



ASSESSMENT OF GENETIC VARIABILITY FOR SEED VIGOUR UNDER HIGH-TEMPERATURE STRESS IN SUNFLOWER USING TEMPERATURE INDUCTION RESPONSE (TIR) TECHNIQUE

T. Raghavendra^{1*}, B.V. Ravi Prakash Reddy², S. Rama Devi³, R. Naseeruddin⁴ and K. Venkata Ramanamma⁵

¹Department of Crop Physiology, ²Department of Genetics & Plant Breeding, ³Department of Genetics & Plant Breeding, ⁴Department of Agronomy, ⁵Department of Plant Pathology, Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Nandyal - 518 501, Andhra Pradesh (India)

*e-mail: t.raghavendra@angrau.ac.in

(Received 9 June, 2026; Accepted 31 August, 2026)

ABSTRACT

Climate change and global warming are intensifying the frequency and severity of heat stress, posing a major threat to crop growth, development, and productivity. Rising temperatures adversely affect key physiological and biochemical processes, particularly during critical stages of crop development. In this context, rapid and reliable screening techniques are essential for evaluating large numbers of genotypes and identifying heat-tolerant sources. The present study was conducted to evaluate the response of thirty sunflower genotypes to temperature induction under laboratory conditions and to identify potential heat-tolerant genotypes. Significant genotypic variation was observed among the genotypes for all the traits evaluated, indicating substantial genetic diversity in response to temperature stress. Seedling survival ranged from 35.0 to 90.0%, while root growth reduction varied from 0.0 to 34.3%. The chlorophyll stability index (CSI) and Superoxide dismutase (SOD) activity declined following temperature induction; however, the magnitude of reduction differed considerably among genotypes. Genotypes 'PHT 6', 'NDI 26', 'NDSH 1012', 'NDI 55', and 'SH 2602' maintained higher chlorophyll stability and antioxidant enzyme activity, suggesting enhanced cellular protection against stress. Genotypes 'PB 1340', 'NDI 30', 'PB 1351', and 'PB 1356' exhibited greater reductions in physiological and biochemical traits, indicating greater susceptibility to heat stress. The study identified 'PHT 6', 'NDI 26', 'NDSH 1012', 'NDI 55', and 'SH 2602' as promising sources of heat tolerance. These genotypes may serve as valuable genetic resources for development of temperature-resilient sunflower cultivars through future breeding programmes.

Keywords: Chlorophyll stability index, heat stress, sunflower, superoxide dismutase, temperature induction

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops cultivated worldwide owing to its high-quality edible oil, wide adaptability, and economic importance. The crop contributes significantly to global vegetable oil production and serves as an important source of healthy unsaturated fatty acids, proteins, vitamins, and antioxidants. However, the productivity of sunflower is increasingly threatened by climate change-induced abiotic stresses, particularly high temperature stress, which has emerged as a major constraint to sustainable crop production in many sunflower-growing areas. Heat stress affects plant growth, development, physiological functions, and ultimately

seed and oil quality (Khajavi *et al.*, 2024). Temperature is a critical environmental factor governing seed germination, seedling establishment, photosynthesis, flowering, and seed filling in sunflower. The optimum temperature for sunflower growth ranges between 25 and 30°C; however, exposure to temperatures above this optimum range can adversely affect cellular metabolism and physiological processes (Guo *et al.*, 2021). High temperature stress disrupts membrane integrity, reduces photosynthetic efficiency, accelerates leaf senescence, and increases the production of reactive oxygen species (ROS), leading to oxidative damage to cellular components. Consequently, plant growth, biomass accumulation, and reproductive success are severely impaired under prolonged heat stress conditions (Hasanuzzaman *et al.*, 2020; Aparna *et al.*, 2023).

One of the primary consequences of heat stress is the excessive ROS generation, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals. These reactive molecules cause lipid peroxidation, protein oxidation, nucleic acid degradation, and chlorophyll destruction, ultimately leading to reduced cellular functionality and premature senescence. To mitigate oxidative damage, plants activate a complex antioxidant defense system comprising enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). Enhanced antioxidant activity is recognized as an important adaptive mechanism contributing to heat stress tolerance in sunflower and other crop species (Gill and Tuteja, 2010; Rahnama *et al.*, 2026).

Chlorophyll stability index (CSI) is widely used as a reliable physiological indicator for assessing stress tolerance in crop plants. Maintenance of chlorophyll under stress reflects the ability of plants to preserve the photosynthetic machinery and sustain carbon assimilation. Genotypes exhibiting higher CSI values generally possess better membrane stability and greater tolerance to temperature-induced injury. Similarly, membrane stability and antioxidant enzyme activity are considered important selection criteria for identifying heat-tolerant genotypes (Sudhakar *et al.*, 2012). Significant genotypic variation in CSI, membrane thermostability, relative water content, and antioxidant responses under high-temperature conditions have already been reported (Aparna *et al.*, 2024; Akhtar *et al.*, 2024).

Inordinate efforts are needed to identify more genetic source of heat tolerance from unexplored landraces and understand the mechanisms of abiotic stress tolerance in crop plants (Sarkar and Bhattacharjee, 2011). It is important to develop a rapid and reliable laboratory protocol that allows simultaneous screening of large number of genotypes. Temperature induction response (TIR) technique provide an efficient approach for evaluating heat tolerance at the seedling stage. The method involves exposing seedlings to controlled temperature stress and assessing their survival, growth reduction, membrane stability, and antioxidant responses. TIR has been used as a screening technique to identify high temperature tolerant cotton lines (Ehab Abou Kheir *et al.*, 2012). Acquired tolerance for a specific abiotic stress has been shown to provide cross protection against other stresses such as salinity, chilling temperatures and drought (Renuka Devi *et al.*, 2013).

An association between crop plant adaptability to high temperature stress and heat acclimation has earlier been reported (Chen *et al.*, 1982), yet the genetic basis of heat tolerance in sunflower is poorly understood. Heat acclimation is a physiological response manifested in acquired thermotolerance of organisms or cells to lethal high temperatures following exposure to a moderate heat stress conditions. Furthermore, reactions of plants to temperature stresses are complex, and adversely affect plant metabolism by disrupting cellular homeostasis and uncoupling major physiological and biochemical processes (Awasthi *et al.*, 2015). Root and shoot growth reduction, seedling survival, chlorophyll stability, and antioxidant enzyme activity have been recognized as effective indicators of thermotolerance in crop plants. These parameters provide valuable information regarding the capacity of genotypes to withstand cellular and physiological damage under heat stress conditions (Blum, 2011; Hasanuzzaman *et al.*, 2020). Considering the increasing incidence of high-temperature stress and the need for resilient sunflower cultivars, the present study was aimed to evaluate thirty sunflower genotypes for their response to temperature induction under laboratory conditions. The study also aimed to assess genotypic variability for seedling survival, root and shoot growth reduction, chlorophyll stability index, and superoxide dismutase activity, and to identify promising heat-tolerant genotypes using physiological, biochemical, and multivariate analytical approaches.

MATERIALS AND METHODS

The experiment was conducted in the year 2025-26 at Regional Agricultural Research Station, ANGRAU, Nandyal, Andhra Pradesh, India with thirty sunflower genotypes obtained from the All India Coordinated Research Project (AICRP) Sunflower of RARS Nandyal.

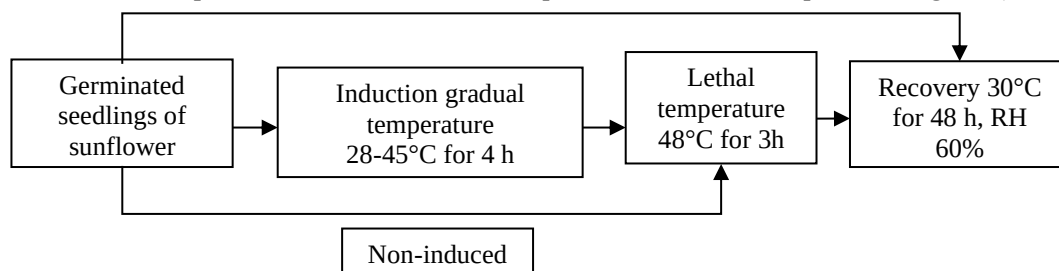
Seedling growth

Sunflower seeds were soaked in water for 24 h to facilitate imbibition and pre-germinated seeds used for the experiment. The uniform seedlings from each genotype were transferred to separate glass beakers. The experiment was conducted in a completely randomised block design with four replications and 5 genotypes per tray.

Standardization of optimum induction temperature and challenge temperature for sunflower seedlings

Identification of lethal (challenge) temperature: The challenge (lethal) temperature is defined as the temperature treatment applied for a specific duration that results in more than a 90% seedling mortality in non-induced sunflower. To determine the challenge temperatures germinated seedlings were exposed to different elevated temperatures (44, 45, 46, 47, and 48°C) for varying durations (1, 2 and 3 h) without prior induction. Following the treatments, the exposed seedlings were allowed to recover at 30°C and 60% relative humidity for 48 h (Fig. 1). At the end of recovery period the temperature at which more than 90% seedling mortality was observed was considered as the challenge temperature for assessing genetic variability in seedling survival. Seedling mortality (%) following the recovery period was recorded. A temperature of 48°C for 3 h was identified as the lethal treatment as it resulted in 100% seedling mortality.

Identifications of sub lethal (induction) temperature: During induction treatment, the seedlings were exposed to a gradual increase in temperature for a specific period. Since the temperature regime and exposure duration vary among the crop species, therefore standardization for each crop is required. The germinated sunflower seedlings were subjected to gradual increasing temperatures i.e. 28 to 45°C for 4 h. After induction treatment, the seedlings were exposed to lethal temperature i.e., 48°C for 3 h and then allowed to recover at 30°C and 60% relative humidity for 48 h. Different temperature regimes and exposure durations were evaluated to standardize the optimum induction protocol. Optimum sub-lethal temperature regime was determined on the basis of percentage survival of seedlings. The sub-lethal treatment that resulted in the highest seedling survival following exposure to the lethal temperature was considered the optimum induction temperature regime (28-45°C).



Standardized protocol to study temperature induction response in sunflower

Thermo induction response (TIR)

Seeds were surface sterilized by treating them with 2 % Bavistin solution for 30 min followed by four to five rinses with distilled water, and kept for germination at 30°C and 60% relative humidity in an incubator. After 48 h, uniform seedlings were transferred to aluminium trays (50 mm) filled with soil. These trays were subjected to sub lethal temperatures by gradually increasing the temperature from 28 to 45°C over a period of 4 h in a programmable plant growth chamber (Witeg Labortechnik, WGC-450). The seedling mortality increased progressively with an increase in temperature and duration of exposure. Overall, the results indicated that both temperature intensity and exposure duration

markedly influenced seedling mortality, with 48°C for 3 h resulting in complete mortality. Both induced and non-induced sunflower seedlings were allowed to recover at 30°C and 60% relative humidity for 48 h. The parameters recorded after recovery period were:

$$\text{a) Percent survival of seedlings} = \frac{\text{Number of seedlings survived at the end of recovery}}{\text{Total number of seedlings sown in the tray}} \times 100$$

$$\text{b) Percent reduction in root growth} =$$

$$\frac{\text{Actual root growth of control seedlings} - \text{Actual root growth of treated seedlings}}{\text{Actual root growth of control seedlings}} \times 100$$

$$\text{c) Percent reduction in shoot growth} =$$

$$\frac{\text{Actual shoot growth of control seedlings} - \text{Actual shoot growth of treated seedlings}}{\text{Actual shoot growth of control seedlings}} \times 100$$

Statistical analysis

An ANOVA was performed for each variable in this experiment; subsequently ANOVA was used to determine whether there were differences among the genotypes. Sunflower genotypes means were separated by use of critical difference at $p \leq 0.05$ obtained by using *OPSTAT* software. The standard error of the mean is the standard deviation of the sample mean estimate of a population mean.

RESULTS AND DISCUSSION

Significant variation was observed among the 30 sunflower genotypes for seedling survival, root growth reduction, and shoot growth reduction under temperature induced stress, indicating substantial genetic variability for thermotolerance. Seedling survival ranged from 35 to 90%, demonstrating differential responses of the genotypes to elevated temperature conditions (Table 1). Genotypes ‘PHT 6’, ‘NDI 26’, and ‘NDSH 1012’ exhibited the highest survival percentages, whereas ‘PB 1357’, ‘RSFH 9’, and ‘CMS 104 B’ recorded comparatively lower survival, indicating greater susceptibility to heat stress.

Root growth reduction varied considerably among genotypes, ranging from 0.0 to 34.3%. Several genotypes, including ‘PHT 6’, ‘NDI 26’, ‘NDSH 1012’, ‘SH 2602’, and ‘PB 1345-1’, exhibited negligible reduction in root growth, suggesting superior maintenance of cell division and elongation processes under stress conditions. In contrast, genotypes ‘PB 1360’, ‘PB 1340’, and ‘PB 1363’ showed substantial reductions in root growth, indicating greater sensitivity to elevated temperatures. Shoot growth reduction also differed significantly among the genotypes. Genotype ‘NDI 30’ exhibited the highest shoot growth reduction, followed by ‘CMS 104 B’ and ‘PHT 13’, whereas tolerant genotypes maintained relatively lower reductions. Reduced shoot growth under heat stress may be attributed to the impaired cell expansion, accelerated respiration, and disruption of metabolic activities. The ability of tolerant genotypes to sustain shoot growth suggests better physiological adaptation to heat stress. The technique of exposing young seedlings to sub-lethal and lethal temperatures has been validated in many crop species Srikanthbabu *et al.* (2002) in pea, Senthilkumar *et al.* (2003) in sunflower, Gangappa *et al.* (2006) in groundnut, Sudhakar *et al.* (2012), Ehab Abou Kheir *et al.* (2012) in cotton, Renuka Devi *et al.* (2013), Sapna Harihar *et al.* (2014) and Vijayalakshmi *et al.* (2015) in rice, Venkateshbabu and Raghavendra (2017) in sorghum, Raghavendra *et al.* (2017) in chickpea and Raghavendra *et al.* (2024) in cotton. This novel temperature induction response (TIR) technique has been demonstrated to effectively reveal genetic variability for intrinsic cellular-level heat tolerance.

The chlorophyll stability index showed substantial variation among genotypes before and after temperature induction. CSI reduction ranged from approximately 5 to 56%, indicating differential stability under stress conditions. Genotypes ‘PHT6’, ‘NDI26’ and ‘NDSH1012’ maintained higher CSI

Table 1: Screening of sunflower genotypes for thermotolerance using temperature induced response (TIR) technique

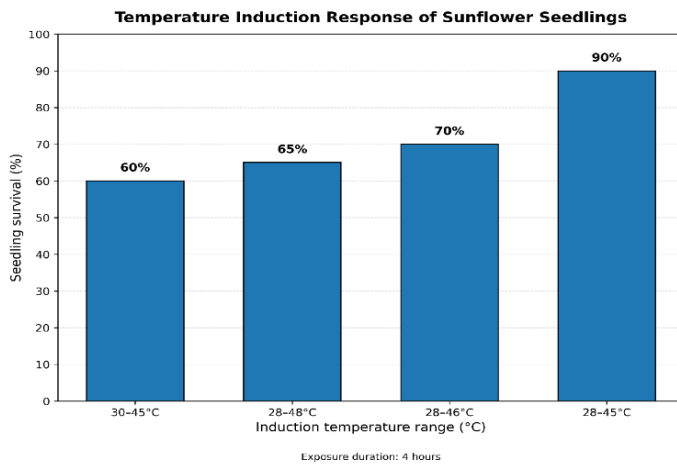
S. No.	Genotypes	Seedlings survival (%)	Actual root growth (cm)		Reduction in root growth (%)	Actual shoot growth (cm)		Reduction in shoot growth (%)
			control	treatment		Control	treatment	
1	PB 1360-1	88.9 (80.82)	11.5	11.8	0.00 (0.00)	10.2	8.0	21.56 (22.55)
2	PB 1360	60.8 (54.51)	13.4	8.8	34.33 (35.66)	11.5	10.0	13.04 (14.52)
3	PB 1355-1	50.0 (48.62)	9.2	8.1	12.32 (13.45)	11.1	9.5	14.41 (13.82)
4	PB 1353	60.0 (64.21)	10.2	8.2	19.20 (23.25)	12.6	10.4	17.46 (18.24)
5	PB 1356	45.0 (39.24)	13.2	9.5	28.28 (26.35)	9.2	8.1	11.95 (12.37)
6	PB 1340	50.0 (46.82)	11.8	8.1	31.64 (33.24)	10.3	6.8	33.98 (31.28)
7	PB 1345-1	80.0 (76.42)	11.5	12.7	0.00 (0.00)	9.4	7.0	25.53 (26.11)
8	PB 1351	70.0 (60.14)	9.5	9.2	3.51 (4.32)	9.7	5.8	40.20 (41.87)
9	PB 1363	60.0 (56.42)	12.6	9.1	27.51 (30.31)	10.8	8.7	19.44 (18.58)
10	PB 1357	35.0 (36.24)	10.2	9.0	11.76 (12.52)	10.4	6.2	40.38 (41.29)
11	PB 1358	80.0 (64.21)	14.2	10.9	23.47 (24.58)	11.8	12.2	0.00 (0.00)
12	PB 1364	70.0 (60.14)	13.4	10.9	18.91 (20.27)	12.6	9.9	21.42 (20.89)
13	PB 1337	70.0 (60.14)	12.8	12.2	4.95 (6.24)	12.7	10.2	19.68 (20.14)
14	SH 2509	50.0 (46.82)	11.7	11.5	1.71 (2.14)	11.7	7.4	36.75 (34.87)
15	SH 2561	70.0 (60.14)	13.5	10.8	19.75 (23.56)	11.8	10.7	9.32 (10.87)
16	SH 2602	80.0 (76.82)	10.8	10.8	1.85 (2.13)	12.5	11.1	11.20 (13.25)
17	RSFH 3	35.0 (36.24)	10.2	9.4	7.84 (10.14)	10.4	6.3	39.42 (40.87)
18	NDI 44	80.0 (81.14)	13.9	10.2	26.38 (25.66)	12.1	9.1	24.79 (22.45)
19	RSFH 9	35.0 (36.24)	11.4	9.6	16.08 (18.35)	10.5	7.8	25.71 (26.98)
20	PHT 6	90.0 (80.82)	13.9	14.8	0.00 (0.00)	11.2	11.8	0.00 (0.00)
21	NDI 30	60.0 (60.14)	12.5	9.8	36.80 (36.41)	9.2	4.3	53.26 (47.25)
22	NDI 55	80.0 (64.21)	14.2	13.5	32.39 (33.02)	10.7	12.0	0.00 (0.00)
23	NDI 26	90.0 (80.82)	15.3	15.6	0.00 (0.00)	11.4	11.9	0.00 (0.00)
24	PHT 13	50.0 (46.82)	11.3	9.9	40.71 (42.35)	8.1	4.8	40.74 (38.45)
25	NDL-B 6	80.0 (71.82)	11.6	10.0	13.79 (15.24)	10.8	10.4	3.70 (5.24)
26	CMS 104 B	50.0 (46.82)	11.2	11.0	14.29 (13.58)	9.4	5.4	42.55 (40.12)
27	NDLB 4	80.0 (64.21)	12.3	12.8	0.00 (0.00)	11.3	9.9	12.38 (10.56)
28	NDLB 3	70.0 (60.14)	13.9	10.2	26.38 (25.66)	10.5	8.8	16.19 (18.97)
29	IB-5	60.4 (58.41)	11.4	9.6	16.08 (18.35)	9.8	7.5	23.46 (25.34)
30	NDSH 1012	85.0 (76.42)	13.9	14.8	0.00 (0.00)	9.2	9.4	0.00 (0.00)
Mean		64.82			22.02			24.68
S.Em		5.62			1.01			1.89
CD _{0.05}		14.90			2.98			3.12

*Values in parenthesis represent arcsine transformed value

values after induction treatment and exhibited minimal chlorophyll degradation. In contrast, the genotypes ‘PB 1340’, ‘PB 1351’, and ‘PB 1356’ exhibited greater CSI reduction, suggesting increased chlorophyll degradation and damage to the photosynthetic machinery (Table 2). High temperature stress accelerates chlorophyll breakdown through enhanced reactive oxygen species (ROS) production and membrane lipid peroxidation. Therefore, the maintenance of chlorophyll stability under stress is considered an important indicator of thermotolerance (Rahnama *et al.*, 2026). Superoxide dismutase activity declined after the induction treatment in all genotypes; however, the magnitude of reduction varied considerably. Tolerant genotypes such as ‘PHT 6’, ‘NDI 55’, ‘NDI 26’ and ‘NDSH 1012’ maintained comparatively higher post-induction SOD activity, reflecting stronger

Table 2: Effect of temperature induction response on chlorophyll stability index and superoxide dismutase of sunflower genotypes

S. No.	Genotypes	Chlorophyll stability index (%)			Superoxide dismutase (units mg ⁻¹ protein)		
		Before induction	After induction	Percent reduction	Before induction	After induction	Percent reduction
1.	PB 1360-1	54.00	35.11	35.23	63	32	49.2
2.	PB 1360	62.11	46.41	25.28	60	28	58.3
3.	PB 1355-1	60.31	48.59	19.43	65	31	52.3
4.	PB 1353	62.31	34.58	44.50	70	38	45.7
5.	PB 1356	70.68	32.25	54.37	66	39	40.9
6.	PB 1340	68.36	31.25	54.29	54	29	46.2
7.	PB 1345-1	66.28	37.25	43.80	60	28	53.3
8.	PB 1351	74.31	32.78	55.89	55	24	56.3
9.	PB 1363	69.58	33.01	52.56	52	31	40.3
10.	PB 1357	76.39	35.14	54.00	57	26	54.3
11.	PB 1358	70.45	40.21	42.92	58	29	50.0
12.	PB 1364	69.25	41.36	40.27	50	28	44.0
13.	PB 1337	68.26	40.25	41.03	66	40	39.3
14.	SH 2509	82.34	63.22	23.22	57	32	43.8
15.	SH 2561	78.21	59.62	23.77	54	31	42.5
16.	SH 2602	71.36	58.37	18.20	50	29	42.0
17.	RSFH 3	63.21	35.67	43.57	52	29	44.2
18.	NDI 44	66.28	37.29	43.74	51	24	52.9
19.	RSFH 9	77.31	41.26	46.63	50	22	56.0
20.	PHT 6	82.31	75.62	8.13	71	48	32.3
21.	NDI 30	79.34	53.21	32.93	54	20	62.9
22.	NDI 55	84.28	60.54	28.17	66	42	36.3
23.	NDI 26	83.30	78.32	5.98	71	45	36.6
24.	PHT 13	70.25	43.20	38.51	54	25	53.7
25.	NDL-B 6	85.32	71.24	16.50	56	20	64.2
26.	CMS 104 B	82.69	63.22	23.55	52	22	57.6
27.	NDLB 4	75.64	56.32	25.54	53	23	56.6
28.	NDLB 3	63.97	54.27	15.16	68	45	33.8
29.	IB-5	84.36	60.34	28.47	56	24	57.1
30.	NDSH 1012	84.37	80.09	5.07	60	42	30.0
Mean		72.89	49.33	33.02	58.37	30.87	47.75
S.Em		2.10	2.75	7.78	1.35	1.55	1.70
CD _{0.05}		5.94	7.78	7.93	3.82	4.39	4.81

**Fig. 1: Effect of seedling survival at different induction temperatures in sunflower**

antioxidant defence systems. Conversely, 'NDI 30', 'NDL-B 6', and 'CMS 104 B' recorded greater reductions in SOD activity, indicating greater oxidative injury. The differential response of antioxidant enzymes among genotypes highlights the importance of ROS-scavenging mechanisms in heat stress tolerance (Akthar *et al.* 2024). Similar enhanced antioxidant enzyme activity in tolerant sunflower genotypes under elevated temperature has earlier been reported (Aparna *et al.* 2023).

Cluster analysis

Hierarchical cluster analysis performed using Ward's minimum variance method grouped the thirty sunflower genotypes into three distinct clusters based on seedling survival, root growth reduction, shoot growth reduction, chlorophyll stability index (CSI), and superoxide dismutase (SOD) activity under temperature induced stress. The clustering pattern clearly demonstrated substantial genetic variability among the evaluated genotypes and reflected differences in their physiological and biochemical responses to elevated temperature stress (Fig. 2).

Cluster I comprised highly heat-tolerant genotypes 'PHT 6', 'NDI 26', 'NDSH 1012', 'NDI 55', 'SH 2602', and 'NDLB 4', which showed higher seedling survival, minimal root growth reduction, lower shoot reduction, greater chlorophyll stability, and higher SOD activity under temperature stress. The superior performance indicates efficient antioxidant defence and maintenance of membrane integrity and photosynthetic activity. Similar findings were reported by Rahnema *et al.* (2026) and Aparna *et al.* (2024), thereby emphasizing the importance of antioxidant activity, chlorophyll retention, membrane stability, and photosynthetic efficiency in sunflower heat tolerance.

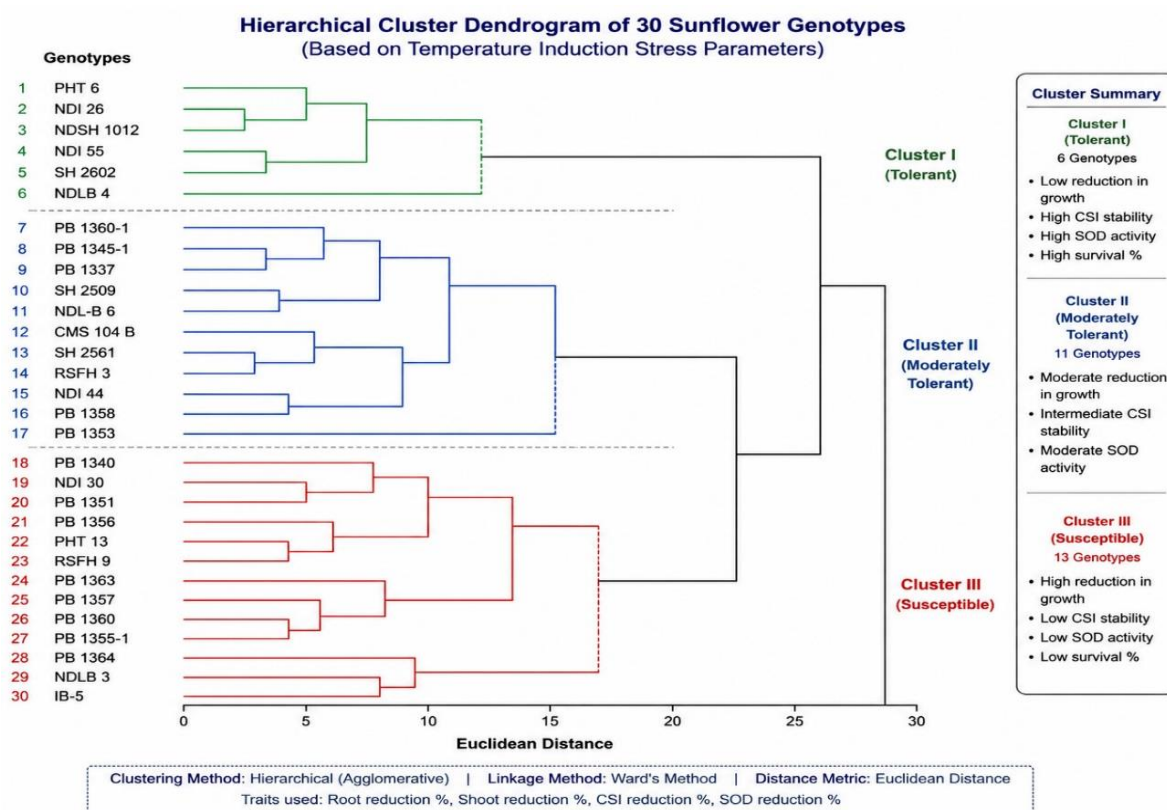


Fig. 2: Cluster analysis revealed substantial genetic variability among sunflower genotypes under temperature induction stress conditions. The tolerant cluster comprising PHT 6, NDI 26, NDSH 1012, and NDI 55 maintained higher physiological stability and antioxidant activity, whereas PB 1340 and NDI 30 were identified as susceptible genotypes due to severe stress-induced reductions

Cluster II consisted of 'PB 1360-1', 'PB 1345-1', 'PB 1337', 'SH 2509', 'SH 2561', 'RSFH 3', 'NDI 44', 'NDL-B 6', and 'CMS 104 B', showing intermediate responses to temperature stress. These genotypes maintained moderate survival, growth, chlorophyll stability, and antioxidant activity, indicating partial heat tolerance. Such moderately resilient genotypes may serve as useful genetic resources for developing climate-resilient cultivars, as also reported by Akhtar *et al.* (2024).

Cluster III comprised susceptible genotypes 'PB 1340', 'PB 1356', 'PB 1351', 'PB 1363', 'PB 1357', 'RSFH 9', 'PHT 13', and 'NDI 30', which exhibited greater reductions in root and shoot

growth, chlorophyll stability, and antioxidant activity. Their poor performance suggests weaker antioxidant defence and greater oxidative damage under heat stress. Similar responses including greater chlorophyll degradation, reduced antioxidant activity, and lower membrane stability in susceptible sunflower genotypes were reported by Aparna *et al.* (2023).

Conclusion: The observed variability among genotypes indicates the existence of exploitable genetic diversity for heat tolerance in sunflower. The maintenance of seedling survival, root growth, chlorophyll stability, and antioxidant activity emerged as key determinants of thermotolerance. The superior performance of ‘PHT 6’, ‘NDI 26’, ‘NDSH 1012’, ‘NDI 55’, and ‘SH 2602’ suggests the presence of effective physiological and biochemical mechanisms that enable these genotypes to withstand temperature-induced oxidative stress. Consequently, these genotypes may serve as valuable donors for developing heat-tolerant sunflower cultivars through future breeding programmes.

Acknowledgement: The authors wish to thank the All India Coordinated Research Project on Sunflower, RARS Nandyal for providing seed material and research facility for this study.

Conflict of interest: The authors declare that they have no competing interests.

REFERENCES

- Akhtar, N., Yousuf, M.A., Shaukat, S., Gul, S., Saleem, U., Mahmood, T., *et al.*, 2024. Appraisal of sunflower hybrids for morpho-phenological traits under normal and terminal heat stress conditions. *SABRAO Journal of Breeding and Genetics*, **56**(6): 2205-2215.
- Aparna, V., Prayaga L., Meena H.P and Guhe A. 2023. Biochemical studies on evaluation of sunflower genotypes for heat stress. *International Journal of Environment and Climate Change*. **13**: 326-332.
- Aparna, V., Prayaga, L., Meena, H.P., and Guhe, A. 2024. Identifying physiological traits associated with high temperature tolerance in sunflower. *International Journal of Plant & Soil Science*, **36**: 396-407.
- Awasthi, R., Bhandari, K. and Nayyar, H. 2015. Temperature stress and redox homeostasis in agricultural crops. *Frontiers in Environmental Science*, **3**: 11. [<https://doi.org/10.3389/fenvs.2015.00011>].
- Blum, A. 2011. *Plant Breeding for Water-Limited Environments*. Springer, New York, USA.
- Chen, H.H., Shen, Z.Y. and Li, P.H. 1982. Adaptability of crop plants to high temperatures stress. *Crop Science*, **22**: 719-725.
- Ehab Abou Kheir., Sheshshayee, M.S., Prasad, T.G. and Udayakumar, M. 2012. Temperature induction response as a screening technique for selecting high temperature-tolerant cotton lines. *Journal of Cotton Science*, **16**: 190-199.
- Gangappa, E., Ravi, K. and Veera Kumar, G.N. 2006. Evaluation of groundnut (*Arachis hypogaea* L.) genotypes for temperature tolerance based on temperature induction response (TIR) technique. *Indian Journal of Genetics*, **66**: 127-130.
- Gill, S.S. and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, **48**(12): 909-930.
- Guo, S., Klinkesorn, U., Lorjaroenphon, Y., Ge, Y., Na Jom, K. 2021. Effects of germinating temperature and time on metabolite profiles of sunflower (*Helianthus annuus* L.) seed. *Food Science and Nutrition*, **9**(6): 2810-2822.
- Hasanuzzaman, M., Borhannuddin Bhuyan, M. H. M., Zulfiqar, F., Raza, A., Mohsin, S.M., Mahmud, J.A., *et al.*, 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants*, **9**(8): 681. [[doi: 10.3390/antiox9080681](https://doi.org/10.3390/antiox9080681)].

- Khajavi, M., Rahnema, A., Meskarbashi, M., Moosavi, S.A. and Harrison, M.T. 2024. Foliar application of cytokinin hormone on morphophysiological and yield traits of sunflower cultivars in late sowing date. *Applied Field Crops Research*, **36**(1): 41-61.
- Raghavendra, T., Jayalakshmi, V. and Venkatesh Babu, D. 2017. Temperature induction response (TIR) - A novel physiological approach for thermotolerant genotypes in chickpea (*Cicer arietinum* L.). *Indian Journal of Agricultural Research*, **51**(3): 252-256.
- Raghavendra, T., Mohan Vishnuvardhan K., Ravi Prakash Reddy B., Lakshmi Kalyani, P. and Umamaheswari, P. 2024. Assessment of acquired thermotolerance and genetic variability in cotton genotypes by temperature induction response (TIR). *Ecology Environment and Conservation*, **30**: S339-S343.
- Rahnema, A., Khajavi, M., Meskarbashee, M., Moosavi, S.A. and Tom Harisson, M. 2026. Cytokinin modulates photosynthesis, antioxidant profiles and fatty acid composition in sunflower plants subjected to extreme heat stress. *Plant Growth Regulation*, **106**: 16 [<https://doi.org/10.1007/s10725-025-01413-4>].
- Renuka Devi, K., Sudhakar, P. and Sivasankar, A. 2013. Screening of paddy genotypes for high water use efficiency and yield components. *Bioinfolet*, **10**(1b): 214-224.
- Sapna, H., Srividhya, S., Vijayalakshmi, C and Boominathan, P. 2014. Temperature induction response technique – A physiological approach to identify thermotolerant genotypes in rice. *International Journal of Agricultural Sciences*, **10**: 230-232.
- Sarkar, R.K. and Bhattacharjee, B. 2011. Rice genotypes with SUB1QTL differ in submergence tolerance, elongation ability during submergence and re-generation growth at re-emergence. *Rice*, **5**: 1-11.
- Senthil-Kumar, M., Srikanthbabu, V., Mohan Raju, B., Ganeshkumar, Shivaprakash, M. and Udayakumar, M. 2003. Screening of inbred lines to develop a thermotolerant sunflower hybrid using the temperature induction response (TIR) technique: a novel approach by exploiting residual variability. *Journal of Experimental Botany*, **54**: 2569-2578.
- Srikanthbabu, V., Krishnaprasad, B.T., Gopalakrishna, R., Savitha, M. and Udayakumar, M. 2002. Identification of pea genotypes with enhanced thermotolerance using temperature induction response technique (TIR). *Journal of Plant Physiology*, **159**(5): 535-545.
- Sudhakar, P., Latha, P., Ramesh babu, P., Sujatha, K and Raja Reddy, K. 2012. Identification of thermotolerant rice genotypes at seedling stage using TIR technique in pursuit of global warming. *Indian Journal of Plant Physiology*, **27**: 185-188.
- Venkatesh Babu, and Raghavendra, T. 2017. Evaluation of sorghum (*sorghum Bicolour* L.) Genotypes for temperature tolerance based on Temperature induction response (TIR) technique. *The Bioscan*, **12**(1): 381-384.
- Vijayalakshmi, D., Srividhya, S., Vivitha, P. and Raveendran, M. 2015. Temperature induction response (TIR) as a rapid screening protocol to dissect the genetic variability in acquired thermotolerance in rice and to identify novel donors for high temperature stress tolerance. *Indian Journal of Plant Physiology*, **20**(4): 368-374.