International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 285-289

Received: 04-11-2018
Accepted: 08-12-2018

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Temperature induction response (TIR): A novel physiological approach for thermo tolerant genotypes in finger millet (*Eleusine coracana* (L.) Gaertn.)

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Abstract
A novel Temperature Induction Response (TIR) technique was standardized for ragi crop. We have standardized the sub lethal *i.e.* challenging temperatures as 37-54ºC (for 5 hours) and lethal temperatures as 58ºC (for 2 and half hours). Using this standardized TIR protocol, highly thermo tolerant genotypes were screened from 50 finger millet germplasm. Among the genotypes, BR-36, TNAU-1214, GPU-28, VR-900, KOPN-933 and PPR-2885 showed highest thermo tolerance in terms of 60 to 95 per cent seedlings survival and lower reduction in root and shoot growth. These genotypes have intrinsic heat tolerance and they can be explored as donor source in breeding programmes aimed for global warming.

Keywords: finger millet, temperature induction response, thermotolerance

Introduction
Finger millet or Ragi (*Eleusine coracana* G.) is the third most important millet crop of India. It is also an important food crop in South Asia and Africa. Its wide adaptability to diverse environments and cultural conditions makes it a potential food crop. Drought coupled with high temperature imposes many adverse effects on plant growth and development. In order to combat these adverse effects, development of thermo tolerant and water saving genotypes is essential.
As ragi crop is mostly cultivated in sub marginal lands and limited moisture conditions, it is prone for recurrent drought, which affects crop growth due to moisture as well as temperature stress.
Therefore information on physiological potential of ragi genotypes is more important in the crop improvement programme to evolve varities suitable for rainfed situations.
The technique of exposing young seedlings to sub lethal and lethal temperatures has been validated in many crop species *viz.*, Sudhakar *et al.* (2012) [4] and Renukadevi *et al.* (2013) [3] in rice, Venkatesh *et al*. (2013) [6] in ragi, Ravi *et al.* (2015) [2] in pearl millet, Raghavendra *et al.* (2017) [1] in chick pea and Venkatesh *et al.* (2017) [1] in sorghum.

Material and Methods
Present investigation was conducted at Phenotyping laboratory, Institute of Frontier Technologies, Acharya N. G. Ranga Agricultural University, Tirupati, Andhra Pradesh with 50 ragi genotypes obtained from Agricultural Research Station, Perumallapalle, Tirupati, Andhra Pradesh.

Identification of lethal temperature
To assess the challenging temperatures for 100 per cent mortality, 24 hour old finger millet seedlings were exposed to different lethal temperatures (55°C, 56°C, 57°C and 58°C) for varying durations(1, 2 and 3 hours)without prior induction. Thus, exposed seedlings were allowed to recover at 30^0 C and 60 per cent relative humidity for 48 hours (Fig 1). At the end of recovery period the temperature at which 90% mortality of the seedlings occurred was taken as the challenging temperature in order to assess the genetic variability for seedling survival. Per cent mortality of finger millet genotypes after recovery was recorded. The lethal temperature of 58°C for 2 and half hours was considered in this context, as maximum

mortality (100%) of seedlings (Table 1).

Identification of sub lethal (induction) temperature

During the induction treatment, the seedlings were exposed to a gradual increase in temperature for a specific period. This temperature regime and duration varies from crop to crop and need to be standardized. The germinated finger millet seedlings (24 hour old seedlings) were subjected to gradually increasing temperatures for a period of five hours. After this induction treatment, seedlings were exposed to lethal temperature *i.e.*, 58^{0}C for two and half hours and then transferred to the normal temperature for recovery. The temperature regimes and durations are varied to arrive at optimum induction protocol. The optimum sub lethal temperatures were arrived based on the per cent survival of seedlings. The sub lethal treatment which recovered least per cent seedlings survival reduction was considered as optimum range of temperatures *i.e.*, 37°C-54°C (Table 2).

A lethal temperature of 58^{0}C for 2 and half hours and induction treatment from 37-54^{0}C for five hours was standardized using TIR (Thermo Induction Response) and considered as best lethal and induction temperatures for phenotyping of finger millet seedlings for intrinsic heat tolerance at cellular level.

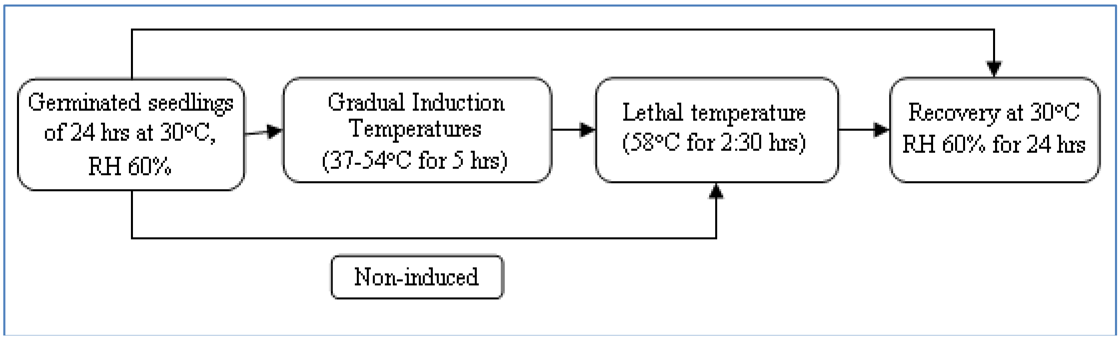


Fig 1: Protocol of the technique: Temperature Induction Response (TIR)

Table 1: Per cent mortality of finger millet seedlings at different lethal temperatures

S. No.	Temperature °C	Per cent mortality of finger millet seedlings after recovery		
		Duration of temperature		
		1 hour	2 hour	3 hour
1	55	0	12	15
2	56	0	25	38
3	57	50	79	85
4	58	65	100	100

Table 2: Per cent survival of finger millet seedlings at different induction (sub lethal) temperature range

S. No.	Temperature range (Induction treatment for 5 hrs) °C	Per cent survival of seedlings
1	34-50	78
2	34-51	86
3	36-52	87
4	36-53	88
5	37-54	95

Assessment of temperature induction response (TIR) and genotypes

Finger millet seeds were surface sterilized by treating with 2 per cent carbendazim solution for 30 minutes and washed with the distilled water for 4-5 times and kept for germination at 30°C and 60 per cent relative humidity in the incubator. After 24 hours, uniform seedlings were selected in each genotype and sown in aluminium trays (50 mm) filled with soil. These trays with seedlings were subjected to sub lethal temperatures (gradual temperatures increasing from 37°C-54°C) for five hours in the environmental chamber (WGC-450 Programmable Plant Growth Chamber). Later these seedlings were exposed to lethal temperatures (58°C) for 2 and half hours (induced).

Induced and non induced finger millet seedlings were allowed to recover at 30°C and 60 per cent relative humidity for 24 hours. The following parameters were recorded from the seedlings 4 days after treatment.

a. $$\text{Per cent survival of seedlings} = \frac{\text{No. of seedlings survived at the end of recovery}}{\text{Total number of seedlings sown in the tray}} \times 100$$

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

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

Results and Discussion

A set of diverse finger millet germplasm comprising of 50 genotypes was screened for intrinsic tolerance using the standardized Thermo Induction Response (TIR) protocol. The experimental data were recorded and presented in Table 3.

The genotypes showed high genetic variability for per cent survival of seedlings, per cent reduction in root and shoot growth respectively. The per cent survival of seedlings varied from 48 to 100 per cent with a mean survival of 59.91 per cent. The per cent reduction in root growth varied from 8.08 (BR-36) to 71.78 (WWN-25) with a mean of 43.35 per cent and the per cent reduction in shoot growth varied from 4.40 (BR-36) to 71.12 (KMR-126) with a mean of 38.92 per cent.

The TIR response of top ten genotypes revealed that BR-36 showed the lowest percent reduction in root (1.99%) and shoot growth (0.55%) with 85% of survival of seedlings followed by TNAU-1214 and GPU-28 (Table 4). Hence, these can be used as a potential donor parents for obtaining thermo tolerant varieties with high grain yield.

Table 4: Thermo tolerance performance of top ten high yielding finger millet genotypes

S. No.	Genotype	Grain yield per plant (g)	% survival of seedlings	% reduction in root growth	% reduction in shoot growth
1	PPR-2885	23.10	70.00	13.2	41.3
2	BR-36	22.72	85.00	1.99	0.55
3	TNAU-1214	22.52	95.00	6.59	8.19
4	GPU-28	21.96	70.00	6.74	24.38
5	VR-900	21.75	60.00	7.92	3.62
6	PR-10-35	20.83	60.00	17.05	54.26
7	IC-519664	20.81	70.00	18.45	31.46
8	PPR-1073	20.68	90.00	21.8	56.55
9	VR-1066	20.67	60.00	24.14	32.2
10	Padmavathi	20.07	90.00	53.79	21.75

Among all the genotypes, BR-36, TNAU-1214, GPU-28, VR-900, KOPN-933 and PPR-2885 showed the highest thermo tolerance ability in terms of 60 to 95 per cent seedlings survival and lower reduction in root and shoot growth (Fig 2). These varieties are able to survive even when they were exposed to lethal temperatures hence these can be used as potent donors in breeding programmes aimed for global warming. These results are in conformity with several studies, which showed that acclimatised plants survive upon exposure to a severe stress, probably due to acquired thermo toleran (Venkatesh *et al.,* 2013) [6].

The seedling survival, shoot and root growth were completely affected in the genotypes WWN-25, IC-382797, Sri Chaithanya and IC-306421 despite of the recovery conditions maintained after exposing to sub lethal to lethal temperature. In spite of exposing to 58°C, germination and seedling growth were not affected in the genotypes BR-36, TNAU-1214, GPU-28, VR-900, KOPN-933 and PPR-2885 probably due to acquired thermo tolerance.

TIR technique is a powerful and constructive technique to identify genetic variability in high temperature tolerance in sorghum within a short period of time and it is suitable for screening a large number of genotypes (Venkatesh *et al.*, 2017) [5]. The present study also revealed that the TIR technique can very well be used in finger millet crop for identification of thermo tolerant genotypes.

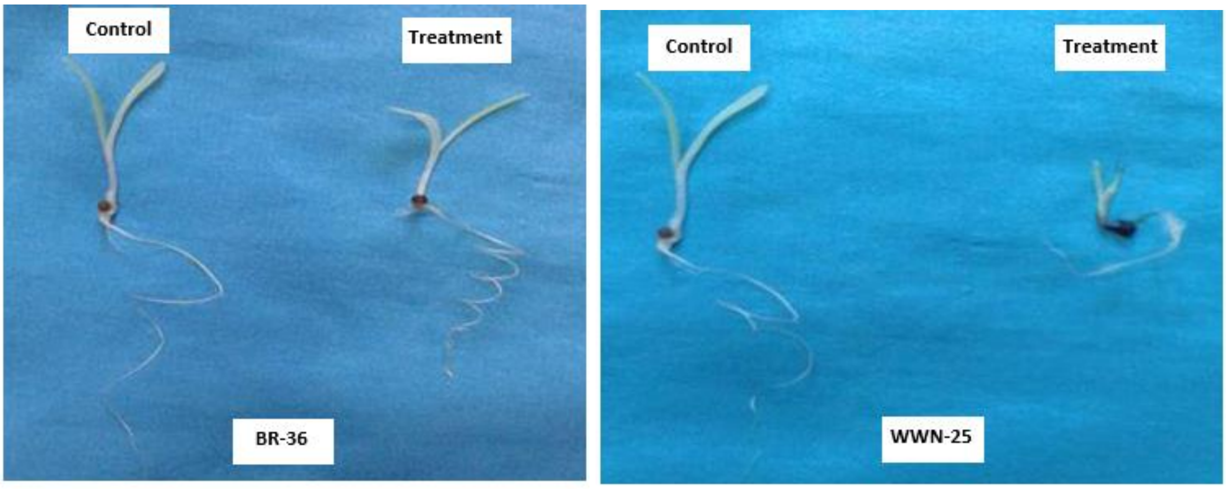
Six finger millet lines *viz.,* BR-36, TNAU-1214, GPU-28, VR-900, KOPN-933 and PPR-2885 which showed 60-95 per cent survival of seedlings, 2 to 13 per cent reduction in root growth and 1 to 41 per cent reduction in shoot growth were selected and needs to be evaluated further under imposed moisture stress conditions. Based on the observations from this technique, six lines were classified as highly tolerant lines and four lines were classified as sensitive lines (Table 5).

Table 5: Identification of promising thermo tolerant finger millet genotypes through TIR technique

Tolerance range	Genotype	Per cent reduction in root growth	Per cent reduction in shoot growth	Per cent survival of seedlings
Highly Tolerant Germplasm Lines	BR-36	1.99	0.55	85.00
	TNAU-1214	6.59	8.19	95.00
	GPU-28	6.74	24.38	70.00
	VR-900	7.92	3.62	60.00
	KOPN-933	12.5	21.95	75.00
	PPR-2885	13.2	41.3	70.00
Highly Sensitive Germplasm Lines	WWN – 25	90.22	72.67	70.00
	IC – 382797	89.20	58.54	65.00
	Srichaithanya	88.46	73.54	55.00
	IC – 306421	85.91	59.37	70.00

Table 3: Mean performance of finger millet germplasm for thermo induction response (TIR) characters

S.No.	Genotype	% survival of seedlings	% reduction in root growth			% reduction in shoot growth		
			Actual root growth in control	Actual root growth in treatment	% reduction in root growth	Actual shoot growth in control	Actual shoot growth in treatment	% reduction in shoot growth
1	VL – 369	95.00	3.93	2.52	35.87	2.23	1.21	45.70
2	Sapthagiri	55.00	4.41	1.18	73.24	1.78	0.89	50.00
3	Champavathi	85.00	5.14	2.41	53.11	2.85	2.42	15.08
4	Padmavathi	90.00	5.80	2.68	53.79	2.62	2.05	21.75
5	VL – 149	75.00	3.97	2.56	35.51	2.40	2.08	13.33
6	Ratnagiri	70.00	5.28	2.95	44.12	2.59	2.29	11.58
7	Maruthi	80.00	4.82	2.45	49.17	2.89	2.57	11.07
8	Bharathi	85.00	5.62	2.96	47.33	2.67	2.25	15.73
9	Kalyani	65.00	3.64	2.31	36.53	2.54	2.22	12.59
10	WN – 259	100.00	4.43	2.40	45.82	2.67	1.42	46.81
11	VR – 900	60.00	2.65	2.44	7.92	1.93	1.86	3.62
12	PPR – 2885	70.00	6.89	5.98	13.20	2.30	1.35	41.30
13	IC – 283853	60.00	4.61	1.82	60.52	2.16	1.27	41.20
14	PR – 10 - 45	70.00	4.80	1.50	68.75	2.58	0.70	72.86
15	TNEC – 1234	60.00	4.20	1.40	66.66	2.27	0.60	73.56
16	VR – 1044	60.00	4.88	1.40	68.55	2.14	0.70	67.28
17	PES -110	55.00	3.88	1.50	61.34	1.80	0.70	61.11
18	DM -1	95.00	5.61	0.73	87.07	2.26	1.03	54.64
19	GPU – 48	55.00	3.42	1.50	56.14	2.06	1.25	39.32
20	DM – 7	65.00	4.11	2.33	43.30	1.66	0.53	68.07
21	VR – 1066	60.00	6.17	4.68	24.14	3.26	2.21	32.20
22	PR – 10 -35	60.00	2.17	1.80	17.05	1.64	0.75	54.26
23	BBM -11	90.00	1.90	0.80	57.89	1.26	0.50	60.31
24	VR -1099	95.00	4.68	2.50	46.58	2.15	1.99	7.44
25	DHRS – 1 – 1	70.00	3.88	2.20	43.29	2.37	1.75	26.16
26	VR – 762	70.00	4.26	2.50	41.31	1.82	0.98	46.15
27	KMR – 118	75.00	5.11	2.30	54.99	2.26	1.00	55.75
28	KOPN -933	75.00	1.20	1.05	12.50	2.46	1.92	21.95
29	PR – 10 -36	75.00	4.90	1.40	71.42	2.13	0.50	76.52
30	KMR – 126	85.00	3.26	1.90	41.71	1.90	0.20	89.47
31	GPU – 82	70.00	1.43	0.90	37.06	1.03	0.92	10.67
32	PPR - 1073	90.00	3.44	2.69	21.80	2.44	1.06	56.55
33	IC – 519664	70.00	6.72	5.48	18.45	3.21	2.20	31.46
34	IC – 426631	65.00	1.52	0.78	48.68	1.84	0.48	73.91
35	WWN – 25	70.00	4.50	0.44	90.22	1.83	0.50	72.67
36	GPU – 28	70.00	5.93	5.53	6.74	2.83	2.14	24.38
37	IC – 382797	65.00	6.67	0.72	89.20	1.93	0.80	58.54
38	PPR – 1012	95.00	1.60	0.50	68.75	1.46	1.21	17.12
39	BR -36	85.00	6.02	5.90	1.99	2.18	1.95	0.55
40	IC - 426617	70.00	3.90	2.00	48.71	2.17	1.00	53.91
41	PR – 10 -21	70.00	5.65	2.40	57.52	2.30	1.85	19.56
42	TNAU -1214	95.00	6.52	6.09	6.59	3.54	3.25	8.19
43	Hima	60.00	5.03	3.40	32.40	1.51	0.50	66.88
44	IC – 414970	75.00	7.34	4.98	32.15	2.65	1.45	45.28
45	IC - 306421	70.00	4.97	0.70	85.91	2.56	1.04	59.37
46	PPR - 2773	75.00	5.38	2.73	49.25	2.38	1.83	23.10
47	GPU – 67	75.00	3.97	2.35	40.80	1.99	1.75	12.06
48	Srichaithanya	55.00	6.07	0.70	88.46	1.89	0.50	73.54
49	Godavari	80.00	3.93	0.94	76.08	1.55	0.72	53.54
50	Vakula	70.00	2.31	0.75	67.53	1.85	0.72	61.08
	General mean	59.91			43.35			38.92
	S.E. (m)	4.04			0.44			0.55
	C.V. (%)	11.68			1.78			2.48
	C.D. (5%)	11.34			1.25			1.56



Highly tolerant genotype Highly sensitive genotype

Fig 2: Thermo induction response of finger millet seedlings in tolerant and sensitive genotypes

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