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Temperature induction responses and its effects on physiological parameters of cotton (*Gossypium Hirsutum* L.)

M Devi[1*], S Vincent[1], V Babu Rajendra Prasad[1], R Anandham[2], L Mahalingam[3]
[1] Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
[2] Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
[3] Department of Cotton, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Abstract
Laboratory experiments were conducted at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during 2019-2020 by using Temperature Induction Response (TIR) technique. The popular cotton (*Gossypium hirsutum* L.) varieties of Tamil Nadu like KC 3, SVPR 2 and MCU 7 were taken for the screening of high temperature tolerance. The identical sized five days and seven days old cotton seedlings were selected and exposed to inductive temperature (gradual temperature raised from 28 to 40℃) for 4 h and non-inductive temperature (46℃ for 3 h, 47℃ for 3 h, 48℃ for 3 h and 48℃ for 4 h) for specific time duration. Among the genotypes, KC 3 had recorded the highest thermotolerance in terms of seedling survival, cell viability, total soluble protein and lipid peroxidation (MDA content) as compared to other two varieties under both inductive and non-inductive temperature.

Keywords: cotton, temperature induction response, cell viability, total soluble protein, malondialdehyde content

Introduction
Cotton (*Gossypium hirsutum* L.) is the world's most important fiber cash crop essential for textile industry and also useful for oils and livestock feed. Eighty percent of world production in cotton comes from seven countries where India is placed in third position (Zahid *et al*., 2016) [15]. Though cotton is a hardy crop can come up in all types of climatic conditions, global warming is a serious emerging thread causing environmental fluctuations in most of the agricultural zones of world including cotton (Solomon, 2007) [11]. Cotton crop responds to various abiotic stresses, especially, high temperature is one which causing severe damages to cotton crop at cellular level in all growth and developmental stages limits the cotton yield (Oosterhuis *et al*., 2002; Sarwar *et al*., 2019) [9][8]. The current study explains the screening of cotton seedlings against thermo tolerance through the Temperature Induction Response (TIR) technique. This technique was widely used for rapid screening of cotton genotypes for high temperature tolerance (Kheir *et al*., 2012) [6]. The current screening study is based on the principle of "acquired thermotolerance" of cotton seedlings of three popular cotton varieties of Tamil Nadu. The accounting data of morphological and biochemical characters of cotton seedlings were considered for the confirmation of high temperature tolerance of varieties.

Materials and methods
The laboratory screening studies through the Temperature Induction Response (TIR) technique was conducted in the Department of Crop Physiology, TNAU, Coimbatore during 2019-2020. Three popular cotton varieties of Tamil Nadu namely, KC 3, SVPR 2 and MCU 7 were obtained from the Department of cotton, TNAU, Coimbatore. The uniform size of five days and seven days old seedlings were grown in paper cup (1:1 ratio of coir pith: vermicompost) where exposed to a gradual temperature from 28 to 40℃ (T_1-Control, T_2-46℃ for 3 h, T_3-47℃ for 3 h, T_4-48℃ for 3 h and T_5-48℃ for 4 h). Also, five days seedlings were exposed directly to the challenging temperature of 46℃ (T_6) for 3 h and 47℃ (T_7) for 3 h.

The exposed seedlings were kept for recovery under room temperature for 48 h and analyzed for seedling survival, cell viability (Gaff and Okong'o – Ogola. 1971) [3], total soluble protein (Lowry *et al*., 1950) [7] and lipid peroxidation (MDA content) (Heath and Pacber, 1968) [4]. All the treatments were maintained with three replications and the data were analyzed under factorial completely randomized design (FCRD) by using SPSS.

Results and Discussion
Seedlings survival
The present study showed that the highest seedlings survival percentage was observed in T_2 - 46^{0}C for 3 h in both 5th and 7th day seedlings as compared to other treatments and the survival percentage was gradually decreased when the seedlings were exposed to induction temperature. The least survival percentage was observed in T_5 - 48^{0}C for 4 h (Fig.1A, B). The five-day old seedlings alone exposed to challenging temperature (without induction temperature) had recorded lower survival percentage (Fig.1A). Among the three cotton varieties, KC 3 had maximum survival rate compared to other varieties of SVPR 2 and MCU 7. When the temperature raised the rate of seedlings survival was decreased. Similar trend of was observed in various crops like cotton (Kheir *et al*., 2012) [6], rice (D. Vijayalakshmi *et al*., 2015) [13], maize (Dar *et al*., 2016) [14] and chickpea (T. Raghavendra *et al*., 2017) [10].

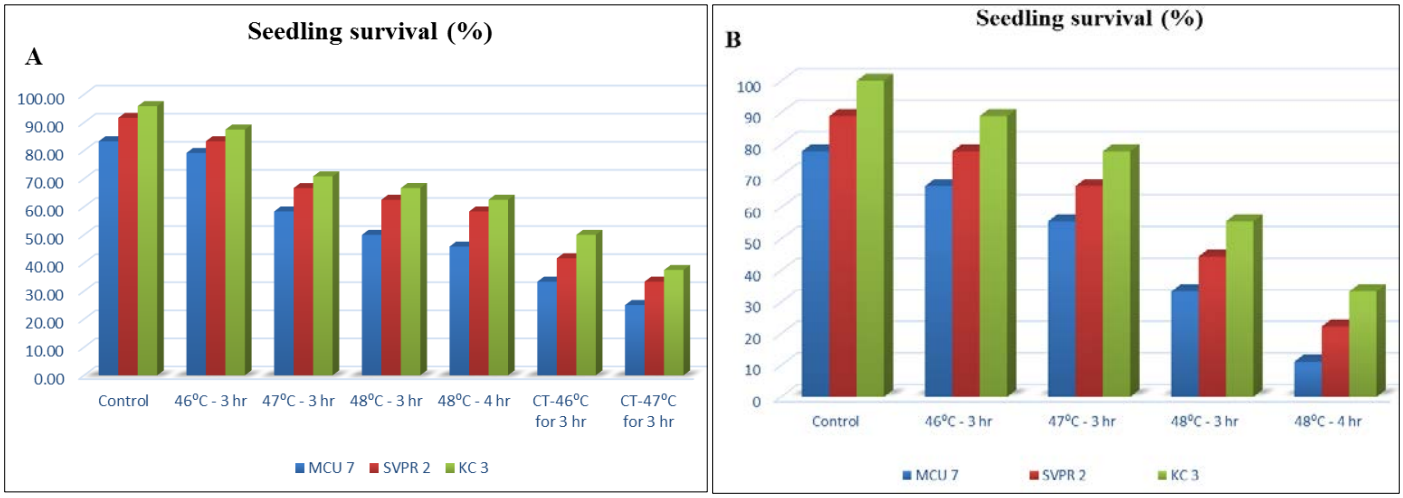

Fig 1: Percentage of seedlings survival rate after exposure of high temperature at the end of recovery period on cotton seedlings (**A.** 5 days old seedling and **B.** 7 days old seedling)

Cell viability

Cell viability was found to be higher in T_2 - 46^{0}C exposed for 3 h showed the least cell viability in 5th and 7th day seedlings. Seedlings of five-day old exposed to the challenging temperature of 46^{0}C for 3 h recorded maximum cell viability when compared to challenging temperature 47^{0}C for 3 h (Fig.2A). Among the treatments, KC 3 had maximum cell viability followed by SVPR 2 and MCU 7. It is observed that the cell viability and temperature were indirectly correlated. The increase in temperature was decreasing the viable cell counts. Kheir *et al*, (2012) [6] reported similar observations cotton showed that the cell viability percentage estimated by Evan's blue method which had higher viable cell count (87.01%) in heat tolerant genotype as that of heat susceptible (34.41%).

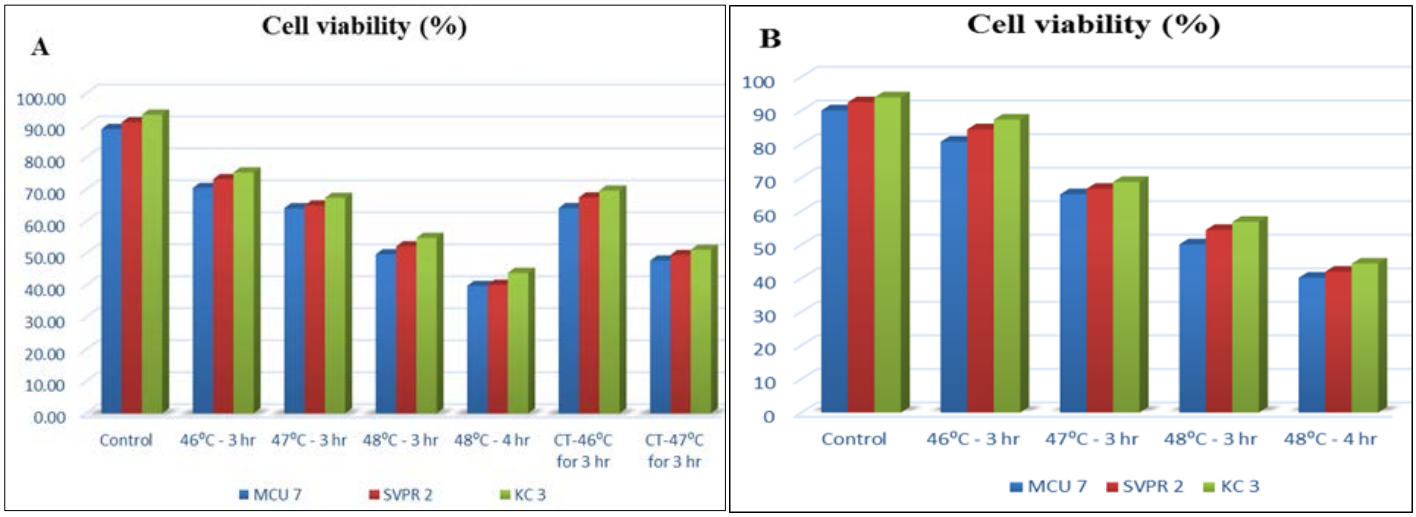

Fig 2: Percent of cell viability on cotton seedlings at different temperatures (**A.** 5 Days old seedling and **B.**7 Days old seedling)

Total soluble protein

Total soluble protein accumulation was increased with increased temperature compared to control (T_1). The highest protein content was observed in T_4 (48^{0}C for 3 h) compared to T_6 and T_7 in 5 days old seedling (Table.1). In 7 days old seedling, T_4 (48^{0}C for 3 hours) treatment had higher content of soluble protein followed by T_5 - 48^{0}C for 4 h, T_3 - 47^{0}C for 3 h and T_2 - 46^{0}C for 3 h (Table.2). Total soluble protein content recorded in KC 3 was higher when compared to SVPR 2 and MCU 7. Increase in protein content with the occurrence of high temperature might be reason of restructuring and accumulation of protein fractions. Similar results were showed in sorghum and barley where the majority of heat shock proteins were associated with the soluble fraction (Ashraf *et al*., 1994) [2].

Table 1: Effect of temperature induction response on total soluble protein **A.** 5 Days old cotton seedling and **B.** 7 Days old seedling

A

Treatments	MCU 7 (mg g^{-1})	SVPR 2 (mg g^{-1})	KC 3 (mg g^{-1})	Mean
T_1 - Control	9.50	11.88	12.05	11.14
T_2 - 46^{0}C for 3 h	5.44	8.30	8.76	7.50

T_3 - 47^{0}C for 3 h	10.68	12.44	12.78	11.96
T_4 - 48^{0}C for 3 h	12.08	13.42	14.45	13.31
T_5 - 48^{0}C for 4 h	9.19	12.24	12.37	11.26
T_6 - Challenging temperature (46^{0}C for 3 h)	9.95	10.85	11.34	10.71
T_7 - Challenging temperature (47^{0}C for 3 h)	10.07	12.89	13.24	12.06
Mean	9.559	11.717	12.141	
	T	V	T*V	
SED	0.004	0.002	0.007	
CD (P=0.05)	0.008	0.005	0.015	

B

Treatments	MCU 7 (mg g^{-1})	SVPR 2 (mg g^{-1})	KC 3 (mg g^{-1})	Mean
T1 - Control	8.50	9.02	9.40	8.97
T2 - 46ºC for 3 h	8.44	9.05	9.90	9.13
T3 - 47ºC for 3 h	8.60	9.43	10.42	9.48
T4 - 48ºC for 3 h	11.17	11.41	12.08	11.55
T5 - 48ºC for 4 h	9.28	9.57	10.35	9.73
Mean	9.19	9.70	10.43	
	T	V	T*V	
SED	0.003	0.002	0.006	
CD (P=0.05)	0.007	0.005	0.012	

Lipid peroxidation (MDA content)

MDA content accumulation was higher in both 5 days and 7 days old seedling in T_5 - 48^{0}C for 4 h and the least MDA content was observed in T_2 - 46^{0}C for 3 h (Fig.3A and Fig.3B). High temperature stress that impairs mitochondrial functions thereby resulting in the induction of oxidative damage that manifests in lipid peroxidation, detected by malondialdehyde (MDA) content (Larkindale and Knight 2002; Vacca *et al*., 2004) [5, 12]. Heat stress causes increased membrane damage due to lipid peroxidation (Amirjani, 2012) [1]. D. Vijayalakshmi *et al*. (2015) [13] observed that lipid peroxidation (MDA content) was lower in inductive temperature when compared to non-inductive temperature.

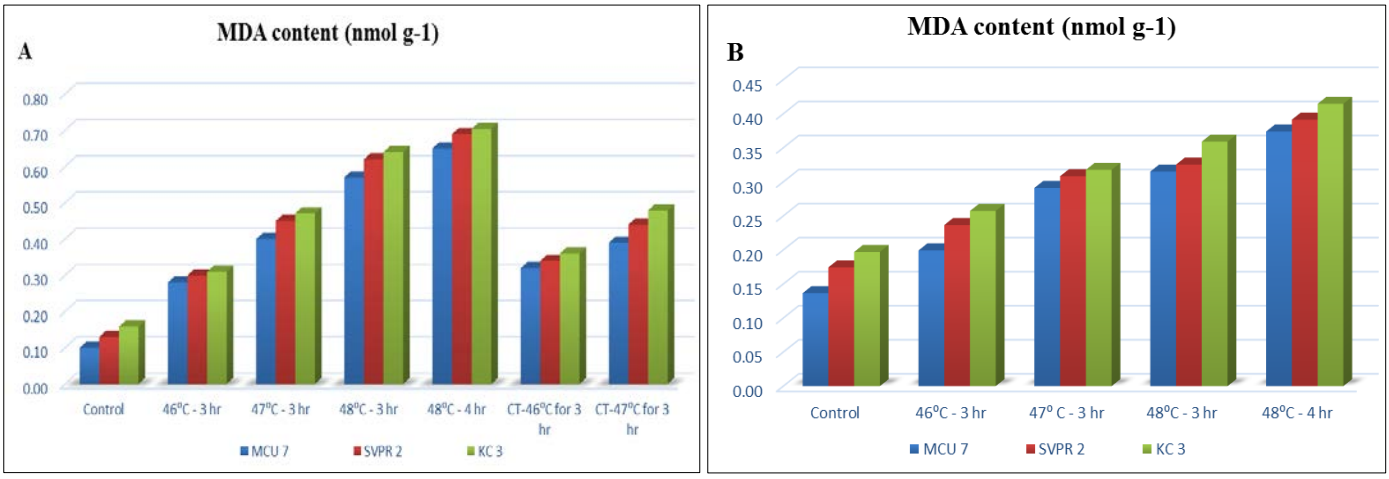

Fig 3: Effect of temperature induction response on lipid peroxidation (MDA content) of cotton seedlings **A.** 5 days old seedling **B.** 7 days old seedling

Conclusion

The present study concluded the screening of 5 days and 7 days old cotton seedling through the temperature induction response (TIR) expressed that the seedling survival, cell viability, total soluble protein and lipid peroxidation (MDA content) was higher in KC 3 as that of SVPR 2 and MCU 7. Further it was inferred that KC 3 variety had shown high thermo tolerance capacity when compared to SVPR 2 and MCU 7.

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