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SCREENING OF THERMOTOLERANT RAGI GENOTYPES AT SEEDLING STAGE USING TIR TECHNIQUE

D. VENKATESH BABU*, P. SUDHAKAR[2] AND Y. SHARATH KUMAR REDDY[1]
[1]Department of Plant Physiology, S.V. Agriculture College, A N G R Agricultural University, Tirupati - 517 502
[2]Regional Agricultural Research Station, ANGRAU, Tiruapti - 517 502
e-mail: venkateshbabu2007ag@gmail.com

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***Corresponding author**

ABSTRACT
Here we report a novel temperature induction response (TIR) technique was standardized for ragi crop. We have standardized the sub lethal *i.e.* challenging temperatures 38-54ºC (for 5 hours) and lethal temperatures as 57ºC (for 2 hours). Using this standardized TIR protocol, highly thermotolerant ragi genotypes were screened from 100 ragi germplasm. Among the genotypes, GP-160 and GP-27 showed highest thermotolerance in terms of 100 per cent seedlings survival and no reduction in root and shoot growth.GP-153, GP-149 and GP-25 also showed higher thermotolerance in terms of 90% seedlings survival and no reduction in root and shoot growth. These genotypes have intrinsic heat tolerance and they can be explored as donar source in breeding programme aimed for global warming.

INTRODUCTION

Finger millet or Ragi (*Eleusine coracana* G.) is the third most important millet crop of India. It is also an important food crop in South Asia and Africa. Finger millet covers an area of 1.307 million hectares in India, with a production of 1.92 million tonnes and productivity of 1,641 kg ha^{-1} (Indian Statistics 2011-12). The crop is mostly cultivated in sub marginal lands and limited moisture conditions. Hence it is prone for recurrent drought, which affects crop growth due to moisture as well as temperature stress.

Drought is coupled with moisture stress and high temperature, which imposes many adverse effects on plant growth and development. Heat stress due to high ambient temperatures is a serious threat to crop production worldwide Hall, (2001). The situation is becoming more alarming with global warming conditions. In order to combat these adverse effects, development of thermotolerant and water saving genotypes is need of the day. From this background ragi genotypes were screened for high temperature tolerance using temperature induction response (TIR) technique. The technique of exposing young seedlings to sub lethal and lethal temperatures has been validated in other crop species viz. rice Sudhakar *et al*. (2012), sunflower Senthil Kumar *et al*. (2003), cotton Ehab Abou Kheir *et al*. (2012), groundnut Gangappa *et al*. (2006) and pea Venkatachalayya *et al*. (2001).

This approach is based on the fact that temperature stress develops gradually from sub lethal to lethal levels of stress. An array of response events were expressed during sub lethal temperatures and give cellular protection at lethal temperatures.

Therefore, evaluating the relative performance of ragi genotypes for high temperature tolerance using TIR technique is main objective

MATERIALS AND METHODS

Present investigation was conducted at Phenotyping laboratory, Institute of Frontier Technologies Acharya N G Ranga Agricultural University. Tirupati, Andhra Pradesh with 100 ragi genotypes obtained from Agricultural research station Perumallapalle, Tirupati, Andhra Pradesh.

Identification of lethal temperature treatment

To assess the challenging temperatures for 100 per cent mortality, 42 hour old ragi seedlings were exposed to different lethal temperatures (52, 54, 56 and 57ºC) for varying durations (1, 2 and 3 hours) without prior induction. Thus, exposed seedlings were allowed to recover at 30ºC 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 ragi genotypes after recovery was recorded (Table 1). The lethal temperature of 57ºC for 2 hour was considered in this text, as maximum mortality (96%) 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 ragi seedlings (42 hour old ragi seedlings) were subject to gradually increasing temperatures for a period of five hours. After this induction treatment, seedlings were exposed to lethal temperature *i. e.,*

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

s.no	Temperature°C	Percent mortality of ragi seedling after recovery Duration of temperatures		
		1 hour	2hour	3 hour
1	50	0	0	10
2	52	0	20	32
3	54	0	34	46
4	56	44	60	81
5	57	52	96	98

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

S.no	Temperature range (Induction treatment for 5hours)	°CPer cent survival of the seedling
1	32-50	75
2	32-52	80
3	36-52	85
4	34-54	90
5	38-56	75

Table 3: Screening of thermotolerant ragi genotypes through TIR technique

s.no	Entries	Per cent survival of the seedlings (%)	Per cent reduction in root growth (%)	Per cent reduction in shoot growth (%)
1	GP-3	80	0	0
2	GP-8	40	39.58	35.83
3	GP-16	70	0	27.43
4	GP-24	80	0	0
5	GP-25	90	0	0
6	GP-27	100	0	0
7	GP-30	60	0	0
8	GP-104	70	0	0
9	GP-109	80	0	26.36
10	GP-111	90	0	0
11	GP-115	90	52.1	4
12	GP-135	70	40.94	3.03
13	GP-141	80	40.81	2.4
14	GP-138	80	49.42	4.81
15	GP-149	80	0	0
16	GP-153	90	0	0
17	GP-159	80	50	8.12
18	GP-160	100	0	0
19	GP-162	70	0	34.36
20	GP-176	40	31.54	26.21

57ºC for two hours and then transferred to the normal temperature for recovery. 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.,* 34ºC-54ºC

Thermo induction response (TIR)

Ragi seeds were surface sterilized by treating with 2 per cent bavistin solution for 30min and washed with the distilled water for 4-5 times and kept for germination at 30ºC 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 34^{0}C-54^{0}C) for five hours in the environmental chamber (WGC-450 Programmable Plant Growth Chamber). Later these seedlings were exposed to lethal temperatures (57^{0}C) for 2 hours (induced). Another set of seedlings were directly exposed to lethal temperatures (non induced).

Induced and non induced ragi seedlings were allowed to recover at 30^{0}C and 60% relative humidity for 48 hours. The following parameters were recorded from the seedlings.

$$\text{a) Percent survival of seedlings} = \frac{\text{NO of sedlings survived at the end of recovery}}{\text{Total number of seedlings sownin the tray}} \times 100$$

$$\text{b) Percent reduction in root growth} = \text{Actual root growth of control seedlings} - \frac{\text{Actualroot growth of treated seedling}}{\text{Actualroot growth of control seedlings}} \times 100$$

$$\text{c) Percent reduction in shoot growth} = \text{Actual shoot growth of control seedlings} - \frac{\text{Actual shoot growth of treated seedling}}{\text{Actual shoot growth of control seedling}} \times 100$$

A lethal temperature of 57ºC for 2 hours and induction treatment from 38-54ºC for five hours was standardized using TIR (Thermo Induction Response) and considered as best lethal and induction temperatures for Phenotyping of ragi 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 only presented in the Table 3. The effect of TIR on genotypes releaved variable results. Such acquired tolerance was variably recorded in other ragi genotypes, where either survival of seedlings was affected in (GP-6,GP-176) or root growth alone was affected in(GP-135, GP-138, GP-115, GP-141, GP-159) or only shoot growth alone was affected in (GP-16, GP-162, GP-109, GP-162).In the genotypes GP-6 and GP-176 the seedling survival ,shoot and root growth were completely affected despite of the recovery conditions maintained after exposing to sub lethal to

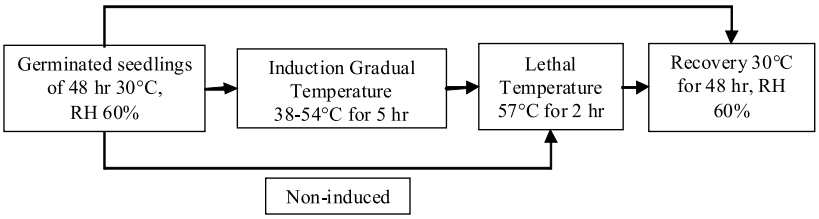

Figure 1: Standardized Temperature Induction Response (TIR) Protocol for Ragi

lethal temperature. In spite of exposing to 57^0C, germination and seedling growth were not affected in GP-27, GP-153 and GP-111 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 Sudhakar *et al*. (2012) and Renukha *et al*. (2013) in rice, Senthil –kumar *et al*. (2003) in sunflower, Ehab Abou Kheir *et al*. (2012) in cotton, Gangappa *et al*. (2006) ,in groundnut, Venkatachalayya et *al*. (2001) in pea. This novel temperature induction response technique has been demonstrated to reveal genetic vaiability in intrinsic stress tolerant at cellular level, Narayanaswamy (2010).

The present study releaved that the TIR technique can very well be used in ragi crop. The identified genotypes GP-3, GP-111 and GP-153 are showed to posses high level of thermotolerance. These genotypes can be used as potent donar source in breeding programmes aimed for global warming.

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