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Deciphering selection criteria for Indian mustard (*Brassica juncea* L.) encountering high-temperature stress during post-reproductive phase

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Abstract
A set of 124 genotypes, which includes released varieties, and diverse fixed lines developed using indigenous and exotic germplasm through various breeding methods, were used to screen for terminal heat stress tolerance. These genotypes along with 5 checks were grown in augmented complete block design in early, timely and late sown conditions. The per cent reductions in mean values of traits recorded in late sown condition in comparison to early sown condition were in decreasing order as: yield per plant (48.6%), thousand seed weight (26.1%), number of primary branches (23.5%), number of siliquae on main shoot (20.8%), and oil (5.9%) whereas, per cent reductions in late sown condition in comparison to timely sown condition were: yield per plant (53.9%), number of siliquae on main shoot (24.3%), thousand seed weight (22.1%), number of seeds per siliqua (12.4%), number of secondary branches (12.4%), siliqua length (11.8%), plant height (6%) and percent oil (3%). Yield per plant was positively and significantly correlated with thousand seed weight (0.39^{**}, 0.58^{**}), number of siliquae on main shoot (0.31^{**}, 0.24^{**}), and number of primary branches (0.26^{**}, 0.18^{*}) in timely and late conditions respectively. Number of seeds per siliqua and yield per plant were non-correlated in timely sown (0.06) condition whereas in the late sown condition it showed significant positive association (0.25^{*}). Under timely sown condition highest direct effect was exerted by 1000 seed weight (0.40) followed by number of seeds per siliqua (0.27), number of secondary branches (0.26), etc Whereas under late sown condition, thousand seed weight (0.47), number of seeds per siliqua (0.20), plant height (0.18), number of secondary branches (0.12) etc.

Keywords: Per cent reductions, traits associations, regression, path analysis and heat susceptibility index (HSI)

Introduction
Oilseeds have an important role in the Indian economy and livelihood. Domestic consumption of edible oils has increased substantially over the years and has touched the level of more than 25 mt in 2017-18, which is likely to increase further with enhancement in per capita income and population. The production of domestic edible oils (10.5 mt) has not been able to keep pace with the growth in consumption; and the gap between production and consumption is being met through imports (15.3 mt) worth of about Rs.74,000 crores (MoA, annual report, 2018-19) [8] which is a huge drain of the foreign exchange reserve of India. So, there is a strong need for increasing domestic oilseed production by mitigating the production constraints without neglecting the challenges of climate change in the 21st century to meet the increasing demand for vegetable oil.
Heat stress due to high ambient temperatures is a serious threat to crop production worldwide (Hall, 2001) [5]. Global earth temperatures have increased by 0.74 °C during the twentieth century, and are likely to increase by 1.1–6.4 °C by the end of the 21st century (IPCC, 2013) [6]. In Asia and the Middle East, crop yields are predicted to fall by 15–35% if the average temperature increases by 3–4 °C (Ortiz *et al.*, 2008) [11]. However, for many crops, including rice, maize, soybean, legumes, sunflower, tomato and brassicas, the reproductive stage appears to be even more vulnerable to temperature increase (Jagadish *et al.*, 2014) [7].
Brassica spp., commonly known as rapeseed-mustard, plays an important role in the Indian economy by providing edible oils, vegetables, condiments and animal feed. Rapeseed-mustard

(8.3 mt) is the third most important annual oilseed crop in India, next to soybean (13.6 mt) and groundnut (9.1 mt) (MoA, annual report, 2018-19) [8]. Rapeseed-mustard grows under diverse agro-ecological situations such as timely, late, rainfed, irrigated, sole and or mixed crop where high temperature is the main constraint not only at germination but also at grain filling stage. Sowing of Indian mustard is highly dependent on the available soil moisture and most of the sowing is done in the soils with conserved moisture. Availability of soil moisture and vacation of fields forces the farmers to stagger the sowing beyond the optimum conditions, therefore, Indian mustard encounters differential high-temperature stress at different stages. Since 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) [17]. 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. It might be so because, the gene constellations responsible for conferring thermo-tolerance at germination stage are different from those conferring tolerance at seedling stage in the Indian mustard genotypes studied (Azharudheen *et al.*, 2013) [1]. More than 90% of the area under oilseed *Brassicas* in India is occupied by the Indian mustard (*Brassica juncea*, 2n=36) because of its relative tolerance to biotic and abiotic stresses in comparison with other oilseed *Brassica* species. Due to the changing climate, the temperature during the last 15 years, except for 2010, was above this limit in the major rapeseed-mustard growing areas of the country.

Areas where assured irrigation is there, high cropping intensity is being followed and mustard is one of the crops in such cropping systems. In Central and North-Western plain zones, where multiple cropping system followed sowing of the mustard crop is delayed up to end of November due to the late vacation of *Kharif* crop which leads to exposure of the crop to high temperature at the time of maturity. Growth and development of late sown crop is more adversely affected by severe winter, foggy and frost conditions during the vegetative stage and high temperature during pod and seed filling stages. It has been estimated that every 1 °C temperature increases from the range optimal for growth and development during the pod setting of canola cause a 10% yield reduction (Nuttall *et al.*, 1992) [10]. Chauhan *et al.* (2009) [2] studied effects of heat stress during the terminal stage on Indian mustard (*Brassica juncea* L.) and reported the reduction in seed yield and its components ranged from 22.2% for seeds/ siliqua to 69.2% for seed yield/plant.

Identification of suitable genotypes and management practices to sustain crop productivity under the climate change scenario is very essential. Many morpho-physiological traits have been suggested for terminal heat tolerance in other crops but the information for *Brassica juncea* is very meager, though a lot of emphases have been made on drought and biotic stresses. Genotypic variability for terminal high-temperature tolerance in Indian mustard is available at IARI, New Delhi. However, these genotypes have not been characterized by different temperature regimes. So the present study was to characterize genotypes and terminal thermotolerance responsive traits which could be helpful to plant breeders in selection for terminal heat tolerant genotypes.

Materials and Methods

A set of 124 genotypes which includes released varieties, and fixed diverse lines developed involving indigenous and exotic germplasm through pedigree selection were used. These genotypes along with five checks were screened during 2016-17 for early, timely and late sown conditions under augmented complete block design at the experimental farm of IARI, New Delhi. The sowing times for early, timely and late sown conditions were the 2nd week of September, 3rd week of October and 2nd week of November respectively. The late sown crop faced an average maximum temperature of >30 ºC at the grain filling stage. Weather data report

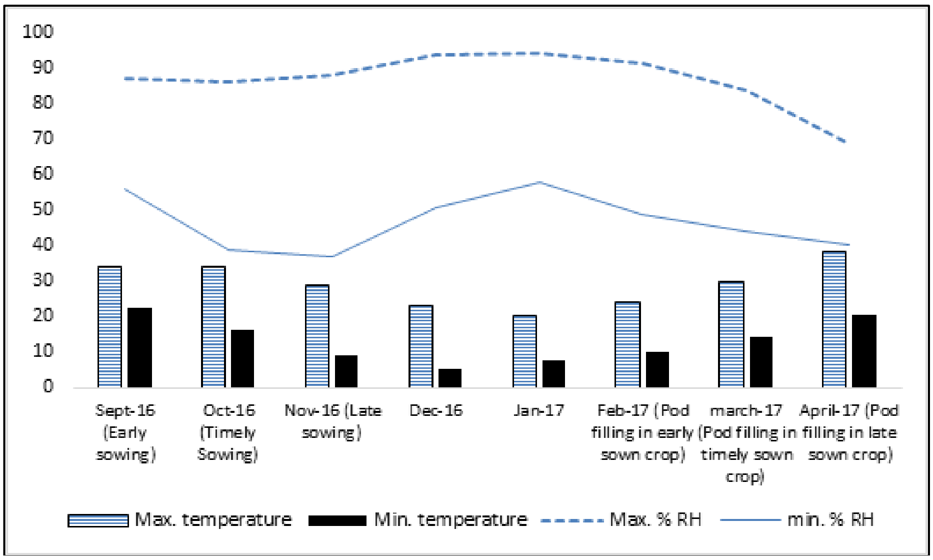


Fig 1: Weather conditions during experiment season.

during the experiment season (*Rabi* 2016-17) is depicted in figure 1. Recommended agronomic package and practices were applied to raise a healthy crop. Data were recorded on five random plants from each entry and their mean was taken for statistical analysis. Adjusted means, obtained from augmented analysis were used for further statistical analysis. Correlations among the yield attributing traits were calculated as par Robinson *et al.*, (1951) [13] and path coefficient analysis was accessed as per Dewey and Lu (1959) [3] using OPSTAT software. The heat susceptibility index (HSI), which determines the adaptability of genotypes to the late sown conditions, was computed using the following formula given by Fisher and Maurer (1978) [4]:

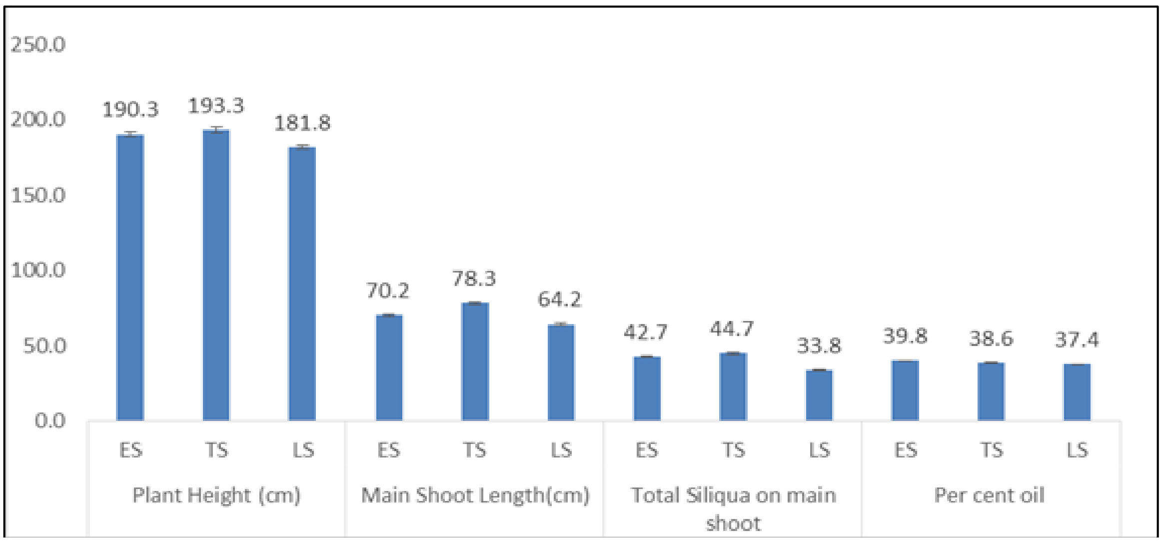
$$HSI = \frac{1 - (\frac{Yt}{Yc})}{1 - (\frac{Ytm}{Ycm})}$$

Where,
Y_t = seed yield/plant of a genotype under the late sown condition,
Y_c = seed yield/plant of a genotype under the timely sown condition,
Y_{tm} = mean seed yield/plant of genotypes under late sown condition and
Y_{cm} = mean seed yield/plant of genotypes under the timely sown condition

Results and Discussion
The range, mean and CV of different traits in favourable (*i.e.* early and timely sown conditions) and unfavourable or terminal stress environments have been depicted in figure 2a, 2b and table 1. The distribution of values has been depicted via box plots which show the variability existing in the genotypes for the traits studied (Fig. 3). The per cent reductions in mean values of traits recorded in late sown condition in comparison to early sown condition were: yield per plant (48.6%), thousand seed weight (26.1%), number of primary branches (23.5%), number of siliquae on main shoot (20.8%), main shoot length (8.6%), per cent oil (5.9%) and plant height (4.5%) whereas, per cent reductions in late sown condition in comparison to timely sown condition were: yield per plant (53.9%), number of siliquae on main shoot (24.3%), thousand seed weight (22.1%), main shoot length (18.1%), number of seeds per siliqua (12.4%), number of secondary branches (12.4%), siliqua length (11.8%) and plant height (6%) (fig. 4). Reduction in these traits due to terminal stage high-temperature stress is probably related to decline in photosynthesis which eventually causes the plant not to reach to its genetic potential. Other researchers Chauhan *et al*., (2009) [2], Singh *et al*., (2014) [15] have also reported similar significant reductions in the traits. The reduction in siliquae on main shoot and seeds/siliqua could be due to floral sterility as > 27 ^{0}C temperature has been reported to induce floral sterility in canola (Morrison and Stewart 2002) [9] as well as development of flowers in to seedless parthenocarpic fruits &/or flower abortion on the stem due to high temperature (Young *et al*. 2004) [18]. Moreover, thousand seed weight is one of the most important determining factors of seed yield and the existence of large seeds that filled well, caused the yield to increase. Results of the present experiment in this regard were consistent with Chauhan *et al*., (2009) [2]. Probably, heat stress through disrupting the plant photosynthesis, decreased assimilates synthesis which is necessary for seed filling, and consequently, it resulted in seed shrinkage and weight loss. In general, the reaction of crops and their evaluation for an optimum yield under different environmental conditions depend on their ability to use the said conditions.

Table 1: Range, mean and C.V of yield attributing traits of different genotypes under early (ES), timely (TS) and late sown (LS) conditions

Traits	Sowing time	Range	Mean±SE	CV	Traits	Sowing time	Range	Mean±SE	CV
Plant height (cm)	ES	141.8-236.7	190.3±1.9	11.1	Siliqua length (cm)	ES	2.4-6.1	3.9±0.05	14.2
	TS	151.8-247.5	193.3±1.8	10.5		TS	3.4-5.8	4.7±0.04	9.9
	LS	145.2-230.63	181.8±1.5	9.2		LS	3-7.2	4.1±0.04	12.2
No. of primary branches	ES	3.6-10.7	6.5±0.1	20.7	No. of seeds per siliqua	ES	10.5-16.5	13.9±0.1	8.7
	TS	-3.420.5	5.3±0.1	29.2		TS	11.9-19.1	15.9±0.1	9.6
	LS	3.1-9.08	5.0±0.1	17.8		LS	7.1-16.6	13.9±0.1	9.1
No. of secondary branches	ES	2-14.1	9.2±0.2	29.9	1000 seed weight	ES	3.6-7.5	5.3±0.1	16
	TS	6.1-14.5	10.2±0.2	18.4		TS	2.3-7.2	5.0±0.1	19.4
	LS	4.6-15.4	8.9±0.2	17.2		LS	1.7-5.8	3.9±0.1	19.4
Main shoot length (cm)	ES	44.4-93.3	70.2±0.8	12.4	Yield per Plant	ES	3.7-32.7	13.9±0.5	42.4
	TS	56.5-108.2	78.3±0.9	12.6		TS	2.6-28	15.5±0.5	34.7
	LS	32.7-93.3	64.2±1.0	17.9		LS	0.8-13.1	7.2±0.2	34
No. of siliquae on main shoot	ES	29.3-62.3	42.7±0.6	16	Per cent oil	ES	37.7-41.8	39.8±0.1	2.3
	TS	26.6-58.9	44.7±0.6	14.1		TS	35-40.9	38.6±0.1	3
	LS	21.9-53.5	33.8±0.6	19.2		LS	34.6-39.7	37.4±0.1	3.1



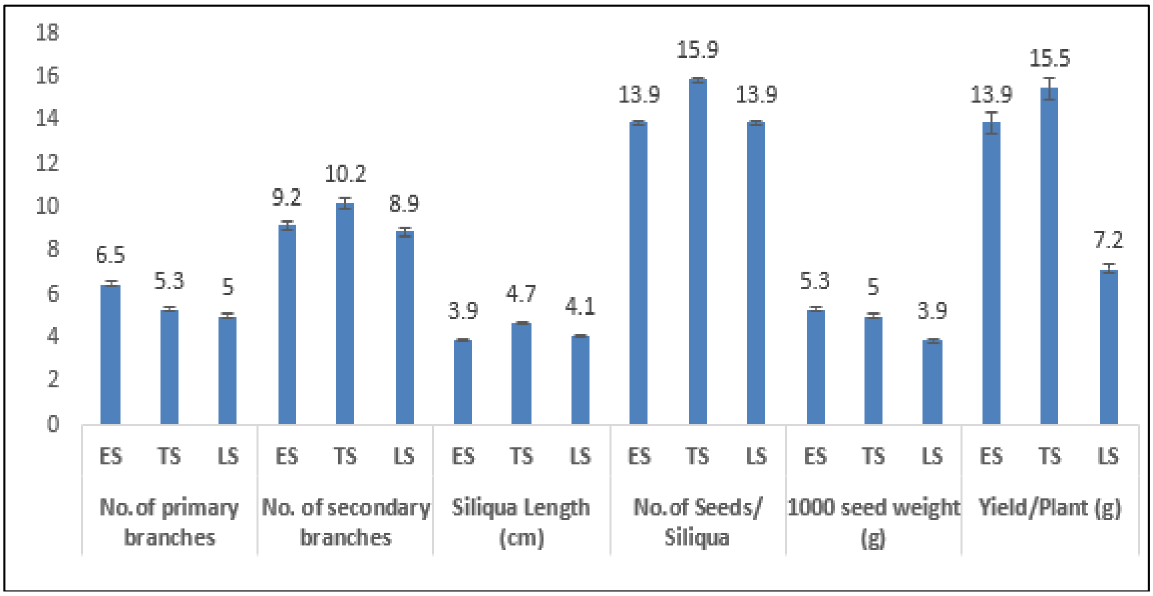


Fig 2a and 2b: Mean values of yield attributing traits in early sown (ES), timely sown (TS) and late sown (LS) condition

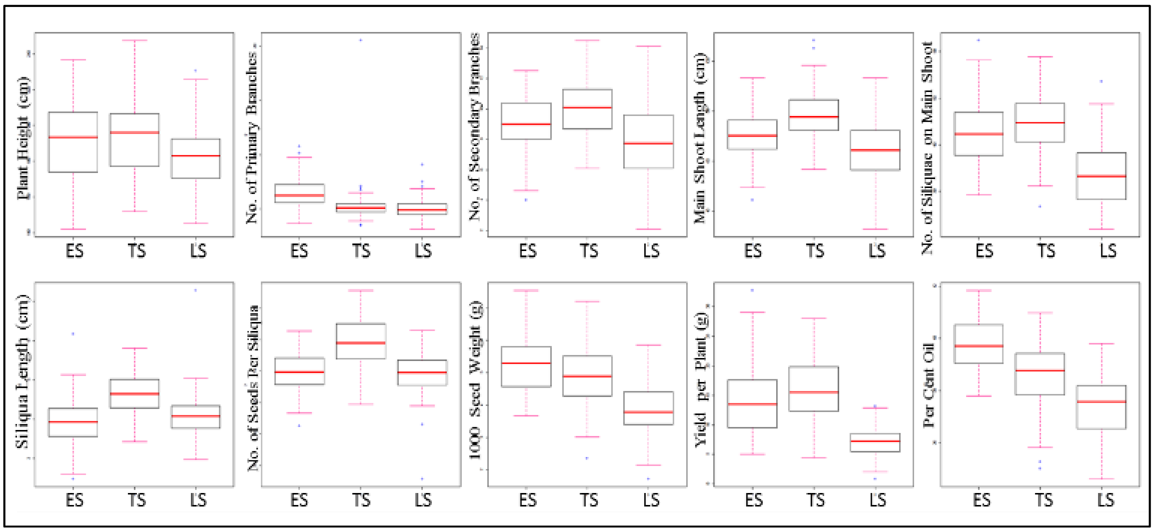


Fig 3: Box plots of all the traits (horizontal line in the box: median)

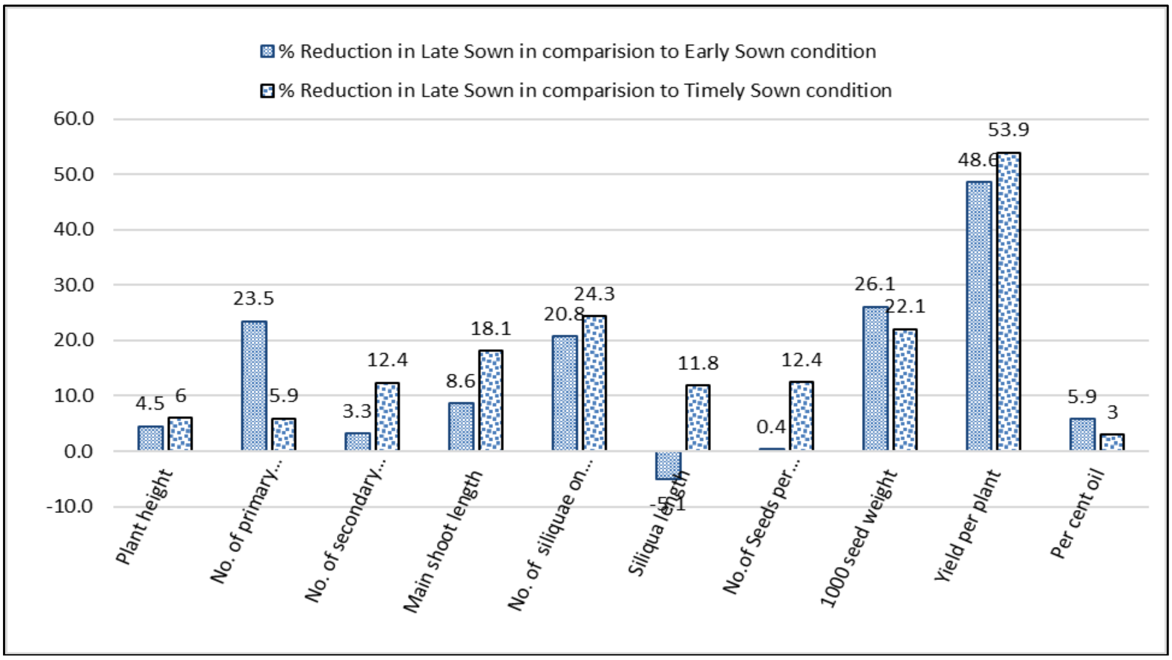


Fig 4: Per cent change in the mean values of yield contributing traits under terminal high temperature in unfavourable environment (*i.e.* late sown) comparison to favourable environments (*i.e.* early and timely sown)

Grain yield, being a complex trait, depends upon yield contributing component traits and their interaction with the environment. Correlation studies provide a better understanding of yield component which helps the plant breeder during selection (Robinson *et al*., 1951) [13]. Yield per plant was positively and significantly correlated with thousand seed weight (0.39^{**}, 0.58^{**}), number of siliquae on main shoot (0.31^{**}, 0.24^{**}), and number of primary branches (0.26^{**}, 0.18^{*}) in timely and late conditions, respectively (Table 2). Number of seeds per siliqua and yield per plant were non-correlated in timely sown (0.06) condition, whereas, in the late sown condition it showed significant positive association (0.25^{*}). In converse to this, main shoot length and yield per plant were significantly and positively correlated in timely sown condition (0.19^{*}), whereas, a non-significant association (0.14) was recorded in terminal heat stress (late sown) condition (Table 2). These reports were in accordance with earlier reports of Singh *et al.* (2013) [15] and Roy *et al.* (2018) [14].

Correlation alone is not sufficient when more characters are involved in the correlation analysis study. It is apparent that many of the characters are correlated because of a mutual association, positive or negative, with other characters. As more variables are considered in the correlation tables, their indirect associations become more complex, less obvious and somewhat perplexing. Under such conditions, the path coefficient analysis provides an effective means of separating the direct and indirect cause of association. In order to identify yield contributing traits with higher direct effects in terminal heat stress condition and normal condition path analysis was carried out of timely and late sown genotypes taking yield per plant as the dependent variable (Table 3).

Table 2: Correlation coefficients among yield and yield contributing traits in timely (TS) and late sown (LS) conditions.

1	Sowing time	No. of primary branches	No. of secondary branches	Main shoot length	No. of siliquae on main shoot	Siliqua length	No. of seeds/ siliqua	1000 seed weight	Yield per plant	Per cent oil
Plant height	TS	0.09	0.16	0.42^{**}	0.45^{**}	-0.1	-0.40^{**}	0.20^{*}	0.14	-0.1
	LS	0.31^{**}	0.17^{**}	0.27^{**}	0.37^{**}	-0.03	0.04	0.50^{**}	0.43^{**}	0.03
No. of primary branches	TS		0.26^{**}	-0.01	0.18^{*}	-0.12	-0.12	0.03	0.26^{**}	-0.01
	LS		0.04^{**}	0.15	0.34^{**}	-0.02	0.1	0.19^{*}	0.18^{*}	0.15
No. of secondary branches	TS			0.06	0.05	-0.17	0.02	0.09	0.38^{**}	0.07
	LS			-0.03	-0.09	-0.24^{**}	0.18^{*}	0.06	0.03	0.08
Main shoot length	TS				0.54^{**}	-0.01	-0.1	0.35^{**}	0.19^{*}	0.12
	LS				0.61^{**}	0.03	-0.08	0.16	0.14	0.05
No. of siliquae on main shoot	TS					-0.18^{*}	-0.16	0.20^{*}	0.31^{**}	0.16
	LS					0.14	0.04	0.19^{*}	0.24^{**}	0.1
Siliqua length	TS						0.47^{**}	0.1	-0.10	0.001
	LS						0.07	-0.16	-0.06	-0.19^{*}
No. of seeds/ siliqua	TS							-0.22^{*}	0.06	0.24^{**}
	LS							0.04	0.25^{**}	-0.08
1000 seed weight	TS								0.39^{**}	0.08
	LS								0.58^{**}	0.12
Yield per plant	TS									0.16^{*}
	LS									0.16

Table 3: Path analysis of timely and late sown condition to find out direct and indirect effects of different yield contributing traits on yield per plant

		Plant height (cm)	No. of primary branches	No. of secondary branches	Main shoot length (cm)	No. of siliquae on main shoot	Siliqua length (cm)	No. of seeds/ siliqua	1000 seed weight	Per cent oil
Plant height (cm)	TS	0.02	0.01	0.04	-0.02	0.10	0.02	-0.11	0.08	0.00
	LS	0.18	-0.01	-0.02	-0.01	0.04	0.00	0.01	0.24	0.00
No. of primary branches	TS	0.00	0.15	0.07	0.00	0.04	0.02	-0.03	0.01	0.00
	LS	0.06	-0.05	0.01	-0.01	0.04	0.00	0.02	0.09	0.02
No. of secondary branches	TS	0.00	0.04	0.26	0.00	0.01	0.03	0.01	0.04	0.00
	LS	-0.02	0.00	0.12	0.00	0.00	-0.01	0.03	-0.04	0.01
Main shoot length (cm)	TS	0.01	0.00	0.02	-0.05	0.12	0.00	-0.03	0.14	0.00
	LS	0.05	-0.01	0.01	-0.05	0.07	0.00	-0.02	0.08	0.01
No. of siliquae on main shoot	TS	0.01	0.03	0.01	-0.03	0.21	0.03	-0.04	0.08	0.00
	LS	0.07	-0.02	-0.01	-0.03	0.12	0.00	0.01	0.09	0.01
Siliqua length (cm)	TS	0.00	-0.02	-0.05	0.00	-0.04	-0.16	0.13	0.04	0.00
	LS	0.00	0.00	-0.02	0.00	0.02	0.03	0.01	-0.08	-0.02
No. of seeds/ siliqua	TS	-0.01	-0.02	0.01	0.01	-0.03	-0.08	0.27	-0.09	0.01
	LS	0.01	0.00	0.02	0.00	0.00	0.00	0.20	0.02	-0.01
1000 seed weight	TS	0.00	0.00	0.02	-0.02	0.04	-0.02	-0.06	0.40	0.00
	LS	0.09	-0.01	-0.01	-0.01	0.02	-0.01	0.01	0.47	0.01
Per cent oil	TS	0.00	0.00	0.02	-0.01	0.03	0.00	0.07	0.03	0.03
	LS	0.01	-0.01	0.01	0.00	0.01	-0.01	-0.02	0.06	0.11

Under timely sown condition highest direct effect was exerted by 1000 seed weight (0.40)which is followed by number of seeds per siliqua (0.27), number of secondary branches (0.26), number of siliquae on main shoot (0.21) and number of primary branches (0.15). Whereas, under terminal heat stress conditions the descending order of direct effects was as thousand seed weight (0.47), number of seeds per siliqua (0.20), plant height (0.18), number of secondary branches (0.12) and number of siliquae on main shoot (0.12). These are the traits which can be used for direct selection for higher yield per plant in normal and terminal heat stress conditions. These results are similar to the reports of Srivastava and Singh (2002) [16], Panth *et al*. (2002) [12] and Singh *et al*. (2013) [15].

Heat susceptibility index (HSI) for the genotypes was calculated using the seed yield per plant of timely and late sown conditions. A linear regression analysis was carried out to quantify the strength of the relationship between the yield per plant (as the dependent variable) and the HSI (as the independent variable). The linear equation obtained was $Y = -3.2x + 10.2$ with R^2=0.29 ($P<0.001$). This showed that HSI is effective in identifying high yielding cultivars under high temperature conditions. The slope is negative for this relationship; it means a lower HSI value is an indicator of higher tolerance to high temperature stress (Fig. 5). Based on this index, G68 (NPJ-112/ NPJ-176), G79 (Varuna/RH 7003//NPJ-112), G102 (NPJ-112/ NPJ-176), G34 (SEJ-8 / NPJ-176) and G25 (PusaAgrani/PusaTarak) were identified as superlative genotypes in respect to terminal high temperature tolerance. These genotypes can further be utilised in breeding programmes for terminal high-temperature tolerance. A similar relationship was also utilised by Singh *et al*., (2014) [15] to identify terminal high-temperature tolerance among genotypes.

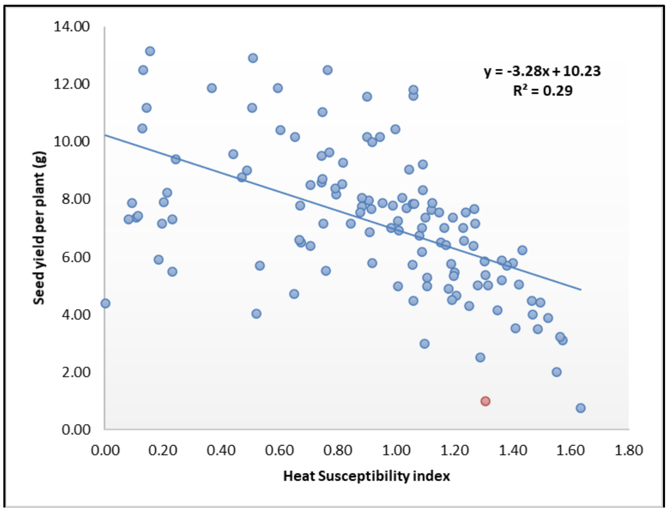


Fig 5: Linear regression of seed yield per plant with heat stress susceptibility index under terminal heat stress condition

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