Physiological Attributes for Screening of Indian mustard 
(\textit{Brassica juncea} L. Czern and Coss) Genotypes during Terminal Heat Stress

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\textbf{A B S T R A C T}

A field experiment was conducted in Rabi 2015 in a randomized block design with the objective to screen advanced breeding lines (genotypes) of Indian mustard (\textit{Brassica juncea} L. Czern and Coss) for different physiological parameters viz, chlorophyll content, membrane stability index (MSI %), relative water content (RWC %) and electrolyte leakage and yield related to terminal heat tolerance. The study indicated that genotypes, ‘Pusa Bold’, ‘NRCHB-101’ and ‘PRO-5222’ had tolerance to high temperature at terminal stage. The correlation coefficient between test weight of genotypes and chlorophyll content (0.843), MSI (0.832) and RWC (0.323) was positive but was negative for electrolyte leakage (- 0.564).

\textbf{Keywords} 
\textit{Brassica juncea}, mustard, relative water content, membrane stability index, electrolyte leakage, heat stress

\textbf{Introduction}

Rapeseed-mustard constitutes an important group of oilseed brassica crop, and is main source of edible oil next to soybean and groundnut in India. Rajasthan, Uttar Pradesh, Madhya Pradesh, Haryana, Gujarat and West Bengal states accounted for nearly 86.5\% area and 91.4\% production of rapeseed-mustard in the country during 2012-13. Indian mustard (\textit{Brassica juncea} L. Czern and Coss) accounted for about 75-80\% of the 6.6 million hectare under these crops during 2013-14 (DRMR, 2015). In North-Western part of India it accounts for about 80\% of the cultivable area (Singh \textit{et al.}, 2014). It is grown under diverse agro ecological situations such as timely/ late sown, rainfed/ irrigated, sole- and/ or mixed crop with cereals and \textit{rabi} pulses. The cultivation is largely carried out under the rainfed farming systems where sowing commences after south-west monsoon rains (Venkateswarlu and Prasad, 2012). Temperature stress is one of the major limitations to crop productivity worldwide. This crop is adversely affected by terminal stage ‘heat stress’ i.e. the sudden increase in ambient maximum temperature, in
a matter of few days, by 5-7 °C with corresponding increase in the minimum temperature, that limits optimal plant performance. Flowering and grain filling are the most sensitive stages for temperature stress damage in Indian mustard probably due to vulnerability during pollen and grain development, anthesis and fertilization leading to reduced crop yield. It is likely that plants have evolved mechanisms through which cellular activity could be maintained at varying temperatures. More frequent weather aberrations and high temperature events are expected due to climate change scenario resulting in greater impact of heat on mustard production. Efforts to strengthen resilience are required by utilizing diverse heat tolerant genotypes in brassica crop improvement programmes. There is need to identify suitable screening indices that would facilitate the brassica crop improvement process for terminal heat tolerance. This will help to stabilize the productivity and to meet the growing demands of edible oil in the country. Therefore, the present study was conducted to screen advanced breeding lines of Indian mustard for different physiological parameters related to terminal heat tolerance.

**Materials and Methods**

Field experiment was conducted in *Rabi* 2015 with 14 advanced breeding lines in randomized block design (RBD) with four replications at Tirhut College of Agriculture, Dholi (25°99’56” N, 85°59’40” E, 52.12 m asl) research farm located in Muzaffarpur district of Bihar, India. Seeds of these advanced lines were received from the ICAR-Directorate of Rapeseed-Mustard Research (ICAR-DRMR), Bharatpur, Rajasthan. The seeds were sown in five rows of 5 m length, with row to row and plant to plant spacing of 30 cm and 10 cm, respectively. Sowing was done on 31st November to expose the crop to high temperature during flowering. Physiological parameters *viz.*, chlorophyll content, leaf membrane stability index (MSI %), relative water content (RWC %), electrolyte leakage, and test weight (100-seeds weight) were recorded. Leaf chlorophyll content was estimated by the method of Hiscox and Israelstam (1979). MSI was determined following the method of Sairam (1994) and RWC as described by Barrs (1968). Electrolyte leakage was determined following method based on *in vitro* desiccation of leaf tissues by a solution of polyethylene glycol (PEG) and a subsequent measurement of electrolyte leakage into deionised water (Bajji et al., 2002). Analysis of variance (ANOVA) was carried out to test the significance of treatment effect and least significant differences (LSDs) between means at *p* ≤ 0.05 and the standard error (SE) of means were computed. Correlation coefficient between test weight and physiological parameters were determined following standard method (Gomez and Gomez, 1984).

**Results and Discussion**

Chlorophyll content in the different genotypes varied significantly from 4.83 to 9.06 mg/g fresh weight, the highest being in terminal heat stress tolerant genotype ‘PRO-5222’ and the lowest in susceptible genotype ‘Pusa bold’ (Table 1). Similar to our findings, Kumar and Srivastava (2003) had also suggested that tall genotype with medium bold seeds and high chlorophyll content was ideal for late sown conditions. MSI ranged from 7.09 to 37.05% highest being in the genotype ‘PRO 5222’. Heat stress 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. Reduction in cell membrane stability under high temperature has also been reported in
The results revealed a significant difference in RWC in Indian mustard genotypes. RWC of genotypes ranged from 52.28 (RGN 330) to 118.61% (PRO 5222) under heat stress conditions. Higher percent relative water content in leaves is a good indicator of heat resistance.

Our findings are in agreement with the earlier studies on Indian mustard by Ram et al., (2012) and Kumar et al., (2013). Electrolyte leakage ranged from 0.89% (PRO 5222) to 1.76 % (RGN 330) highest being in the susceptible genotypes. This is frequently related to an increase in membrane permeability, affecting membrane integrity and cell compartmentation under stress conditions (Campos et al., 2003).

Reduction in test weight (1000-seed weight) occurred under terminal heat stress which varied significantly among genotypes and ranged between 3.11 g (Pusa Bold) to 5.57 g (PRO 5222). Though the test weight of ‘Pusa Bold’ was lowest, the seed yield was highest (1619 kg/ha). The highest temperature during reproductive phase (50 days prior to maturity) was between 24.5 to 33 °C (Fig. 1). The elevated temperature adversely affected yield of the crop. Angadi et al., (1999) had reported that the optimum daytime temperature for B. juncea cultivar was above 20 °C and close to 28 °C. Efficient photosynthetic response occurred at 15-20 °C temperature (Shekhawat et al., 2012). Heat stress during the post-anthesis (seed filling) negatively influences the movement of photosynthetic products to the developing sinks and inhibits the synthetic processes, thus lowering seed weight and seed yield and may alter seed quality (Bhullar and Jenner, 1985). A low reduction in the physiological parameters like MSI, RWC and chlorophyll are some of the simple indices for screening and identifying heat stress tolerant genotypes as also suggested by Kumar et al., (2013).

Table 1 Physiological parameters related to terminal heat tolerance vis-à-vis yield of Indian mustard genotypes

| Hybrid Genotypes  | Chlorophyll content (mg/g fresh wt.) | MSI (%) | RWC (%) | Electrolyte leakage (%) | Test weight (g) | Yield (kg/ha) |
|-------------------|-------------------------------------|--------|---------|-------------------------|----------------|--------------|
| Pusa Bold         | 7.92                                | 11.15  | 94.32   | 1.35                    | 4.60           | 1146         |
| Kranti            | 6.94                                | 35.37  | 90.77   | 1.24                    | 4.52           | 1228         |
| RH 923            | 7.37                                | 25.73  | 84.38   | 1.36                    | 3.94           | 1249         |
| DRMR 1153-12      | 7.81                                | 14.29  | 81.83   | 1.27                    | 4.17           | 1196         |
| DRMRIJ 13-38      | 8.00                                | 18.55  | 85.58   | 1.24                    | 4.71           | 860          |
| JD 6              | 7.61                                | 17.62  | 85.86   | 1.15                    | 5.40           | 1042         |
| Pusa Bold         | 4.83                                | 12.50  | 88.62   | 1.28                    | 3.11           | 1619         |
| RH 919            | 6.22                                | 8.78   | 92.14   | 1.33                    | 3.53           | 1331         |
| RH 749            | 8.23                                | 6.83   | 88.12   | 1.36                    | 3.59           | 1114         |
| RGN 337           | 6.97                                | 15.11  | 90.08   | 1.28                    | 3.87           | 1267         |
| RGN 368           | 8.74                                | 7.09   | 86.48   | 1.45                    | 4.13           | 1050         |
| NRCHB 101         | 8.80                                | 17.84  | 84.36   | 1.15                    | 5.39           | 1693         |
| RGN 330           | 7.35                                | 20.04  | 52.28   | 1.76                    | 4.09           | 1167         |
| PRO 5222          | 9.06                                | 37.05  | 118.61  | 0.89                    | 5.57           | 1362         |
| LSD (p=0.05)      | 0.34                                | 0.62   | 0.60    | 0.23                    | 0.81           | 273          |
| SEm ±             | 0.01                                | 0.22   | 0.21    | 0.08                    | 0.28           | 11           |

2910
Fig.1 Daily mean maximum minimum temperature during reproductive phase at TCA, Dholi, Muzaffarpur

Fig.2 Correlation between test weight and different physiological traits of Indian mustard genotypes
The significant co-efficient of correlation between test weight (1000-seed weight) and physiological traits were observed (Fig. 2). The correlation coefficient ‘r’ value was significant and positive for chlorophyll content (0.843**), MSI (0.832**) and RWC (0.323*) while it was negative for electrolyte leakage (-0.564**). Similar correlation between seed yield and physiological traits had also been reported in Indian mustard by Ram et al., (2012) and Sharma and Sardana (2013). The present findings show that since the traits are highly correlated, selections based on correlations may be a useful for indirect selections for higher seed yield potential.

In conclusion, we report that the genotypes found best with respect to physiological traits vis-à-vis test weight and yield should be selected for late shown conditions where there is likelihood of terminal heat stress tolerance to plants. The study revealed that genotypes ‘NRCHB-101’ and ‘PRO-5222’ had tolerance to high temperature at terminal stage.

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