



High temperature stress tolerance in Indian mustard (*Brassica juncea*) germplasm as evaluated by membrane stability index and excised-leaf water loss techniques

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Abstract

A total of 796 Indian mustard [*Brassica juncea* (L.) Czern and Coss.] germplasm accessions including 4 checks were evaluated in augmented block design for their *per se* performance with respect to their high temperature stress tolerance at seedling stage. The traits assessed were percent population survival at 10 and 25 days after sowing, percent membrane stability index, percent relative water content, percent excised-leaf water loss, percent oil content, 1000- seed weight (g) and seed yield per plant (g). Eighty seeds of each germplasm including four checks were sown in the field under heat stress (26th September) conserved moisture conditions during *Rabi* 2012-13 in single rows of two metre length. Among all germplasm accessions tested, only 48 germplasm accessions were identified on the basis of percent population survival at 10 DAS (41.0°C maximum temperature at 0-10 cm depths), and 25 DAS (40.2°C maximum temperature at 0-10 cm depths). Correlation coefficients between seed yield per plant and heat stress traits indicated that seed yield per plant was positively associated with membrane stability index ($r=0.282^*$), and 1000 seed weight ($r=0.417^{**}$). On the basis of *per se* performance, germplasm accessions DRMR-1574, DRMR-1624, DRMR-1600, DRMR-1799 and Urvashi found to be tolerant to heat stress could be included in the breeding programme genotypes for high temperature stress conditions.

Key words: *Brassica juncea*, heat stress tolerance, membrane stability index

Introduction

Rapeseed- mustard constitutes an important group of oilseed Brassica crops, and of these, Indian mustard [*Brassica juncea* (L.) Czern and Coss] is an important edible oil yielding crop accounting for about 80% of the cultivated area in North- Western parts of India (Singh *et al.*, 2014). Amongst all the states in India, Rajasthan is an important producer of Indian mustard. Indian mustard is very sensitive to heat stress at early seedling stage. Although, early sowing has many advantages, the early sown-crop encounters high temperature stress, which results in a significant yield loss. High temperature stress is the most important abiotic stress affecting plant productivity around the world (Hall, 1992). Recent studies estimate 10-40% loss in crop production in

India due to high temperature stress (IPCC 2007). The rising atmospheric CO₂ and temperature are the two important factors of climate change which are likely to impact agriculture and food security across the globe. Despite some projected increase in photosynthesis due to higher atmospheric CO₂, increased temperature results in reduced productivity (Wassmann *et al.*, 2009). The global average air temperature is expected to rise by 1.8 to 4.0°C by the end of this century. The *Rabi* season temperature is expected to increase more than the *kharif* season (Aggarwal and Mall, 2002). Studies determining response of Indian mustard are lacking in India to climate change.

Comparing three species of oilseed Brassica for variation in critical temperature and the most

sensitive crop growth stage for high temperature stress, Angadi *et al.*, (2000) identified Indian mustard to have greater tolerance to heat and water stress than the Canola quality Indian mustard. Niknam and Turner (1999), Wright *et al.*, (1996), Kirk and Oram, (1978) and Parker (1999) also reported Indian mustard to possess several agronomic advantages over Canola. Research on the direct effect of high temperature stress at the seedling stage in *Brassica juncea* is lacking. The present study, therefore, was undertaken to identify Indian mustard germplasm accessions superior for high temperature stress tolerance at seedling stage.

Materials and Methods

Seven hundred ninety six Indian mustard germplasm accessions for the present study were procured from the Directorate of Rapeseed-Mustard Research (DRMR), Bharatpur. Eighty seeds of each germplasm accession including four checks, were sown under heat stress condition (maximum temperature 40.1°C at 0- 10 cm depth on seeding date on September 26, 2012) in augmented block design at the DRMR research farm, Bharatpur (77.27°E longitude; 27.12°N latitude and 178.37 m above mean sea level), India. The soil of the experimental site was sandy loam with EC 1.5 dSm⁻¹, low organic carbon (0.25 – 0.30%), poor available N (125-135 kg/ha), medium P (20-22 kg/ha), and available K of 240-260 kg/ha and a pH of 8.1. The Indian mustard crop was raised strictly under conserved moisture conditions. All the germplasm accessions were grown in a single row of three metre length. The distance between row to row and plant to plant was 30 cm and 10 cm, respectively. The percent population survival (PPS) at 10 days (41.0°C maximum temperature at 0-10 cm depths) and 25 days after sowing (DAS) (40.2°C maximum temperature at 0-10 cm depths) were recorded from each plot. Growth and physiological characters, including, percent membrane stability index (PMSI), percent excised- leaf water loss (PELWL), percent relative water content (PRWC), percent oil content, 1000-seed weight (g) and seed yield per plant (g) were recorded from five randomly selected germplasm accessions which had PPS of more than 37.5.

Determination of growth and physiological parameters:

Leaf membrane stability index (MSI) was determined following the method of Premachandra *et al.*, (1990) as modified by Sairam, (1994). Leaf stripes (0.2g) of uniform size were placed in test tubes containing 10 ml of double distilled water in two sets. Test tubes in one set were kept at 40°C in a water bath for 30 min and electrical conductivity of the water containing the sample was measured (C₁) using a conductivity bridge. Test tubes in the other set were incubated at 100°C in boiling water in water bath for 15 min and electrical conductivity was measured as above (C₂). Leaf membrane stability index (MSI) was calculated using the following formula :

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

For determining excised- leaf water loss (ELWL) the leaves were weighed at three stages viz. immediately after sampling (fresh weight); after drying in an incubator at 28°C and 50% R.H. for 6 h; and after oven drying for 24 h at 70°C as suggested by Clarke, (1987): ELWL was calculated using the following formula :

$$ELWL = [Fresh\ weight - Weight\ after\ 6\ h] / (Fresh\ weight - Dry\ weight) \times 100$$

The samples for RWC were also weighed immediately to obtain fresh weight (FW); 2 cm leaf sections were floated in distilled water for 4 h, blot-dried and weighed to obtain turgid weight (TW); The 2 cm leaf sections were oven dried at 60°C for 24 h and weighed to obtain dry weight (DW). The RWC was calculated using the formula of Barrs (1968):

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

All mature siliquae from five randomly selected plants were threshed and average grain weight per plant was calculated.

Statistical analysis:

Analysis of variance (ANOVA) was performed on the data following the procedure suggested by Abhishek *et al.*, (2004) and critical difference (CD)

calculated at 5% probability level. Correlation coefficient between seed yield per plant and physiological parameters were determined according to Gomez and Gomez, (1984).

Results and Discussion

Temperature is an important factor which affects growth and development of plants. All plants require a certain amount of heat units during growth periods and the duration to achieve heat units depends upon the climatic conditions. For the present

experiment, high temperature stress was created by seeding in the last week of September under conserved moisture conditions. Results indicate that fifty two Indian mustard germplasm accessions responded differently under high temperature stress condition. Although, the population survival percentages at 10 and 25 DAS decreased continuously with increasing heat stress in all accessions, the percent decrease was lower in DRMR-1798, DRMR-2332, DRMR-2264 and DRMR-2341 (Table 1) and (Fig 1).

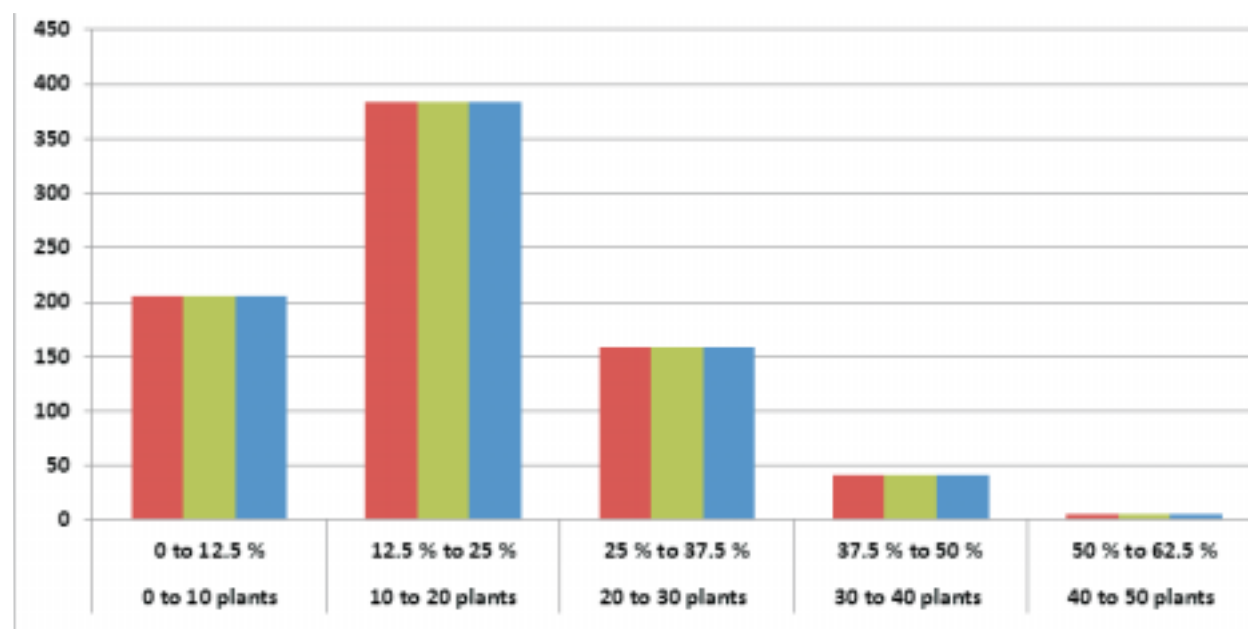


Fig. 1 Frequency distribution of population survival percentage (25 DAS) for high temperature stress tolerance evaluation at seedling stage in 796 germplasm accessions of Indian Mustard during *rabi* 2012-13

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 the present investigation, membrane stability index (MSI) decreased under heat stress in all Indian mustard germplasm; MSI of the germplasm accessions ranged from 5.22 to 45.36 %. The

germplasm accessions DRMR-1624 (45.4 %), DRMR-1313 (37.8 %), DRMR-1674 (34.9 %), DRMR-1662 (34.3 %) and DRMR-1118 (32.8 %) recorded significantly higher MSI under heat stress condition compared to DRMR-1575 (5.2 %), DRMR-1998 (5.8 %), DRMR-1098 (6.3 %) and DRMR-403 (6.8 %) (Table 1).

Since membrane damage increases with increase in stress level, MSI can be considered as a very important tool for evaluating heat tolerance potential in Indian mustard germplasm. Similar reduction in cell membrane stability under high temperature stress has also been reported in cowpea (Ismail and Hall, 1999).

Table 1: Relative performance of Indian mustard germplasm accessions for high temperature stress tolerance parameters during *rabi*-2012-13

Germplasm	Population survival (%) (10 DAS)	Population survival (%) (25 DAS)	Membrane stability index (%)	Excised-Leaf Water Loss (%)	Relative Water content (%)	Oil content (%)	1000-seed weight (g)	Seed yield per plant (g)
DRMR-30	56.3	37.5	9.6	30.2	74.3	40.4	4.8	7.2
DRMR-111	55.1	36.3	22.8	35.1	73.3	42.5	4.9	13.1
DRMR-403	53.1	43.8	6.8	39.1	74.3	40.7	4.8	9.9
DRMR-945	53.0	42.5	11.9	39.4	71.1	40.6	4.3	8.1
DRMR-957	56.4	45.0	16.7	28.4	65.8	40.2	4.9	12.2
DRMR-1077	50.5	41.3	9.6	34.2	73.9	39.2	4.3	7.1
DRMR-1098	56.1	46.3	6.3	37.2	70.9	42.2	5.3	12.2
DRMR-1105	57.9	38.7	18.6	27.9	68.9	41.5	4.8	9.8
DRMR-1118	64.5	53.7	32.8	19.6	73.9	41.5	5.6	9.1
DRMR-1386	57.1	36.3	7.9	36.1	71.2	40.5	5.5	14.6
DRMR-1394	55.8	37.5	12.8	29.2	68.9	43.1	5.5	14.4
DRMR-1570	56.6	37.5	27.2	20.2	76.4	41.7	4.7	10.1
DRMR-1574	56.9	37.5	27.8	22.3	70.1	42.3	5.4	31.0
DRMR-1575	51.4	42.5	5.2	39.1	79.1	41.2	6.3	17.5
DRMR-1600	47.9	36.5	31.9	23.9	65.9	39.2	4.8	19.4
DRMR-1616	54.1	42.5	7.4	38.4	65.6	42.6	4.9	15.4
DRMR-1623	60.1	41.3	23.2	24.8	70.9	42.4	5.9	15.9
DRMR-1624	59.1	38.8	45.4	20.4	62.4	42.3	5.5	20.5
DRMR-1626	56.7	38.8	12.4	38.4	66.8	43.9	4.5	12.1
DRMR-1656	59.2	40.0	33.7	28.5	76.0	42.5	4.6	16.2
DRMR-1662	56.1	38.7	34.3	23.7	67.5	42.8	5.0	15.6
DRMR-1674	54.1	43.2	34.9	24.8	79.8	42.2	5.8	10.4
DRMR-1690	59.2	38.8	11.4	39.3	67.7	41.9	5.6	9.3
DRMR-1691	54.1	38.7	22.8	29.4	72.2	41.7	5.5	12.9
DRMR-1724	56.4	36.3	7.9	34.3	71.6	41.7	4.2	14.4
DRMR-1777	57.1	38.7	7.7	35.4	75.0	42.7	5.6	16.0

DRMR-1783	56.4	38.7	9.9	39.8	71.4	42.6	5.5	12.5
DRMR-1795	61.1	42.5	9.0	38.5	72.7	40.5	4.7	10.6
DRMR-1798	76.9	55.0	13.6	32.5	66.0	40.6	4.1	13.1
DRMR-1799	62.3	41.3	23.3	33.9	69.3	40.6	4.1	18.1
DRMR-1801	62.1	43.8	11.6	28.3	67.6	42.0	4.1	8.3
DRMR-1998	54.1	36.3	5.8	36.8	65.9	41.4	4.0	7.1
DRMR-2000	55.1	36.2	15.8	22.3	73.6	41.4	4.1	8.9
DRMR-2001	54.1	36.2	6.4	35.6	73.2	41.9	5.1	7.9
DRMR-2008	59.6	41.2	10.1	35.1	69.8	41.4	4.0	9.1
DRMR-2057	55.1	36.2	9.5	33.6	74.9	42.2	4.2	8.2
DRMR-2072	54.9	38.7	17.9	22.3	71.8	42.1	4.0	15.1
DRMR-2208	55.7	38.6	10.8	34.1	71.9	39.9	4.5	10.2
DRMR-2214	57.1	36.2	7.4	38.1	79.2	42.8	4.3	7.5
DRMR-2258	55.1	37.5	8.9	39.1	77.8	41.4	4.5	10.3
DRMR-2264	66.1	47.5	10.9	30.2	79.5	41.4	4.4	15.6
DRMR-2272	61.1	42.5	16.1	28.2	74.7	42.8	4.4	10.2
DRMR-2332	67.1	46.3	6.9	36.1	83.7	40.1	4.1	17.9
DRMR-2341	65.2	46.3	20.8	20.2	76.9	40.8	4.1	11.5
DRMR-2359	64.1	36.3	15.5	29.2	74.9	42.3	4.0	14.1
DRMR-1263	60.1	41.3	9.9	32.1	78.0	41.9	4.2	11.1
DRMR-1313	55.3	37.5	37.8	20.5	78.7	41.6	3.9	4.7
DRMR-1444	58.2	36.2	8.4	35.1	76.5	43.8	4.0	10.1
BPR-541-4(C)	61.3	42.8	8.1	34.1	66.8	40.6	3.9	5.8
BPR-543-2(C)	65.1	43.9	28.4	20.9	71.1	43.9	4.2	7.9
Urvashi ©	61.1	40.6	17.0	23.1	77.4	42.9	5.1	18.0
NRCDR-2 ©	63.5	42.6	14.0	21.1	79.1	41.4	4.8	15.5
Mean	58.1	40.6	16.2	30.8	72.6	41.7	4.7	12.4
Range	50.5-76.9	36.3-55.0	5.2-45.4	19.6-39.8	62.4-83.7	39.2-43.9	3.9-6.3	4.7-31.0
CV (%)	1.78	1.79	2.77	2.03	1.06	1.85	2.09	5.89
CD at 5%	2.85	1.99	1.24	1.64	2.10	2.09	0.26	1.29

Thermo-tolerant plants have less excised leaf water loss compared to thermo-susceptible-plants. Under the high temperature stress conditions, accessions DRMR-1118, DRMR-2341 and DRMR-1570, with their respective values of 19.6, 20.2 and 20.3 %, (20.3 %) gave significantly lower ELWL values than accessions DRMR-1783 (39.9 %) and DRMR-945 (39.4 %) (Table 1). This finding is in good agreement with the Sorghum genotypes at seedling and post-anthesis stages (Ali *et al.*, 2009).

The results revealed a significant difference in relative water content in Indian mustard germplasm (Table 1). In general, heat stress adversely affects relative water content of mustard germplasm; RWC of the germplasm accessions ranged from 62.4 % (DRMR-1624) to 83.7 % (DRMR-2332) under heat stress condition. The germplasm accession DRMR-2332 (83.7 %), DRMR-1674 (79.8%), DRMR-2264 (79.5 %) and DRMR-1575 (79.1 %) recorded maximum percent relative water content under high temperature stress condition compared to accessions DRMR-1624 (62.4 %), DRMR-957 (54.4 %) and DRMR-1616 (55.6%). Higher percent relative water content in leaves is a good indicator of heat – drought resistance. Our present findings are in agreement with the earlier studies on wheat (Dhanda and Sethi, 1998) and Indian mustard (Bhagirath Ram *et al.*, 2012; Sudhir *et al.*, 2013), oil content of the accessions evaluated ranged from 39.2 to 44.0 % (Table 1). Under high temperature stress conditions, accession DRMR-1626 produced the maximum percent oil content of 44 followed by DRMR-1444 (43.8%), BPR-543-2 (43.2 %) and DRMR- 1394 (43.1 %). Similarly, accession DRMR-1600 produced the lowest oil content of 39.2 percent (Table 1). These findings are in agreement with several earlier studies on *Brassica* at seedling stage (Zada *et al.* 2013; Heenam and Armstrong, 1993; Mendham *et al.* 1981, 1990).

Significant reduction in 1000 seed weight occurred under heat stress at seedling stage. The 1000 seed weight of the germplasm accessions ranged from 6.3 g (DRMR-1575) to 3.9 g [BPR-541-49 (C)] (Table 1). Among the germplasm accessions,

Table 2: Correlation coefficient among seed yield per plant and heat stress tolerance physiological parameters

Character	Population survival (%) (10 DAS)	Population survival (%) (25 DAS)	Membrane stability index (%)	Excised-Leaf Water Loss (%)	Relative Water content (%)	Oil content (%)	1000-seed weight (g)	Seed yield per plant (g)
Population survival (%) 10 DAS	1.000	0.642**	-0.017	-0.148	0.121	-0.002	-0.326*	0.031
Population survival (%) 25 DAS		1.000	-0.009	-0.031	0.091	-0.249	-0.079	-0.041
Membrane stability index (%)			1.000	-0.750**	-0.132	0.176	0.204	0.282*
Excised- Leaf Water Loss (%)				1.000	0.020	-0.064	0.007	-0.186
Relative Water content (%)					1.000	0.007	-0.131	-0.017
Oil content (%)						1.000	0.241	0.188
1000- seed weight (g)							1.000	0.417**
Seed yield per plant (g)								1.000

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

DRMR-1575 gave the maximum 1000 seed weight of 6.3 g followed by DRMR-1623 (6.0 g), DRMR-1674 (5.8 g) and DRMR-1118 (5.7 g) under high temperature stress conditions, whereas accession BPR-541-4 (C) produced the least 1000 seed weight of 3.9 g.

Although, studies regarding effect of heat stress on 1000 seed weight in Brassica are lacking, Ahamed *et al.*, (2010) reported that the heat stress of 35–40°C, reduced the 1000-grain weight in heat sensitive rice variety Shuanggui by 7.0%–7.9% compared to only 3.4–4.4 % in heat tolerant Huanghuazhan variety.

The seed yield per plant varied significantly between germplasm accessions and it ranged from 4.7 g to 31.0 g (Table 1). Amongst the accessions tested, accessions DRMR-1574, DRMR-1624 and DRMR-1600 produced seed yield per plant in the amount of 31, 20.5 and 19.4 g, respectively (Table-1). Under the high temperature stress conditions, accessions, DRMR-1313 (4.7g), BPR-541-4 (C) (5.8 g), DRMR-1998 (7.1 g) and DRMR-1077 (7.1 g) yielded the least seed yield per plant. The reduction in seed yield per plant might be attributed to reduction in total biomass of the plant as well as adverse effect on yield parameter in early sown crops. Similar reduction in seed yield and genotypic differences in early sown *Brassica* crops (Chauhan *et al.*, 2009, Singh *et al.*, 2010, Lallu *et al.*, 2010; Bhagirath Ram *et al.*, 2012) and in chickpea (Khetarpal *et al.*, 2009) have been reported.

The significant co-efficient of correlation between seed-yield and other physiological traits ranged from 0.282* to -0.750** (Table-2). Under high heat stress conditions, the seed yield plant⁻¹ had significant positive correlation with 1000 seed weight ($r=0.417^{**}$) (Table 2). The percent oil content was negatively correlated with PPS 25 DAS ($r=-0.249$) (Table 2). The PELWL had highly significant negative correlation with MSI ($r=-0.750^{**}$) under the high temperature stress conditions, the PPS at 10 DAS ($r=0.642^{**}$) had significant positive correlation with the PPS at 25 DAS. Similar correlation between seed yield plant⁻¹ and physiological traits had also been reported in Indian mustard by

Bhagirath Ram *et al.* (2012) and Sharma and Sardana (2013).

Holland (2006) observed that genetic correlations between traits are due to linkage and/or pleiotropy indicating the magnitude and direction of correlated responses to selection. He also emphasized the relative efficiency of correlations facilitating indirect selection. The present findings show that since the traits are highly correlated, selections based on correlations may be a useful breeding strategy in indirect selections for higher seed yield potential (Ojaghi and Akhundova, 2010).

In conclusion, we report that out of the fifty two germplasm accessions evaluated, seven accessions DRMR-1574, DRMR-1624, DRMR-1600, DRMR-1799, Urvashi, DRMR-2332 and DRMR-1575 were found to be tolerant to heat stress. The present study also suggests that germplasm accessions DRMR-1574, DRMR-1624, DRMR-1600, DRMR-1799 and Urvashi could be included in future breeding programme aimed in developing high yielding genotypes for high temperature stress conditions.

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