



Screening of Finger Millet (*Eleusine coracana* L. Gaertn.) Genotypes for Heat Tolerance Using the Temperature Induction Response (TIR)

K. Tressa Naidu ^{a*}, G. Rama Rao ^{b++}, P. Sandhya Rani ^{c#},
Y. Amaravathi ^{d†} and L. Madhavi Latha ^{e‡}

^a Department of Crop Physiology, S. V. Agricultural College, Tirupati, ANGRAU-517502, India.

^b Department of Crop Physiology Agricultural College, Pulivendula, ANGRAU -516391, India.

^c Department of Crop Physiology, Agricultural Research Station, Darsi-523247, India.

^d Molecular Biology and Biotechnology, Regional Agricultural Research Station -51750, India.

^e Agriculture Research Station, Kadiri-51559, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A lab experiment was carried out at the Phenotyping Laboratory, Institute of Frontier Technologies, ANGRAU, Tirupati, to test thirty finger millet (*Eleusine coracana* L. Gaertn) genotypes collected from the Millet Breeder at ARS Perumallapalle. The aim was to identify which genotypes can tolerate high temperatures by using Thermotolerance Induction Response (TIR) technique. The finger millet seedlings were gradually exposed to increasing temperatures from 37°C to 54°C for five hours, and then to a lethal temperature of 58°C for two and a half hours. After the heat

⁺⁺ Professor and Head; [#] Principal Scientist & Head; [†] Scientist; [‡] Principal Scientist;
^{*}Corresponding author: E-mail: tressa.trinity@gmail.com;

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treatment, the seedlings were allowed to recover for two days at 30°C and 60% relative humidity. Among the genotypes after the recovery, their survival percentage, and the reduction in root and shoot growth were measured. Based on these observations, PPR-2773, PPR-1279, PPR-22-1296, and PPR-1211 performed well, showing lower reductions in root and shoot growth, indicating better heat tolerance. This TIR method makes it easier to quickly identify heat-tolerant finger millet lines at the seedling stage itself from a large number of genotypes.

Keywords: Finger millet; temperature induction response; survival percentage; heat-tolerant.

1. INTRODUCTION

Climate change has emerged as one of the most critical threats to global agricultural sustainability, as rising temperatures, erratic rainfall, prolonged droughts, and frequent extreme weather events increasingly disrupt crop productivity. Major cereals such as rice, wheat, and maize already show measurable yield declines under heat and water stress, raising concerns over global food security (Sujatha *et al.*, 2018). India, with nearly 60% of its agriculture dependent on rainfed systems, is particularly vulnerable to shifts in monsoon patterns, recurrent dry spells, and steadily increasing mean temperatures. These climatic fluctuations reduce crop stability, lower yield reliability, and intensify production risks across many states.

Within this context, small millets—especially finger millet—have regained prominence due to their exceptional resilience and nutrient richness. Finger millet is valued for its high calcium, iron, dietary fiber, and phytochemical content, contributing to bone health, antidiabetic effects, hormonal balance, and the management of chronic disorders (Kumar *et al.*, 2016; Chaudhary & Mudgal, 2020; Singh *et al.*, 2022; Nakarani *et al.*, 2021; Mukherjee *et al.*, 2023). In Andhra Pradesh, the crop is cultivated over 0.23 lakh hectares, producing 0.31 lakh tonnes with a productivity of 1348 kg/ha (Third Advance Estimates 2024–25, www.indiastat.com).

Despite being traditionally resilient, finger millet is now exposed to longer dry spells, frequent heatwaves, and delayed monsoons, which disturb germination, reduce tillering, impair grain filling, and ultimately affect yield stability. These unpredictable stress episodes highlight the urgent need to identify and develop climate-resilient genotypes capable of maintaining productivity under extreme temperatures. High temperature particularly affects photosynthesis, reproduction, respiration, and membrane stability across cereals, underscoring the necessity of developing heat-tolerant cultivars (Wahid, 2007; Sujatha *et al.*, 2018).

Screening for heat tolerance at the seedling stage offers a rapid and cost-effective approach to differentiate tolerant and susceptible genotypes before field evaluation. Several methods exist for assessing temperature tolerance, including constant high-temperature germination, alternating day–night temperature regimes, thermal time analysis, electrolyte leakage assays, radicle emergence tests, seedling vigor measurements, and molecular profiling of heat shock proteins. Among these, the Temperature Induction Response (TIR) technique has emerged as one of the most effective and reliable tools for heat tolerance assessment. TIR mimics natural heat acclimation by exposing seedlings to a sublethal rise in temperature followed by a lethal heat shock, allowing precise identification of genotypes capable of acquiring thermotolerance. The method is rapid, reproducible, and suitable for screening large genotype sets, making it ideal for breeding programs focused on climate-resilient varieties. Its efficiency has been demonstrated across crops such as sorghum (Parveen *et al.*, 2024), rice (Vijayalakshmi *et al.*, 2015), sunflower (Senthil Kumar *et al.*, 2003), and finger millet (Venkatesh Babu, 2013).

Under heat stress, plants experience a significant increase in reactive oxygen species (ROS), which can oxidize cellular components such as DNA, lipids, and proteins (Pooja & Munjal, 2019). Lipid peroxidation results in elevated malondialdehyde (MDA) levels, a key indicator of membrane damage and oxidative injury. To counteract these effects, plants activate multiple protective mechanisms involving heat shock proteins (HSPs), antioxidants, osmolytes, and membrane-stabilizing molecules. Heat stress rapidly alters membrane fluidity, triggering lipid-derived signaling cascades, cytoskeletal reorganization, Ca²⁺ influx, and ABA-mediated pathways that collectively initiate protective gene expression (Bita & Gerats, 2013). Through these responses, plants acquire thermotolerance—an adaptive state that enhances their ability to survive otherwise lethal temperatures (Song *et al.*, 2012).

Given the rising intensity of heatwaves and increasing variability in climatic conditions, identifying finger millet genotypes with superior acquired thermotolerance is crucial for developing future-ready cultivars. In this context, the Temperature Induction Response (TIR) technique serves as a valuable tool to screen a large number of genotypes efficiently and reliably, enabling the selection of promising heat-tolerant lines that can sustain productivity under climate stress.

2. MATERIALS AND METHODS

The present study was conducted at Phenotyping laboratory, Institute of Frontier Technologies, Acharya N. G. Ranga Agricultural University, Tirupati, Andhra Pradesh with thirty finger millet genotypes obtained from Agricultural Research Station, Perumallapalle, Tirupati, Andhra Pradesh. The experimental protocol in this work was adapted from (Venkatesh Babu, 2013), with modifications made to optimize the conditions for the genotypes and laboratory setup used in this study.

2.1 Identification of Lethal Temperature

To determine the challenging lethal temperature for finger millet, three day old seedlings were exposed to a range of high temperatures (50, 52, 54, 56 and 58°C) for varying durations of 1, 2, and 3 hours without prior induction. Following heat treatment, the seedlings were transferred to recovery conditions maintained at 30°C and 60% relative humidity for 72 hours. At the end of the recovery period, the percentage mortality was calculated. The temperature–time combination that resulted in approximately 90% mortality was identified as the lethal (challenging) temperature for subsequent TIR screening, and the

corresponding mortality values were recorded in Table 1.

Per cent mortality of seedlings =

$$\frac{\text{No. of seedlings died at the end of recovery}}{\text{Total no. of seedlings sown in the tray}} \times 100$$

2.2 Identifications of Sub Lethal (induction) Temperature

To standardize the sublethal (induction) temperature for finger millet, uniformly germinated three-day-old seedlings were subjected to a gradual increase in temperature for a fixed duration. Seedlings were exposed to stepwise temperature regimes for five hours, with a 4°C increment every hour. This gradual rise in temperature facilitated the induction of adaptive heat-response mechanisms prior to severe stress. Immediately after the induction phase, seedlings were transferred to the predetermined lethal temperature of 58°C for 2.5 hours. Following heat treatment, all sets were allowed to recover at 30°C and 60% relative humidity for 72 hours.

At the end of the recovery period, observations on percent survival over the control were recorded in Table 2. A control set maintained at 30°C throughout the experiment served as the absolute reference. The induction regime that resulted in the least reduction in survival and growth was considered optimal. Based on the differential recovery responses, the temperature range of 37–54°C for five hours was identified as the optimum sublethal (induction) treatment, and 58°C for 2.5 hours was standardized as the lethal temperature (Raghavendra *et al.*, 2017). These standardized parameters were adopted as the TIR protocol for phenotyping finger millet seedlings for intrinsic heat tolerance at the cellular level.

Table 1. Percent mortality of finger millet seedlings across varying lethal temperature treatments

S. No.	Temperature °C	Percent mortality of finger millet seedlings after the recovery period across different temperature durations.		
		1 hour	2 hour	3hour
1	50	0	0	18
2	52	0	15	32
3	54	20	58	72
4	56	49	73	86
5	58	62	94	98

Table 2. Percent survival of finger millet seedlings at varying sublethal induction temperatures

S. No.	Sublethal induction temperature range for 5-hour exposure (°C)	Percent survival of the seedling
1	30-42	80
2	32-46	78
3	34-48	90
4	36-52	84
5	38-54	89

2.3 Evaluation of Temperature Induction Response (TIR) and Genotypic Performance

Seeds of thirty finger millet genotypes were surface sterilized using 2% bavistin solution for 30 minutes, thoroughly rinsed 4–5 times with distilled water, and placed for germination at 30°C and 60% relative humidity in an incubator. After five days, uniform and healthy seedlings from each genotype were transplanted into aluminium trays (50 mm) filled with a soil:vermicompost:vermiculite mixture in a 2:1:1 ratio. The trays were then subjected to a standardized sublethal induction treatment involving a gradual temperature increase from 37°C to 54°C for five hours in a programmable environmental chamber. Immediately after induction, seedlings were exposed to the lethal temperature of 58°C for 2.5 hours (induced treatment).

A separate set of seedlings for each genotype was directly exposed to the lethal temperature without prior induction (non-induced treatment). All induced and non-induced were allowed to recover at 30°C and 60% relative humidity for 72 hours. Four days after treatment, growth and survival parameters were recorded for comparative evaluation of thermotolerance across genotypes.

$$\text{a) Percent survival of seedlings} = \frac{\text{No. of seedlings survived at the end of the recovery}}{\text{Total no. of seedlings grown in the tray}} \times 100$$

$$\text{b) Per cent reduction in root growth} = \frac{\text{Actual root growth of control seedlings} - \text{actual root growth of treatment seedlings}}{\text{Actual root growth of control seedlings}} \times 100$$

$$\text{c) Per cent reduction in shoot growth} = \frac{\text{Actual shoot growth of control seedlings} - \text{actual shoot growth of treatment seedlings}}{\text{Actual shoot growth of control seedlings}} \times 100$$

2.4 Statistical Analysis

The study used a Completely Randomized Design (CRD) with three replications to maintain proper experimental control and statistical reliability. The data from the experiment went through analysis of variance (ANOVA) to assess the significance of treatment effects. Mean separation occurred wherever the ANOVA showed significant differences. We included measures of variability like standard errors and critical difference (CD) values to improve the transparency, strength, and reproducibility of the findings.

3. RESULTS AND DISCUSSION

Significant genetic variability was observed among the thirty finger millet genotypes evaluated under the Temperature Induction Response (TIR) protocol (Table 3). The percent reduction in root growth ranged from 4.81% (PPR-1279) to 81.19% (PPR-1112), with a mean of 41.74%, indicating considerable differences in root thermosensitivity. Genotypes such as PPR-

1279, PPR-22-1296, PPR-2773, and PPR-1170 exhibited the lowest reductions, suggesting superior maintenance of root integrity under lethal heat exposure. In contrast, severe root inhibition in PPR-1112 and PPR-1096 reflects higher vulnerability to thermal damage.

Shoot growth reduction varied from 6.25% (PPR-1279) to 75.40% (PPR-1112), with a mean of 39.13%. Genotypes such as PPR-1279, PPR-22-1296, PPR-1285, and KOPN-942 recorded minimal shoot inhibition, indicating greater resilience of shoot meristems and photosynthetic tissues under stress. Conversely, genotypes including PPR-1112, PPR-1252, and PPR-1209 exhibited substantial shoot reduction, which is often associated with impaired cell expansion and chlorophyll degradation at elevated temperatures. Similar results were reported by Raghavendra *et al.*, (2017).

Seedling survival after lethal temperature exposure also varied widely, ranging from 20 to 95% with a mean of 70.3%. Highest survival was

Table 3. Mean response of finger millet genotypes for thermotolerance traits under TIR screening

S.No.	Genotype	Root growth in control	Root growth in treatment	% reduction in root growth	Shoot growth in control	Shoot growth in treatment	% reduction in shoot growth	% survival of seedlings
1	PPR-1333	3.40	2.02	40.59	2.23	1.23	44.84	45
2	PPR-2187	5.14	2.34	54.47	2.75	2.35	14.55	20
3	PPR-1205	5.94	4.45	25.08	1.96	1.50	23.47	77
4	PPR-1216	3.98	2.66	33.17	2.40	0.85	64.58	25
5	PPR-1285	4.83	2.46	49.07	2.78	2.47	11.15	80
6	PPR-1339	3.54	2.41	31.92	2.44	2.12	13.11	65
7	PPR-1112	6.54	1.23	81.19	1.87	0.46	75.40	40
8	PPR-1279	6.24	5.94	4.81	2.08	1.95	6.25	95
9	TIRUMALA	5.98	2.46	58.86	2.68	2.36	11.94	87
10	PPR-1170	1.60	0.51	68.13	1.47	1.11	24.49	95
11	PPR-22-1296	6.40	5.95	7.03	3.82	3.48	8.90	90
12	KMR-206	6.08	1.72	71.71	1.84	0.80	56.50	75
13	VAKULA	2.45	1.23	49.80	1.98	1.14	42.42	70
14	PPR-1330	3.54	2.70	23.73	2.54	1.10	56.69	70
15	PPR-1252	4.90	2.51	48.78	2.13	0.61	71.36	70
16	GPU-67	3.64	2.25	38.19	1.87	0.64	65.78	75
17	KOPN-942	4.00	2.23	44.25	1.98	1.74	12.12	80
18	PPR-1332	2.20	1.07	51.36	2.54	2.25	11.42	50
19	PPR-1333	4.98	3.50	29.72	1.75	0.68	61.14	65
20	PPR-1211	4.20	1.40	66.67	2.28	0.65	71.49	90
21	PPR-1222	3.94	2.22	43.65	2.41	1.85	23.24	55
22	PPR-1152	3.78	1.64	56.61	1.80	0.80	55.56	60
23	PPR-1280	4.65	2.65	43.01	2.64	1.65	37.50	75
24	PPR-1329	4.16	2.80	32.69	1.94	0.88	54.64	80
25	PPR-1096	6.45	1.34	79.22	2.64	1.05	60.23	55
26	PPR-1170	1.24	1.08	12.90	2.56	1.94	24.22	95
27	PPR-2773	3.12	2.72	12.82	2.40	2.01	16.25	90
28	PPR-1217	6.17	4.50	27.07	3.27	2.30	29.66	60
29	PPR-1206	2.18	1.80	17.43	1.64	0.75	54.27	65
30	PPR-1209	1.53	0.79	48.37	1.90	0.55	70.84	70
	Mean			41.74			39.13	70.3
	S.E(m)			0.54			0.65	1.53
	C.D (P=0.05)			1.30			1.62	5.96

observed in PPR-1279, PPR-1170, PPR-22-1296, PPR-1211, and PPR-2773, demonstrating strong recovery potential and effective acquisition of thermotolerance. Low survival in PPR-2187, PPR-1216, and PPR-1332 corroborated their heightened sensitivity to heat stress.

By considering the root growth reduction, shoot growth reduction, and seedling survival—genotypes PPR-1279, PPR-22-1296, PPR-1170, Tirumala, PPR-2773, PPR-1329, and GPU-67 were identified as the most thermotolerant. Their consistent performance suggests robust cellular thermoprotection mechanisms, likely involving heat-shock protein induction, membrane stabilization, and enhanced antioxidant activity. Genotypes such as PPR-1112 and PPR-1096 were the most susceptible across traits. These findings were in agreement with the observations reported by Sujatha *et al.*, (2018).

The study confirmed the efficiency of the TIR technique in differentiating thermotolerant and thermosensitive genotypes within finger millet. The identified tolerant lines represent valuable genetic resources for developing heat-resilient cultivars suited to climate-change-affected production environments.

4. CONCLUSION

The present study illustrated that TIR is an efficient and rapid technique for the identification of heat-tolerant finger millet genotypes at the seedling stage. The thirty genotypes assessed exhibited considerable genetic variability, thus clearly distinguishing between tolerant and susceptible lines. Those genotypes, namely PPR-1279, PPR-22-1296, PPR-1170, Tirumala and PPR-2773, exhibited least reduction in root and shoot growth with high percent survival, indicating a strong thermotolerance. On the other hand, PPR-1112 and PPR-1096 showed more sensitive responses to heat stress. The results indicated the potential of these tolerant lines to act as donors for breeding programmes aimed at developing heat-resilient cultivars. Overall, the TIR method provides a reliable platform for early-stage screening and supports future efforts towards climate-smart finger millet improvement.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image

generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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