

Evaluating thermotolerant sunflower genotypes with temperature induction response (TIR) technique

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ABSTRACT

High temperature affects various physiological processes of the plant. Delayed sowing and changing climate both subject the crop to increasing temperatures during the crop growth period. There is a need to take on a technique to screen the wide number of genotypes for high-temperature tolerance. In the present study, a screening protocol was followed based on the principle of “acquired tolerance” in which 47 sunflower seedlings were exposed to sub-lethal heat stress to induce tolerance before subjecting to subsequent lethal stress and the second set were directly exposed to lethal stress. Significant variation was observed for the traits - survival percentage, total seedling length, and seedling weight. Tolerant inbreds were identified using Z distribution and PCA. Results suggested that TIR is a rapid and powerful technique that can be used to screen large number of germplasms to identify thermotolerant lines.

Introduction

Sunflower (*Helianthus annuus* L.) crop is grown worldwide under a wide range of agro-environments. The world Sunflower area accounts for 27.37 m ha, production of 56.07 m t, and productivity of 2049 kg ha⁻¹ (FAOSTAT, 2019). India is 0.26 m ha, with a production of 0.22 m t and productivity of 826 kg ha⁻¹. In India, sunflower cultivation is concentrated mainly in the states, of Karnataka, Maharashtra, Odisha, Andhra Pradesh, and Haryana. Sunflower is primarily grown for its edible oil, protein-rich residual cake for livestock as both irrigated and rainfed crop. Because of its low input requirement and limited GHG emission, it is labeled as an “environmentally friendly crop” (Debaeke *et al.*, 2017). Induced stress tolerances to high temperature (HT) are complex traits dependent on many attributes (Harihar *et al.*, 2014). Heat stress (HS) alters the morpho-physiological processes resulting oxidative stress by reactive oxygen species

(ROS) (Hasanuzzaman *et al.*, 2012). 25–30°C is the optimal temperature for sunflower germination and growth, while temperatures exceeding 30°C pose stress on the plant (Qadir *et al.*, 2007). The development of a heat-resistant sunflower breeding population is important for sustainable yield under HS. Plants can tolerate heat stress by physical and biochemical modifications by gene expression changes to some extent (Moreno and Orellana, 2011). Plants show strong responses to HS depending upon the duration, extremity, and also surrounding environmental factors, but the traits identification to HS tolerance should be consideration. Plant scientists are aiming to discover the responses that lead to HT and to look into how the crop can be sustained in HT environments. The objective of this study was 1) to evaluate the performance of sunflower inbreds under increasing temperature at the seedling stage and to correlate the

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data with yield under field condition, 2) to investigate variability in the response of inbreds under induced and lethal treatments, and 3) to identify the tolerant lines under heat stress at seedling.

Material and Methods

Plant Material

A set of 47 promising parental lines of sunflower (*Helianthus annuus* L.) genotypes including 42 inbreds and 5 hybrid checks were used as experimental materials to assess their tolerance to temperature at seedling stage.

Experimental conditions

The lab experiment was conducted during *rabi*, 2021 in ICAR-IIOR, Rajendranagar for a period of 7 days using a factorial CRD design to evaluate the thermotolerance in sunflower seedlings of 0.5 cm radicle length, i) control seedlings maintained at 25 °C ii) induction seedlings were subjected to a sub-lethal level of temperature stress from 35°C for 1h, 40°C for 2h and 45°C for 1h (induction treatment) and then to a subsequent lethal level of 49°C for 2h and allowed to recover for 72 hours at room temperature (25°C) iii) Lethal seedlings with control seedlings directly subjected to the lethal temperature of 49°C for 2h and allowed to recover (Fig 1). Earlier studies indicate that the TIR technique is an effective technique to identify genotypes tolerant to HT in various crops (Kheir *et al.*, 2012), and therefore, adopted in the present study.

Morphological and yield

Data on morphological parameters such as survival percentage (SP), shoot length (SL), root length (RL), total seedling length (TSL), seedling weight (SW), and seed vigor index (SVI) were noted from 3 randomly selected sunflower seedlings of each genotype in the replication of each treatment and then averaged.

Statistical analysis

The data were summarized using descriptive statistics and analyzed using Z distribution and PCA. The trait's survival percentage and percent reduction in total seedling length are subjected to Z distribution analysis to identify the tolerant genotypes. In PCA the shoot length, root length, seedling weight, and survival measured in different units are standardized to eliminate the scale bias. Based on equation 1, the indices are computed using eigen values and factor loadings obtained from the analysis. Genotypes with the efficient performance of shoot length, root length, seedling weight, and survival are ranked top under induction and lethal treatments. PCA by R (version 3.1.3) was used to characterize trait variation. IBM SPSS was used for Z distribution and correlation.

$$I = \frac{\sum_{i=1}^n X_i [\sum_{j=1}^n |L_{ij}| \cdot E_j]}{\sum_{i=1}^n [\sum_{j=1}^n |L_{ij}| \cdot E_j]}$$

Where, I=Index, X_i = i^{th} indicator, L_{ij} =factor loading value of the j^{th} variable on the j^{th} factor, and E_j = eigen value of the j^{th} factor.

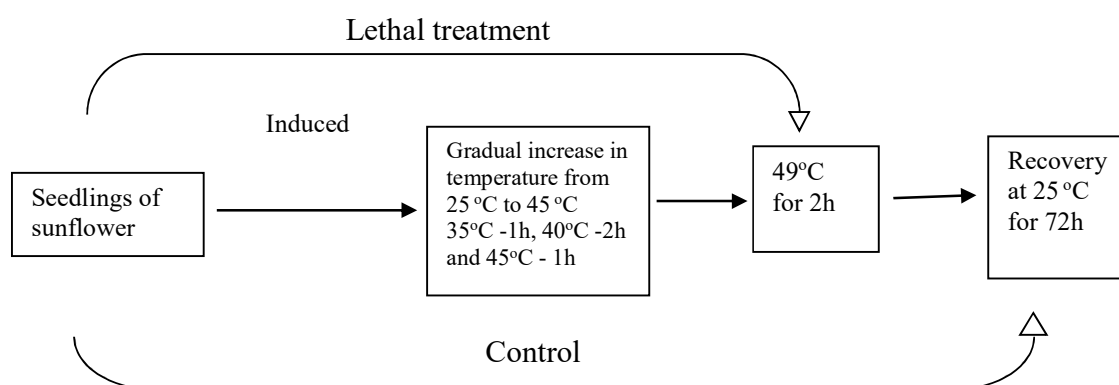


Fig 1. Protocol to study high-temperature stress tolerance through TIR technique

Results and Discussion

Among the 47 sunflower genotypes evaluated, the reduction in growth ranged from 24% in CMS 2023B to 82% in ARM 248B. CMS 2023B showed less reduction in recovery growth when compared to other lines. The induced seedlings of 47 genotypes performed better than the lethal seedlings as they are exposed slowly to the lethal temperature. TIR is a good technique to evaluate the genetic variability for HT tolerance from the earlier studies (Harihar *et al.*, 2014, Kheir *et al.*, 2012).

Genetic variability in sunflower upon induction treatment

Significant variation was observed among the temperature treatments, genotypes and interaction or the trait's SP, RL, SL, TSL, and SW. The genetic variability for thermo tolerance among the 47 sunflower genotypes was analyzed through Z distribution in which the whole set of genotypes is distributed into four different quadrants. Quadrant I included the genotypes with highest SP over control and are considered tolerant while quadrant IV has a

higher reduction percent in growth and is considered the most susceptible. CMS lines-17B, 58B, 59B, 127B, 135B, 144B, DSF2B, NDCMS 4B, NDL 3B, HA 291B and check DRSH 1 were grouped in quadrant I and showed a low reduction in growth and higher survival percentage (Fig. 2). Genotypes grouped in Quadrant II had the highest percent reduction, while genotypes grouped in quadrant III had a low survival percentage compared to the control, therefore, these were considered moderately tolerant genotypes. Genotypes AKSF 6-3B, CMS lines-107B, 108B, 122B, 234B, 275B, 302B, 519B, 607B, 850B, 2023B, Pet 2-7-1B, ARM lines-240B, 243B, HA 248B, HA 292B, NDL lines 5B, 6B, 7B, checks CO-2, CSFH 12205, KBSH 44 were grouped in quadrant II and III. The genotypes CMS lines-11B, 38B, 42B, 70B, 103B, 104B, 125B, 853B, NDCMS 2B, ARM lines- 248B, 249B, FMS 400B were grouped in quadrant IV as susceptible genotypes. The genotypes falling in quadrants I and IV distinctly differ in thermo tolerance. The genotypes classified based on Z distribution is presented in Supplementary table 1.

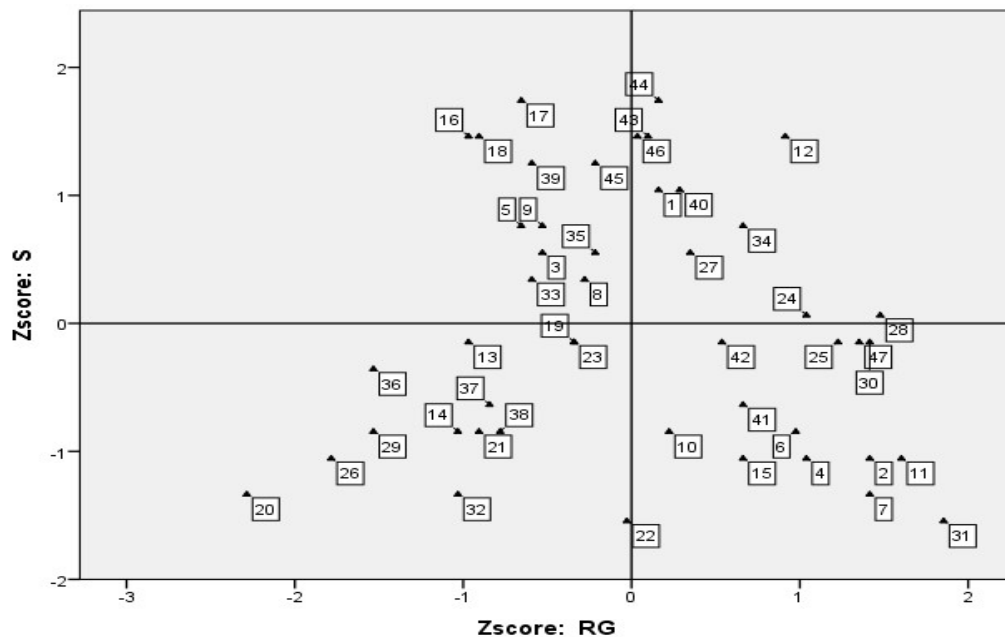


Fig 2. Classification of sunflower genotypes

Table 1 Genotypes found tolerant in Z distribution and PCA

Z distribution			PCA		
Tolerant	Moderately tolerant	Susceptible	Control	Induction	Lethal
CMS 17B	AKSF 6-3B	CMS 11B	CMS 42B	CSFH 12205	NDL 6B
CMS 58B	CMS 107B	CMS 38B	CMS 107B	CO 2	CMS 17B
CMS 59B	CMS 108B	CMS 42B	KBSH 44	CMS 127B	CMS 144B
CMS 127B	CMS 122B	CMS 70B	CMS 850B	ARM 240B	DSF2B
CMS 135B	CMS 234B	CMS 103B	CSFH 12205	CMS 58B	NDL 3B
CMS 144B	CMS 275B	CMS 104B	AKSF 6-3B	CMS 17B	CMS 58B
CMS DSF2B	CMS 302B	CMS 125B	ARM 243B	CMS 135B	CMS 275B
NDCMS 4B	CMS 519B	CMS 853B	CMS 70B	CMS 59B	CMS 59B
NDL 3B	CMS 607B	NDCMS 2B	CMS 108B	CMS 144B	CMS 122B
HA 291B	CMS 850B	ARM 248B	DRSH 1	NDL 3B	
DRSH 1	CMS 2023B	ARM 249B	CMS 144B		
	COSF 12B	FMS 400B			
	ARM 240B	RSFH 130			
	ARM 243B				
	HA 248B				
	HA 292B				
	NDL 5B				
	NDL 6B				
	NDL 7B				
	CMS Pet 2-7-1B				
	CO 2				
	CSFH 12205				
	KBSH 44				

The %SP varied from 43 to 87% under IT and from 0 to 57% under LT. Among the 47 genotypes screened, 11 showed >75% survival under IT. TSL varied from 4.1 to 12.9 cm under IT and genotypes CMS 108B, 135B, 144B, 275B, 302B, 2023B, ARM 249B, HA 248B, HA 292B, NDL 3B, 5B has shown TSL on par with checks. The %RRG ranged from 16 to 82. The genotypes CMS 275B (16%), and 2023B (27%), showed minimum % RRG (less than 30%), whereas the genotypes CMS 103B (78%), CMS Pet SP after IT indicating the seedling growth performance after the recovery period plays an important role in screening sunflower genotypes

2-7-1B (76%), NDCMS 2B (75%), CMS 42B (75%), ARM 243B (75%), CMS 853B (72%), recorded a maximum reduction compared to checks. Inbreds CMS 135B and 144B has recorded SP on par with checks and less reduction in SP under IT and LT. Among inbreds ARM 249B (948) followed by CMS 144B (772), CMS 135B (763), and among checks CSFH 12205 (901), KBSH 44 (828) has recorded maximum SVI under IT. A significant positive correlation was observed between SVI and (Fig 3). In previous studies also the tolerant, intermediate, and susceptible genotypes were screened based on percent recovery growth. The Z

distribution analysis demonstrated a standard method of identifying tolerant genotypes in rice (Vijyalakshmi *et al.*, 2015)

Principal Component Analysis (PCA)

The shoot length, root length, seedling weight, and survival measured in different units are standardized to eliminate the scale bias. The indices are computed through factor loadings and eigen values obtained from the analysis. Genotypes with the efficient performance of SL, RL, SW, and S are ranked top under induction and lethal treatments (Table). Among checks CSFH 12205 (1.2), CO 2 (1.2), and among inbreds CMS 127B (1), followed by ARM 240B (0.9), CMS 58B (0.8), CMS 17B (0.8), CMS 135B (0.7), CMS 59B (0.7), CMS 144B (0.7), NDL 3B (0.6) under induction treatment (Table 3).

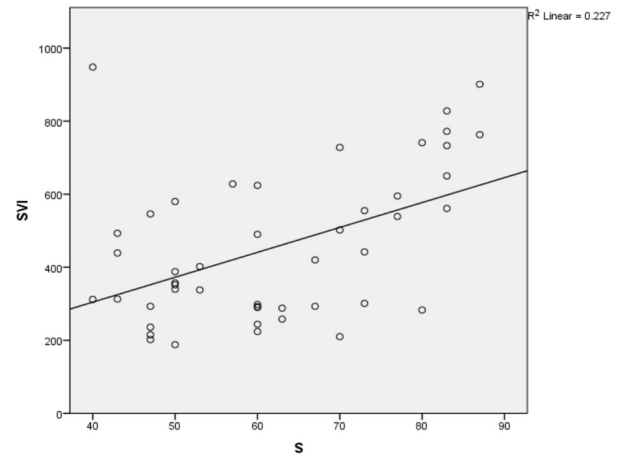


Fig 3. Correlation between seed vigor index and survival

Table 2: PCA index ranks at induction and lethal treatments and seed yield reduction rank under field condition

Ranks									
S.No	Genotypes	Induction rank	Lethal rank	SY rank	S.No	Genotypes	Induction rank	Lethal rank	SY rank
1	AKSF 6-3B	25	27	45	25	CMS 853B	11	9	23
2	NDCMS 2B	41	46	27	26	CMS 2023B	7	7	16
3	NDCMS 4B	18	27	13	27	CMS DSF2B	14	2	10
4	CMS 11B	39	28	9	28	CMS Pet 2-7-1B	14	7	11
5	CMS 17B	6	2	11	29	ARM 240B	3	3	5
6	CMS 38B	37	36	26	30	ARM 243B	15	18	3
7	CMS 42B	37	41	41	31	ARM 248B	17	17	5
8	CMS 58B	5	5	31	32	ARM 249B	6	4	11
9	CMS 59B	6	6	38	33	HA 248B	10	11	1
10	CMS 70B	33	20	9	34	HA 291B	6	9	9
11	CMS 103B	30	24	3	35	HA 292B	7	12	9
12	CMS 107B	26	22	32	36	NDL 3B	3	2	1
13	CMS 108B	21	10	29	37	NDL 5B	4	3	3
14	CMS 122B	16	6	17	38	NDL 6B	5	1	5
15	CMS 125B	27	26	19	39	NDL 7B	3	4	7
16	CMS 127B	3	14	31	40	COSF 12B	6	6	2
17	CMS 135B	4	13	29	41	FMS 400B	6	6	4
18	CMS 144B	4	2	30	42	104B	5	6	4
19	CMS 234B	10	16	12	43	CO 2	2	3	5
20	CMS 275B	5	4	21	44	CSFH 12205	1	1	2
21	CMS 302B	9	24	27	45	DRSH 1	1	1	2
22	CMS 519B	23	6	5	46	KBSH 44	1	2	2
23	CMS 607B	16	19	2	47	RSFH 130	1	1	1
24	CMS 850B	20	16	2					

Table 3: Seedling parameters of the selected inbreds and checks under induction and lethal treatments for indexing

Induction treatment							Lethal treatment					
S.No	Genotypes	Shoot length	Root length	Seedling Weight	Survival %	Index	Genotypes	Shoot length	Root length	Seedling weight	Survival %	index
1	CSFH 12205	78.1	24.4	94.8	86.7	1.2	NDL 6B	47.9	71.7	36.5	20	1.2
2	CO 2	92.3	17	92.1	83.3	1.2	CMS 17B	67	20.3	73.8	40	1.1
3	CMS 127B	72.8	46.9	85.5	83.3	1	CMS 144B	70.6	18.7	49	63.3	0.9
4	ARM 240B	98.8	49.5	93.9	50	0.9	DSF2B	32.7	24.4	80.7	56.7	0.8
5	CMS 58B	49.7	54	99.5	66.7	0.8	NDL 3B	47.9	49.5	48	10	0.7
6	CMS 17B	88.3	33.9	76.7	73.3	0.8	CMS 58B	33.9	25.6	86.6	26.7	0.7
7	CMS 135B	79.9	22.4	72.4	86.7	0.7	CMS 275B	41.2	41.4	64.1	10	0.7
8	CMS 59B	56.6	56.3	83	73.3	0.7	CMS 59B	30.1	27.2	74.3	46.7	0.7
9	CMS 144B	86.3	32.3	65.8	83.3	0.7	CMS 122B	39.1	51	42.9	13.3	0.6
10	NDL 3B	73.4	71.1	78.6	56.7	0.6	ARM 240B	43.4	13.7	86.6	23.3	0.5
11	CMS 275B	82.4	86.2	78.8	43.3	0.5	CSFH 12205	47.2	17.2	54.9	53.3	0.5
12	NDL 7B	66.7	38.3	68.9	80	0.5	CMS 519B	31.6	54.2	37.5	16.7	0.5
13	NDL 5B	65.7	55.9	84.3	53.3	0.5	CMS 108B	33.6	36.9	40.2	33.3	0.3
14	CMS 2023B	80.6	72.5	74.1	46.7	0.4	ARM 249B	51.6	12.3	75.6	0	0.2
15	ARM 249B	75.8	55.9	84.3	43.3	0.4	CMS 2023B	33.8	35.2	48.2	13.3	0.2
16	CMS 234B	44	61.7	84	60	0.3	NDL 5B	31.9	33	51.4	16.7	0.2
17	CMS 302B	55	65.6	84.4	50	0.3	CMS 853B	32.8	20.4	67.8	20	0.2
18	NDCMS 4B	54.7	59.4	68	70	0.3	CMS 135B	22.6	18.2	51.2	66.7	0.2
19	HA 291B	40.5	32.5	80.6	73.3	0.3	CMS 127B	35.1	24.8	33	56.7	0.2
20	CMS 122B	62.1	67.1	77.2	50	0.3	CMS Pet 2-7-1B	24.3	34.3	44.1	30	0.1
21	DRSH 1	67.8	26.4	60.8	80	0.3	DRSH 1	35	16.2	47.6	50	0.1
22	CMS 853B	32.4	23.1	94.8	60	0.2	CO 2	35.1	9.8	53.1	53.3	0.1
23	HA 292B	59.7	43	65.4	70	0.2	CMS 70B	43.1	24.9	41.8	20	0.1
24	NDL 6B	50	81.5	74.7	50	0.2	NDL 7B	38.1	18.3	37.1	46.7	0

Evaluating thermotolerant sunflower genotypes with temperature induction

25	AKSF 6 3B	60.4	25.8	58.3	76.7	0.1	CMS 234B	24.1	25.9	30.8	50	-0.1
26	CMS 108B	51.1	79	58.3	60	0	CMS 107B	43.1	9.6	30.6	56.7	-0.1
27	HA 248B	56.6	57.1	48.9	66.7	-0.2	AKSF 6 3B	22.6	14.2	50.7	46.7	-0.1
28	KBSH 44	57.9	28.2	42.5	83.3	-0.2	NDCMS 4B	32.1	21.7	46.8	16.7	-0.2
29	CMS 607B	36.4	71.6	56.2	60	-0.3	RSFH 130	19.7	27.2	25	50	-0.2
30	DSF2B	47.1	32.6	53	70	-0.3	CMS 11B	38.7	14.2	41.4	20	-0.3
31	CMS 107B	47.5	14.4	46.7	83.3	-0.3	CMS 103B	24.5	11.1	65.3	13.3	-0.3
32	CMS Pet 2-7-1B	34.5	11.6	69.1	63.3	-0.3	HA 291B	23.9	25	30.6	33.3	-0.3
33	104B	49.6	22.2	57.3	60	-0.4	CMS 850B	16.7	32.3	15	46.7	-0.4
34	COSF 12B	39.9	48.7	39.8	76.7	-0.4	KBSH 44	18.9	11.6	36.7	56.7	-0.4
35	CMS 103B	31	13.6	78.3	46.7	-0.5	HA 248B	29	22.3	21.3	33.3	-0.4
36	CMS 125B	54.8	22.3	57.2	46.7	-0.6	CMS 607B	17.4	27.9	28.9	26.7	-0.4
37	CMS 850B	23.1	40.1	52.3	63.3	-0.7	CMS 125B	39.9	17.3	28.1	13.3	-0.5
38	CMS 70B	58	27.2	46.5	50	-0.7	CBE COSF 12B	21.2	14.5	34.3	40	-0.5
39	ARM 243B	25.2	25.1	53.8	60	-0.8	FMS 400B	8.4	14.9	52.1	30	-0.5
40	CMS 38B	41	19.1	54.5	50	-0.8	CMS 38B	30.3	12.4	47.9	3.3	-0.5
41	CMS 11B	53	15.6	51.2	46.7	-0.8	HA 292B	18.9	14	41.8	23.3	-0.6
42	NDCMS 2B	29.6	20.1	60.4	46.7	-0.8	CMS 302B	31.6	10.4	33.3	16.7	-0.7
43	CMS 42B	29.5	21.4	60.6	43.3	-0.9	104B	27.1	9.7	33.7	23.3	-0.7
44	CMS 519B	34	71.7	45.4	40	-0.9	ARM 248B	25.8	7	46.7	3.3	-0.8
45	FMS 400B	42.1	32.7	30	53.3	-1.1	ARM 243B	12.6	18.1	26.4	26.7	-0.8
46	RSFH 130	22.8	30.6	35.4	60	-1.1	CMS 42B	20.8	14.5	24.5	20	-0.8
47	ARM 248B	26.1	9.1	49.3	40	-1.3	NDCMS 2B	22.2	8.6	26.8	10	-1

Among checks CSFH 12205 (1.2), CO 2 (1.2), and among inbreds CMS 127B (1), followed by ARM 240B (0.9), CMS 58B (0.8), CMS 17B (0.8), CMS 135B (0.7), CMS 59B (0.7), CMS 144B (0.7), NDL 3B (0.6) under induction treatment (Table 3), checks CSFH 12205 (0.5) and among inbreds, NDL 6B (1.2) followed by CMS 17B (1.1), CMS 144B (0.9), CMS DSF2B (0.8), NDL 3B (0.7), CMS 58B (0.7), CMS 275B (0.7), CMS 59B (0.7), CMS 122B (0.6) under lethal treatment (Table 1) recorded higher index of >0.5. Genotypes that were found tolerant in PCA were found moderately tolerant to tolerant under Z distribution (Table 3).

Survival and recovery growth

Different abiotic stresses affect crop growth and productivity thus reducing the yield. Over the years considerable efforts have been made to determine the adaptive mechanism of crops to heat stress and to identify traits, which would help in the selection of identification of tolerant genotypes for temperature stress. In the present study, despite the exposure of inbreds to IT and LT, the survival percentage differed amongst the inbreds (Table 2) because of variations in the stress adaptive mechanisms for thermo tolerance among the genotypes. Earlier standardized the TIR protocol has been modified (ACRIP annual report 2014-2015) for sunflower. The modification was followed to screen 42 inbreds and 5 checks of sunflower in the present study for cellular level tolerance. A similar study was conducted in other crops such as groundnut, cotton (Kheir *et al.*, 2012), and banana (Vidya *et al.*, 2017). In field conditions, plants acquire thermotolerance to go through subsequent LT as in pulses (Partheeban *et al.*, 2017), sunflower (Senthil Kumar *et al.*, 2007). The higher TSL of induced seedlings as a consequence of altered metabolism is seen in a sunflower (Senthil Kumar *et al.*, 2007),

Several physiological and biochemical processes occur in response to IT. Further, the threshold temperature for induction and lethal tolerance differs among species as in sunflower, 49°C for 2 h (Senthil Kumar *et al.*, 2007), in genetic variability for acquired thermotolerance can be observed after exposure to IT for the traits SP, TSL, and SW (Table 3). IT alters the gene expression and thus accompanies greater adaptation and genetic variability to HT (Table 3). This could be due to the different inbreds used which vary in their acquired thermotolerance, which plays an important role as reported in sunflower (Senthil-Kumar *et al.*, 2007); cotton (Kheir *et al.*, 2012). As expected, differences in stress response were noticed among sunflower genotypes. Genetic variation in SP during recovery from LT ranged significantly ($P < 0.01$) from 0 to 66.7 % in non-induced seedlings and 40 to 86.7% in induced seedlings. The genetic variation in SL and SW has shown the same trend. The variability in basal tolerance was narrow for the trait's SL and SW, while the variability in acquired tolerance was narrow for SP (Table 3). The thermotolerant genotypes selected based on the TIR technique have less yield reduction in field conditions under HT indicating their tolerance at the plant level;. Selection based on SP, TSL, and %RG at the seedling stage is more convenient as it is a reflection of the sum of variation in intrinsic tolerance brought by different tolerance mechanisms (Senthil-kumar *et al.*, 2007). With IT, CMS 135B (87%), CSFH 12205 (87%), KBSH 44 (83%), CO 2 (83%), CMS 107B (83%), CMS 127B (83%), CMS 144B (83%), DRSH 1 (80%), AKSF 6-3B (77%) has recorded survival percentage of >75% indicating the effect of IT on the survivability even at LT. The use of the TIR approach is relevant only when there is adequate genetic variability in the genotypes under study.

Table 3: Genetic variability in thermo tolerance among 47 sunflower genotypes

	Survival (%)		Seedling length		Seedling weight	
	Induced	Non-induced	Induced	Non-induced	Induced	Non-induced
Mean	62.1±2.1	30.8±2.6	7.2±0.3	4.1±0.2	1.3±0.1	0.9±0.04
Max	86.7	66.7	12.9	7.1	2.5	1.6
Min	40	0	3.4	1.6	0.6	0.3

Conclusion

Here we demonstrate the TIR technique as a reliable and rapid screening technique for the preliminary evaluation of the large number of sunflower genotypes for heat tolerance at a very early stage and to assess the genotypic variability in acquired thermotolerance. It helps to narrow down the genotypes by grouping them into tolerant, moderately tolerant, and susceptible genotypes. In this investigation using the TIR approach it is found that AKSF 6-3B, CMS lines 59B, 107B, 127B, 135B, and 144B were consistently found as tolerant and inbreds HA 248B, CMS lines 103B, 607B as susceptible for HT stress. Using this technique it was also evident that there is sufficient genetic variability

present among sunflower lines at 49 °C. Lines found tolerant to high temperatures are useful in breeding programs seeking to overcome this yield limitation. Tolerant inbreds can be studied further to identify the candidate genes for high-temperature tolerance.

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Conflict of interest

The authors declare that they have no conflict of interest.

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