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Assessment of acquired thermo tolerance and genetic variability in cotton genotypes by Temperature Induction Response (TIR)

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ABSTRACT

Cotton has global importance, being a major fiber crop with a high commercial value. It is grown in tropical and sub-tropical regions and plays pivotal role in economy in many countries. It thrives well under optimal temperature. Too high and too low temperatures affect badly its growth and yield. Too low temperature affects its germination and seedling establishment stages. Particularly, high temperatures influence many physiological and biochemical processes within cotton plant that result in poor seed cotton yield. Plants, when exposed to sub-lethal stress (induction stress), develop the ability to withstand severe temperatures and this phenomenon is often referred to as acquired thermo tolerance. In this context, a lab experiment was conducted to standardize the temperature induction response (TIR) protocol for cotton seedlings using WGC-450 programmable plant growth chamber. Temperatures were standardized as sub lethal, i.e. challenging temperatures as 28-40 ^{0}C (for 4 hours) and lethal temperatures as 47 ^{0}C (for 3 hours). A set of diverse cotton germplasm comprising of 35 genotypes were screened for intrinsic tolerance using the standardized Thermo Induced Response (TIR) protocol. Among the genotypes CNH-09-073, BGDS-1077, NDLH-2057-1, ARBH-1851, ABD-645, RAH-1075 and NDLH 2028-2 showed highest thermo tolerance in terms of higher seedling survival with no reduction in root and shoot growth.

***Key words**: Cotton, Thermotolerance, Lethal temperature, Sub- lethal temperatures*

Introduction

Heat stress significantly restricts crop growth and compromises plant production around the world despite the several mechanisms evolved by the plants in order to alleviate adverse environmental conditions. According to climate reports, average global temperature has been rising over the last century and at the current rates of greenhouse gas emissions, a further increase is projected by the end of the 21st century. Higher than optimum temperatures during the day and their negative effects on plant physiology, biochemistry, and yield have been extensively investigated by many workers.

To alleviate this threat, it is necessary to improve the adaptability of cotton genetically to cope with the warming climates. Association between adaptability of crop plants to high temperature stress and heat acclimation has been reported (Chen *et al.*, 1982). However, the genetic basis for heat tolerance or adaptability to heat stress in cotton is poorly understood. Heat acclimation is a physiological response manifested in the acquired thermo-tolerance of organisms or cells to lethal high temperatures following exposure to a moderate heat stress condition. Furthermore, reactions of plants to temperature stresses are complex and have adverse effects on plant metabolism by disrupting cellular homeostasis and uncoupling major physiological and biochemical processes (Awasthi *et al*., 2015). One of the approaches to improve thermo-tolerance is to transfer superior alleles from intrinsically thermo-tolerant wild relatives. A large number of genetic variations exist for intrinsic tolerance among the hirsutums and arboreums of cotton.

Inordinate efforts are needed to identify more sources from the unexplored landraces and understand the bases of abiotic stress tolerance Sarkar and Bhattacharjee (2011). It is important to develop a rapid and reliable lab protocol that allows simultaneous screening of large number of genotypes. Temperature induction response (TIR) has been used as a screening technique to identify high temperature tolerant cotton lines (Ehab abou Kheir *et al*., 2012). Acquired tolerance for a specific abiotic stress has been shown to give cross protection for other stresses such as salinity, chilling temperatures and drought (Renuka Devi *et al*., 2013). The present study, therefore, was planned to assess the genetic variability of cotton germplasm and land races using temperature induction response (TIR) to identify more sources for intrinsic tolerance and to identify the genotypes that combine temperature tolerance with tolerance of drought stresses.

Materials and Methods

Research work was carried at Regional Agricultural Research Station, Acharya N G Ranga Agricultural University. Nandyal, Kurnool District Andhra Pradesh with 35 cotton genotypes obtained from AICCP Cotton scheme of RARS Nandyal.

Seedling growth: The cotton seeds were soaked in water for 24 hr and allowed to imbibe in glass beaker. Pre germinated seeds were selected for the experiment and the uniform seedlings from each genotype were transferred to different glass beakers

Standardization of Optimum Induction Temperature and Challenging High Temperature for Cotton Seedlings

Identification of lethal or challenging temperature: Challenging or lethal temperature is the temperature treatment for a specific time duration (in hours) required to cause more than a 90% reduction in seedling survival in non induced seedlings of cotton. To assess the challenging temperatures for 100 per cent mortality, germinated cotton seedlings were exposed to different challenging temperatures (44, 45, 46, and 47 ^{o}C) for varying durations (1, 2 and 3 hours) without prior induction. Thus, exposed seedlings were allowed to recover at 30 ^{o}C and 60 per cent relative humidity for 48 hours. 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 cotton genotypes after recovery was recorded (Table 1). The lethal temperature of 47 ^{o}C for 3 hour was considered in this context, as maximum mortality (100%) of seedlings.

Identifications 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 regimes and duration are varied from crop to crop and are to be standardized. The germinated cotton seedlings were subject to gradually increasing temperatures, i.e 28 to 40 ^{o}C for a period of four hours. After this induction treatment, seedlings were exposed to lethal temperature i.e., 47 ^{0}C for three hours and then allowed to recover at 30 ^{o}C and 60% relative humidity for 48h. 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 seedlings survival reduction was considered as optimum temperatures i.e., 28 ^{0}C-40 ^{0}C.

Thermo induction response (TIR): Cotton seeds were surface sterilized by treating with 2 per cent bavistin solution for 30 min and cleaned with the distilled water for 4-5 times and kept for germination at 30^{0}C and 60% relative humidity in the incubator. After 48 hours, uniform seedlings were selected in each genotype and sown in aluminum trays (50 mm) filled with soil. These trays with seedlings were subjected to sub lethal temperatures (gradual temperatures increasing from (28 ^{0}C-40 ^{0}C) for four hours in the environmental chamber (WGC-450 Programmable Plant Growth Chamber). Later these seedlings were exposed to lethal temperatures, i.e 47 ^{0}C for 3 hours (induced). Another set of seedlings were directly exposed to lethal temperatures (non induced). Induced and non induced cotton seedlings were allowed to recover at 30 ^{0}C and 60%

relative humidity for 48 hours. The following parameters were recorded from the seedlings.

a) Percent 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) Percent reduction in root growth =

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

c) Percent reduction in shoot growth =

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

A lethal temperature of 47 ^{0}C for 3 hours and induction treatment from 28-40 ^{0}C for four hours was standardized using TIR (Thermo Induction Response) and considered as best lethal and induction temperatures for phenotyping of cotton seedlings for intrinsic heat tolerance at cellular level (Table 1 and 2).

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

Temperatures	Percent Mortality of cotton seedlings after recovery		
	Duration of temperatures		
	1 hour	2 hour	3 hour
44 ^{0}C	0	20	30
45 ^{0}C	0	30	50
46 ^{0}C	50	65	86
47 ^{0}C	75	90	100

Table 2. Per cent mortality of cotton seedlings at different induction (sub lethal)

Temperature range	Temperature range Percent survival of the seedling Induction Temperature ^{0}C (4 hours)
30-45	60
28-48	65
28-46	70
28-40	90

Results and Discussion

The experimental data were recorded and the genotypes which showed contrasting values for survival of seedlings, reduction in root and shoot growth were presented in (Table 3). The effect of TIR on genotypes revealed variable results, such as acquired tolerance was variably recorded in cotton genotypes, where either survival of seedlings was affected in 24 genotypes (Sri Rama, NDLH 2035-5, BS6-18, Sivanandi, RHC-1306, NH-704, BS 5-18, CNH-1131, Suraj, CNH 2052, CPD 1852, CNH 16300, RAH-1076 AKH-10-3, RHC-1307, CNH-2073, CPD-1851, GBHV-201, H-1525, CNH-09111, NDLH 2010 , NDLH 2985, NDLH 2051-1 **and** Narasimha**) or root growth alone was affected in 1 genotypes (**NDLH 2005-4**)** or only shoot growth alone was affected in 2 genotypes (CNH 2002 and GBHV-200). In spite of exposing to 47^{0}C, germination and seedling growth were not affected in CNH-09-073, BGDS-1077, NDLH-2057-1, ARBH-1851, ABD-645, RAH-1075 and NDLH 2028-2 probably due to acquired thermo tolerance.

The technique of exposing young seedlings to sub lethal and lethal temperatures has been validated in many crop species Venkatachalayya *et al.* (2001) in pea, Senthil-kumar *et al*. (2003) in sunflower, Gangappa *et al.* (2006) in groundnut, Sudhakar et *al.* (2012), Renukha devi *et al*. (2013), Sapna Harihar *et al*. (2014) and Vijayalakshmi *et al.* (2015) in rice, Ehab abou kheir *et al.* (2012) in cotton, Venkatesh Babu and Raghavendra (2017) in Sorghum and Raghavendra *et al.* (2017) in Chickpea. This novel temperature induction response technique has been demonstrated to reveal genetic variability in intrinsic stress tolerant at cellular level, Narayanaswamy (2010).

Conclusion

The above results suggest that the TIR technique is a powerful and constructive technique to identify genetic variability in high temperature tolerance in cotton within a short period of time and it is suitable

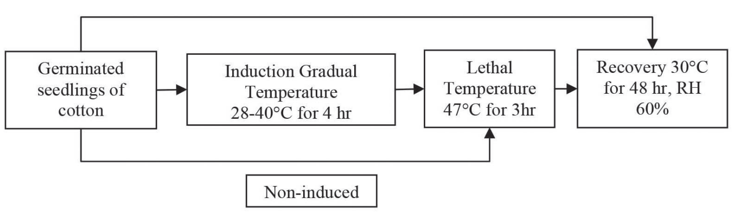

Table 3. Screening of thermo tolerant Cotton genotypes through Thermo Induced Response (TIR) technique

S. No.	Genotypes	Germination percent	Percent reduction in root growth	Percent reduction in shoot growth
1	Sri Rama	50(45.02)	45.12(42.22)	19.52(26.23)
2	NDLH 2035-5	50(45.02)	33.64(35.47)	21.64(27.74)
3	BS6-18	100(90.05)	40.22(39.38)	19.05(25.89)
4	Sivanandi	100(90.05)	26.43(30.95)	21.67(27.76)
5	RHC-1306	90(71.60)	29.84(33.13)	34.73(36.13)
6	NH-704	70(56.82)	25.87(30.59)	32.33(34.67)
7	CNH-09-073	100(90.05)	0.00(0.00)	0.00(0.00)
8	BS 5-18	80(63.47)	30.71(33.67)	17.24(24.55)
9	BGDS-1077	80(65.02)	0.00(0.00)	0.00(0.00)
10	CNH-1131	30(33.23)	24.78(29.87)	13.10(21.23)
11	Suraj	90(71.60)	15.32(23.05)	32.18(34.58)
12	CNH 2052	100(90.05)	7.83(16.26)	30.35(33.45)
13	CPD 1852	90(71.60)	21.23(27.45)	28.37(32.20)
14	CNH 16300	90(71.60)	20.34(26.82)	24.88(29.94)
15	GBHV-200	40(39.25)	0.00(0.00)	29.17(32.71)
16	RAH-1076	80(63.47)	13.89(21.89)	30.67(33.65)
17	AKH-10-3	80(63.47)	29.76(33.08)	35.90(36.83)
18	RHC-1307	50(45.02)	20.28(26.78)	24.24(29.51)
19	CNH-2073	100(90.05)	4.91(12.81)	9.09(17.56)
20	CPD-1851	40(39.25)	46.21(42.85)	53.41(46.98)
21	GBHV-201	90(71.60)	6.76(15.08)	11.76(20.07)
22	NDLH-2057-1	70(56.82)	0.00(0.00)	0.00(0.00)
23	ARBH-1851	90(71.60)	0.00(0.00)	0.00(0.00)
24	H-1525	20(26.58)	6.76(15.08)	22.35(28.23)
25	ABD-645	30(33.23)	0.00(0.00)	0.00(0.00)
26	RAH-1075	70(56.82)	0.00(0.00)	0.00(0.00)
27	CNH-09111	40(39.25)	24.44(29.64)	32.97(35.06)
28	Narasimha	90(71.60)	10.78(19.18)	15.04(22.83)
29	CNH 2002	80(63.47)	0.00(0.00)	10.35(18.78)
30	NDLH 2028-2	70(56.82)	0.00(0.00)	0.00(0.00)
31	NDLH 2005-4	100(90.05)	16.67(24.11)	0.00(0.00)
32	NDLH 2010	70(56.82)	28.21(32.10)	34.52(36.00)
33	NDLH 2985	70(56.82)	8.12(16.56)	11.69(20.00)
34	NDLH 2051-1	70(56.82)	23.81(29.22)	26.25(30.84)
35	NDLH 2056-4	100(90.05)	0.00(0.00)	0.00(0.00)
	Mean	72.5	17.45	16.60
	S.Em±	6.61	0.681	0.892
	CD (P=0.05)	17.2	2.58	3.01

*Values in parenthesis represent arcsine transformed values.

for screening a large number of genotypes. The identified 7 genotypes CNH-09-073, BGDS-1077, NDLH-2057-1, ARBH-1851, ABD-645, RAH-1075 and NDLH 2028-2 of cotton can be used as donor source for developing high temperature tolerant cotton genotypes. The genotypes with intrinsic heat tolerance can be explored for the development of varieties suitable for high temperature conditions where cotton is prone to terminal heat stress.

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Conflict of Interest: None

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