The Bioscan 12(1): 381-384, 2017 (Supplement on Agronomy)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

EVALUATION OF SORGHUM (*SORGHUM BICOLOUR* L.) GENOTYPES FOR TEMPERATURE TOLERANCE BASED ON TEMPERATURE INDUCTION RESPONSE (TIR) TECHNIQUE

D.VENKATESH BABU* AND T.RAGHAVENDRA
Department of Crop Physiology,
Regional Agricultural Research Station, ANGRAU, Nandyal - 518 501
e-mail: venkateshbabu2007ag@gmail.com

KEYWORDS
Sorghum
Thermotolerance
Lethal temperature
Sub- lethal temperatures

Received on :
21.10.2016

Accepted on :
09.02.2017

***Corresponding author**

ABSTRACT

Plants, when exposed to sub lethal stress (induction stress), develop the ability to with stand severe temperatures and this phenomenon is often referred to as acquired thermotolerance. In the present study, a novel temperature induction response (TIR) technique was standardized for sorghum crop. We have standardized the sub lethal i.e. challenging temperatures 38-52^{0}C (for 4 hours &30 min) and lethal temperatures as 56^{0}C (for 3 hours) Using this standardized TIR protocol, highly thermotolerant sorghum genotypes were screened from 40 sorghun germplasm. Sufficient genotypic variability was noticed from zero level to 100% level of tolerance. Among the genotypes, GP-2626 and GP-2564 showed highest thermo tolerance in terms of 100 per cent seedlings survival and no reduction in root and shoot growth. GPW-M35 and GPW-278 showed higher thermotolerance in terms of 95 percent seedlings survival and no reduction in root and shoot growth. It is proposed that this technique can be used as a potential tool to identify and select temperature tolerant lines at the seedling stage from a large population. These genotypes have intrinsic heat tolerance and they can be explored as donar source in breeding programme aimed for global warming.

INTRODUCTION

Prevalence of high temperature is the major limitation for the cultivation of crops in tropical conditions. 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. The day temperatures to which the plants are exposed in many tropical areas are often above their optimal growth temperature and a small increase above optimum has large effect on growth rate (Howarth, 1996). Development of genotypes that are capable to survive better under high temperature stress is paramount important and inevitable.

Sorghum (*Sorghum bicolour* L. Moench) is most sensitive to high temperature stress around the reproductive development stage (Prasad et al. 2008; Nguyen *et al.,* 2013; Singh *et al.*< 2015), although high temperatures can also affect plant height (Prasad *et al.,* 2008; Nguyen *et al.,* 2013), leaf growth, and phenology (Hammer *et al.,* 2010). 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 tolerances to temperature extremes are complex traits dependent on many attributes. One of the approaches to improve thermotolerance is to transfer superior alleles from intrinsically thermotolerant wildrelatives, which require precise screening methods to measure the variability in thermotolerance. 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 thermotolerance at field level (Selmani and Wasson, 1993), but these measurements are highly influenced by environmental factors which are the major limitation. The best alternative, therefore, would be 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.The technique of exposing young seedlings to sub lethal and lethal temperatures has been validated in other crop species viz. rice (Sapna harihar *et al.* 2013; Sudhakar *et al.* 2012), ragi (Venkatesh babu *et a.l* 2013), cotton (Ehab Abou Kheir *et al.* 2012), groundnut (Gangappa *et al.* 2006), sunflower (Senthil Kumar *et al.* 2003)and pea (Venkatachalayya *et al.* 2001).

In the present study, an attempt was made to standardize the temperature induction response technique which is prerequisite for screening the temperature tolerance in sorghum genotypes.

MATERIALS AND METHODS

Present investigation was done at Regional Agricultural Research Station, Acharya N G Ranga Agricultural University. Nandyal, Kurnool District Andhra Pradesh with 40 sorghum genotypes obtained from Millets Scheme of RARS Nandyal.

Seedling growth

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

Identification of lethal 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^{0}C) for varying durations (1, 2 and 3 hours) without prior induction. Thus, exposed seedlings were allowed to recover at 30^{0}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 sorghum genotypes after recovery was recorded (Table 1). The lethal temperature of 56^{0}C for 3 hour was considered in this text, as maximum mortality (98%) 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 are to be standardized. The germinated sorghum seedlings (42 hour old sorghum seedlings) were subject to gradually increasing temperatures for a period of four and half hours. After this induction treatment, seedlings were exposed to lethal temperature *i.e.,* 56^{0}C for three hours and then transferred to the normal temperature for recovery. The temperature regimes and durations are varied to arrive at optimum induction protocol (Table2).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., 38^{0}C-52^{0}C.

Thermo induction response (TIR):

Sorghum 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 42 hours, uniform seedlings were selected in each genotype and sown in aluminium trays (50mm) filled with soil. These trays with seedlings were subjected to sub lethal temperatures (gradual temperatures increasing from 38ºC-52ºC) for four and half hours in the environmental chamber (WGC-450 Programmable Plant Growth Chamber). Later these seedlings were exposed to lethal temperatures (56ºC) for 3 hours (induced). Another set of seedlings were directly exposed to lethal temperatures (non induced).

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

$$\text{Percent survival of seedlings} = \frac{\text{No. of seedlings survivedat the end of recovery}}{\text{Total number of seedlings sownin the tray}} \times 100$$

$$\text{Percent rediction in root growth} = \frac{\text{Actual root growth of treated seedlings}}{\text{Actual root growth of control seedlings}} \times 100$$

$$\text{Percent reduction on shoot growth} = \frac{\text{Actual shoot growth of treated seedlings}}{\text{Actual shoot growth of control seedlings}} \times 100$$

A lethal temperature of 56^{0}C for 3 hours and induction treatment from 38-52^{0}C for four and half 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. (Table 1 and Table 2).

RESULTS AND DISCUSSION

The experimental data were recorded and the genotypes which showed contrast values for survival of seedlings, reduction in root and shoot growth were presented in the Table 3. The effect of TIR on genotypes releaved variable results.

Such as acquired tolerance was variably recorded in other sorghum genotypes, where either survival of seedlings was affected in 14 genotype (GP-2414,GP-2597,GP-2598,GP-2615,GP-2630,GP-2631, GP-2632, GP-2633, GP-2641,GP-2642, GP-2645, GP-2649, GPW-296, GPW-300) or root growth alone was affected in 2 genotypes (GP-2641,GP-2649) or only shoot growth alone was affected in 15 genotypes (GP-2327-2, GP-2403, GP-2608,GP-2611,GP-2612,GP-2613,GP-2617, GP-2618, GP-2629, GP-2625, GP-2634, GP-2635,GP-2637, GP-2640, GP-2644). Inthegenotypes GP-2632 and GP-2633 the seedling survival, shoot and root growth were completely affected despite of the recovery conditions maintained after exposing to sub lethal to lethal temperature. In spite of exposing to 57^{0}C, germination and seedling growth were not affected in GP-2626, GP-2564, GPW-M35 and GPW278 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) and Bharani *et al*.(2016) in groundnut, Sudhakar et *al*. (2012) Renukha *et al*.(2013)

Table 1: Per cent mortality of sorghum seedlings at different lethal temperatures

Temperatures	Percent Mortality of sorghum seedlings after recovery Duration of temperatures 1hour	2 hour	3hour
50 ^{0}C	0	0	0
52 ^{0}C	0	20	40
54 ^{0}C	40	60	86
56 ^{0}C	60	88	98

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

Temperature range Induction Temperature ºC (4 hours 30min)	Percent survival of the seedling
32-50	70
32-52	80
34-54	70
38-52	90
35-56	60

Table 3: screening of thermotolerant sorghum genotypes through TIR technique

Sl.No.	Genotypes	Germination %	Percent Reduction in root growth	Percent Reduction in shoot growth
1.	GP-2327-2	95.0 (80.82)	0.00 (0.00)	27.36(31.55)
2.	GP-2403	75.0 (60.14)	0.00 (0.00)	13.14(21.26)
3.	GP -2414	95.0 (80.82)	1.75 (7.61)	23.26(28.85)
4.	GP 2597	75.0 (60.14)	13.09 (21.22)	31.38(34.36)
5.	GP 2598	95.0 (80.82)	8.90 (17.36)	25.59(30.46)
6.	GP 2600	90.0 (71.60)	29.24 (32.75)	27.75(31.80)
7.	GP 2608	90.0 (71.60)	0.00(0.00)	19.01(25.86)
8.	GP 2611	100.0 (90.05)	0.00(0.00)	25.24(30.17)
9.	GP 2612	95.0 (80.82)	0.00(0.00)	22.94 (28.63)
10.	GP 2613	100.0 (90.05)	0.00(0.00)	23.37 (28.92)
11.	GP 2615	00.0 (90.05)	18.39 (25.41)	14.92 (22.73)
12.	GP 2617	90.0 (71.60)	0.00(0.00)	22.68 (28.45)
13.	GP 2618	90.0 (71.60)	0.00(0.00)	29.58 (32.96)
14.	GP 2629	65.0 (53.81)	0.00(0.00)	30.19 (33.34)
15.	GP 2625	85.0 (67.53)	0.00 (0.00)	41.61 (40.19)
16.	GP 2626	100.0(90.05)	0.00 (0.00)	0.00(0.00)
17.	GP 2627	90.0 (71.60)	0.00 (0.00)	29.56 (32.95)
18.	GP 2628	95.0 (80.82)	0.00 (0.00)	23.76 (29.19)
19.	GP 2630	85.0 (67.53)	28.07 (22.12)	31.10(33.91)
20.	GP 2631	80.0 (64.21)	35.25 (33.23)	41.78 (40.29)
21.	GP 2632	95.0(80.82)	29.21(32.73)	32.64(34.86)
22.	GP 2633	100.0(90.05)	34.11(35.75)	33.49(35.37)
23.	GP 2634	75.0(60.14)	0.00(0.00)	11.80(20.10)
24.	GP 2635	95.0(80.82)	0.00(0.00)	29.39(32.85)
25.	GP 2636	85.0(67.53)	42.71(40.83)	38.48(38.36)
26.	GP 2637	70.0(56.82)	0.00(0.00)	11.97(20.25)
27.	GP 2638	60.0(50.79)	45.66(42.53)	41.91(40.36)
28.	GP 2640	95.0(80.82)	0.00(0.00)	17.71(24.90)
29.	GP 2641	75.0(60.14)	35.55(36.62)	0.00(0.00)
30.	GP 2642	55.0(47.91)	45.40 (42.38)	7.74(16.16)
31.	GP 2644	65.0(54.24)	0.00 (0.00)	3.15 (10.22)
32.	GP 2645	45.0(42.01)	19.81 (26.44)	25.83(30.56)
33.	GP 2648	35.0(36.24)	0.00 (0.00)	4.00(11.54)
34	GP 2649	40.0(39.13)	5.54 (13.61)	0.00 (0.00)
35.	GP 2650	100.0(80.82)	0.00(0.00)	20.36(26.83)
36.	GP 2564	95.0(90.05)	0.00(0.00)	0.00(0.00)
37.	GPW-M35	95.0(80.82)	0.00(0.00)	0.00(0.00)
38.	GP W278	95.0(80.82)	0.00 (0.00)	0.00(0.00)
39.	GP W296	95.0(80.82)	28.78(32.46)	31.59(34.17)
40.	GP W300	75.0 (60.14)	21.84(27.87)	38.5(38.42)
	Mean	70.42	12.47	25.02
	S.Em ±	5.925	0.599	1.438
	CD (P=0.05)	16.997	1.718	0.709

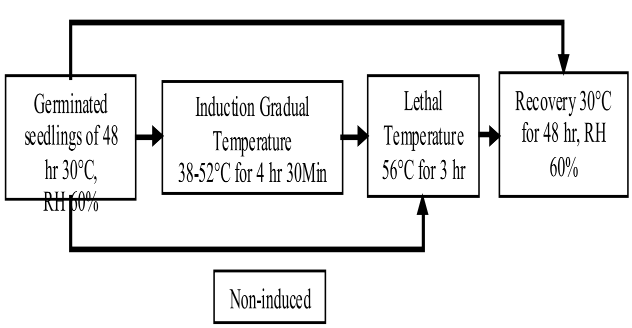

Figure 1: Standardized Temperature Induction Response (TIR) Protocol for Sorghum

and 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 vaiability in intrinsic stress tolerant at cellular level, Narayanaswamy (2010).

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 a large number of genotypes. The identified 4 genotypes GP-2626, GP-2564, GPW-M35 and GPW-278 of sorghum can be used as donor source for developing high temperature tolerant sorghum genotypes to resist global rise.

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