, with SONALIKA continuing to flower earliest at 25.87±0.448 days. The most considerable reduction in days to flower initiation was observed in JKMS 8532, which decreased from 31.60±0.416 days under control to 24.23±0.186 days under heat stress. In contrast, RH-30 experienced a more modest decrease, from 42.33±0.504 to 36.00±0.833 days. Statistical analysis revealed significant difference between heat stress (T) and genotype (G) differences, and their interactions (T×G) on the timing of flower initiation.

Table 3: Impact of heat stress on days to flower initiation and days to 50% flowering of different Indian mustard genotypes

Genotypes	Days to flower initiation		Days to 50% flowering	
	Control	**Heat Stress**	**Control**	**Heat Stress**
ADVANCE-414	40.33±0.120	32.83±0.384	51.80±0.833	41.80±0.058
ANMOL	38.57±0.953	33.47±0.448	45.27±0.593	40.63±0.441
BOND	37.40±0.569	29.23±0.034	44.30±0.945	38.13±0.121
COREL-432	33.70±0.057	29.47±0.033	41.30±0.681	38.57±0.536
JKMS 8532	31.60±0.416	24.23±0.186	39.90±0.777	37.00±0.473
MRR-8030	30.97±0.176	28.97±0.745	39.03±0.656	29.27±0.384
NMS-2018	29.83±0.240	25.90±0.208	38.60±0.681	37.10±0.777
RAJSHREE	29.63±0.448	27.40±0.681	37.70±0.153	31.10±0.361
RH-30	42.33±0.504	36.00±0.833	54.17±0.809	44.50±0.153
SONALIKA	27.23±0.689	25.87±0.448	29.30±0.681	26.47±0.240
Factors	**C.D.**	**SE(m)**	**C.D.**	**SE(m)**
Treatment (T)	0.444	0.155	0.526	0.183
Genotypes (G)	0.992	0.346	1.176	0.410
Interaction (TxG)	1.403	0.489	1.664	0.580

Days to 50% flowering: The effect of heat stress on days to 50% flowering was evaluated for the 10 different genotypes, and results revealed the significant variation in response. Under control conditions, flowering times ranged from 29.30±0.681days for SONALIKA to 54.17±0.809 days for RH-30. Heat stress generally accelerated flowering, with the most pronounced reduction observed in MRR-8030, which flowered in just 29.27±0.384 days compared to 39.03±0.656 days under control conditions. Conversely, genotypes like RH-30 and ADVANCE-414 showed a more modest decrease in flowering time under heat stress, with RH-30 taking 44.50±0.153 days versus 54.17±0.809 days under control, and ADVANCE-414 reducing from 51.80±0.833 to 41.80±0.058 days. Statistical analysis indicated that significant difference was observed with respect to terminal heat stress (T) and genotype (G) and their interactions (T×G) (Table 3).

Table 4: Impact of heat stress on main shoot length and plant height of different Indian mustard genotypes

Genotypes	Main shoot length (cm)		Plant Height (Root+Shoot; cm)	
	Control	Heat Stress	Control	Heat Stress
ADVANCE-414	172.00±4.297	104.00±1.894	183.50±1.431	121.00±1.511
ANMOL	171.00±1.957	89.00±1.853	180.00±1.125	116.50±2.727
BOND	169.00±0.089	101.00±0.369	179.75±3.367	100.00±1.299
COREL-432	167.00±3.387	96.00±0.348	176.75±1.389	110.50±0.057
JKMS 8532	165.00±3.435	100.00±2.243	175.00±1.823	116.50±2.971
MRR-8030	165.00±0.426	93.00±0.921	174.25±3.539	106.50±0.720
NMS-2018	162.00±3.625	98.00±0.663	172.75±4.226	113.00±0.999
RAJSHREE	160.00±3.997	91.00±1.231	169.00±3.343	104.00±2.489
RH-30	175.00±0.562	106.00±1.103	187.00±0.291	124.00±1.936
SONALIKA	159.00±0.993	90.00±1.686	168.25±0.436	102.00±2.290
Factors	**C.D.**	**SE(m)**	**C.D.**	**SE(m)**
Treatment (T)	0.145	0.05	2.015	0.702
Genotypes (G)	0.324	0.113	4.506	1.571
Interaction (TxG)	0.458	0.16	6.372	2.221

Main shoot length (cm): The main shoot length of plants was significantly affected by heat stress across various genotypes. Under control conditions, RH-30 had the longest main shoot at 175.00±0.562 cm, while SONALIKA had the shortest at 159.00±0.993 cm. Heat stress led to a marked reduction in shoot length for all genotypes, with RH-30 retaining the longest shoot under stress at 106.00±1.103 cm, though this was a considerable decrease from its control length. SONALIKA also experienced a substantial decline, with shoot length reducing from 159.00±0.993 to 90.00±1.686 cm. The results were statistically significant, terminal heat stress (T) and genotype (G) and their interactions (T×G) (Table. 4).

Plant height (cm): Results showed the heat stress significantly impacted on plant height, encompassing both root and shoot growth, varied across different genotypes. Significant differences were observed with respect to terminal heat stress (T), genotype (G) and T×G (Table 1). Under control conditions, RH-30 exhibited the tallest plants at 187.00±0.291 cm, while SONALIKA had the shortest at 168.25±0.436 cm. Heat stress led to a notable reduction in plant height for most genotypes, with RH-30 and ADVANCE-414 showing the smallest declines, dropping to 187.00±0.291 to 124.00±1.936 cm and 183.50±1.431 to 121.00±1.511cm, respectively. In contrast, BOND experienced the most substantial reduction, with plant height decreasing from 179.75±3.367 cm under control to 100.00±1.299 cm under heat stress.

Table 5: Impact of heat stress on number of primary branches and number of secondary branches of different Indian mustard genotypes

Genotypes	**Number of primary branches**		**Number of secondary branches**	
	Control	**Heat Stress**	**Control**	**Heat Stress**
ADVANCE-414	15.40±0.063	12.00±0.021	33.62±0.577	12.50±0.057
ANMOL	13.60±0.199	11.30±0.078	31.54±0.657	11.00±0.030
BOND	12.40±0.051	9.43±0.161	27.60±0.318	10.00±0.223
COREL-432	13.00±0.321	10.50±0.167	24.10±0.265	9.50±0.099
JKMS 8532	11.00±0.167	8.12±0.062	22.40±0.036	9.00±0.078
MRR-8030	10.70±0.062	8.00±0.125	20.72±0.054	8.50±0.217
NMS-2018	10.20±0.217	7.04±0.128	18.46±0.146	8.00±0.021
RAJSHREE	9.54±0.026	6.00±0.104	13.85±0.158	7.00±0.140
RH-30	16.00±0.401	13.30±0.021	36.80±0.096	13.00±0.208
SONALIKA	8.00±0.050	5.24±0.098	11.00±0.036	11.30±0.047
Factors	**C.D.**	**SE(m)**	**C.D.**	**SE(m)**
Treatment (T)	0.145	0.050	0.220	0.077
Genotypes (G)	0.324	0.113	0.493	0.172
Interaction (TxG)	0.458	0.160	0.697	0.243

Number of primary branches: The number of primary branches per plant was remarkably impacted by heat stress, with significant variability observed among different genotypes. Under control conditions, RH-30 had the highest number of primary branches at 16.00±0.401, while SONALIKA had the lowest at 8.00±0.050. Heat stress reduced branch numbers across all genotypes, with RH-30 maintaining the highest count under stress at 13.30±0.021 branches, although this was a considerable decrease from its control value. SONALIKA showed the most significant decline, with the number of branches dropping from 8.00±0.050 to 5.24±0.098. Statistical analysis indicated significant effects of treatment with respect to the control, which were observed with respect to terminal heat stress (T), genotype (G) and T×G (Table 5).

Number of secondary branches: The number of secondary branches per plant exhibited a marked decline under heat stress across all genotypes. Under control conditions, RH-30 had the highest count of secondary branches at 36.80±0.096, whereas RAJSHREE had the lowest at 13.85±0.158. Heat stress significantly reduced the number of secondary branches, with RH-30 still showing the highest count under stress at 13.00±0.208 branches, although this was a notable decrease from its control value. In contrast, RAJSHREE's count dropped from 13.85±0.158 to 7.00±0.140 branches. Interestingly, SONALIKA showed minimal change, with its number of secondary branches remaining relatively stable, increasing slightly from 11.00±0.036 to 11.30±0.047 under heat stress. Statistical analysis confirmed significant effects of treatment (T), genotype differences (G), and their interactions (T×G) on the number of secondary branches (Table 5).

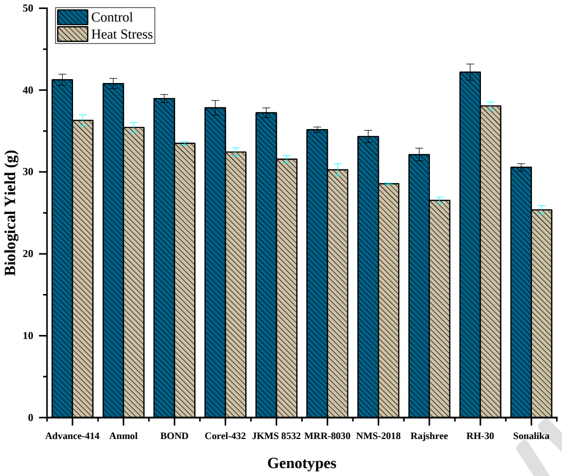

Figure 2: Impact of heat stress on biological yield (g) of different Indian mustard genotypes

Biological yield: The biological yield per plant was significantly affected by heat stress, with all genotypes showing a decrease under stress conditions. Under control conditions, RH-30 achieved the highest biological yield at 42.20±0.985 grams per plant, while SONALIKA had the lowest at 30.57±0.448 grams. Heat stress reduced biological yield across all genotypes, with RH-30 still showing the highest yield under stress at 38.07±0.176 grams, though this was a decrease from its control value. SONALIKA experienced a considerable reduction, with its yield dropping from 30.57±0.448 grams to 25.37±0.504 grams. The analysis confirmed significant effects of treatment (T) and genotype differences (G) on biological yield, although interaction effects were not applicable.

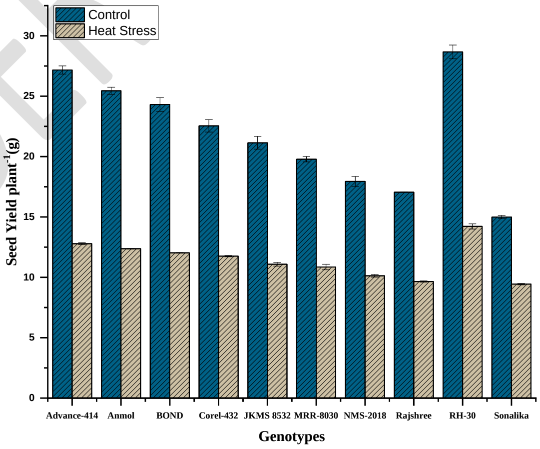

Figure 3: Impact of heat stress on seed yield per plant (g) of different Indian mustard genotypes

Seed yield: Seed yield per plant was significantly affected by heat stress across various genotypes. Under control conditions, RH-30 achieved the highest seed yield at 28.67±0.568 grams per plant, while SONALIKA produced the lowest yield at 14.99±0.134 grams. Heat stress led to a notable decrease in seed yield for all genotypes, with RH-30 maintaining the highest yield under stress at

14.22±0.215 grams, although still considerably reduced from its control value. The most substantial yield reduction was observed in SONALIKA, decreasing from 14.99±0.134 grams to 9.44±0.059 grams. Statistical analysis indicated significant effects of treatment (T), genotype differences (G), and their interactions (T×G) on seed yield.

Discussion

Terminal heat stress significantly affects flowering, growth and yield in Indian mustard (Brassica juncea), influencing essential physiological and morphological traits across genotypes. Relative water content (RWC) and water saturation deficit (WSD) are important indicators of a plant's ability to maintain hydration under stress. Under terminal heat stress, RWC is significantly reduced across genotypes, possibly due to increased transpiration and limited water absorption [16]. These findings suggest that RWC maintenance under heat stress plays a critical role in maintaining physiological functions, which agrees with recent research showing that genotypes with lower WSD exhibit greater heat resilience [17]. Heat stress showed a reduction in RDW across genotypes, whereas RH-30 maintain relatively higher RDW as compare to Sonalika [18].

The membrane stability index (MSI) is a measure of cellular integrity under high temprature stress. RH-30 and Advance-414 maintained relatively stable MSI values, indicating better heat tolerance [19]. Genotypes with high MSI benefit from improved protective mechanisms, possibly through upregulation of heat shock proteins and antioxidants, which help maintain cellular stability [20]. High temperature promotes faster phenological development, reducing the number of days to flowering in most genotypes. RH-30 showed the longest flowering onset time under control conditions, but under heat stress, genotypes such as JKMS 8532 showed a reduction in days to flowering [21]. Days to 50% flowering were also similarly affected, with MRR-8030 showing the greatest reduction under heat, indicating faster flowering [22]. These results underscore the genotype-dependent nature of flowering responses to heat stress, with resilient genotypes such as RH-30 controlling the speed of flowering, which may be beneficial for maintaining yield potential in a changing climate [23]. Reduction in plant height and shoot length limits the plant's ability to photosynthesize and accumulate nutrients. RH-30 and ADVANCE-414 maintained plant height and shoot length, while BOND had minimal plant height. Plant height is closely linked to yield; tall plants generally have greater photosynthetic capacity, which supports seed development and indicates better adaptation to elevated temperatures [24]. This study was published by Mishra et al. This is aligned with Negussu et al., [25], who suggested that branching reduction under stress is possibly due to hormonal imbalance and resource reallocation that impairs branching meristem. Sonalika showed significant reduction in branching, suggesting reduced resource resilience under heat stress and RH-30 highlights its suitability with maintaining branching in high temperature environments where maintaining branches under stress is beneficial [27]. The low seed yield is attributed to poor flowering, low branch number and low photosynthetic efficiency under high temperature. RH-30 had highest in seed yield under heat stress, while Sonalika showed significant reduction due to heat stress. 1000-seed weight and biological yield also had same reductional pattern across all the tested genotypes, while RH-30 maintaining test weight under heat stress conditions [27].

Summary and conclusion

In this work, we evaluated the impact of terminal heat stress on mustard genotypes. displaying tolerance to heat stress. RH-30 maintained high relative water content along with a steady membrane stability index and high yielding. Overall, genotypes RH-30 and Rajshree found superior to stress tolerance in all tested parameters and further used in the breeding programme to develop the heat resilience cultivar in mustard.

References

1. Kaur, G., Singh, V. V., Singh, K. H., Priyamedha, Rialch, I., Gupta, M., & Banga, S. S. (2022). Classical genetics and traditional breeding in *Brassica juncea*. In The *Brassica juncea* Genome (pp. 85-113). Cham: Springer International Publishing.
2. Valladares-Diestra, K., de Souza Vandenberghe, L. P., & Soccol, C. R. (2020). Oilseed enzymatic pretreatment for efficient oil recovery in biodiesel production industry: A review. BioEnergy Research, 13, 1016-1030.
3. USDA (2020). Center for Nutrition Policy and Promotion. Official USDA Food Plans: Cost of Food at Home at Four Levels, U.S. Average, December 2020. January 2021
4. DA&FW (2022-23..) Department of Agriculture and Farmers Welfare. Final Estimates of 2022-23 of Area and Production of Horticultural Crops.
5. Toromade, A. S., Soyombo, D. A., Kupa, E., & Ijomah, T. I. (2024). Reviewing the impact of climate change on global food security: Challenges and solutions. *International Journal of Applied Research in Social Sciences*, *6*(7), 1403-1416.
6. Pillai, A. J., & Walia, P. (2024). Heat Stress in Indian Mustard (Brassica juncea L.): A Critical Review of Impacts and Adaptation Strategies. PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY, 25(5-6), 1-11.
7. Chand, S., Patidar, O. P., Chaudhary, R., Saroj, R., Chandra, K., Meena, V. K., ... & Vasisth, P. (2021). Rapeseed-mustard breeding in India: Scenario, achievements and research needs. *Brassica breeding and biotechnology*, *174*.
8. Chaudhary, S., Devi, P., Bhardwaj, A., Jha, U. C., Sharma, K. D., Prasad, P. V., ... & Nayyar, H. (2020). Identification and characterization of contrasting genotypes/cultivars for developing heat tolerance in agricultural crops: current status and prospects. *Frontiers in Plant Science*, *11*, 587264.

9. Lohani, N., Singh, M. B., & Bhalla, P. L. (2020). High temperature susceptibility of sexual reproduction in crop plants. *Journal of Experimental Botany*, *71*(2), 555-568.
10. Ren, H., Bao, J., Gao, Z., Sun, D., Zheng, S., & Bai, J. (2023). How rice adapts to high temperatures. *Frontiers in plant science*, *14*, 1137923.
11. Khan, A. H., Min, L., Ma, Y., Zeeshan, M., Jin, S., & Zhang, X. (2023). High‐temperature stress in crops: male sterility, yield loss and potential remedy approaches. *Plant Biotechnology Journal*, *21*(4), 680-697.
12. Samantaray, S. S., Misra, A., Shaw, S., Prakash, J., Pandey, V. S., & Nayak, M. K. (2023). Investigating to chemically reactive and radiative Darcy/non-Darcy stagnation point flow of ternary composite nanofluids with moderate Prandtl numbers. International Journal of Modelling and Simulation, 1-17.
13. Sakpal, A., Yadav, S., Choudhary, R., Saini, N., Vasudev, S., Yadava, D. K., ... & Yadav, S. K. (2023). Heat-Stress-induced changes in physio-biochemical parameters of mustard cultivars and their role in heat stress tolerance at the seedling stage. *Plants*, *12*(6), 1400.
14. Ram, B., Priyamedha, M. S., Sharma, H. K., Rani, R., Singh, K. H., Singh, V. V., ... & Rai, P. K. (2021). Development and evaluation of early maturing thermo-tolerant Indian mustard (Brassica juncea L. Czern & Coss) genotypes for cultivation in semi-arid region of India. *Electronic Journal of Plant Breeding*, *12*(1), 200-206.
15. Singh, V. V., Sharma, H. K., Sharma, L., & Rai, P. K. (2024). Morpho-physiological studies and selection criteria in Indian mustard (*Brassica juncea* L.) under rainfed condition. *Vegetos*, 1-10.
16. Sattar, A., Sher, A., Abourehab, M. A., Ijaz, M., Nawaz, M., Ul-Allah, S., ... & Javaid, M. M. (2022). Application of silicon and biochar alleviates the adversities of arsenic stress in maize by triggering the morpho-physiological and antioxidant defense mechanisms. Frontiers in Environmental Science, 10, 979049.
17. Thakur, A., Kumar, A., Kumar, D., Warghat, A. R., & Pandey, S. S. (2024). Physiological and biochemical regulation of Valeriana jatamansi Jones under water stress. *Plant Physiology and Biochemistry*, *208*, 108476.
18. Sonia, Kaur, V., Yadav, S. K., Arya, S. S., Aravind, J., Jacob, S. R., & Gautam, R. K. (2024). Development and evaluation of barley mini-core collection for salinity tolerance and identification of novel haplotypic variants for HvRAF. *Plant and Soil*, *497*(1), 317-337.
19. Hongal, D. A., Raju, D., Kumar, S., Talukdar, A., Das, A., Kumari, K., ... & Dey, S. S. (2023). Elucidating the role of key physio-biochemical traits and molecular network conferring heat stress tolerance in cucumber. *Frontiers in Plant Science*, *14*, 1128928.
20. Kumar, P., Kirti, S., & Krishnagowdu, S. (2024). Effect of heat stress on a physio-biochemical characteristic of Brassica (Mustered) Species. *IIP Iterative International Publisher*, *1*, 194-218.

21. Gupta, D., Singh, G., Tiwari, S., Patel, A., Fatima, A., Dubey, A., & Prasad, S. M. (2021). Salt stress toxicity amelioration by Phytohormones, synthetic product, and nutrient amendment practices. *Physiology of salt stress in plants: perception, signalling, omics and tolerance mechanism*, 198-228.
22. Yadav, B., Soni, A. K., Meena, A. R., Jakhar, M., & Meena, S. (2023). Impact of foliar boron and fertilizer management systems on growth, yield and quality of brinjal. *International Journal of Environment and Climate Change*, *13*(9), 1369-1375.
23. Kumar, S., Bhushan, B., Wakchaure, G. C., Dutta, R., Jat, B. S., Meena, K. K., ... & Pathak, H. (2023). Unveiling the impact of heat stress on seed biochemical composition of major cereal crops: Implications for crop resilience and nutritional value. Plant Stress, 9, 100183.
24. Chug, P., & Sharma, P. (2021). Effect of Heat Stress on the Relationship between SPAD and Chlorophyll Content in Indian Mustard Genotypes. Curr. J. Appl. Sci. Technol, 40, 38-47.
25. Negussu, M., Ventimiglia, M., Vergata, C., Buti, M., Bonfil, D. J., Bar-Zvi, D., ... & Martinelli, F. (2024). Unveiling heat stress responses in chickpea: a transcriptomic insight in early-and late-flowering cultivars.
26. Kathuria, D., Thakur, S., & Singh, N. (2024). Advances of metabolomic in exploring phenolic compounds diversity in cereal and their health implications. *International Journal of Food Science & Technology*.
27. Singh, R., Saffeullah, P., Ahmad, S., & Umar, S. (2024). Linseed (Linum usitatissimum L.) accessions depict genetic variations in growth and yield characteristics, biochemical parameters, antioxidant potential, and secoisolariciresinol diglucoside content. *Genetic Resources and Crop Evolution*, 1-24.