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Standardisation of Temperature Induction Response Technique: A Promising Method for Screening of Maize Genotypes for Thermotolerance at Seed Level

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Climate change adversely affects global agriculture. Heat stress is a major problem for crop production, which detrimentally affects plant growth and development. Maize is a diverse crop widely used as food, feed and fodder. Heat stress affects maize growth and development significantly, reducing the yield and quality of the crop. Crop improvement is critical for heat stress.

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With the advancement in omics technologies, developing tolerant genotypes has become a great option. The crop improvement involves screening and evaluation of the diverse maize genotypes, which is laborious and time-consuming. The temperature induction response technique (TIR) is one of the reliable and robust techniques that can be used for screening a large population of genotypes. It is based on the concept of acquired thermotolerance and lethal dose 50, but it is crop-specific. Hence, the present study standardized the TIR technique in maize at the seed level. The Lethal temperature standardised for maize seeds was 50°C for three hours, while the induction or sublethal temperature standardised was gradual exposure of maize seeds to 32-50°C for 4.5 hours. After a recovery period of 24 hours under room conditions, the seeds were tested for germination and recovery growth was measured as a means of thermotolerance. The standardized TIR in maize can be a reliable and robust technique for evaluating and screening the maize genotypes for thermotolerance at the seed stage itself.

Keywords: Heat stress; maize; screening technique; acquired thermotolerance.

1. INTRODUCTION

According to a new report by the IPCC, global crop production is under serious threat from heat stress. The world has warmed by 1.09°C (±0.1°C) since the pre-industrial era and the temperature is rising by 0.3°C every 10 years. By the end of this century, the planet could be 3°C hotter than it is now [1]. This is mainly caused by human activities, especially the release of greenhouse gases such as CO_2, CH_4 and N_2O. The IPCC warns that the temperature will continue to increase until at least the mid-21st century, regardless of the emission scenarios. Suppose the temperature exceeds the crop's optimum temperature, it will result in the diminishing growth and development of crop plants, resulting in a loss of yield and quality of agricultural produce [2].

Maize (*Zea mays* L.) is a diverse and adaptable cereal crop that grows in various climates and has many types and uses. It is the second most-grown crop in the world, with different varieties such as sweet corn, field corn, baby corn and popcorn. Field corn also includes particular types such as quality protein maize, waxy maize and high-oil maize. Maize is an essential crop for food, feed and industry for billions of people. Most of the maize production (83%) is used for industrial purposes such as feed, starch and bio-fuel. The rest of the maize is consumed as food (17%) or feed (61%) [3,4].

Heat stress causes irreversible damage to the growth and development of Maize. Excessive heat causes a reduction in net photosynthesis, leaf area, poor seedlings establishment and reduced biomass accumulation and test weight, which induces alterations in the metabolic and cellular mechanisms [5,6]. The reproductive phase of the plant is highly susceptible to heat stress, which impairs the process of pollen dehiscence, fertility, silk emergence and stigma receptivity, seed setting and grain filling, ultimately dropping the grain yield [7]. The studies suggested that genetic improvement, evaluation and tolerant line selection for heat stress tolerance are viable solutions. Otherwise, maize yields would decrease by 7.4% for every 1°C rise in temperature [8,9]. Breeding for heat tolerance involves identifying and using heat-tolerant germplasm, molecular markers and biotechnological tools to develop maize genotypes that cope with high temperatures [10].

Thermotolerance is the ability of plants to withstand high temperatures and maintain their growth and productivity. It is an essential trait for crop plants, especially under climate change scenarios. Thermotolerance can be either intrinsic or acquired. Intrinsic thermotolerance is the inherent capacity of plants to cope with heat stress, whereas acquired thermotolerance is the enhanced tolerance that plants develop after exposure to mild or sublethal temperatures [11].

Temperature induction response (TIR) technique is widely used to screen and identify thermotolerant genotypes in various crop species. It involves exposing plantlets to a sublethal temperature (induction temperature) followed by a lethal temperature (challenging temperature) and measuring the survival and growth of the plantlets after a recovery period [12]. The principle behind this technique is that the induction temperature induces the heat shock proteins (HSPs) and other stress responses that protect the plantlets from the lethal temperature [13,14]. The TIR has been successfully standardised at the seedling stage and employed in many crops, such as Rice [15,14], Cotton [16], Soybean [17], Millets [18,19,20].

It has been discovered that there is a research gap in standardizing the TIR technique at the seed level in maize. Although it has been studied and standardized in maize at the seedling stages [21], no research has been conducted at the seed level. Therefore, it is essential to develop a TIR technique at the seed level that is more straightforward and faster than TIR standardized at the seedling level. This will allow breeders to carry out evaluation and screening of a large pool of genotypes for thermotolerance at the seed level itself, saving time, energy and resources. As a result, the present study was planned to develop the TIR technique at the seed level in maize.

2. MATERIALS AND METHODS

2.1 Plant Materials

The RCRMH-2 is a high-yielding, heat-stress resilient, medium-duration maize hybrid released from the University of Agricultural Sciences, Raichur, Karnataka, India and was used for the standardization of TIR at the seed level.

2.2 Terms Used

Lethal temperature: The temperature at which 100 per cent or the total mortality of seeds was noticed.

Induction temperature (Sub-lethal temperature): The induction temperature is a non-lethal low temperature for a specific duration or a gradual increase in temperature for a certain period of time.

Recovery period: The period provided to seeds after exposure to temperature treatment during which seed recovery occurs.

Standardization of Lethal Temperature and Lethal dose 50 (LD_{50}) in Maize Seed: The 100 viable maize seeds were taken in triplicates and immersed in water in a glass beaker for 10-12 hours until they were fully imbibed. Then, seeds were placed on moist filter papers in aluminium trays and subjected to different temperatures (32°C to 60°C) for various durations (1,2 and 3 hrs) in a growth chamber without any prior induction. After the exposure, the seeds were allowed to recover at room temperature with 60 per cent RH for 24 hours. Finally, the seeds were sown in germination paper and kept under room conditions. Further, the germination was evaluated, and based on the results of germination, the temperature that caused 100 per cent seed mortality was considered as the lethal temperature for maize [13]. The temperature that caused 50 per cent seedling mortality was considered as the LD_{50} temperature for maize seeds [22].

Standardization of Induction of Temperature or Sublethal Temperature in Maize at Seed Level: The viable maize seeds were soaked in water for 10-12 hours in a glass beaker until they were fully imbibed. Then, seeds were subjected to a range of gradual induction temperatures for different durations in a TIR chamber and then to the standardised lethal temperature and duration (Table 2). After the lethal temperature treatment, the seeds were transferred to room conditions and allowed to recover for about 24 hours. Another set of seeds with no temperature treatment was used as an absolute control. Then, seeds were sown in germination paper and further evaluated for germination, survival, recovery growth and seedling vigor as recovery growth for each induction treatment.

The List of Parameters Used to Determine Recovery Growth as a Means of Acquired Thermotolerance [23,13,24]

Germination percentage:

$$\text{Germination (\%)}: \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \text{ x } 100$$

Root and shoot length: The ten normal seedlings were randomly selected from each replication at the final count. The root length of 10 seedlings were measured from the collar region to the tip of the longest or primary root and from the shoot length base to the tip of the main stem with the help of a centimetre scale and the average root and shoot length was computed and expressed in centimetre.

Per cent reduction in root growth:

$$\frac{\text{Root length of control seedlings} - \text{Root length of treated seedlings}}{\text{Root growth of control seedlings}} \text{x} 100$$

Per cent reduction in the shoot growth:

$$\frac{\text{Shoot length of control seedlings} - \text{Shoot length of treated seedlings}}{\text{Shoot length of control seedlings}} \text{ x } 100$$

Per cent reduction in total growth (Root + Shoot) of seedlings:

$$\frac{\text{Total length of control seedlings} - \text{Total length of treated seedlings}}{\text{Total length of control seedlings}} \text{ x } 100$$

Seedling vigour index-1 (SVI-1):

$$\text{SVI-1} = \text{Seedling length x Germination \%}$$

2.3 Statistical Analysis

The data obtained in the experiments was subject to analysis of variance (ANOVA) for different treatments and the least significant difference (LSD) was calculated [25]. Statistical analysis was carried out by using the online tool OPSTAT [26] to calculate the level of significance at probability 1% ($P < 0.01$), critical difference (CD) and coefficient of variance (CV%).

3. RESULTS

3.1 Identification of Lethal dose Fifty (LD_{50}) and Lethal Temperature

The results showed (Table 1) that the LD_{50} of RCRMH-2 was noticed at 50°C for 3 hours of exposure (T_{11}), where only 50 % of the maize seeds were germinated, or 50 % of the seed's mortality was recorded. Further, the lethal temperature was recorded at 56°C for 3 hours (T_{14}), with zero seed germination. The germination per cent decreases significantly as the temperature increases above 40°C. Thus, suggesting that the seeds were sensitive to high temperatures and had a limited capacity to cope with heat stress. The results also revealed that the duration of exposure had a significant effect on seed germination. For example, at 48°C (T_{10}), the germination percentage drops from 88.67% for 1 hour to 52% for 3 hours. This indicates that the longer the duration of exposure to high temperatures, higher the damage it causes and leads to the decreased seed germinations.

3.2 Standardization of Thermotolerance Induction Temperature cycle or Sub-lethal Temperature

The maize seeds were exposed to different temperature induction cycles to standardize the appropriate TIR cycle or sub-lethal temperature. The gradual increment in temperature and duration of exposure was carried out from the initial temperature to the final temperature dose. The one-hour increment in duration of exposure was carried out for a rise in every 4°C temperature, followed by exposure to lethal temperature (56°C for 3 hrs). The details of the temperature induction cycles are presented in Table 2.

To quantify the degree of acquired thermotolerance, the germination per cent, reduction in root length, shoot length and seedling growth over control was calculated for each induction treatment, which was significantly affected. The results were presented in Table 3. The per cent reduction in germination, root and shoot length was varied with the temperature induction treatments. The treatment T_7 (32-50°C 4.5 hrs and 56°C for 3 hrs) showed the significantly highest germination (87.33%), while the lowest per cent reduction in root length (12.67%), shoot length (4.72%), total seedling growth (9.84%) and seedling vigor index-I (1866.13) compared to other induction treatments. Therefore, the T_7 is considered and standardized as the optimum induction temperature or sub-lethal temperature for RCRMH-2 at the seed level and is diagrammatically presented in Fig. 1.

Table 1. Effect of temperature on germination of maize seeds to identify LD_{50} and lethal temperature

Treatment (°C)	Germination (%)		
	1 hour	**2 hours**	**3 hours**
T_1 -30	100.00	99.33	96.67
T_2 -32	99.33	98.67	98.00
T_3 -34	98.67	99.33	97.33
T_4 -36	99.33	98.67	97.33
T_5 -38	98.67	98.67	88.67
T_6 - 40	97.33	99.33	80.00
T_7 -42	96.67	92.67	74.67
T_8 -44	94.00	87.33	68.67
T_9 -46	91.33	81.33	58.00
T_{10} -48	88.67	77.33	52.00
T_{11} -50	84.00	73.33	**49.33***
T_{12} -52	78.00	67.33	30.00
T_{13} - 54	74.00	63.33	12.67
T_{14} - 56	69.33	59.33	**00.00** #
T_{15} - 58	65.33	57.33	0.00
T_{16} -60	58.00	52.67	0.00
C.D.	2.41	1.93	2.60
SE(m)	0.83	0.67	0.90

*Note: *LD_{50}: 50 °C for 3 hours of duration, #Lethal Temperature: 56°C for 3 hours of duration. Recovery period: 24 hours of duration under room conditions.*

Table 2. The range of temperature and duration of exposure followed for standardization of the thermotolerance induction cycle or sub-lethal temperature in maize seeds

Treatment (°C)	Induction temperature (°C) and duration of exposure (hours)						
T_1-Control	Room Conditions						
T_2 -32-40	32-36°C-1hr	36-40°C-1hr	LT- 56°C- 3hr	-	-	-	-
T_3- 32-42	32-36°C-1hr	36-40°C-1hr	40-42°C- 0.5 hr	LT- 56°C- 3hr	-	-	-
T_4 -32-44	32-36°C-1hr	36-40°C-1hr	40-44°C - 1 hr	LT- 56°C- 3hr	-	-	-
T_5 -32-46	32-36°C-1hr	36-40°C-1hr	40-44°C - 1 hr	44-46°C-0.5 hr	LT- 56°C- 3hr	-	-
T_6 -32-48	32-36°C-1hr	36-40°C-1hr	40-44°C - 1 hr	44-48°C- 1hr	LT- 56°C- 3hr	-	-
T_7 -32-50	32-36°C-1hr	36-40°C-1hr	40-44°C - 1 hr	44-48°C- 1 hr	50°C- 0.5 hr	LT- 56°C- 3hr	-
T_8- 32-52	32-36°C-1hr	36-40°C-1hr	40-44°C - 1 hr	44-48°C-1 hr	48-52°C - 1 hr	LT- 56°C- 3hr	-
T_9- 32-54	32-36°C-1hr	36-40°C-1hr	40-44°C - 1 hr	44-48°C- 1 hr	48-52°C - 1 hr	52-54°C- 0.5 hr	LT- 56°C- 3hr
T_{10} -32-56	32-36°C-1hr	36-40°C-1hr	40-44°C - 1 hr	44-48°C- 1 hr	48-52°C - 1 hr	52-56°C- 1 hr	LT- 56°C- 3hr

Note: hr- hours and LT- Lethal temperature

Table 3. Standardisation of thermotolerance induction or sub-lethal temperature based on seed germination and seedling growth parameters in maize at seed level

Treatment (°C)	Germination (%)	Root length (cm)	Reduction in root (%)	Shoot length (cm)	Reduction in shoot (%)	Seedling growth (Root+shoot)	Reduction in seedling growth over control (%)	Seedling vigor index-I
T_1 -Control	100.00	15.27	NA	8.43	NA	23.70	NA	2370.00
T_2 -32-40	50.67	5.97	60.92	4.03	52.15	10.00	57.80	506.73
T_3- 32-42	52.67	6.07	60.26	4.23	49.78	10.30	56.54	542.53
T_4 -32-44	57.33	7.27	52.40	5.37	36.37	12.63	46.69	724.40
T_5 -32-46	61.33	9.27	39.30	6.20	26.47	15.47	34.73	948.60
T_6 -32-48	70.67	11.20	26.63	7.10	15.81	18.30	22.77	1293.20
T_7 -32-50 *	**87.33**	**13.33**	**12.67**	**8.03**	**4.72**	**21.37**	**9.84**	**1866.13**
T_8- 32-52	41.33	6.23	59.17	6.23	26.08	12.47	47.40	515.20
T_9- 32-54	10.67	4.17	72.71	5.51	34.66	9.68	59.17	103.25
T_{10} -32-56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C.D.	1.77	0.25	8.74	0.24	20.33	0.31	12.75	37.18
SE(m)	0.60	0.09	2.94	0.08	6.84	0.11	4.29	12.52

**Thermotolerance induction temperature or Sublethal temperature: 32-50 °C + Lethal temperature (32-36 °C for1 hr, 36-40 °C for 1hr, 40-44 °C for 1hr, 44-48 °C for1hr and 50 °C for 0.5 hr followed by exposure to lethal temperature of 56 for3 hours*

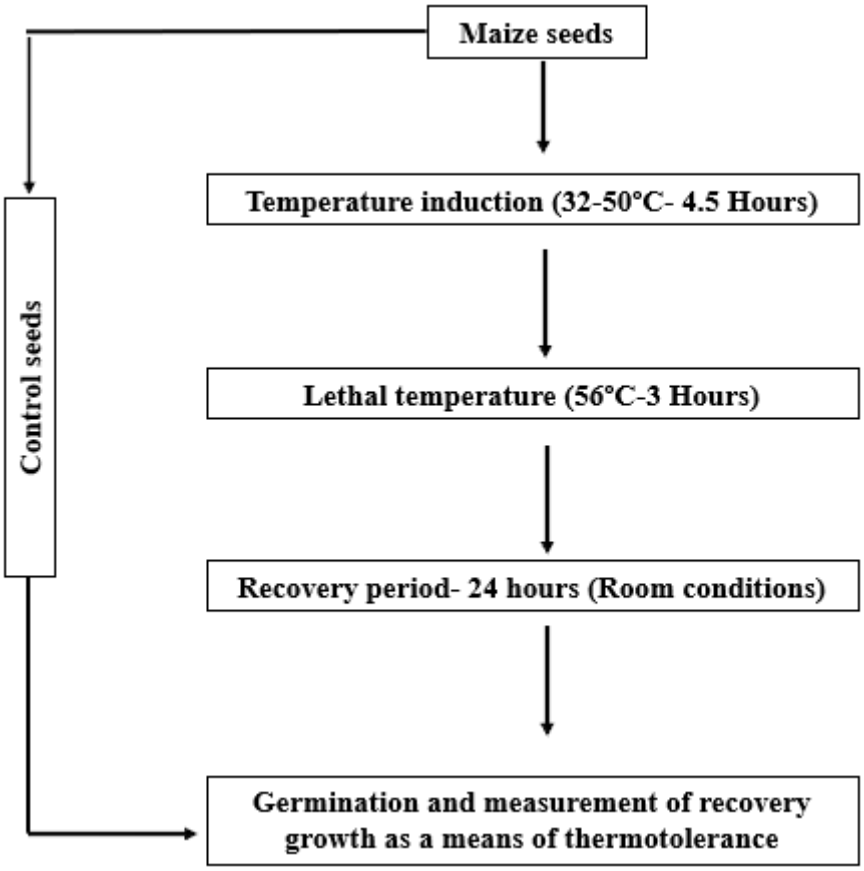

Fig. 1. The Diagrammatic representation of the standardised temperature induction response technique in maize at the seed level

4. DISCUSSION

Acquired thermotolerance is the ability of plants to cope with lethal high temperatures after acclimatization at sub-lethal temperatures. Acquired thermotolerance reflects a natural thermotolerance mechanism in plants and has been used to identify thermotolerant lines [27]. Acquired thermotolerance involves various biochemical, physiological and molecular changes in plants, such as the induction of heat-shock proteins, the activation of heat-shock transcription factors and the modulation of stress-related antioxidants and hormones [28]. The synthesis of heat shock proteins (HSPs) increased significantly during induction stress, which helped the tomato seedlings to cope with lethal stress by enhancing their physiological and biochemical functions [11]. The protein profile of the induced seedlings, as revealed by SDS-PAGE, showed a marked and sustained expression of HSPs even after three days of recovery. These HSPs enabled the seedlings to adjust to high-temperature stress [29]. A previous study by Gomathi et al [30] reported that the settlings of the sugarcane that were treated with TIR resulted in an increased accumulation of phenolic compounds and expression of antioxidant enzymes. Since phenolics have been synthesized in cells and acts as ROS scavengers in their soluble form. The proline and glycine betaine, which were well-known osmolytes and are involved in the protection of the cells from high-temperature damage, were also found to be increased significantly in the induced settlings of the sugarcane compared to the non-induced settlings. It is also reported that the expression of hsp70 and hsp18.1 transcripts and the accumulation of HSP104 and HSP90 proteins were higher in the tolerant genotype of pea than in the susceptible genotypes when treated with TIR [31]. There is strong evidence to suggest that the induction of thermotolerance in seeds involves the predominant expression of the stress-responsive proteins and genes during the sub-lethal stress that induces the necessary changes in the plant metabolism to cope with the subsequent severe or lethal stress [29]. The seedlings of sunflowers that were TIR-treated

exhibited a higher expression of HSP 90 and HSP 104 in the induced seedling [32]. These HSPs are responsible for various processes, such as maintaining, chaperoning proteins and membrane stability, that are essential for the plant's biochemical and physiological functions [33].

The present study also noted the prominent improvement in root and shoot growth after induction treatment indicating the acquisition of thermotolerance. The seed is a miniature plant with an embryo enclosed inside. Seeds carry a genetic material that determines the traits and characteristics of the plants that grow from them [34]. Harnessing the thermotolerance at the seed level itself has more advantages than later stages. Which can endure and sustain the acquired thermotolerance more effectively for a longer period under the heat stress conditions in the field right from emergence to maturity. Similar studies were conducted by Dar et al. [21] in maize. They standardized the TIR protocol using the same method but at the seedlings level. They reported an induction temperature of 30-45°C for five hours and a lethal temperature of 50°C for three hours. Therefore, the present study has an advantage over the former research, where the TIR technique was standardized at the seedlings stage. which is laborious and time-consuming and involves raising the seedlings and carefully handling them as they are delicate. In divergence, the present study standardized the TIR technique at the seed level, which allows for direct use of seeds and saves time in raising seedlings. Furthermore, seeds are firmer and easier to handle than seedlings.

5. CONCLUSION

The study standardized TIR in maize at the seed level, the induction or sublethal temperature standardised was gradual exposure of maize seeds to 32-50°C for 4.5 hours followed by exposure to the standardised lethal temperature of 50°C for three hours and a recovery period of about of 24 hours under room conditions, further assessing germination and the recovery growth as a means of thermotolerance. The standardized TIR can be a reliable screening technique for evaluating maize genotypes for heat tolerance and assessing the genotypic variability in thermotolerance at the seed level itself. Genetic makeup and variability among maize genotypes confer heat tolerance. The main benefit of this technique is its reproducibility and quickness in screening a vast population of maize genotypes at the seed stage itself. It also assists the breeder in narrowing down tolerant genotypes in a big population, saving the energy and time required for screening. Later, identified tolerant genotypes can be utilized for additional documentation of traits conferring heat tolerance and can also be used for mapping the QTLs or genes, which will benefit breeders in marker-assisted selection for heat stress and other crop improvement programs in maize.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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