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Assessment of Sorghum (*Sorghum bicolor* L.) Genotypes for Temperature Tolerance Based on Temperature Induction Response (TIR) Technique

S. Isha Parveen [a*], P. Shanthi [b++], C. V. Chandra Mohan Reddy [c#], R. Madhusudhana [d†], Shaik Nafeez Umar [b‡] and P. Latha [e^]

[a] Department of Genetics and Plant Breeding, ANGRAU, S.V Agricultural College, Tirupati -517502, India.
[b] ANGRAU, S.V Agricultural College, Tirupati – 517502, India.
[c] Regional Agricultural Research Station, ANGRAU, Lam, Guntur- 034, India.
[d] ICAR- IIMR, Hyderabad- 030, India.
[e] Regional Agricultural Research Station, ANGRAU, Tirupati- 517 502, 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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[++] *Associate Professor (GPBR);*
[#] *Principal Scientist (Millets);*
[†] *Project Coordinator (Sorghum and Millets);*
[‡] *Assistant Professor (Agril. Statistics);*
[^] *Principal Scientist (Crop Physiology);*
**Corresponding author: E-mail: s.ishaparveen@angrau.ac.in;*

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ABSTRACT

A novel temperature induction response (TIR) technique in Sorghum was standardized as sub lethal i.e. challenging temperatures of 38-56ºC (for 5 hours) and lethal temperatures of 57ºC (for 3 hours). Using this standardized TIR protocol, highly thermo tolerant genotypes were screened from 27 sorghum entries (parents and hybrids). Amongthem, 104A, M 35-1, PVK 801, 104 A x R 91012, 104 A x PVK 801, AKMS-66-2A x SPV 2758 and AKMS-66-2A x SPV 2468 showed the highest thermo tolerance ability in terms of 75.0 to 87.0 per cent seedlings survival and lower reduction in root and shoot growth. These entries have intrinsic heat tolerance and they can be explored as donor source in breeding programmes aimed.

Keywords: Sorghum; temperature induction response; thermotolerance.

1. INTRODUCTION

"Sorghum [*Sorghum bicolor* (L.) Moench] currently ranks fifth in production of the staple cereals behind maize, rice, wheat, and barley. Originating in Africa, sorghum is a C4 grass that can tolerate dry soils and high temperatures than many other cultivated cereal crops" (Abou Kheir, et al., 2012).

"Prevalence of high temperature is the major limitation for the cultivation of crops in tropical. The frequency and severity of high temperature events are predicted to increase over the next decade (IPCC 2007) and this will likely exacerbate the adverse effects of high temperature stress on crop yields. Development of genotypes that are capable to survive better under high temperature stress is important and inevitable. Sorghum (*Sorghum bicolor* L. Moench) is most sensitive to high temperature stress around the reproductive stage" (Singh et al., 2015), "although high temperatures can also affect plant height, leaf growth and phenology. Importantly, significant genotypic variation in seed set response to high temperature has been observed for sorghum, both in the threshold temperature and in the tolerance to high temperatures above the threshold" (Singh et al., 2015). "These differences in threshold temperature around anthesis are likely to cause complex genotype by location interactions for grain yield. Acquired stress tolerance to temperature extremes are complex traits dependent on many attributes" (Raviteja et al., 2023; Raviteja et al., 2023).

Various screening techniques based on specific physiological parameters such as single leaf photosynthetic capacity, quantification of chlorophyll fluorescence under stress are being used to screen thermo tolerance at field level, but these measurements are highly influenced by environmental factors which are the major limitation. The best alternative is to develop suitable laboratory protocol for screening acquired thermo tolerance of sorghum genotypes. From this perspective, a protocol called temperature induction response (TIR) technique has been developed and standardised for Sorghum.

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 two male sterile lines (104 A and AKMS 66-2A), eight potential restorer lines (RSLG 262, M 35-1, SPV 2758, SPV 2468, R 3777, PVK 801, R 196 and R 91012) chosen based on their yield performance and adaptation along with standard check (CSH 15R).

Seedling growth: The sorghum seeds (about 30-40) were soaked in water for 18 hr and then allowed to germinate in petri plates. Three days old seedlings were selected for the experiment. The uniform seedlings from each genotype were transferred to different sets of petri plates for further studies.

2.1 Identification of Lethal (Challenging) Temperature Treatment

"To assess the challenging temperatures for 100 per cent mortality, 42-hour old sorghum seedlings were exposed to different lethal temperatures (52, 54 and 56°C) for varying durations (1, 2 and 3 hours) without prior induction. Thus, exposed seedlings were allowed to recover at 30°C and 60 per cent relative humidity for 48 hours. At the end of recovery period, the temperature at which 90 per cent 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 sorghum genotypes after recovery was recorded (Table 1). The lethal temperature of 56°C for 3 hours was considered in this study, as maximum mortality (98 %) of seedlings" (Bheemesh et al., 2018).

2.2 Identification of Sub Lethal (induction) Temperature

During the induction treatment, the seedlings were exposed to a gradual increase in temperature for a specific period. The germinated sorghum seedlings (42-hour old sorghum seedlings) were subject to gradually increasing temperatures for a period of five hours. After this induction treatment, seedlings were exposed to lethal temperature i.e., 56°C for two hours and then transferred to the normal temperature for recovery. The temperature regimes and durations are varied to arrive at optimum induction protocol (Table 2). The optimum sub lethal temperatures were arrived based on the per cent survival of seedlings. The sub lethal treatment which recovered least per cent seedling survival reduction was considered as optimum temperatures i.e., 38°C to 52°C.

2.3 Thermo Induction Response (TIR)

"Sorghum seeds were surface sterilized by treating with 2 per cent bavistin solution for 30 min 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 48 hours, uniform seedlings were selected in each genotype and sown in aluminium trays filled with soil. These trays with seedlings were subjected to sub lethal temperatures (gradual temperatures increasing from 38°C to 54°C) for five hours in the environmental chamber (WGC-450 Programmable Plant Growth Chamber). Later these seedlings were exposed to lethal temperatures (57°C) for 2 hours (induced). Another set of seedlings were directly exposed to lethal temperatures (non induced)" (Bhavana and Rao, 2017).

Induced and non induced sorghum seedlings were allowed to recover at 30°C and 60 per cent relative humidity for 48 hours. The following parameters were recorded from the seedlings.

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

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

A lethal temperature of 57°C for 3 hours and induction treatment from 38 to 56°C for five hours was standardized using TIR (Thermo Induction Response) and considered as best lethal and induction temperatures for phenotyping of sorghum seedlings for intrinsic heat tolerance at cellular level.

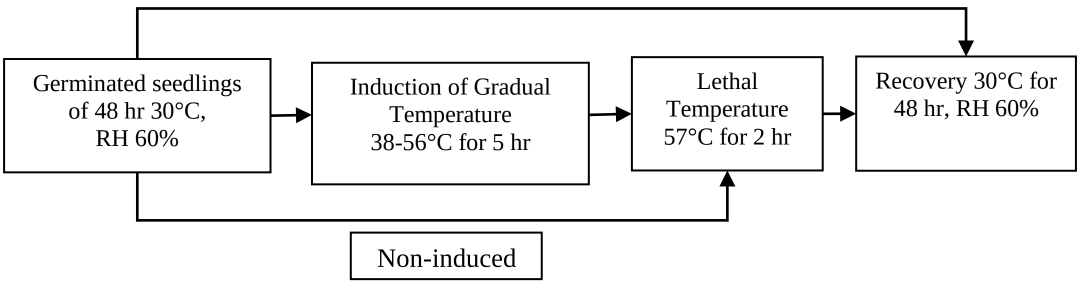

Fig. 1. Standardized Temperature Induction Response (TIR) protocol for sorghum

Table 1. Per cent mortality of Sorghum seedlings at different lethal temperatures

S. No.	Temperature °C	Per cent mortality of sorghum seedling after recovery		
		Duration of temperatures		
		1 hour	2 hour	3 hour
1	50	0	0	10
2	52	0	20	32
3	54	0	34	46
4	56	44	60	81
5	57	52	96	98

Table 2. Per cent mortality of sorghum seedlings at different induction (sub lethal) temperature range

S. No.	Temperature range (Induction treatment for 5 hours) °C	Per cent survival of the seedling
1	32-50	75
2	32-52	80
3	36-52	85
4	38-54	90
5	38-56	75

3. RESULTS AND DISCUSSION

The results pertaining to the findings from a screening experiment evaluating the response of various sorghum genotypes to TIR under controlled laboratory conditions were presented in Table 3. The sorghum entries showed high genetic variability for per cent survival of seedlings as well as per cent reduction in root and shoot growth. The per cent survival of seedlings varied from 58 to 88 per cent with a mean survival of 76 per cent. The per cent reduction in root growth varied from 15.2 (AKMS-66-2A x SPV 2758) to 75.3 (R 196) with a mean of 44.9 per cent and the per cent reduction in shoot growth varied from 7.8 (AKMS 66-2A x SPV 2758) to 68.2 (AKMS 66-2A x RSLG 262) with a mean of 36.4 per cent.

Among all the sorghum entries studied, the parents 104A, M 35-1, PVK 801 and the crosses 104 A x R 91012, 104 A x PVK 801, AKMS-66-2A x SPV 2758, AKMS-66-2A x SPV 2468 showed the highest thermo tolerance ability in terms of 75.0 to 87.0 per cent seedlings survival and lower reduction in root and shoot growth. These entries are able to survive even when they were exposed to lethal temperatures hence these parents can be used as potent donors in breeding programmes. The technique of exposing young seedlings to sub lethal and lethal temperatures has been validated in many crop species by Venkatachalayya et al., (2001) in pea, Senthil–kumar et al*.,* (2003) in sunflower, Gangappa et al*.,* (2006) in groundnut.

The seedling survival, shoot and root growth were more affected in the tester RSLG 262 and crosses AKMS x PVK 801, AKMS x R 3777 despite of the recovery conditions maintained after exposing to sub lethal to lethal temperature. Inspite of exposing to 57°C, germination and seedling growth were not affected in the parents viz., 104A, M 35-1, PVK 801 and crosses 104 A x R 91012, 104 A x PVK 801, AKMS 66-2A x SPV 2758, AKMS-66-2A x SPV 2468 which showed 75.0 to 87.0 per cent survival of seedlings, 15.2 to 20.9 per cent reduction in root growth and 7.4 to 20.4 per cent reduction in shoot growth. These thermo tolerant genotypes were selected and needs to be evaluated further under imposed moisture stress conditions. Similar studies were conducted by Vijayalakshmi et al., (2015) in rice, Venkatesh babu et al., (2013) in ragi, Ehab Abou Kheir et al., (2012) in cotton, This novel temperature induction response technique has been demonstrated to reveal genetic viability in intrinsic stress tolerant at cellular level (Narayana swamy, 2010).

Table 3. Mean performance of sorghum germplasm for thermo induction response (TIR) characters

S.No	Genotypes	% survival of seedling	% reduction in root growth			% reduction in shoot growth		
			Root growth in control	Root growth treatment	% reduction in root growth	Shoot growth in control	Shoot growth treatment	% reduction in shoot growth
1	104A	87.0	2.54	2.01	20.9	2.30	1.87	18.7
2	AKMS 66-2A	85.0	2.67	1.70	36.3	2.84	1.85	34.9
3	RSLG 262	63.0	4.50	1.85	58.9	3.25	1.85	43.1
4	M 35-1	87.0	4.35	3.55	18.4	3.24	2.77	14.5
5	SPV 2758	82.0	4.60	1.69	63.3	4.23	2.42	42.8
6	SPV 2468	86.0	4.32	2.14	50.5	3.52	2.09	40.6
7	R 3777	73.0	3.25	1.52	53.2	2.80	1.12	60.0
8	R 196	85.0	4.61	1.22	73.5	3.75	2.54	32.3
9	R 91012	78.0	3.52	1.92	45.5	2.59	1.79	30.9
10	PVK 801	78.0	3.55	2.85	19.7	3.18	2.66	16.4
11	104 A X RSLG 262	64.0	3.74	1.24	66.8	3.87	2.12	45.2
12	104 A X M35-1	88.0	4.14	1.81	56.3	3.50	1.97	43.7
13	104 A X SPV 2758	84.0	3.57	1.89	47.1	3.50	1.89	46.0
14	104A X SPV 2468	69.0	3.44	2.07	39.8	3.55	2.14	39.7
15	104 A X R 3777	88.0	2.20	1.84	16.4	2.01	1.60	20.4
16	104 A X R 196	78.0	2.77	1.66	40.1	2.74	1.89	31.0
17	104 A X R 91012	81.0	2.74	1.87	31.8	3.05	1.68	44.9
18	104 A X PVK 801	75.0	2.30	1.87	18.7	4.78	3.85	19.5
19	AKMS 66-2A x RSLG 262	84.0	4.05	2.35	42.0	2.74	0.87	68.2
20	AKMS-66-2A X M35-1	66.0	3.22	1.20	62.7	2.96	1.33	55.1
21	AKMS-66-2A X SPV 2758	82.0	2.89	2.45	15.2	2.04	1.88	7.80
22	AKMS-66-2A X SPV 2468	80.0	2.63	2.12	19.4	2.13	1.87	12.2
23	AKMS-66-2A X R3777	61.0	3.84	1.32	65.6	3.84	2.04	46.9
24	AKMS-66-2A X R 196	68.0	3.87	1.34	65.4	2.07	1.05	49.3
25	AKMS-66-2A X R 91012	65.0	2.78	1.35	51.4	3.66	2.04	44.3
26	AKMS -66-2A X PVK 801	58.0	4.72	2.04	56.8	2.78	1.75	37.1
27	CSH 15R	76.0	3.74	1.49	60.2	5.10	3.16	38.0
	General mean	**75.0**			**44.9**			**36.4**
	S.E. (m)	**3.46**			**2.23**			**1.90**
	C.D. (5%)	**10.04**			**6.48**			**5.51**

4. CONCLUSION

The above results suggest that the 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 large number of genotypes at a time. The best proven parents 104A, M 35-1, PVK 801 and crosses 104 A x R 91012, 104 A x PVK 801, AKMS 66-2A x SPV 2758, AKMS-66-2A x SPV 2468 in sorghum can be used as donor source for developing high temperature tolerant sorghum genotypes to resist global rise.

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 writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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