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Phenotyping of Thermotolerant Finger Millet (*Eleusine coracana* L.) Genotypes using Temperature Induction Response at the Seedling Stage

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Abstract— *The Temperature Induction Response (TIR) technique was standardized and employed to identify thermotolerant finger millet (Eleusine coracana L.) genotypes at the seedling stage. The technique involves exposing seedlings to a gradual sub-lethal temperature induction followed by a lethal temperature treatment, and subsequently assessing seedling recovery. Optimization of induction and lethal temperature regimes was based on percent seedling survival and percent reduction in root and shoot growth after a 72-h recovery period. An induction treatment ranging from 37°C to 52°C over five hours, followed by exposure to a lethal temperature of 58°C for two hours, was identified as optimal for screening. Fifteen finger millet genotypes were evaluated under induced and non-induced conditions. Genotypes were classified based on seedling survival percentage and growth reduction parameters. Cultivar Tirumala and genotype VR-1099 exhibited the least reduction in root and shoot growth along with higher survival rates, indicating superior thermotolerance. The study demonstrates that the TIR technique is an effective and rapid phenotyping tool for identifying thermotolerant finger millet genotypes at the seedling stage, facilitating the efficient selection of parental lines for breeding heat-resilient varieties.*

***Keywords—* Finger millet, Temperature induction response, Thermotolerance, Heat stress, Seedling screening.**

I. INTRODUCTION

High temperature stress is a major abiotic constraint affecting crop productivity, particularly in semi-arid and tropical regions. Plants exhibit both inherent (basal) thermotolerance and acquired thermotolerance, the latter being rapidly induced by prior exposure to moderately high temperatures. The Temperature Induction Response (TIR) technique was developed to exploit this adaptive mechanism by subjecting seedlings to sub-lethal temperature stress prior to lethal temperature exposure, thereby enabling the identification of genotypes with superior heat tolerance. Acquired thermotolerance is associated with cellular acclimation processes, including the synthesis and accumulation of heat shock proteins (HSPs), which function as molecular chaperones that maintain protein stability under stress conditions. Previous studies have demonstrated that seedlings exposed to induction temperatures prior to lethal stress show enhanced survival and recovery compared to directly stressed seedlings. Therefore, standardization of induction and lethal temperature regimes is crucial for accurately screening genotypes for intrinsic heat tolerance. Given the increasing frequency of heat stress episodes under climate change scenarios, identifying crops with enhanced thermotolerance is essential for sustainable crop improvement programs. Finger millet, a nutrient-dense, climate-resilient cereal crucial for food security in arid regions, is a prime candidate for such thermotolerance screening. The objectives of this study were to (1) standardize the TIR protocol for finger millet seedlings, and (2) employ this protocol to screen and classify fifteen genotypes for thermotolerance.

II. MATERIALS AND METHODS

2.1 Experimental location and plant material:

The experiment was conducted at the Phenotyping Laboratory, Institute of Frontier Technology, Regional Agricultural Research Station, Tirupati. Fifteen finger millet genotypes, representing local cultivars and advanced breeding lines with putative variability for stress response, were obtained from the Millet Breeding Programme, Agricultural Research Station, Perumallapalle, Chittoor district, Andhra Pradesh.

2.2 Temperature Induction Response (TIR) protocol:

Seeds were surface-sterilized using fungicide (Mancozeb 63% + Carbendazim 12% WP) at 2 g L^{-1} for 30 minutes and thoroughly rinsed 4–5 times with distilled water. Seeds were germinated in an incubator maintained at 30°C and 60% relative humidity. After 48 hours, uniform seedlings were selected and transplanted into aluminium trays containing soil. The TIR protocol was optimized through preliminary experiments. For the final screening, seedlings were subjected to an induction treatment where temperature was gradually increased from 37°C to 52°C over a period of five hours (a rate of 3°C per hour) in a programmable Plant Growth Chamber (WGC-450). Immediately thereafter, induced seedlings were exposed to a lethal temperature of 58°C for two hours. A parallel set of seedlings was directly exposed to the lethal temperature without induction (non-induced control). An absolute control was maintained at optimal 30°C conditions throughout. Following treatment, seedlings were allowed to recover for 72 hours under normal growth conditions.

2.3 Observations and data analysis:

Root length, shoot length, and percent seedling survival were recorded after the recovery period. Percent reduction in root and shoot growth under stress conditions was calculated relative to the absolute control seedlings. The experiment was laid out in a Completely Randomized Design (CRD) with five replications. Data were analyzed using analysis of variance (ANOVA), and treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% probability level with appropriate statistical software.

III. RESULTS AND DISCUSSION

The Temperature Induction Response (TIR) technique effectively differentiated finger millet genotypes for thermotolerance under controlled laboratory conditions, revealing significant genetic variability in root growth, shoot growth, and seedling survival following exposure to high temperature stress (Table 1). Similar effectiveness of TIR-based phenotyping has been reported in small millets and cereals, confirming its reliability for early-stage screening of heat tolerance (Reddy et al., 2023; Kumar et al., 2022).

Under control conditions, root growth among the genotypes ranged from 3.60 cm in PPR 1216 to 6.60 cm in PPR 2885, reflecting inherent genotypic differences in seedling vigor. Exposure to induction and lethal temperature treatments caused a marked reduction in root growth across all genotypes; however, the magnitude of reduction varied significantly. Root growth under stress ranged from 1.08 cm in PPR 1216 to 5.18 cm in cultivar Tirumala. The percent reduction in root growth was lowest in Tirumala (19.06%), followed by VR 1099 (20.20%), PPR 1160 (22.57%), and PPR 1272 (23.39%), indicating superior thermotolerance. In contrast, PPR 1216 (70.00%), PPR 1094 (65.02%), and PPR 2885 (64.85%) exhibited severe reduction in root growth, suggesting higher sensitivity to temperature stress. Reduced root growth under heat stress has been associated with impaired cell elongation and membrane destabilization in susceptible genotypes (Sharma et al., 2024). The maintenance of root growth in tolerant genotypes like Tirumala and VR 1099 is critical, as a robust root system supports water and nutrient uptake under combined heat and drought stress scenarios common in semi-arid regions.

Shoot growth also exhibited significant genotypic variation under both control and stress conditions. Under control conditions, shoot length ranged from 1.82 cm in VR 1099 to 2.68 cm in PPR 1216. Following high temperature exposure, shoot growth declined substantially, ranging from 1.04 cm in genotype ID to 1.94 cm in Tirumala. The percent reduction in shoot growth varied from 17.80% in Tirumala to 60.82% in PPR 1216. Genotypes Tirumala, VR 1099 (18.68%), and PPR 1160 (18.56%) recorded significantly lower shoot growth reduction, indicating better maintenance of physiological and metabolic processes under stress. Similar observations were reported in finger millet and other millets, where tolerant genotypes maintained shoot growth through effective heat shock protein (HSP) synthesis and improved antioxidant defense mechanisms (Kumar et al., 2022; Trivedi et al., 2023).

Seedling survival percentage further reinforced the differential thermotolerance among genotypes. Survival under stress conditions ranged from 69% in PPR 1216 to 93% in cultivar Tirumala. Higher survival percentages were recorded in Tirumala (93%), VR 1099 (90%), PPR 1272 (88%), and PPR 1160 (87%), while lower survival was observed in PPR 1216, PPR 1094 (72%), and VR 1171 (74%). Higher survival in tolerant genotypes may be attributed to effective cellular acclimation during induction treatment, enabling rapid recovery following lethal temperature exposure, as also reported in recent heat-stress studies in small millets (Reddy et al., 2023; Sharma et al., 2024).

Overall, the present findings confirm that the TIR technique is a rapid and reliable phenotyping tool for identifying thermotolerant finger millet genotypes at the seedling stage. Based on minimal reduction in root and shoot growth and higher seedling survival, cultivar Tirumala and genotype VR 1099 were identified as highly thermotolerant, whereas PPR 1216 and PPR 1094 were highly susceptible. These results align with recent reports emphasizing the importance of early-stage physiological screening for developing heat-resilient millet cultivars under climate change scenarios (Kumar et al., 2022; Trivedi et al., 2023).

TABLE 1
SCREENING OF FINGER MILLET GENOTYPES THROUGH TEMPERATURE INDUCTION RESPONSE (TIR) TECHNIQUE UNDER LABORATORY CONDITIONS

SL. No	Genotypes	Root growth in control (cm)	Root growth in treatment (cm)	Percent reduction in root growth	Shoot growth in control (cm)	Shoot growth in treatment (cm)	Percent reduction in shoot growth	Percent survival in treatment
1	PPR 1096	4.6 i	2.34 g	49.13	2.16 d	1.12 ef	48.15	75
2	ID	4.82 h	2.9 e	39.83	1.95 f	1.04 g	46.67	77
3	PPR 1243	4.56 i	2.7 f	40.79	1.87 gh	1.08 fg	42.25	78
4	VR 1099	5.94 d	4.74 b	20.2	1.82 h	1.48 c	18.68	90
5	PPR 1160	5.76 e	4.46 c	22.57	1.94 fg	1.58 b	18.56	87
6	VR 1192	6.2 c	4.2 d	32.26	1.9 fg	1.22 d	35.79	82
7	VR 1171	4.9 g	2.1 i	57.14	2.08 e	1.22 d	41.35	74
8	PPR 2885	6.6 a	2.32 gh	64.85	2.08 e	1.18 de	43.27	76
9	PPR 1279	5.4 f	2.86 e	47.04	2.16 d	1.18 de	45.37	74
10	PPR 1272	5.9 d	4.52 c	23.39	2.1 de	1.6 b	23.81	88
11	PPR 1216	3.6 k	1.08 j	70	2.68 a	1.05 fg	60.82	69
12	VR 1188	3.68 j	2.29 gh	37.77	1.94 fg	1.1 fg	43.3	80
13	PPR 1094	6.46 b	2.26 h	65.02	2.54 b	1.1 fg	56.69	72
14	Tirumala	6.4 b	5.18 a	19.06	2.36 c	1.94 a	17.8	93
	SE m ±	0.007	0.005		0.005	0.005		0.316
	CD 5%	0.02	0.364		0.014	0.014		0.898

Means within a column followed by the same lowercase letter are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test (DMRT). Data represent the mean of five replications.

IV. CONCLUSION

The Temperature Induction Response (TIR) technique proved to be an effective and rapid method for screening finger millet (Eleusine coracana L.) genotypes for thermotolerance at the seedling stage. Significant genotypic variation was observed for root and shoot growth reduction and seedling survival under high temperature stress. Cultivar Tirumala and genotype VR 1099 exhibited minimal growth reduction and higher survival, indicating superior thermotolerance, whereas PPR 1216 and PPR 1094 were identified as susceptible. The study confirms the usefulness of TIR-based phenotyping for early-stage selection of heat-tolerant genotypes. The identified thermotolerant genotypes are recommended for use as donor parents in hybridization programs and for further validation under field-level heat stress conditions to develop climate-resilient finger millet varieties

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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