



Screening of Indian mustard (*Brassica juncea*) for thermo tolerance at seedling and terminal stages

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Abstract

Effect of heat stress was visualized at seedling stage in terms of seedling mortality and at terminal stage on growth traits and yield in 25 promising mustard (*Brassica juncea* L.) genotypes grown in randomized block design with three replications in two environments viz. timely (3rd week of October) and late sown (3rd week of November). Genotypes RB-10, PR-2004-2, NPJ-93, NRCDR-2, CS-810-5-2-SP showed >30% seedling mortality and were rated as thermo tolerant. Significant genotypic differences existed for all growth traits at two sowing dates, and environment had a profound impact not only on morphological traits but also on the reproductive behavior and seed filling which in turn was associated with yield reduction under terminal heat stress. Two genotypes RL-2047 and RGN-152 registered yield reduction less than 30% in the present investigation. RL-2047 showed terminal heat tolerance for seed yield/plant and was moderately tolerant for biological yield/plant, seeds/silique, and silique length while RGN-152 possessed terminal heat stress tolerance only for biological yield/plant in addition to moderate tolerance for siliques on main shoot, and silique length. Seed yield/plant exhibited significant and positive association with heat susceptible index (HSI, $r=0.58^*$), and negative association with heat tolerance efficiency (HTE, $r=-0.57^*$) in the timely sown cultivars. Reverse association was found under high temperature stress with delayed sowing. Furthermore, average heat susceptible index (HSI) was 0.28 and heat tolerance efficiency (HTE) was 84.8 % in the resistant genotypes. Heat tolerance parameters studied in this investigation may be used for screening traits, and the identified genotypes as suitable donor for crossing programmes to develop heat tolerant mustard genotypes.

Key words: Heat susceptible index, heat tolerance efficiency, seedling mortality, 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 which eventually leads to 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 many advantages it offers. Early harvest helps to avoid disease infestation and pest attack that normally coincides with the flowering stage. Shattering of siliques can be avoided during the time of harvest when the crop encounters high temperature and thus the 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 help in early sowing of the crop.

High temperature affects rapeseed-mustard not only at seedling but also at the pod filling stage. Due to multiple cropping systems, the sowing of Indian mustard gets delayed upto end of October to mid November due to late harvesting. Growth and development of late sown crop is more adversely affected by severe winter, foggy and frost conditions during vegetative stage and high temperature during pod and seed filling stages. 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). The problem of heat stress at flowering stage is observed in all the major mustard growing countries including China, Australia, Canada and Europe, but heat stress at seedling and terminal stage is a unique problem to India (Salisbury and Gurung, 2011). There are many studies related with thermo tolerance in oilseed brassicas at the flowering, but very little information is available in understanding the heat tolerance at seedling and terminal stages. Further, heat tolerance in crop plants is developmentally regulated, and is known to be stage specific phenomena, meaning tolerance at one stage of plant development may not be correlated with tolerance at the other developmental stages (Wahid *et al.* 2007). Therefore, the present study was undertaken with the objectives to assess the genetic variability for seedling and terminal thermo tolerance in Indian mustard genotypes, and to characterize the selected thermo tolerant genotypes in order to discover reliable criteria for thermo tolerance at seedling and terminal high temperature stress.

Material and Methods

Twenty five *B. juncea* genotypes were screened during 2006-07 for high temperature at seedling stage under laboratory conditions and for terminal heat stress under field conditions.

Laboratory experiment: Genotypes were grown in the trays filled with 5 kg soil, which was thoroughly mixed with known volume of water for germination. Seeds of each of 25 genotypes were

sown in rows and trays were placed in seed germinator at $25^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 4 days at 70% RH. For each genotype, three replications were kept. Twenty seedlings per genotype per replicate were used for recording the observations. Known volume of water was sprinkled on the 3rd day of germination. Four day old seedlings were subjected to high temperature ($40^{\circ}\text{C}\pm 1^{\circ}\text{C}$) regime for 2hrs. Experiments were repeated twice and seedling mortality was recorded after heat shock.

Field Experiments: The same set of 25 genotypes were sown in the field at two dates (1st at optimum time in third week of October, normal sowing (NS), and 2nd at late sown (LS) third week of November, to allow high temperature at terminal stage. Each genotype was sown in paired rows with three replications in random block design at two sowing dates. All the recommended agronomic package and protection measures were adopted for raising healthy crop. Yield and yield components were recorded at physiological maturity and data was analyzed statistically by CPCS (2008) software, and simple correlation was also worked out. Heat susceptibility index (HSI) was calculated by using Fischer and Maurer (1978) formula, and heat tolerance efficiency (HTE) was calculated by the equation of Fischer and Wood (1981).

Results and Discussion

Seedling mortality

Seedling mortality varied significantly within the genotypes (Fig 1). NPJ-93 registered the minimum seedling mortality of 25%, while PBR-1188 the maximum mortality of 59.7%. Genotypes with seedling mortality less than 30% were RB10, RK-05-01, PR-2004-2, NPJ-93, NRC DR-2 and CS-610-5-2-SP and, therefore, rated as tolerant to heat stress. Genotypes with 45% seedling mortality were categorized as susceptible.

Growth parameters

Significant variation existed for plant height among the cultivars in timely sown crop (NS). Plant height varied from 186.6 cm (SKM450) to 244.2cm (RK-03-02) with a mean of 211.4 cm under normal sown conditions (Table 1). Under LS conditions plant height ranged from 167.5 (CS-1900-1-4) to 205 (RK-

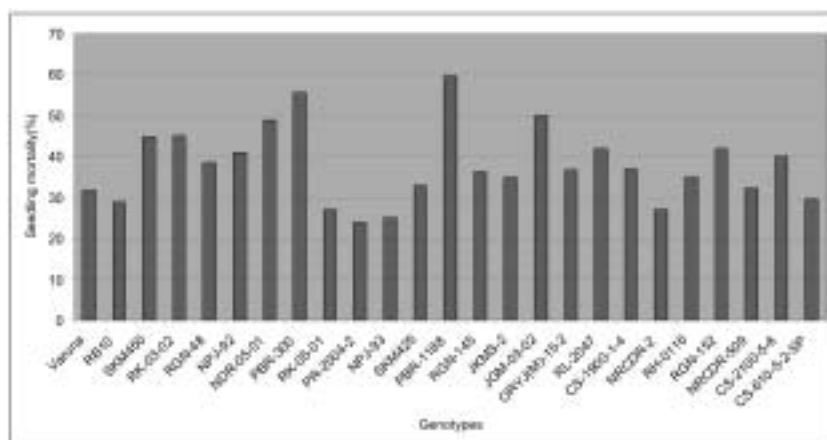


Fig1: Effect of heat stress on seedling mortality in Indian mustard genotypes (critical differences at 5% =19.1)

Table1: Range, mean and critical difference for morph -physiological characters in Indian mustard under two contrasting environments

Traits	Normal sown(NS)			Late sown (LS)		
	Range	Mean	Critical differences (5%)	Range	Mean	Critical differences (5%)
Flowering behavior						
Flower initiation	37.5- 50.5	42.5±3.8	1.68	57.5-73.0	63.3±3.3	5.37
50% flowering	47.0-59.0	51.8±3.4	2.7	63.5-76.5	69.6±4.6	6.1
100% flowering	55.0-67.5	60.4±3.3	3.04	68.5-85.5	75.4±4.9	6
Flowering duration	16.5-20.0	18.1±0.94	NS	8.0-15.5	12.2±1.9	3.69
Fruiting behavior						
Siliqua initiation	40.0-55.5	47.9±3.4	2.53	63.5-77.5	68.6±3.8	5.14
50% siliquae formation	83.0-92.5	87.2±2.8	4.74	81.5-98.0	88.6±4.3	6.4
100% siliquae formation	106.5-117.5	110.3±3.1	NS	100.5-113.0	105.9±3.7	5.57
Siliquae/podding duration	49.0-70.0	62.1±4.5	NS	32.0-42.5	37.3±2.3	4.78
Days to maturity	144-152	148.6±2.0	NS	136.5-144.5	140±2.8	4.79
Growth parameters						
Plant height(cm)	186.6-244.2	211.4±15.2	34.1	167.5-205	186.5±9.0	NS
Main shoot length(MSL, cm)	70.2-98.5	83.4±7.3	NS	52.7-83.1	67.9±6.4	NS
Primary branches	4.2-6.60	5.3±0.51	NS	3.7-5.6	4.5±0.48	NS
Secondary branches	13.5-26.9	18.6±2.9	NS	12.6-19.5	15.4±1.4	NS
Yield components and Seed yield						
Siliqua on main shoot	37.7-58.5	47.2±4.6	NS	34.5-48.1	40.9±2.2	NS
Siliqua length(cm)	4.4-6.3	5.4±0.35	NS	3.9-5.1	4.63±0.28	NS
1000 seed weight(g)	4.44-7.15	5.34±0.51	1.25	3.84-5.83	4.8±0.50	NS
Seed filling						
Seeds/siliqua	9.3-13.1	11.6±0.80	3.22	8.1-11.5	10.1±0.56	NS
Shriveled seeds/siliqua	0.3-1.0	0.6±0.15	NS	0.2-0.6	0.3±0.09	NS
Fully developed seeds/siliqua	9.0-13.1	11±0.85	3.26	7.6-11.0	9.6±0.63	NS
Biological yield/plant(g)	75.4-132.2	103.6±20.5	NS	35.1-74.1	49.4±8.0	20.1
Seed yield/plant(g)	17.4-36.0	25.4±5.3	NS	8.0-19.1	11.9±2.1	5.43
HI (%)	19.9-33.5	24.9±3.3	8.34	18.2-29.8	24.1±2.4	NS
Oil content (%)	40.2-43.4	41.6±0.8	NS	30.5-41.8	38.7±1.7	NS

03-02) with an average plant height of 186.5 cm. Plant height was reduced by 11.4% in the late sown crop. Four genotypes registered minimum reduction in plant height. Main shoot length (MSL) did not vary significantly in both the sowing dates. Maximum MSL was 98.5 cm (PBR-300) and lowest 70.2cm (JGM-03-02) under normal sown whereas in the late sown it was 83.1cm (CS-610-5-2-SP) and lowest 52.7 cm (SKM450). Mean reduction in main shoot length was 18.6% in the late sown crop and PBR-1188, JGM-03-02, RL-2047, NRCDR-2 and CS-610-5-2-SP recorded minimum reduction in this growth trait. Number of branches, both primary and secondary showed non-significant differences between the genotypes and also for the sowing dates. Number of primary branches were maximum in cultivar RK-05-01 (6.6) followed by RB10 (6.1) and least in RGN-152 (4.2) in timely sown crop, whereas primary branches were lower in late sown crop ranging from 5.6 (PBR-1188) to 3.7 (RGN-48). Minimum reduction in primary branches was registered in SKM450, RGN-145, ORYJ (M)-15-2, NRCDR-2, RH-0116 and RGN-152. Number of primary branches was reduced by 15.1% under late sown conditions. Mean number of secondary branches in 25 cultivars were 17.2% higher in normal sown than the late sown crop. Secondary branches varied from 26.9 (RK-05-01) to 13.5 (NRCDR-509), whereas under late sown conditions, the variation was from 19.5 (PBR-1188) to 12.6 (NPJ-93, NRCDR-509). Eleven genotypes registered minimum reduction in secondary branches.

Seed filling

Number of seeds per siliqua significantly varied only under normal sowing which ranged from 9.3 (CS-2100-5-6) to 13.1 (Varuna). However, In the late sown crop number of seeds varied from 8.1 (CS-2100-5-6) to 11.5 (CS-610-5-2-SP). Seed filling in terms of aborted/ shriveled and fully formed seeds is depicted in Table 1. Non-significant differences occurred for shriveled seeds at both the sowing dates, whereas fully developed seeds varied significantly only in NPJ-92 and NPJ-93 in the normal sown crop. Fully developed seeds were highest in cultivar PBR-300 (13.1 NS and 11.0 LS) and CS-610-5-2-SP (11.1 NS 11.0 LS) and lowest

in cultivar CS-2100-5-6 (9.8 NS and 7.0 LS). Number of shriveled seeds varied from 1.0 (RGN-48) to 0.3 (ORYJ (M)-15-2) in NS and 0.6 (SKM450, PBR-1188) to 0.2 (RK-05-01, JKMS-2, ORYJ (M)-15-2, RH-0116, RGN-152) in LS.

Yield and yield components

Siliquae on the main shoot (SMS) varied from 37.7(Varuna) to 58.5 (ORYJ (M)-15-2) in the normal sown crop, and from 34.5 (SKM450) to 48.1 (RK-03-02) in the late sown crop (Table 1). However, genotypes showed non significant differences for this trait in both the sowing dates. SMS were reduced by 14.9% in the 2nd date of sowing. Siliqua length ranged from 4.4 (SKM425) to 6.3 (NRCDR-2) and from 3.9 (SKM450) to 5.1 (RK-05-01) in normal and late sown crop respectively. Non-significant differences in the genotypes were accorded to siliqua length under both the sowing dates. Siliqua length was reduced by 14.2% in the late sowing. 1000 seed weight differed significantly in the genotypes of the normal sown crop whereas non-significant differences were accorded in the delayed sowing. 1000 seed weight in the normal sowing varied from 4.44 (NRCDR-509) to 7.15 (CS-1900-1-4) and 3.84 (RGN-48) to 5.83 (NPJ-93) in the delayed sown crop. Reduction in mean 1000 seed weight was 10.1% in the late sown genotypes. Biomass (biological yield/ plant) expressed on per plant basis varied from 75.4g (NPJ-93) to 132.2g (RK-03-02), and 35.1g (PR-2004-2) to 74.1g (RL-2047) in the NS and LS genotypes, respectively. Seed yield varied from 17.4g (RGN-48) to 36.0g (JKMS-2) in the normal sown crop and from 8.0g (NDR-05-01) to 19.1g (RL-2047) in the delayed sown crop. Biomass and seed yield did not vary significantly within the cultivars in the normal sown crop while significant differences existed for these traits in the late sown cultivars. Mean reduction in the biomass was 52.3% and seed yield 53.1% in the late sown crop. HI varied from 19.9 (JGM-03-02) to 33.5 (JKMS-2), and from 18.2 (NDR-05-01) to 29.8 (RGN-152) in the normal and late sown crops, respectively. Non-significant differences were recorded within the cultivars for oil content at the two dates of sowing. Oil content was reduced by 6.97% with the delayed sowing. Ferrel *et al.* (2002) in assessment of canola

phenology and yield reaction to sowing date in west Australia reported that per week sowing delay led to 1-7% reduction in yield which could be due to limited growth period due to terminal heat. Earlier, Taylor and Smith (1992) reported that sowing date affected the yield and yield component significantly and optimum sowing date depends on variety and climate. Late sowing in canola as reported by Robertson and Holland (2004) led to flowering and filling stage face terminal heat which resulted in grain and oil yield reduction. Findings of Shengri *et al.* (2012) also supports our results. Impact of high temperature stress has been studied in various crops and for details references be made to studies in Brassicas (Angadi *et al.* 2000, Kaur *et al.* 2011a,

Sharma *et al.* 2012), Bread wheat (Stone and Nicolas 1984, Kaur *et al.* 2011b, Spring wheat (Bahar and Yildirim 2010) and tomatoes (Srivastava *et al.* 2011)

Heat resistance parameters (Table 2)

Genotypes were characterized based on the lower heat susceptible index (HSI) and higher heat tolerance efficiency (HTE %) for growth traits, yield components and seed yield. Genotypes SKM-450 and PR-2004-2 were rated as highly tolerant based on HSI and HTE for plant height and number of primary branches, whereas PBR-1188 had heat tolerance for main shoot length and for both primary and secondary branches. Heat susceptible

Table 2 : Heat tolerance parameters associated with growth traits in Indian mustard

Genotypes	Plant height		MSL		Primary branches		Secondary branches	
	HSI	HTE (%)	HSI	HTE (%)	HSI	HTE (%)	HSI	HTE (%)
Varuna	1.39	83.8	1.3	76.6	2.09	69.6	0.50	91.5
RB10	0.93**	89.3**	1.4	74.6	2.26	67.2	0.50	91.5
SKM450	0.11*	98.8*	1.1	80.3	0.44*	93.6*	1.41	75.7
RK-03-02	1.37	84.1	1.1	80.2	0.78**	88.6**	0.04*	99.4*
RGN-48	0.54**	93.8**	0.6**	88.5**	0.77**	88.8**	0.50*	91.4*
NPJ-92	0.94**	89.1**	1.2	77.6	1.99	71.2	0.31*	94.7*
NDR-05-01	1.20	86.0	1.9	65.4	0.44*	93.6*	1.00	82.8
PBR-300	1.36	84.3	1.4	74.8	0.93**	86.5**	0.07*	98.8*
RK-05-01	1.44	83.3	0.1*	97.7*	1.57	77.3	2.52	56.5
PR-2004-2	0.03*	99.6*	0.9**	84.2**	0.25*	96.4*	1.49	74.2
NPJ-93	0.48*	94.5*	1.0	80.8	0.55**	92.0**	1.67	71.2
SKM425	0.50*	94.2*	1.3	76.6	0.63**	90.9**	0.82**	85.8**
PBR-1188	1.38	84.0	0.4*	91.8*	0.24*	96.6**	0.36*	93.8*
RGN-145	1.34	84.5	0.9**	83.4**	0.26*	96.3*	1.35	76.8
JKMS-2	0.98**	88.6**	1.3	76.6	1.60	76.8	0.42*	92.7*
JGM-03-02	1.34	84.5	0.3*	94.7**	1.53	77.8	0.58**	90.1**
ORYJ(M)-15-2	1.22	85.8	1.8	66.7	0.27*	96.1*	1.30	77.6
RL-2047	1.08	87.5	0.2*	96.4*	0.73**	89.5**	0.55**	90.5**
CS-1900-1-4	0.88**	89.8**	1.4	74.1	0.60**	91.3**	1.40	75.8
NRCDR-2	0.74**	91.4**	0.5**	89.9**	0.44*	93.6*	0.96**	83.4**
RH-0116	1.28	85.1	1.1	79.6	0.52**	92.5**	0.24*	95.8*
RGN-152	0.43*	95.0*	0.8**	84.9**	0.16*	97.6*	1.36	76.6
NRCDR-509	1.68	80.5	1.2	77.6	1.29	81.3	0.39*	93.3*
CS-2100-5-6	0.83**	90.3**	1.3	75.4	1.75	74.6	1.77	69.5
CS-610-5-2-SP	0.99**	88.5**	0.1*	98.3*	2.08	69.8	1.93	66.8

≤ 0.5: Highly tolerant*, 0.5 – 0.99: moderately tolerant **, ≥ 1: Susceptible

index for seed yield categorized RL-2047 and RGN-152 as highly tolerant while 10 genotypes were rated as moderately tolerant. Heat tolerance efficiency in these two cultivars was >89%. HSI was >0.5 for siliqua length in NPJ-93, SKM425, JKMS-2, JGM-03-02 and CS-1900-1-4 with HTE of >94%, whereas for cultivars ORYJ(M)-15-2 and CS-610-5-2-SP it was for seeds per siliqua with HTE >93%. Seven genotypes registered low HSI for siliquae on main shoot with HTE >95%. HSI for biomass indicated RGN-152 to be highly tolerant with HTE of 76%. Ten genotypes were moderately tolerant to heat stress for biomass. Only RGN152 and RL-2047 had lowest HSI indicating heat tolerance for seed yield. Mean HSI for seed yield in tolerant genotypes was 0.284 and HTE 84.8%, moderately tolerant had average HSI of 0.824 and HTE of 56.4% whereas for susceptible genotypes HSI of 1.16 and HTE 38.1%.

Correlations

Positive and significant correlation of seed yield with biological yield at harvest in normal ($r=0.80^*$) and late ($r=0.84^*$) sown conditions, however, seed yield/plant showed positive association with 1000 seed weight ($r=0.47$) only under normal sowing, but had negative correlation ($r=-0.02$) under delayed sowing. Positive and significant correlation of seed yield with dry matter has also been reported previously (Kachroo *et al.* 1997, Yadav *et al.* 1990 and Meena *et al.* 2013). Seed yield showed highly positive correlation with HIS ($r=0.58^*$) and negative correlation with HTE ($r=-0.57^*$) under normal sown conditions. On the contrary, seed yield had negative correlation with HIS ($r=-0.64^*$) but positive correlation with THE ($r=0.64^*$) in late sown crop. In the normal sown crop highly positive correlation existed between biomass and HSI ($r=0.57^*$) but biomass was negatively correlated with HTE ($r=-0.57^*$). Biomass was negatively correlated with HSI ($r=-0.38$) but positively related with HTE ($r=0.38$) under late sown conditions. Results of our study have showed a parallelism with the recent findings of Sharma *et al.* 2013. They reported that minimum yield reduction was realized in the genotypes which had the highest HSE and the lowest HSI. In parallel most of the findings (Ozkan *et al.* 1998, Golabadi *et al.* 2006, Sio-Se *et al.*, 2006) showed that genotypes with lowest HSI was most

tolerant than the highest HSI. In the same way Sharma *et al.* (2011, 2012) have reported heat susceptible varieties had high values ($HSI > 1$) while resistant varieties had lower values. Conclusively, HSI and HTE are the most important resistant parameters to evaluate genotypes under high temperature. These indices can be easily used to find heat tolerant/ resistant genotypes in the mustard breeding programmes. Interestingly, cultivars NRCDR-2 and CS10-5-2-SP were thermo tolerant as seedling mortality was less than 30% and were also rated as moderately tolerant to terminal heat stress.

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