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PHYSIOLOGICAL TRAITS VERSUS SEED YIELD DERIVED PARAMETERS BASED HEAT STRESS STUDY IN INDIAN MUSTARD

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SUMMARY

Constantly rising global temperature (@ 0.2 °C per decade) has been one of the most detrimental stresses in crop growth and development. Indian mustard as being susceptible to temperature stress mostly targeted for commercial cultivation as early sown or late sown crop with the expectation of higher seed yield. A set of 45 Indian mustard genotypes was studied for their response to heat stress by following randomized block design with 3 consecutive experiments. Cell membrane stability, acquired thermal tolerance and % reduction of relative water content was emerged as preferred traits to classify the genotypes for their response to high temperature stress. Based on physiological parameters, 13 genotypes were categorized as tolerant. Further on the basis of % yield reduction, 8 out of 13 tolerant genotypes identified earlier were found to be heat tolerant at seedling stage and 2 genotypes as tolerant at terminal stage. Cell membrane stability, acquired thermal tolerance and % reduction of relative water content exhibited significant correlations with yield and derived parameters. In comparison to 'mean productivity' vs. 'tolerance', 'stress tolerance efficiency' vs. 'stress susceptibility index' was observed to be more effective in this study.

Key words: Indian mustard, heat stress, thermo-tolerance, physiological traits, seed yield derived parameters, correlation-coefficient

Key findings: The identified parameters and their standardized data along with well characterized 13 tolerant genotypes can be used for heat tolerance cultivar development in Indian mustard. The findings of this study are reliable and practically feasible.

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INTRODUCTION

Rapeseed-mustard has now become the second largest produced edible oilseed crop in the world after soybean (FAO, 2013). Of these, Indian mustard (*Brassica juncea* L. Czern & Coss) is the most important oilseed crop in India occupying more than 80% of total area under cultivation of rapeseed-mustard. Being a C3 plant, it has efficient photosynthetic response at 15 °C – 20 °C temperatures (Chauhan *et al*., 2009). However, the constantly rising global air temperature @ ~0.2 °C per decade is raising apprehension regarding crop productivity and food security. This is expected up to ~4.0 °C higher than the current level by 2100 (IPCC, 2007; Rana *et al*., 2011; Rao *et al*., 2011; Srivastava *et al*., 2011).

Indian mustard is grown with annual precipitation of 500 to 4200 mm, annual temperature of 6 °C to 27 °C and soil pH of 4.3 to 8.3 (Singh, 2013) as rainfed or irrigated crop planted through September (early), October (timely) to November (late). Early sowing avoids diseases, aphid attack and fruit shattering; however, heat stress severely affects germination, seedling establishment and finally the yield. The inter-/ mixed cropping with wheat as well as late sowing after rice and cotton exposes this crop to high temperature stress during reproductive stage (Chauhan *et al.,* 2009). Losses due to heat stress at seedling and flowering stages are attributed to enhanced plant development and flower abortion with appreciable losses in seed yield (Morrison and Stewart, 2002). High temperature stress causes loss of cell water content and cellular membrane disruptions. The basic mechanism of cellular membrane disruption under heat stress can affect photosynthetic or mitochondrial activity or even decrease the ability of the plasma membrane to retain solutes (Mohammadi *et al.,* 2007).

The uniqueness of Indian mustard growing environments (early sowing and late sowing) and constantly increasing global temperature necessitates the search for effective selection criteria involving- morphological (yield), physiological and/or molecular parameters. There is near absence of studies employing physiological traits for the screening of genotypes against temperature stress in Indian mustard. However, physiological traits namely cell membrane stability (CMS %), acquired thermal tolerance (ATT %) and relative water content have been effectively used for discriminating drought- as well as thermo-tolerant and susceptible genotypes in other crops (Begum and Paul, 1993; Mondal and Paul, 1996; Shafeeq *et al*., 2006; Paul *et al*., 2006). Considering the significance of high temperature on seedling as well as reproductive stages, this investigation was planned with the objectives to determine the suitability of physiological traits and yield derived parameters for assessing the heat-tolerance of mustard genotypes.

MATERIALS AND METHODS

Plant materials and crop culture

Forty five diverse genotypes of Indian mustard were assessed in field and laboratory at G.B. Pant University of Agriculture and Technology, Pantnagar (29°N and 79.3°E, 243.8 m above sea level), Uttarakhand, India to determine their response to seedling as well as terminal stage heat stresses. All the 45 genotypes were evaluated in randomized complete block design with 3 replications during 2010-11 under early sown (last week of September, E1), timely sown (last week of October, E2) and late sown (last week of November, E3) conditions to expose the crop at seedling as well as terminal heat stress. All the recommended agronomic practices (Singh, 2013) were followed to raise the good crop except the changes in sowing time. Irrigations were given as per the schedule to avoid any water stress. Data on all aspects of studied traits were recorded from 5 randomly selected competitive plants of each replication.

Meteorological Data

Weather data on temperature (°C, maximum and minimum), relative humidity (%, at 07.15 am and 14.15 pm), rainfall (mm), number of rainy days, sun-shine hours, wind velocity (km/hr) and evaporation were recorded for years 2010-11 (averaged over meteorological week, Figure 1). Weather was as usual as North Indian environment. Since, experiments were conducted under well irrigated conditions; so only differences in aerial temperature were the major consideration. There were 2.8 °C to 7.0 °C differences between E1 & E2 and 3.5 °C to 4.8 °C differences between E3 and E2 (Figure 1). This temperature differences among E1, E2 and E3 was ideal for study of heat stress effect on seedling stage and terminal stage in Indian mustard genotypes.

Physiological traits vis-a-vis supra-optimal temperature

Physiological traits were studied by following standard procedures with some modifications. Fully developed green leaves were used for analyses from timely sown field crop at vegetative to pre-siliqua stages. The values of concerned physiological traits i.e. Cell Membrane Stability (Blum, 1988), Relative Water Content (Barrs and Weatherley, 1962), chlorophyll contents (AOAC, 1980) and acquired thermal tolerance (Porter *et al*., 1994) were calculated as per the standard procedure. However, % Reduction of Relative Water Content (% RRWC) was calculated as:

$$\frac{(\text{RWC \% at 8 am} - \text{RWC \% at 2pm})}{\text{RWC \% at 8am}} \times 100$$

Where, RWC % is the relative water content.

Yield derived stress parameters vis-a-vis supra-optimal temperature

The seed yield (g/plant) of all 45 genotypes under normal and stress (seedling as well as terminal stage heat stress) environments were recorded and used to determine different stress tolerance attributes i.e. Mean Productivity (Rosielle and Hamblin, 1981), Tolerance (Rosielle and Hamblin, 1981), Stress Susceptibility Index (Fischer and Maurer, 1978) and Stress Tolerance Efficiency (Fischer and Wood, 1981). These parameters have been used by other researchers (i.e. Blum *et al.*, 2001; Chauhan *et al.*, 2009; Golabadi *et al.*, 2006; Kirigwi *et al.*, 2004; Singh *et al.*, 2007) for characterization of genotypes in different crops.

Data were analyzed for selection parameters i.e. mean, range, variance, coefficient of variance, heritability, genetic advance and correlation coefficients using Genstat 5 (Genstat 5 Committee, 1987) to determine the significance of each aspect.

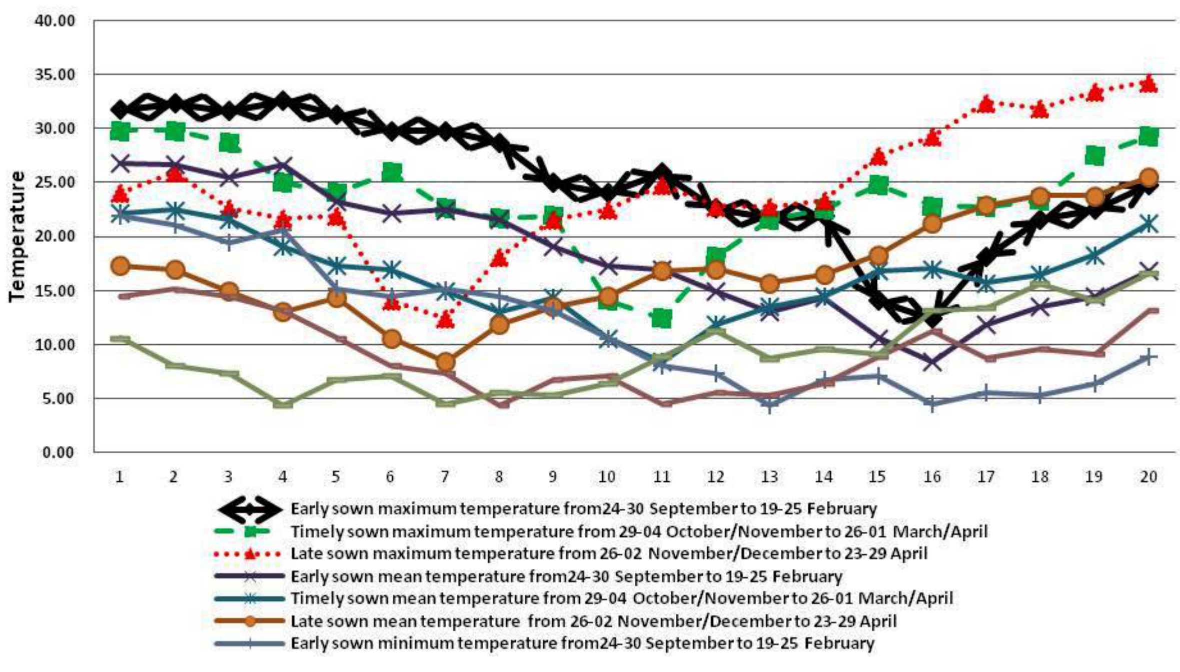

Figure 1. Temperature during 2010-11

RESULTS

Variability, heritability and genetic advance

The variance analyses showed significant ($P < 0.01$) mean squares due to genotypes for all the traits (Table 1) indicating substantial variability for the traits. Phenotypic coefficient of variance (PCV %) was higher than genotypic coefficient of variance (GCV %) with close correspondence between them (data not shown). All the 7 physiological traits exhibited higher magnitude of GCV % (< 20%). The moderate (10% to 20%) to higher (< 20%) estimate of GCV % was observed for seed yield under all 3 environments and also for seed yield derived stress parameters. All the 7 physiological traits, seed yield as well as yield derived parameters exhibited higher magnitude of heritability (h^2_{bs}) i.e. < 60% and genetic advance in per cent (GA %) i.e. < 30%.

Table 1. Selection parameters for different heat stress related traits in Indian mustard.

Selection parameters	CMS	% RRWC	ATT	Chl a	Chl b	Chl a/b	TCC	MP	TOL	STE	SSI	SYN	SYS	SYT
Mean	57.8	13.1	57.9	1.0	0.4	2.7	1.4	8.3	3.7	63.2	1.1	10.2	6.8	6.0
Range	23.0-97.5	4.3-19.2	23.2-85.1	0.4-2.0	0.1-0.7	1.0-8.1	0.6-2.8	6.0-9.9	1.9-5.9	46.2-82.3	0.5-1.6	8.0-11.9	4.3-9.4	3.1-8.6
Variance	1000.1**	58.1**	955.7**	0.5**	0.1**	6.2**	0.6**	3.1**	3.3**	261.3**	0.3**	3.7**	5.4**	6.6**
GCV %	31.6	33.7	30.8	40.2	35.8	50.3	33.6	12.3	27.4	14.5	25.7	10.8	19.7	24.3
Heritability (broad sense)	99.8	99.3	99.9	90.3	96.8	82.1	98.6	96.4	86.6	88.9	89.5	92.3	95.1	93.3
Genetic advance	37.6	9.0	36.8	0.8	0.3	2.6	0.9	2.1	2.0	17.8	0.6	2.2	2.7	2.9
GA in % of mean	65.0	69.1	63.4	78.8	72.5	93.9	68.8	24.8	52.5	28.1	50.0	21.4	39.5	48.3

* and ** Significant at 5% and 1% level, respectively.
CMS, Cell membrane stability; %RRWC, Reduction in relative water content (%); ATT, Acquired thermal tolerance; Chl a, Chlorophyll a; Chl b, Chlorophyll b; Chl a/b, Ratio of Chl a and Chl b; TCC, Total Chlorophyll content; MP, Mean productivity; TOL, Tolerance; SSI, Stress susceptibility index; STE, Stress tolerance efficiency; SYN, Seed yield in normal condition; SYS, Seed yield in seedling heat stress condition; SYT, Seed yield in terminal heat stress condition.

Heat stress effect vis-a-vis heat stress parameters

Temperature during early sown and late sown crop season was 2.8 °C to 7.0 °C and 3.5 °C to 4.8 °C higher than the timely sown crop season at seedling stage and terminal stage, respectively. This clearly affected the plant establishment and seed yield. Seed yield of seedling stage and terminal stage heat stressed crop was reduced by 32.8% and 40.9%, in relation to timely sown crop, respectively. The lowest and highest yield reduction due to seedling as well as terminal heat stress was found for genotypes PRL 08-6 (12.7%) and PR 08-3 (59.2%) as well as PRL 06-37 (16.2%) and RH 0209 (60.1%), respectively. The pattern of yield reduction of genotypes was in accordance to the results obtained on physiological parameters (Table 1, Figures 2 to 5). The genotypes observed on the basis of physiological parameters as 'tolerant'; comparatively, were observed superior either in seedling heat stress or terminal heat stress or in both the environments on the basis of "% yield reduction" (Table 2). The mean values of mean productivity (MP)- to seedling heat stress (SHS), to terminal heat stress (THS) and to average of both stresses (Av.) were ranged from 6.4 for RH 0209 to 10.4 for Rohini, 5.6 for RH 0209 to 9.9 for PR 08-3 and 6.0 for RH 0209 to 9.9 for PRL 07-2, respectively (Table 1). The mean values of tolerance (TOL)- to SHS, to THS and to Av. ranged from 1.3 for PRL 08-6 to 7.2 for PR 07-5, 1.6 for PRL 06-37 to 6.3 for Divya 22, 1.9 for PRL 08-6 to 5.9 for Divya 22, respectively (Table 1). The mean values of stress tolerance efficiency (STE)- to SHS, to THS and to Av. ranged from 37.7 for PR 07-5 to 87.4 for PRL 08-6, 37.7 for PRE 08-1 to 83.8 for PRL 06-37, 46.2 for Divya 22 to 82.3 for PRL 08-6, respectively (Table 1). The mean values of stress susceptibility index (SSI)- to SHS, to THS and to Av. ranged from 0.4 for PRL 08-6 to 2.1 for PR 07-5, 0.4 for PRL 06-37 to 1.7 for PRE 08-1, 0.5 for PRL 08-6 to 1.6 for Divya 22, respectively (Table 1). The higher value of MP as well as STE and lower value of TOL as well as SSI represent more tolerant to stress; while for susceptible genotypes, these values are *vice* *versa.* The observed patterns of genotypes to be scaled as tolerant or susceptible on the basis of physiological traits were similar as per MP, STE, TOL and SSI (Figures 2 to 5).

Table 2. Categorization of Indian mustard genotypes in heat stress tolerant and susceptible groups based on physiological traits.

No.	Classes	CMS %	ATT %	% RRWC	Genotypes
1	Tolerant	>70%	>70%	<10%	Vardan, NRCM 803, Rohini, PR 06-1, Urvashi, PRL 08-6, PRL 07-3, EJ 20, PRL 06-37, PR 08-5, RRN 631, HYT 33, PRL 08-7
2	Moderately Tolerant	50% to 70%	50% to 70%	10% to 20%	RH 0447, PR 08-3, PRL 08-8, PR 08-13, Krishna, NDRE 7, NDRE 22, RGN 241, PRKS 28, Kanti, Kranti
3	Susceptible	<50%	<50%	>20%	PRL 08-2, RH 0216, EJ 17, JD 06, PRE 08-2, NDRE 4, RH 0209, RH 0304, SKM 0526, PRL 08-5, Divya 22, PR 07-5, PRE 07-6, Ashirwad, RK 08-2, PR 08-2, PRL 07-2, JMWR 08-3, PRE 08-1, PRE 08-2, Maya

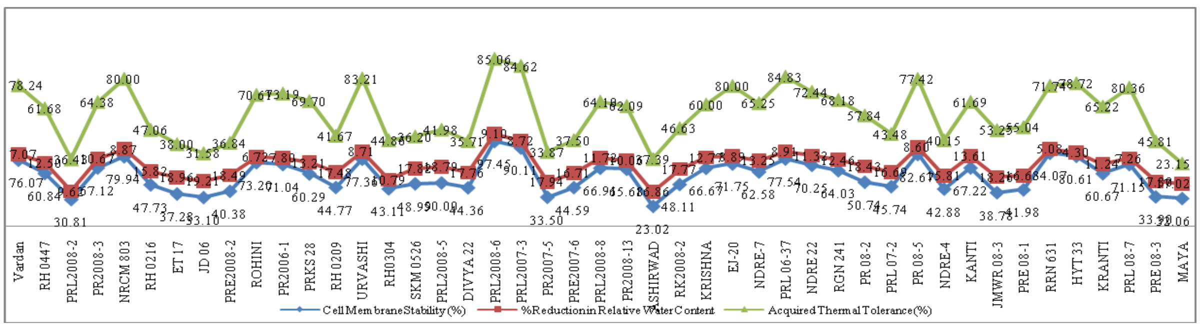

Figure 2. Mean values for CMS (%), %RRWC and ATT% in Indian mustard

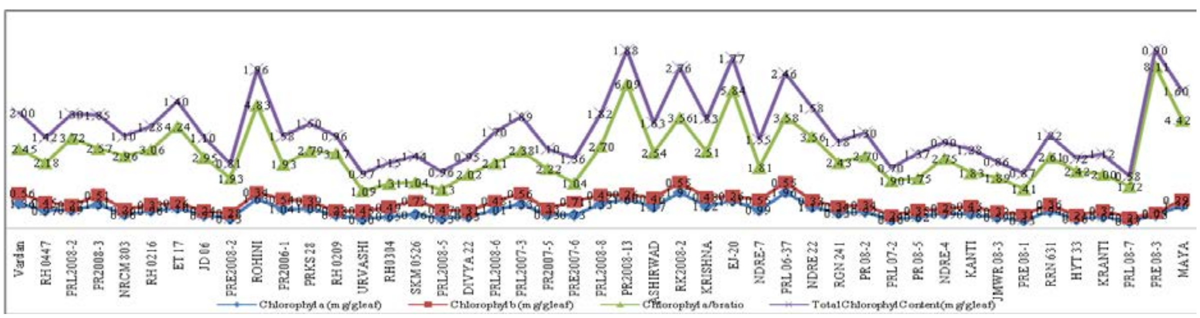

Figure 3. Mean values for different chlorophyll contents in Indian mustard

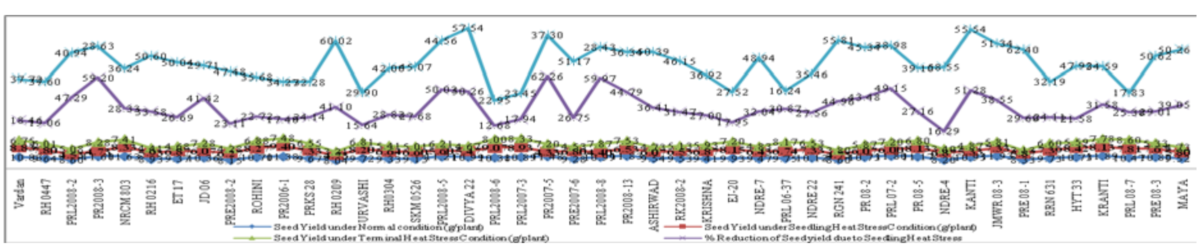

Figure 4. Mean values for seed yield under different environments as well as their per cent reduction under heat stress in Indian mustard

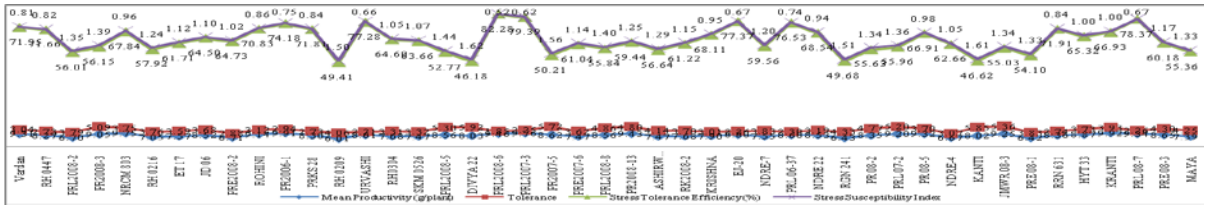

Figure 5. Mean values for different yield based heat stress related parameters in Indian mustard

Correlation among heat stress parameters

Among the physiological traits, CMS % and ATT % had significant ($P < 0.05$) positive correlation with seed yield in normal (SYN) (r = 0.3 and r = 0.3), seedling (SYS) (r = 0.6 and r = 0.6) and terminal (SYT) (r = 0.6 and r = 0.6) heat stress environments (Table 3). Per cent RRWC had negative correlation with seed yield in all environments. All the 4 chlorophyll content based parameters failed to manifest significant ($P < 0.05$) correlation with seed yield; barring TCC with SYT (r = 0.3). Among yield based stress parameters (Table 3), the genotypic correlation coefficient of MP and STE were observed significantly ($P < 0.01$) positive correlated with SYN, SYS and SYT. TOL and SSI were negatively correlated with seed yield traits. Genotypic correlation coefficient of all physiological traits with yield derived stress parameters were observed desirable ($P < 0.01$) (Table 4). Among physiological traits, CMS %, % RRWC and ATT % had highly significant (P < 0.01) genotypic correlation with MP, TOL, STE and SSI while among chlorophyll content traits, only TCC had significant ($P < 0.05$) correlation with STE.

Table 3. Correlation coefficients between seed yield and stress related traits in different environments in Indian mustard.

Seed yield	Physiological parameters							Stress related yield parameters			
(gm/plant)	CMS	% RRWC	ATT	Chl a	Chl b	Chl a/b	TCC	MP	TOL	STE	SSI
SYN	0.3*	-0.2	0.3*	0.1	-0.1	0.1	0.1	0.9**	0.4**	0.1	-0.1
SYS	0.6**	-0.6**	0.6**	0.1	0.1	0.1	0.1	0.6**	-0.7**	0.8**	-0.9**
SYT	0.6**	-0.6**	0.6**	0.3	0.1	0.1	0.3*	0.9**	-0.2	0.6**	-0.5**

Table 4. Correlation coefficient between physiological traits and yield based stress related parameters in *B. juncea* (L.).

Stress related	Physiological traits						
parameters	CMS	% RRWC	ATT	Chl a	Chl b	Chl a/b	TCC
MP	0.6**	-0.5**	0.6**	0.1	0.1	0.1	0.2
TOL	-0.5**	0.5**	-0.5**	-0.2	-0.2	-0.1	-0.3
STE	0.7**	-0.7**	0.7**	0.3	0.2	0.1	0.3*
SSI	-0.6**	0.6**	-0.7**	-0.2	-0.2	-0.1	-0.3

* and ** Significant at 5% and 1% level, respectively.
CMS, Cell membrane stability; % RRWC, Reduction in relative water content (%); ATT, Acquired thermal tolerance; Chl a, Chlorophyll a; Chl b, Chlorophyll b; Chl a/b, Ratio of Chl a and Chl b; TCC, Total Chlorophyll content; MP, Mean productivity; TOL, Tolerance; SSI, Stress susceptibility index; STE, Stress tolerance efficiency; SYN, Seed yield in normal condition; SYS, Seed yield in seedling heat stress condition; SYT, Seed yield in terminal heat stress condition.

DISCUSSION

Physiological traits vis-a-vis supra-optimal temperature via mean performance

The values of each physiological trait were grouped into 3 classes for categorization of genotypes into tolerant, medium tolerant and susceptible classes (Table 2). For instance, CMS % were grouped as < 50%, 50% to 70% and > 70% for susceptible, medium tolerant and tolerant type, respectively, following Blum *et al*. (2001); Shafeeq *et al.* (2006) in wheat for drought tolerant. The genotypes judged as tolerant or susceptible on the basis of CMS % were also observed in the similar category when judged on the basis of %RRWC as well as on the basis of ATT % (Figures 2 to 5). The values of % RRWC were grouped as < 10%, 10% to 20% and >20% for tolerant, medium tolerant and susceptible type, respectively. Since, Barr and Weatherley (1962) stated that the normal values of relative water content (RWC) range between 98% in fully turgid transpiring leaves to about 30-40% in severely desiccated and dying leaves, depending upon the plant species. In most crop *species*, the typical leaf RWC at around initial wilting is about 60% to 70% (Barr and Weatherley, 1962). The genotypes showing up to 20% RRWC were observed to have about > 70% RWC (relative water content) at 2.00 pm. Following these, we set out a criterion that up to 20% RRWC represent tolerant to medium tolerant and > 20% as for susceptible one (Begum and Paul, 1993; Mondal and Paul, 1996; Paul *et al*., 2006). The value of ATT% were grouped into 3 categories i.e. < 50%, 50% to 70% and > 70% similar to that of CMS% for susceptible, medium tolerant and tolerant type, respectively. Because, both parameters have the same criteria of measurement i.e. amount of electrolyte leakage from leaf cell after exposing to a particular set of temperature. Regarding chlorophyll content, it is known that genotypes having high chlorophyll, carotenoids as well as chlorophyll a in comparison to chlorophyll b are more stress tolerant especially to drought and temperature (Sairam, 1994; Kraus *et al*., 1995). Following aforesaid criteria, genotypes were categorized into 3 groups (Table 2). However, the assessments based on the observed values of chlorophyll contents were inconsistent with those of other physiological traits for different genotypes (Figures 2 to 5).

Yield derived stress parameters vis-a-vis supra-optimal temperature via mean performance

Results based on yield reduction due to seedling as well as terminal heat stress suggested that 28 °C to 29 °C could be considered as the threshold temperature for Indian mustard (Figure 1). Morrison & Stewart (2002) also reported 27 °C for *Brassica napus* while 29.5 °C for other *Brassica species* as the threshold temperature. It is interesting to mention that the genotypes categorized on the basis of physiological parameters as ‘tolerant’, were exhibited less yield reduction in comparison to those categorized as ‘moderately tolerant’ as well as ‘susceptible’ genotypes . Similarly, ‘moderately tolerant’ genotypes were observed superior to ‘susceptible’ genotypes. Eight genotypes namely Vardan, RH 0447, PR 06-1, Urvashi, PRL 08-6, PRL 07-3, EJ 20 and NDRE 4 were judged tolerant to seedling stage heat stress while 2 genotypes namely PRL 06-37 and PRL 08-7 as tolerant to terminal stage heat stress because of exhibiting < 20% yield reduction (AICRP-ICAR, 2010). The genotypes Rohini, PRE 08-2 and HYT 33, and PRL 08-6 and PRL 07-3 exhibited 22% to 23% yield reduction under seedling and terminal heat stress, respectively. So, PRL 08-6 and PRL 07-3 were observed more outstanding across the environments. The higher values of MP and STE while lower values of TOL and SSI and *vice versa* represent more tolerant and susceptible genotypes (Fischer and Maurer, 1978; Fischer and Wood, 1981; Rosielle and Hamblin, 1981). The physiological traits based criterion were corroborated with MP, STE, TOL and SSI based criteria in grouping genotypes as tolerant, moderately tolerant and susceptible (Figures 2 to 5).

Variability, heritability and genetic advance

Since, the mere existence of variability does not necessarily ensure its transmission to the offspring. Therefore, heritability and genetic advance were also estimated. This helps in true assessment of selection efficiency on the basis of phenotypic performance. All the 7 physiological traits, seed yield and yield derived stress parameters exhibited higher magnitude of h^2_b% and GA% (Johnson *et al*., 1955) i.e. > 80% and > 20% (Table 1). Wide ranges of GA% coupled with higher magnitude of h^2_b indicated the better scope of genetic improvement through selection for all the concerned traits.

Correlation-coefficients among physiological traits and yield based stress parameters

Significant (P < 0.01) positive association of CMS %, ATT %, Chl a, Chl a/b ratio, TCC, MP and STE with seed yield whereas negative associations of %RRWC, Chl b, TOL and SSI with seed yield indicated that these parameters could be used as stress parameters for screening of genotypes (Blum, 1986; Porter *et al*., 1994; Barrs and Weatherley, 1962; AOAC, 1980; Rosielle and Hamblin, 1981; Fischer and Maurer, 1978; Fischer and Woods, 1981). The genotypic correlation coefficient of MP, STE and SSI were observed highly significant (r = -0.5 to 0.9, P < 0.01) with SYN, SYS and SYT (Golabadi *et al*., 2006). But differing association of TOL in normal and stress environments was indicative of their poor reliance in such studies (Table 3) (Singh *et al*., 2007). However, yield based stress parameters and physiological parameters manifested stronger genotypic correlation with stressed seed yield than the normal one. In comparison to 'MP vs. TOL' (as the concept given by Rosielle and Hamblin, 1981), 'STE vs. SSI' (as the concept given by Fischer, Maurer and Wood 1978, 1981) was observed to be more effective in present study (Kirigwi *et al*., 2004; Singh *et al*., 2007).

Results of this study indicate that CMS %, ATT % and % RRWC among physiological traits while STE and SSI among yield based traits were emerged as reliable practically feasible parameters in discriminating the mustard genotypes for thermo-tolerance. The standardized data (Table 2) of these parameters could be used as preliminary selection tool in Indian mustard breeding programs. Two genotypes namely PRL 08-6 and PRL 07-3 among 45 mustard genotypes were observed more outstanding across environments exhibiting seedling as well as terminal heat stress tolerance. Hence, this study is very useful in strategizing breeding programs for high temperature stress tolerance in Indian mustard.

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