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Screening Indian mustard [*Brassica juncea* (L.) Czern and Coss)] germplasm for seedling thermo-tolerance using a new screening protocol

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An experiment was conducted to study the genetic variability for seedling thermo-tolerance in the Indian mustard germplasm. 165 genotypes of Indian mustard were sown in two seasons (2011 and 2012) in the early conditions when the soil temperature is in the range of 40 to 42°C. The same set of genotypes were screened in the controlled growth chamber using a screening protocol that simulates the temperature fluctuations experienced during the seedling stage of Indian mustard in many of the mustard growing areas of the country. Analysis of the data revealed significant variation for seedling thermo-tolerance. There was a high consistency in the tolerant and susceptible genotypes identified between the two screening procedures. The phytotron screening protocol was effective in differentiating and identifying high temperature tolerant and susceptible genotypes; and for the further studies, field screening can be replaced with phytotron screening. Re-evaluation of selected thermo-tolerant and thermo-susceptible genotypes in the controlled growth chamber revealed significant differences between the two groups regarding the specific leaf area and in the extent of lipid peroxidation, indicating that these two parameters can be used as selection criterion for seedling thermo-tolerance in Indian mustard.

Key words: Lipid peroxidation, screening, seedling thermo-tolerance index (STI), thermo-tolerance.

INTRODUCTION

Oilseed brassicas are the second most important edible oilseed crop of India after soybean in terms of the acreage and production. More than 90% of the area under oilseed brassicas in India is occupied by the Indian mustard (*Brassica juncea*) because of its relative tolerance to biotic and abiotic stresses in comparison with other oilseed *Brassica* species. The recommended sowing time of rapeseed-mustard in India is the first 3 weeks of October. An optimum average temperature of 26°C is required for the proper germination and establishment of seedlings (Lallu and Dixit, 2008). Due to the changing climate, the temperature during the last 15 years except 2010 was above this limit in the major rapeseed-mustard growing areas of the country, thus, affecting the seedling survival and resulting in poor plant stand in the field which eventually leads to a decline in the production and productivity of rapeseed-mustard. Prevalence of high temperature at seedling stage also prevents the early sowing of mustard, which is a recommended practice because of the many advantages it offers. Early harvest of mustard helps to avoid disease infestation and pest attack that normally coincide with the

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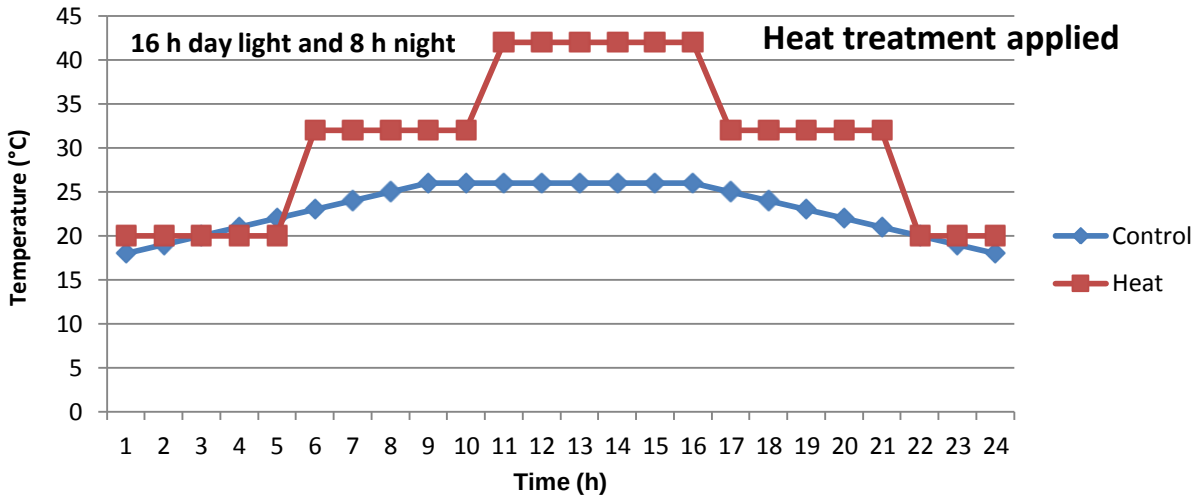

Figure 1. Heat treatment applied in the treatment and control experiments.

flowering stage. Shattering of siliquae can be avoided during the time of harvest when the crop encounters high temperature and short duration early mustard is suitable for multiple cropping (Kaur et al., 2009). It is therefore imperative to develop seedling thermo-tolerant Indian mustard varieties to mitigate the losses occurring due to the stress which will also helps in the early sowing of the crop.

High temperature affects rapeseed-mustard both at seedling and pod filling stage. Whereas the problem of heat stress at flowering stage is observed in all the major mustard growing countries including China, Australia, Canada and European; heat stress at seedling stage is a problem unique to India (Salisbury and Gurung, 2011). This is because of the fact that mustard is sown in the post-monsoon season in India, when average day time temperatures are well above the recommended limit. There are many studies (Morrison, 1993; Angadi et al., 2000; Morrison and Stewart, 2002; Young et al., 2004) related with thermo-tolerance in oilseed brassicas at the flowering stage, but little progress has been made so far in understanding the heat tolerance at seedling stage in *Brassica*. Heat tolerance in crop plants is a developmentally regulated, stage-specific phenomenon; tolerance at one stage of plant development may not be correlated with tolerance at other developmental stages (Wahid et al., 2007). So, individual stages throughout the ontogeny of the plant should be evaluated separately for the assessment of tolerance and for the identification, characterization and genetic manipulation of tolerance traits. Therefore, the present study was proposed with the objectives to assess the genetic variability for seedling thermo-tolerance in the Indian mustard germplasm and to characterize the selected thermo-tolerant and thermo-susceptible genotypes in order to discover reliable selection criteria for seedling thermo-tolerance in Indian mustard.

MATERIALS AND METHODS

A set of 165 *B. juncea* genotypes including 84 released varieties and 81 advanced lines was utilized for the study. Pusa Mustard 25, Pusa Mustard 27, Pusa Agrani and Pusa Karishma were used as the checks. Early sowing of the genotypes was carried out for two seasons (2011 and 2012) in augmented design in the experimental field of the Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi. The maximum temperature was around 38°C during the experimental period and the soil temperature was in the range of 40 to 42°C.

The same set of genotypes were screened for heat tolerance in the controlled growth chamber in the National Phytotron Facility (NPF) in IARI, New Delhi, using a protocol that simulates the temperature fluctuation experienced in the field during the seedling stage of Indian mustard in the major mustard growing states in India (Figure 1). Five (5) days old seedlings were exposed to a temperature of 20°C for 8 h in dark. Then the temperature was gradually elevated to 32°C and maintained for 5 h in light. The temperature was then elevated to 42°C and maintained for 6 h in light. Then the temperature was decreased to 32°C, and maintained for 5 h in light; and then the entire cycle is repeated. A control experiment was carried out in the glass house where optimum temperature for germination and seedling survival was given (Figure 1). Both the experiments were carried out in augmented design with 5 blocks, such that there were 37 genotypes (33 test genotypes + 4 checks) in each block. In both the experiments, the sowing medium was prepared by mixing autoclaved soil and vermi-compost in 3:1 ratio. One hundred (100) seeds of each genotype were sown in different trays and supplement irrigation was given to avoid water stress. The entire experiment was conducted at a constant R.H. of 80%.

The germination and seedling survival in the genotypes in both field and phytotron experiments were recorded every day from the 3rd day after sowing up to 14th day after sowing, when the first true leaf emerges in more than 50% of the seedlings. Susceptible and tolerant genotypes were identified based on the seedling survival. Three parameters viz., days to 50% mortality, seedling thermo-tolerance index (STI) and seed to STI (SSTI) were used to assess a genotype's ability to germinate and survive under heat stress. STI was calculated as the ratio of number of seedling survived to the number of seedling emerged, expressed in percentage. SSTI was calculated as the ratio of seedling survival to the number of

Table 1. Analysis of variance for seedling thermo-tolerance indices under high temperature treatment.

Traits/source of variation	Mean sum of squares						General mean	Critical differences (5%)			
	Blocks	Entries	Checks	Genotypes	C × G	Error		CD_1	CD_2	CD_3	CD_4
df	4	168	3	164	1	12					
Days to 50% mortality (%)	0.08	11.85**	249.20**	7.48**	16.36**	0.08	11.25	0.52	1.18	1.32	0.98
STI (%)	10.15	460.33**	4807.57**	358.87**	4057.04**	22.72	29.77	6.57	14.69	16.42	12.29
SSTI (%)	21.44	231.42**	1984.18**	194.97**	951.55**	12.05	19.92	4.78	10.70	11.96	8.95

Critical differences (CD): CD_1: CD between two control treatments; CD_2: CD between two test treatments (same block); CD_3: CD between two test treatments (different blocks); CD_4: CD between a test treatment and a control treatment.

seedlings expected to emerge, expressed in percentage (Yadav et al., 2011). The SSTI is an extension of STI by taking expected germination into account. It was necessary to correct the effect of under soil mortality (USM). The germination under the control experiment of the same lot of spare seed was taken as expected germination in this study. To make homogeneity in the variance, the data were transformed by angular transformation. The transformed values were analyzed by the statistical software SPAD (Rathore et al., 2004). The data obtained from the screening experiments conducted in the phytotron and field was compared.

Morphological and biochemical characterization of high temperature tolerant and susceptible genotypes

A major challenge in traditional breeding for heat tolerance is the identification of effective selection criteria to facilitate detection of heat-tolerant plants. With an objective to find out an appropriate selection criteria for seedling thermo-tolerance in mustard, the identified tolerant and susceptible genotypes were re-evaluated in the controlled growth chamber in completely randomized design with three replications. Two morphological (specific leaf area and shoot length from soil level to the first true leaf) and two physiological parameters, viz., chlorophyll content (Lichtenthaler and Wellburn, 1983) and the extent of lipid peroxidation (Heath and Packer, 1968) were used to characterize these genotypes. Regression analysis was carried out between correlated traits to measure the average relationship between them.

RESULTS AND DISCUSSION

Screening studies

There were no significant differences among the genotypes for all the three parameters studied in the control experiment carried out under ambient conditions in the Glasshouse Facility of the NPF. Whereas, analysis of variance showed significant variation for all the three parameters studied in both the field experiments and the experiment conducted in controlled Growth Chamber; indicating that different genotypes displayed a wide range of heat tolerance responses in seedlings to high temperature stress, suggesting wide genetic variability (Table 1). The genotypes were classified in to three categories based on the seedling thermo-tolerance. Assessment was based on the time needed for a line to reach 50% mortality, with the longer the time, the more tolerant to high temperature. Based upon this result, the lines were grouped into thermo-tolerant (more than 9 days of treatment to reach 50% mortality), moderately thermo-tolerant (6 to 8 days), and thermo-susceptible genotypes (less than 5 days of treatment to reach 50% mortality). Eighteen (18) genotypes, 9 from released varieties and 9 from advanced lines, were found to be thermo-tolerant. One of the thermo-tolerant variety identified in the present study, EJ 22 was also reported to be thermo-tolerant by Singh (2011) based on a screening study involving 43 rapeseed-mustard genotypes. Thirty-six (36) released varieties and 53 advanced lines were classified as moderately thermo-tolerant and the remaining 58 lines (39 varieties and 19 advanced lines) were classified as thermo-susceptible genotypes. The heat tolerance indices, STI and SSTI were also calculated for each genotype on 9 days after treatment or 14 days after sowing. There was no significant correlation for the three parameters between the field and growth chamber screening when the entire set of genotypes are considered, which can be explained by the fact that the seedling survival in the field can be affected by many factors other than high temperature. Due to this reason, the data obtained from the phytotron screening was used as the baseline to identify heat-tolerant and heat-susceptible genotypes. The genotypes which were identified to be thermo-tolerant in the phytotron screening were also found to be tolerant in the field. The same was true for the thermo-susceptible genotypes. Since there is a high consistency in the tolerant and susceptible genotypes identified between the two screening

Table 2. Selected genotypes for seedling thermo-tolerance/thermo-susceptibility.

Genotype	Source	Days for 50% seedling mortality	STI (%)	SSTI (%)	Number of times line recorded as thermo-tolerant at seedling stage
EJ 22	India	-	59.09	56.27	3
NPJ 113	India	-	59.37	59.37	3
NPJ 124	India	-	67.56	65.67	3
P.Bahar	India	-	55.17	46.35	3
5011 (Pusa Agrani × Laxmi)	India	11	45.71	44.36	2
Pusa Karishma	India	5	0.00	0.00	0
6018 (RGN-48 x Laxmi)	India	5	0.00	0.00	0
BEC 286	Poland	3	0.00	0.00	0

STI = Seedling thermo-tolerance index, SSTI = Seed to seedling thermo-tolerance index.

procedures, the above described phytotron screening protocol is effective in differentiating and identifying high temperature tolerant and susceptible genotypes; and for the further studies, field screening may be replaced with phytotron screening.

Morphological and biochemical characterization of high temperature tolerant and susceptible genotypes

Five selected thermo-tolerant (EJ 22, NPJ 113, NPJ 124, Pusa Bahar and 5011) and three thermo-susceptible (Pusa Karishma, 6018 and BEC 286) genotypes were used in the study (Table 2). Among the five parameters studied, two parameters, specific leaf area and lipid peroxidation, clearly differentiate between thermo-tolerant and thermo-susceptible genotypes (Table 3). Thermo-tolerant genotypes have less specific leaf area compared to thermo-susceptible genotypes, indicating that reduced leaf area and the resultant reduction in the transpiration rate can be one of the reasons for thermo-tolerance in the selected mustard genotypes. Generation and reactions of reactive oxygen species (ROS) is one of the important symptoms of cellular injury due to high temperature (Liu and Huang, 2000). ROS cause the autocatalytic peroxidation of membrane lipids and pigments thus leading to the loss of membrane semi-permeability and modifying its functions (Xu et al., 2006). The level of lipid peroxidation is measured in terms of thiobarbituric acid reactive substances (TBARS) content (Heath and Packer, 1968). In the present study, the tolerant genotypes showed a reduced extent of lipid peroxidation indicating that these genotypes were able to prevent the detrimental effects of ROS by scavenging with various anti-oxidant enzymes and thereby maintaining the cell membrane stability. Chlorophyll content and the shoot length from soil level to the first true leaf did not differentiate the seedling thermo-tolerance as there is no definite trend for these parameterrs between thermo-tolerant and susceptible genotypes (Table 3), though chlorophyll content was recommended as a criteria to screen seedling thermo-tolerance in wheat (Mullarkey and Jones, 2000). Correlation analysis indicated a negative association between the seedling thermo-tolerance indices (STI, SSTI and days to 50% seedling mortality) with the specific leaf area ($r = -0.904$, -0.924 and -0.910, respectively) and the extent of lipid peroxidation, measured in terms of the TBARS content ($r = -0.605$, -0.649 and -0.610, respectively). Having established the association between these traits, regression analysis was carried out to measure the average relationship between the variables (Figure 2) in order to further explore the relationship among them. In the regression analysis, the seedling thermo-tolerance indicators were taken as the dependent variables, which were expressed as a function of the independent variables, that is, specific leaf area and the TBARS content. In all the combinations of various traits, the regression coefficients were negative (Figure 2) indicating that the relationship between the independent and dependent variables are negative, so that reduced specific leaf area and less extent of lipid peroxidation led to an increased seedling thermo-tolerance in the genotypes studied. The amount of increase in a genotypes' thermo-tolerance with a unit reduction in the specific leaf area or TBARS content can be quantified in terms of the corresponding regression coefficients. Based on these observations, it can be concluded that specific leaf area and the extent of lipid peroxidation can be used as parameters to differentiate between thermo-tolerant and thermo-susceptible genotypes at seedling stage in Indian mustard.

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Table 3. Morphological and biochemical characterization of high temperature tolerant and susceptible genotypes.

Genotype	Specific leaf area (cm^2/g)		Length to the I^{st} true leaf (cm) from soil level (cm)		Chl. a (µg/ml)		Chl. b(µg/ml)		Total Chl. (µg/ml)		TBARS content (nano moles/g)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
EJ 22	235.57	1.00	3.33	0.18	8.13	0.19	2.44	0.14	10.57	0.33	269.08	16.95
NPJ 113	207.55	9.54	2.51	0.20	6.33	0.13	1.92	0.04	8.25	0.17	322.41	46.38
NPJ 124	266.20	11.34	2.86	0.21	11.19	0.31	3.35	0.10	14.53	0.40	448.71	14.26
P.Bahar	324.98	12.56	2.07	0.18	12.21	2.15	3.68	0.47	15.89	2.62	623.66	13.14
5011	323.81	4.76	2.49	0.15	11.97	0.79	3.71	0.23	15.68	1.01	566.89	3.45
Pusa Karishma	381.28	21.62	1.66	0.10	12.65	2.22	3.80	0.68	16.45	2.89	561.40	18.15
6018	434.10	8.67	1.90	0.15	11.13	0.83	3.34	0.31	14.47	1.13	591.88	4.75
BEC 286	436.72	11.76	2.69	0.10	11.82	0.80	3.63	0.20	15.44	1.00	611.07	6.17
C.D.	30.23		0.47		3.78		1.08		4.84		63.96	
SE(m)	9.87		0.17		1.23		0.35		1.58		20.53	
SE(d)	13.96		0.23		1.75		0.50		2.23		29.03	

TBARS = Thiobarbituric acid reactive substances, C.D. = Critical difference, SE(m) = Standard error mean, SE(d) = Standard error difference.

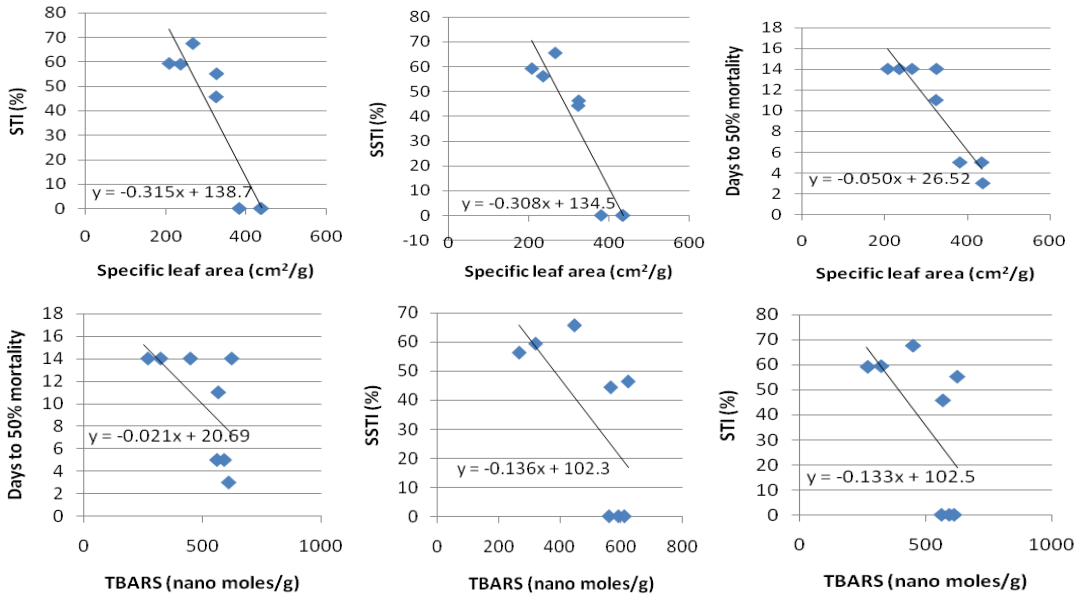

Figure 2. Regression of the three seedling thermo-tolerance indices on specific leaf area and TBARS content.

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