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RESEARCH ARTICLE

In Vitro Selection of Recombinant Inbred Lines for Thermotolerance Using Temperature Induction Response in Finger Millet (*Eleusine coracana* L.)

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ABSTRACT

Climate change is leading to rising global temperatures, causing shifts in weather patterns and more frequent extreme heat events. These temperature shifts disrupt plant growth and development, threatening global ecosystems and food security. Finger millet is a climate-resilient crop, gaining attention for its relative ability to tolerate high temperatures and drought conditions compared to other cereal food crops, but yield improvement is stagnated. The newer sources of stress tolerance in finger millet can be obtained through the development of Recombinant Inbred Lines (RIL's). In the present study, 222 RIL's (F_6) developed for thermotolerance were screened using Temperature induction response (TIR). The study revealed wide variability in the seedling traits such as seedling survival (%), reduction in the shoot, root, and total seedling length (%), as well as the seedling vigour index (SVI). By employing the standardized Z distribution, the RIL's were categorized into tolerant and susceptible based on the percent seedling survival and percent reduction in recovery growth. Five RIL's viz. 6.1.11, 6.5.10, 6.12.5, 6.13.8, and 6.20.24 were identified as tolerant while five viz. 6.3.2, 6.4.12, 6.7.2, 6.10.14 and 6.17.8a were recognized as susceptible. The identified RILs can be further used as donor source for crop improvement studies.

Keywords: *High-temperature; Recombinant inbred lines; Finger millet; Temperature induction response*

INTRODUCTION

According to model-based predictions for climate change, the average global surface air temperatures are likely to increase by 4.0-5.8°C by 2100. In India, the average increase in temperatures from 1901 till 2018 was 0.7°C and expected to rise by 4.4°C by the end of the 21st century (Krishnan *et al*., 2020). Higher atmospheric temperatures often associated with heat waves pose a significant challenge to plant growth and development (http://climate.ec.), especially in the arid and semi-arid regions of the world (Varun *et al.,* 2023). Among the key physiological processes, chlorophyll synthesis, RUBP carboxylase, carbonic anhydrase and photosynthesis are more affected (Prasad and Djanaguiraman, 2011; Opole *et al*., 2018; Ignatova *et al*., 2019) which in turn leads to decreased growth rate and yield of crops (Fahad *et al*., 2017). However, the response and susceptibility of plants to high temperatures vary between genotypes and developmental stages (Bita and Gerats, 2013; Wahid *et al*., 2007).

To meet the increasing demand for food production under changing climatic conditions, a careful selection of climate-resilient crops is mandatory. Finger millet is one such climate-resilient crop able to tolerate harsh weather conditions like high temperatures and low soil moisture conditions (Gupta *et al*., 2017). Despite the possibility of having newer sources of abiotic stress tolerance traits among the finger millet germplasm, the time and ease of access could impede the progress. Therefore, using a stable population derived specifically for high-temperature tolerance through recombination breeding could be more suitable for evaluation (Anil and Nanja Reddy, 2023).

Plants adapt to heat stress by an inherent basal level tolerance and through acquired tolerance from severe temperature stress. Acquired thermotolerance is fast and induced by cell acclimation to moderate high temperature (HT) periods (Hikosaka *et al*., 2006), which can be assessed at the seedling stage itself using a screening technique called temperature induction response (TIR). It has been used as an efficient screening technique to identify high-temperature tolerant genotypes in millets like finger millet (Sujatha *et al*., 2018; Bhavana *et al*., 2019) and foxtail millet (Bheemesh *et al*., 2018) apart from other cereals and pulses. The best-characterized aspect of acquired thermotolerance is the production of heat shock proteins (HSP's) which confer thermotolerance through an acclimation process (Bourgine and Guihar, 2021).

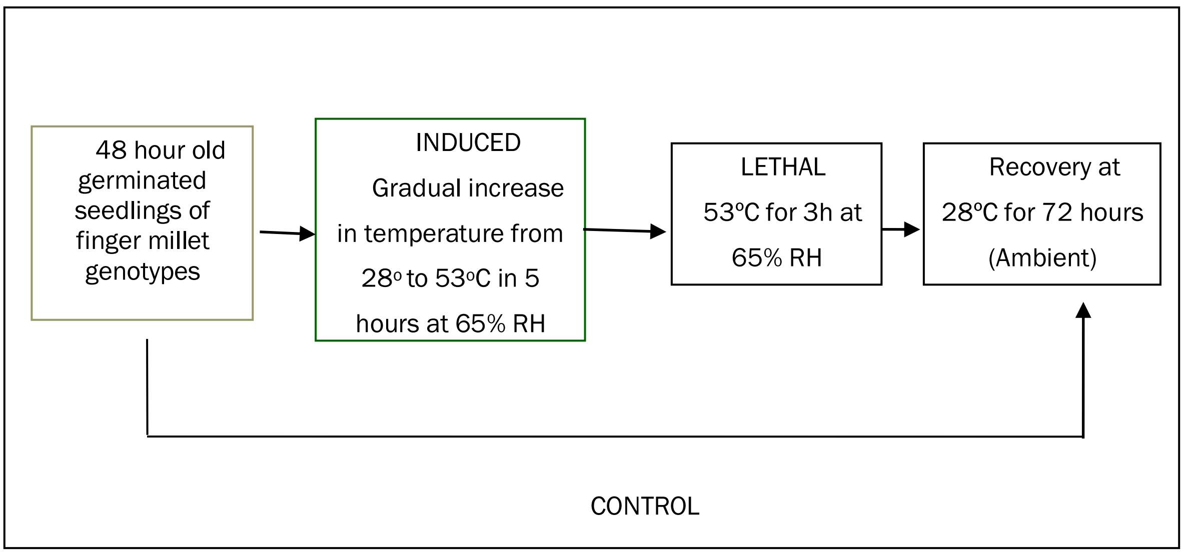
It is imperative that such methods be used for the identification of abiotic stress-tolerant crop lines. Therefore, in the present study, we screened 222 RIL's using the TIR technique followed by a statistical tool, the standardized Z distribution to identify the RIL's with contrasting responses for high temperature.

MATERIAL AND METHODS

A recombinant inbred line population consisting of 222 lines (F_6 generation) was screened at the seedling level for HT tolerance based on a standardized technique called temperature induction response (TIR). Parents of this population were temperature-tolerant PR-202 and susceptible KJNS-46, developed for chlorophyll and grain yield. The induction and lethal temperatures used for the present study were 28-53°C for 5 hours and 53°C for 3 hours, respectively (Vinaykumar, 2015).

The seeds were soaked overnight and placed on a Petri plate lined with two layers of blotting paper for 48 hours to achieve seed germination. From these, 14 uniform seedlings of approximately 1 cm in length were placed in aluminum trays lined with two layers of blotting paper wetted sufficiently. Such plates were exposed to induction cum lethal temperature, wherein the temperature was gradually increased from 28^{o}C to 53^{o}C for 5 hours followed by exposure to lethal temperature of 53 ^{o}C for 3 hours in TIR chamber. The RH inside the TIR chamber was 65%. In the control, the plates were kept at an ambient temperature of 28^{o}C with 65% RH throughout the experimental period (**Figure 1**).

Figure 1. Schematic representation of temperature induction response (TIR) protocol used to assess the thermotolerance of RIL's



At the end of the temperature treatment, aluminum plates were removed from the TIR chamber, added adequate quantity of water, and kept under ambient room conditions for 72 hours for recovery of growth. Following this, observations were recorded on seedling survival, root length, and shoot length. The seedling vigour index and percent reduction in the root, shoot and total seedling length was calculated as per the following formula (**Eqn. 1, 2 and 3**).

$$\text{i) Seedling survival (\%)} = \frac{\text{No. of seedlings survived at the end of recovery}}{\text{Total number of seedlings placed in the tray}} \times 100 \quad (1)$$

$$\text{ii) \% Reduction in seedling growth} = \frac{\text{Observed length in Control} - \text{Observed length in Induction}}{\text{Observed length in Control}} \times 100 \qquad (2)$$

iii) Seedling Vigour Index (SVI) = Germination percent x Seedling length (3)

After recording the data of 222 RILs, the data was subjected to standardized normal Z distribution to classify the RIL's into tolerant and susceptible, based on seedling survival and the percent reduction in recovery growth of seedling. The values of percent seedling survival and percent reduction in recovery growth were transformed to generate standardized normal distribution values (Z-values) and plotted against each other. Based on the Z-values, the lines with higher seedling survival with less percent reduction in recovery growth were selected as tolerant RIL's whereas, those exhibiting a lower seedling survival with higher percent reduction in recovery growth rate were selected as susceptible RIL's for heat stress (Srikanthbabu *et al.*, 2002).

The Z-values for different parameters were computed as

$$\text{Z-value} = \frac{\text{Individual mean} - \text{Overall mean}}{\text{Standard deviation}}$$

The statistical design adopted was completely randomized design with two treatments in three replications. All statistical analysis was carried out in the software OPSTAT (Sheoran *et al.*, 1998) and the normal Z distribution graph was plotted in Microsoft Excel.

RESULTS AND DISCUSSION

Genetic variability for seedling traits in the population

Based on the identified induction and lethal temperatures, a population consisting of 222 RIL's was screened for seedling traits such as seedling survival (%), reduction in the shoot, root, and total seedling length (%) as well as Seedling Vigour Index (SVI).

Seedling survival (%): The seedling survival ranged from 50.0% to 96.4% with a mean of 83.9% across 222 RIL's of finger millet (**Table 1**), implying that the induction temperature led to acclimation of seedlings to high temperature. Similar variability from 72% to 100% (Sujatha *et al.*, 2018), 55% to 100% (Bhavana *et al.*, 2019) in finger millet, 70% to 100% in foxtail millet (Bheemesh *et al.*, 2018), 30% to 88% in rice (Vijayalakshmi *et al.*,

2015) has been reported. This variability in seedling survival is mainly due to the differential expression of stress-responsive genes during the sub-lethal induction stress that would bring the required changes in the plant metabolism necessary for withstanding the subsequent severe lethal stress (Senthil Kumar *et al*., 2003; Ahanger *et al*., 2017).

Table 1. Genetic diversity in the seedling traits for acquired thermotolerance in 222 RIL's of finger millet

Parameter	Min.	Max.	Mean	SD	SE(m) ±	CD	CV (%)
Seedling survival (%)	50.0	96.4	83.9	7.3	3.7	10.4	7.7
Reduction in shoot length (%)	1.24	83.4	44.9	18.0	2.9	8.2	11.3
Reduction in root length (%)	17.7	88.3	66.5	16.0	1.9	5.3	5.0
Reduction in total seedling length (%)	9.0	78.9	58.4	14.8	1.8	4.9	5.2
Seedling vigour index (SVI)	72.9	579	238	8.6	13.0	36.2	3.2

Reduction in shoot length (%): The percent reduction of shoot length over control varied from 1.24% to 83.4%, with a mean of 44.9% (**Table 1**). The percent reduction of total seedling length ranged from 9.0% to 78.9%, with a mean of 58.4% over control. Similar to the variability shown by the RIL's for seedling survival, there is significant variability noticed for total seedling growth on exposure to HT compared to control conditions in many crops, which varied from -23.54 to 38.70% (Sujatha *et al*., 2018) and 0.55 to 89.47% (Bhavana *et al*., 2019) in finger millet, 7-18% in foxtail millet (Bheemesh *et al*., 2018), 7.56 to 51.09% in rice (Vijayalakshmi *et al*., 2015).

Reduction in root length (%): In the present study, wide variability in root length from 17.7% to 88.3% was observed, with a mean of 66.5% (**Table 1**) among the RIL's of finger millet. Earlier reports also showed similar reduction in root length with induction temperature, which ranged from 6.74 to 90.22 % in finger millet (Bhavana *et al*., 2019), 2 to 72% in foxtail millet (Bheemesh *et al*., 2018), -36 to 53.3% in rice (Wahab *et al*., 2020) and -47.05 to 42.85% in finger millet (Sujatha *et al*., 2018). This indicates the existence of wide genetic variability for acquired thermotolerance among the RIL population (Reshma *et al*., 2021).

Seedling Vigour Index (SVI): SVI is the quality parameter that needs to be measured to supplement the germination in any crop. The advantages of high seedling vigour are most apparent in early seedling growth and are often associated with a rapid rate of emergence and crop stand establishment (Kumar *et al*., 2006). The Seedling Vigour Index (SVI) in the present study ranged from 72.9 to 579 with a mean of 238 (**Table 1**), which is due to large variability in seed survival and seedling length as these two are the components of seedling vigour.

Overall study indicates the existence of wide variability for the percent seedling survival, percent reduction in shoot length, root length, total seedling length, and seedling vigour index among the 222 RIL's of finger millet. These variations are mainly due to the acclimation of these RIL's to HT, indicating the ability of acquired thermo tolerance to provide an opportunity for selection (Mishra *et al*., 2020; Reshma *et al*., 2021).

Identification of contrasting RIL's based on TIR study

The standardized normal Z distribution is an efficient tool employed to cluster the genotypes/ RIL's into different groups (susceptible and tolerant) based on the percent seedling survival and the percent reduction in recovery growth under induction temperature over the control (Srikanthbabu *et al*., 2002; Bhavana *et al*., 2019; Kokkanti *et al*., 2019; Chaudhary *et al*., 2020). The RIL's having a higher seedling survival with a lesser percent reduction in recovery growth are considered tolerant; and the RIL's with a lower seedling survival percentage and having a higher percent reduction in recovery growth are considered susceptible to high temperature (**Figure 2**). Accordingly, the RIL's identified as tolerant are 6.1.11, 6.5.10, 6.12.5, 6.13.8, and 6.20.24. In contrast, the susceptible RIL's are 6.3.2, 6.4.12, 6.7.2, 6.10.14, and 6.17.8a (**Table 2**). The tolerant RIL's were superior to better parent PR-202 (**Figure 2**). Higher recovery growth observed in tolerant RIL's could be due to altered metabolism in response to acclimation (Larkindale *et al*., 2005). These identified RIL's can be used as donor lines for further crop improvement studies.

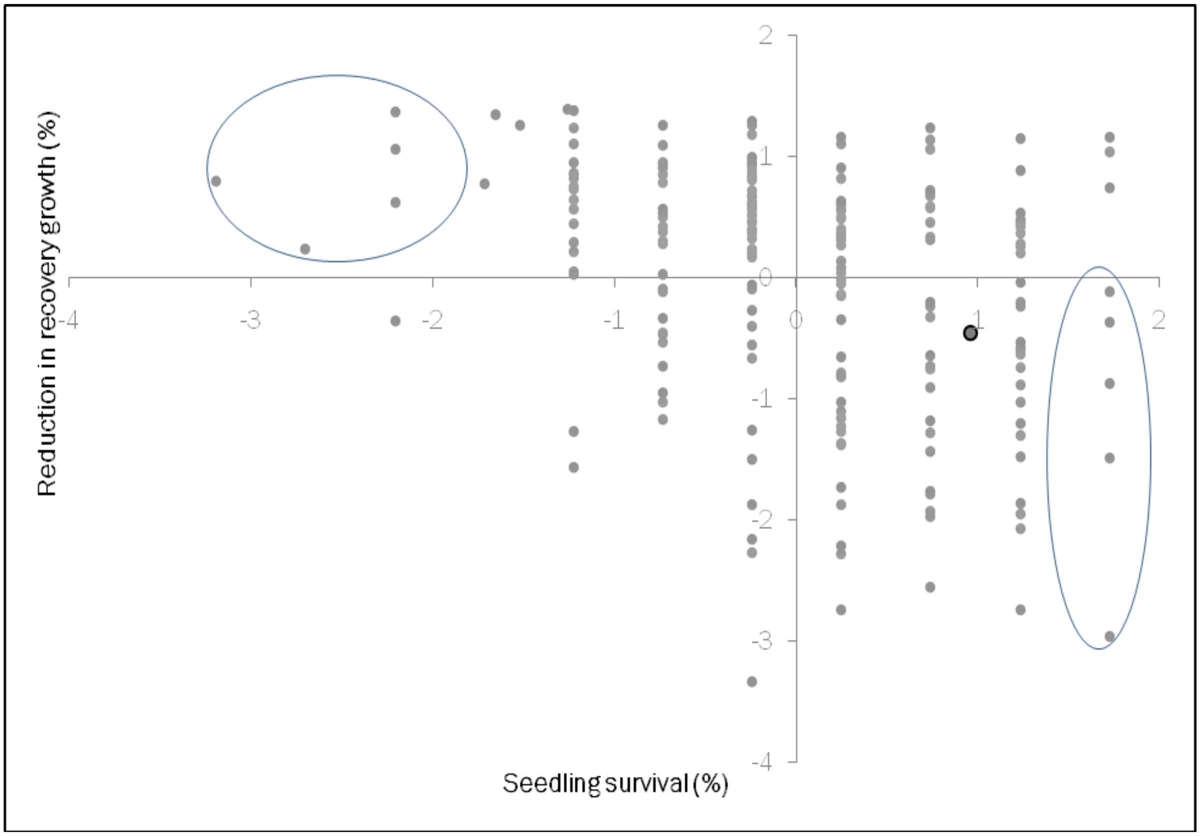


Figure 2. Normal Z distribution of RIL's based on percent seedling survival and percent reduction in recovery growth. Black dot indicates tolerant parent PR-202.

Table 2. Tolerant and susceptible RIL's of finger millet selected based on normal Z distribution

RIL's	% seedling survival	Shoot length			Root length			Total seedling length			Seedling vigour index (SVI)	
		Control (cm)	Stress (cm)	% Reduction	Control (cm)	Stress (cm)	% Reduction	Control (cm)	Stress (cm)	% Reduction	control	stress
Tolerant RIL's												
6.1.11	92.9	2.97	2.43	17.9	3.48	2.86	17.7	6.44	5.29	17.8	644.0	490.5
6.5.10	96.4	2.56	2.43	4.7	4.25	3.39	20.3	6.81	5.82	14.5	680.5	560.5
6.12.5	92.9	2.31	1.69	26.6	3.81	2.54	33.2	6.12	4.23	30.8	611.5	392.6
6.13.8	92.9	1.98	1.90	4.1	3.89	2.20	43.4	5.79	4.18	27.7	578.5	387.9
6.20.24	92.9	2.69	2.13	20.6	3.57	2.28	36.2	6.26	4.41	29.5	625.5	409.3
Mean	**93.6**	**2.50**	**2.12**	**14.8**	**3.80**	**2.65**	**30.16**	**6.28**	**4.79**	**24.1**	**628.0**	**448.2**
Susceptible RIL's												
6.3.2	72.9	3.35	0.91	72.9	3.94	0.76	80.6	7.28	1.67	77.1	728.0	121.8
6.4.12	50.0	3.76	1.12	70.2	6.12	1.03	83.2	9.87	2.15	78.3	987.0	107.1
6.7.2	50.0	3.20	0.59	81.4	4.91	1.11	77.3	8.10	1.71	78.9	810.0	85.3
6.10.14	67.9	2.25	1.15	48.6	4.80	0.67	86.0	7.05	1.82	74.1	704.5	124.1
6.17.8a	67.9	2.33	0.62	73.2	4.30	0.79	81.6	6.63	1.41	78.7	663.0	96.0
Mean	**61.7**	**2.98**	**0.89**	**69.3**	**4.81**	**0.87**	**81.7**	**7.79**	**1.75**	**77.4**	**778.5**	**106.8**

CONCLUSION

The Temperature Induction Response (TIR) was found to be a robust and powerful technique to screen the RIL or genotypes for temperature responses at a seedling level in a short period of time. The standardized normal Z distribution could be an easy and efficient tool to classify the genotypes using selected traits. The identified tolerant RIL's based on the seedling survival (%) and reduction in recovery growth (%) were 6.1.11, 6.5.10, 6.12.5, 6.13.8, 6.20.24, which can be used in crop improvement for regions with high temperatures.

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Competing interest: There was no conflict of interest in the publication of this content.

Data availability: The thesis containing this data will be available at the University Library, UAS, Bangalore.

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