Original Research Article

EVALUATION OF FOXTAIL MILLET VARIETIES FOR THERMOTOLERANCE AT SEEDLING STAGE USING TEMPERATURE INDUCTION RESPONSE TECHNIQUE(TIR)

ABSTRACT

A lab experiment was conducted to standardize the temperature induction response (TIR) protocol for foxtail millet seedlings using WGC-450 programmable plant growth chamber. Temperatures were standardized as sub lethal i.e. challenging temperatures as 38°C – 54°C for 5 hours and lethal temperatures as 56°C for 3 hours. Seven foxtail millet varieties (Suryanandi, SiA 3156, Srilakshmi, SiA 3085, Narasimharaya, Krishnadevaraya and Prasad) were screened for intrinsic tolerance using the standardized Thermo Induced Response (TIR) protocol. Out of the seven varieties evaluated, SiA 3085, Prasad, Suryanandi, Srilakshmi were identified to possess high level of thermo tolerance in terms of higher seedling survival with less reduction in root and shoot growth. The varieties with intrinsic heat tolerance can be explored for the development of heat stress tolerant varieties.

Keywords: temperature induction response technique, Heat stress; thermo tolerance, Foxtail millet

1. INTRODUCTION

Foxtail millet is the world's second most produced millet crop. It is grown in more than 23 African, Asian, and American countries, and it plays an important role in global agriculture by feeding millions of people in arid and semi-arid locations. Climate change makes it the ideal crop for dryland farming due to its C4 plant type, resistance to abiotic stresses, ability to regenerate after stress, low input requirements, ease of cultivation, and resistance to pests and diseases. However, all foxtail millet cultivars may not withstand significant moisture and temperature stress, especially during the germination and seedling growth stages. Developing screening technologies to identify thermo-tolerant foxtail millet variants is crucial as global temperatures rise. In this context, a lab experiment was carried out to standardize the temperature induction response (TIR) procedure for foxtail millet seedlings in the WGC-450 programmable plant growth chamber. The TIR method is developed from the widely accepted principle of Lethal Dose50 (LD50), proposed by Trevan in 1921 for the biological standardization of insulin, toxins and drugs. Still, nowadays, it is widely used in clinical research and toxicology to determine the toxicity of drugs, pesticides and fungicides [Pillai *et al.,* 2021]. The temperature induction response (TIR) technique is a robust and widely recognised empirical and non-destructive method for rapidly assessing the heat tolerance in crop plants at the seedling stage [Raghavendra *et al.,* 2017)]. Based on this novel temperature induction response technique, genetic variability for cellular level tolerance has been validated in many crop species by Venkatachalayya*et al.* (2001) in pea, Gangappa*et al*. (2006) in groundnut, Sudhakar *et al*. (2012), Harihar (2014) and Vijayalakshmi et al.(2015) in rice, Senthilkumar *et al*. (2004) in sunflower, Venkatesh *et al*. (2013) in ragi, Kheir *et al* (2012) in cotton ,Sairekha*et al*. (2016) in mung bean, Raghavendra *et al.* (2017) in chickpea, Ange *et al*(2016) in soy bean. In the present investigation, an attempt was made to standardize the temperature induction response technique in foxtail millet which is necessary for screening the thermotolerant foxtail millet varieties

2. MATERIAL AND METHODS

Research work was carried at Institute of frontier technology located at Regional Agricultural Research Station, Acharya N G Ranga Agricultural University, Tirupati District Andhra Pradesh with seven popular varieties of foxtail millet (SiA 3085, Prasad, Suryanandi, Krishnadevaraya, NarasimharayaSiA 3156 and Srilakshmi) which were collected from Regional Agricultural Research Station, Nandyal.

2.1 Seedling growth :Foxtail millet seeds were surface sterilized by treating with 0.1 per cent carbendazim for 30 minutes and washed with distilled water for 4 to 5 times followed by 1 per cent sodium hypochlorite for 1 to 2 minutes. Then the seeds were washed with distilled water for 4 to 5 times and kept for germination at 30°C and 60 per cent relative humidity in the incubator. After 72 hours, uniform seedlings were selected in each variety and sown in aluminum trays (50 mm) filled with soil.

2.2. Screening of foxtail millet varieties for heat tolerance by TIR technique

Temperature Induction Response (TIR) technique has been developed to identify and select the thermo tolerant genotypes. It involves exposing of seedlings or plants to induced heat stress and subsequently challenging with severe temperature and selection is based on seedling survival at the end of recovery period. This approach of TIR involves, first the identification of challenging temperature and induction temperature and later standardizing them before using for screening the material for intrinsic stress tolerance.

2.2.1. ***Identification of lethal temperature treatment***

To assess the challenging temperatures for 100 per cent mortality, 3 days old foxtail millet seedlings were exposed to different lethal temperatures (50^0C, 52^0C, 54^0C, 56^0C and 58^0C) for varying durations (1, 2 and 3 hours) without prior induction. Thus, exposed seedlings were allowed to recover at 30^0C 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. Percent mortality of foxtail millet genotypes after recovery was recorded. The lethal temperature of 56^0C for 3 hours was considered in this context, as maximum mortality (93%) of seedlings (Table 1).

2.2.2 ***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 temperature regimes and duration varies from crop to crop and need to be standardized. To assess and standardize the sub lethal (induction) temperature, the germinated foxtail millet seedlings (72 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., 56^0C for three hours and then transferred to the normal temperature for recovery. The temperature regimes and durations 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., 38^0C-54^0C (Table 3).

A lethal temperature of 56^0C for 3 hours and induction treatment from 38-54^0C for five hours was standardized using TIR (Thermo Induction Response) and considered as best lethal and induction temperatures for phenotyping of foxtail millet seedlings for intrinsic heat tolerance at cellular level.

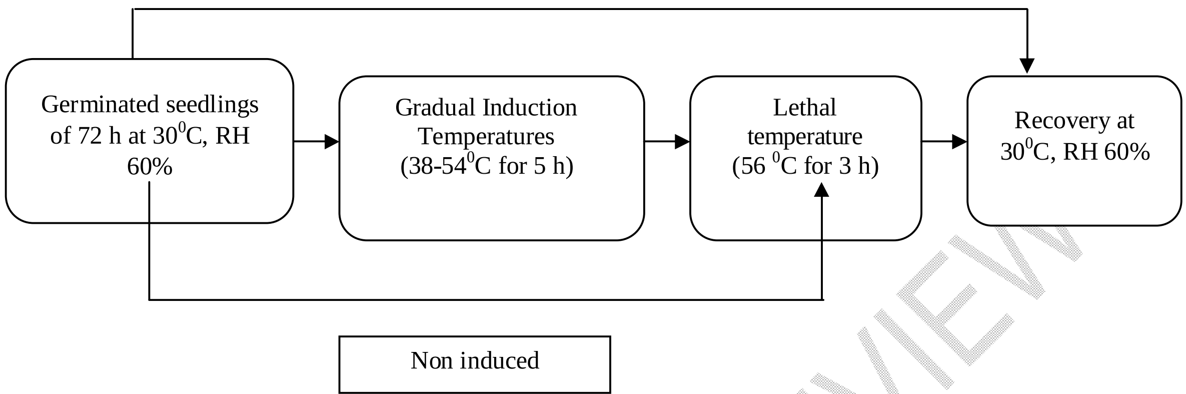

Fig. 1 Protocol of the technique: Temperature Induction Response (TIR)

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

S.No.	Temperature (^{0}C)	Per cent mortality of foxtail seedlings afterrecovery		
		Duration oftemperature		
		1 hour	2 hour	3 hour
1	50	0	0	7
2	52	0	0	23
3	54	0	33	45
4	56	35	66	93
5	58	49	94	100

Table 2 Percent survival of foxtail millet seedlings different induction (sub lethal) temperature range

S. No.	Temperature range (Induction treatment for 5 h) ^{0}C	Per cent survival of seedlings
1	34-50	77
2	34-51	82
3	36-52	84
4	36-53	86
5	38-54	92
6	38-55	76

3.2.3 *Assessment of Thermo Induction Response (TIR)*

Foxtail millet seeds were surface sterilized by treating with 0.1 per cent carbendazim for 30 minutes and washed with distilled water for 4 to 5 times followed by 1 per cent sodium hypochlorite for 1 to 2 minutes. Then the seeds were washed with distilled water for 4 to 5 times and kept for germination at 30°C and 60 per cent relative humidity in the incubator. After 72 hours, uniform seedlings were selected in each variety and sown in aluminum trays (50 mm) filled with soil. These trays with seedlings were subjected to sub-lethal temperatures (gradual temperature increasing from 38°C to 54°C for 5 hours in the environmental chamber (WGC-450 Programmable Plant Growth Chamber). Later these seedlings were exposed to lethal temperatures (56^{o} C) (induced) for 3 hours. Another sub set of seedlings were directly exposed to lethal temperatures (noninduced). Induced and non induced foxtail millet seedlings were allowed to recover at 30^{o} C and 60 percent relative humidity for 48 hours. A control tray was maintained at 30°C, without exposing to sub-lethal and lethal temperatures.

The following parameters were recorded at 7 days after treatment 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=

$$\frac{\text{Actual root growth of control seedlings - actual root growth of treated seedlings}}{\text{Actual root growth of control seedlings}} \times 100$$

c) Per cent reduction in shoot growth=

$$\frac{\text{Actual shoot growth of control seedlings - actual shoot growth of treated seedlings}}{\text{Actual shoot growth of control seedlings}} \times 100$$

3. RESULTS

A set of seven foxtail millet varieties viz., SiA 3085, Prasad, Suryanandi, Krishnadevaraya, NarasimharayaSiA 3156 and Srilakshmi were screened for high temperature stress tolerance employing the standardized thermo induction response (TIR) protocol. The experimental data was recorded and presented in Table 4 and depicted in Fig 2

The varieties showed significant genetic variability for per cent survival of seedlings, per cent reduction in root growth and shoot growth, respectively. The present investigation also revealed that the temperature induction response (TIR) technique can be employed in foxtail millet crop, four foxtail millet varieties *viz.*, SiA 3085, Prasad, Suryanandi, Srilakshmi were identified to possess higher level of thermo tolerance. They showed 85-95 per cent survival of seedlings, 14.3 to 39 per cent reduction in root growth and 21.9 to 30.5 per cent reduction in shoot growth and the remaining three varieties *viz.*, Narasimharaya, Krishnadevaraya, and SiA 3156 showed 70-85 per cent survival of seedlings, 33.3 to 43.3 per cent reduction in root growth and 36.6 to 42.1 per cent reduction in shoot growth. Thus, these varieties can be considered as highly heat sensitive varieties. Based on the observations from this technique, four varieties were classified as highly tolerant varieties and the three varieties were classified as sensitive varieties.

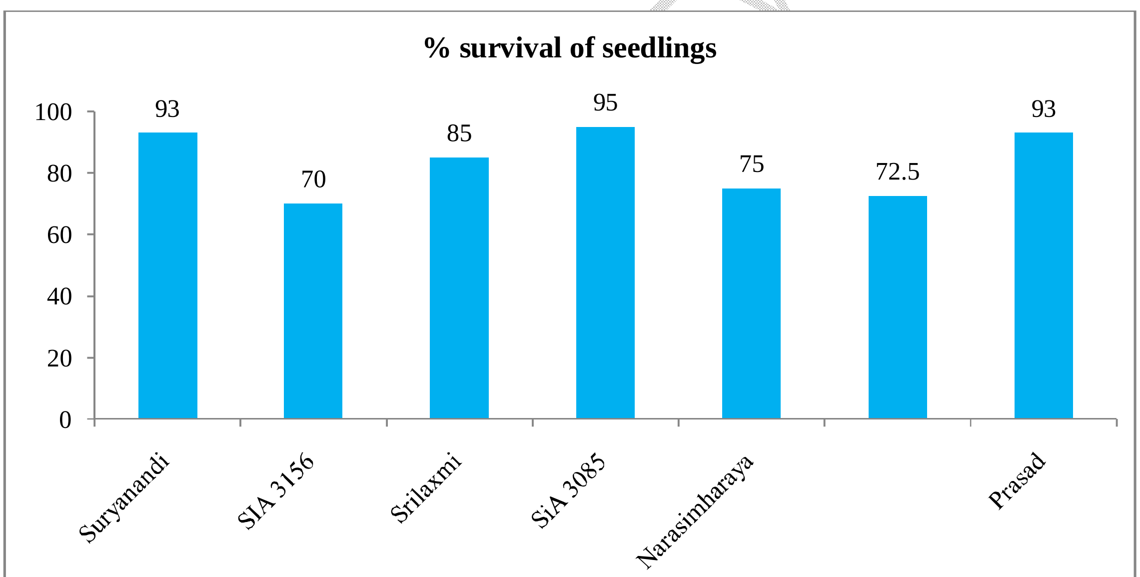

Fig 2 Bar graph showing % survival of seedlings

4.DISCUSSION:

In spite of exposing to lethal temperature of 56°C, germination and seedling growth were lessaffected in the varieties viz., SiA 3085, Prasad, Suryanandi and Srilakshmi which might be probably due to acquired thermo tolerance. The results are in line with the findings of several studies, which showed that acclimated plants survive upon exposure stress, which otherwise could be lethal and are considered as heat tolerant (Senthilkumar *et al.*, 2004).

The remaining three varieties viz., Krishnadevaraya, Narasimharaya and SiA3156, the seedling survival, shoot and root growth were more affected despite of the recovery conditions maintained after exposing to sub lethal to lethal Temperature. Thus, these varieties can be considered as highly heat sensitive varieties.

Table 4 Identification of promising stress tolerant foxtail millet varieties through TIR technique

Tolerance Range	Varieties
Highly Stress Tolerant varieties	**SiA 3085**
	Prasad
	Suryanandi
	Srilaxmi
Highly Stress Sensitive varieties	**Krishnadevaraya**
	Narasimharaya
	SiA 3156

Table 5 Performance of foxtail millet varieties for thermo Induction Response (TIR) characters

*Data was subjected to arcsine transformation. Figures in parenthesis are original values

S.No.	Varieties	Percent survival of seedlings*	% reduction in shoot growth			% reduction in root growth		
			Actual shoot growth in control	Actual shoot growth in treatment	Percent reduction in shoot growth*	Actual root growth in control	Actual root growth in treatment	Percent reduction in root growth*
1	**Suryanandi**	76 (93)	4.80	3.37	33.1 (29.9)	4.79	3.87	23.2 (19.2)
2	**SIA 3156**	57 (70)	4.85	3.37	40.4 (42.1)	4.16	2.36	41.1 (43.3)
3	**Srilaxmi**	67 (85)	3.88	2.24	33.4 (30.5)	4.42	2.70	38.6 (39.0)
4	**SiA 3085**	81 (95)	4.03	3.10	27.8 (23.0)	4.38	3.76	21.2 (14.2)
5	**Narasimharaya**	60 (75)	5.21	3.30	37.2 (36.6)	4.88	3.47	32.5 (28.9)
6	**Krishnadevaraya**	59 (72.5)	4.10	2.55	37.9 (37.8)	4.40	2.94	35.1 (33.3)
7	**Prasad**	76 (93)	4.09	3.19	30 (21.9)	3.90	3.29	22.0 (15.8)
	SEm ±	3.91	-	-	1.4	-	-	1.6
	CD (P=0.05)	11.7	-	-	4	-	-	4

4. CONCLUSION

Critical perusal of the data pertaining to the experiment revealed that the varieties SiA 3085, Prasad, Suryanandi, Srilakshmi were highly tolerant to heat stress. . These varieties with intrinsic heat tolerance can be explored for the development of heat stress tolerant varieties. The results also revealed that the TIR technique is a powerful and constructive technique for identifing the foxtail millet varieties for their genetic variability in thermo tolerance within a short period of time and it is suitable for screening a large number of genotypes.

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