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STANDARDIZATION OF OPTIMUM TEMPERATURE CONDITIONS AND SCREENING OF THERMOTOLERANT MUNGBEAN GENOTYPES USING THERMO INDUCTION RESPONSE (TIR) TECHNIQUE

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ABSTRACT

A screening protocol for thermotolerance in mungbean genotypes was standardised based on the principle of "acquired tolerance" in which exposure of seedlings to sub lethal and lethal temperature stress in order to induce the tolerance in genotypes. We have standardized the sub lethal temperature of 38-54ºC for 5 hours and lethal temperature as 56ºC for 2 hours. Among the genotypes, ML 267 and TLM 7 showed highest thermotolerance in terms of 100 percent seedling survival and no reduction in root growth and less reduction in shoot growth (54.72%). Results suggested that TIR is a robust and powerful technique and can be used to screen breeding lines or germplasm lines to identify high temperature tolerant lines.

KEY WORDS
Mungbean
Lethal temperature
Sub lethal temperature
Thermotolerance

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INTRODUCTION

Pulses are good sources of proteins and commonly considered as the poor man's meat. In India, the frequency of pulses consumption is much higher than any other source of protein and about 89 per cent of persons consume pulses at least once a week, while only 35.4 per cent of persons consume fish or chicken/ meat at least once a week (Indian Institute of Pulses Research, 2007). In India, among the pulses, mungbean occupied third place in production and productivity and is the major exporter and importer of mungbean globally. However, the present varieties could not be able to meet the production targets due to impact of various biotic and abiotic stresses. Among the abiotic stresses, drought and temperature stresses are the most important ones to be considered while planning the breeding programmes, as there are no readily available sources of germplasm for drought and temperature stresses. In general, drought is also the result of low precipitation and high temperature. In addition to this, the most worrying part of prediction is the estimated increase in the winter and summer temperatures by 3.2 degrees and 2.2 degrees Celsius, respectively by 2050 (Agropedia). Hence, in order to overcome these adverse effects, development of thermo and drought tolerant genotypes is necessary. How ever, there is lack of reliable screening techniques available for screening thermotolerance in mungbean. The technique of exposing young seedlings to sub lethal and lethal temperatures has been validated in the crops like Wheat (Ravindra *et al.,* 2014), Ragi (Venkatesh Babu *et al.,* 2013), Rice (Renuka *et al.,* 2013 and Sudhakar *et al.,* 2012), Cotton (Ehab Abou Kheir *et al.,* 2012), Groundnut (Gangappa *et al.,* 2006), Sunflower (Senthil Kumar *et al.,* 2003) and Pea (Venkatachalayya *et al.,* 2001). However, limited work has been done in mungbean to screen the germplasm for thermotolerance, which is very essential in the present day context. Based on the previous experiments, Temperature Induction Response (TIR) technique, a potent technique for screening thermotolerance in mungbean is considered. This TIR technique involves exposing seedlings or plants to induction stress and subsequently challenging with severe temperature and selecting the surviving seedlings at the end of the recovery period. Hence, the present investigation has been carried to standardise the TIR technique and screen the mungbean genotypes for thermotolerance, which could be further useful in the breeding programmes aimed at developing the genotypes with high yield coupled with drought and temperature stress tolerance.

MATERIALS AND METHODS

The experimental material for the present investigation consisted of thirty one mungbean genotypes obtained from Regional Agricultural Research Station, Lam, Guntur and Agricultural Research Station, Madhira and the study was conducted at Phenotyping laboratory, Institute of Frontier Technologies, Acharya N G Ranga Agricultural University, Tirupathi, Andhra Pradesh. The details of the methodology followed the Venkatesh Babu *et al.,* 2013 in Rice protocol with little modifications.

Identification of lethal temperature treatment

To assess the challenging temperatures for 100 per cent mortality, 24 hour old mungbean seedlings were exposed to different lethal temperatures (53°C, 55°C, 56°C, 57°C and 58°C) for the 1 hour, 2 hours and 3 hours without prior induction. Thus, exposed seedlings were allowed to recover at 30°C and 60 per cent relative humidity for 48 hours. At the end of recovery period the temperature at which 95% or above 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 mungbean genotypes after recovery was recorded (Table 1) (Fig 1). The lethal temperature of 56°C for 2 hours 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. The temperature regimes and duration varies from crop to crop and need to be standardized. The germinated mungbean seedlings (24 hour old mungbean seedlings) were subjected to gradually increasing temperatures for a period of five hours.

Figure 1: Performance of selected genotypes under standardization process at Control (a), Lethal temperature 54°C (b) and Lethal temperature 56°C (c) respectively

After this induction treatment, seedlings were exposed to standardised lethal temperature *i.e.,* 56°C for two hours and then transferred to the normal temperature for recovery. The temperature regimes are varied at 5 hours duration to arrive at optimum induction protocol (Table 2). The optimum sub lethal temperatures were arrived based on the per cent survival of seedlings. Among the temperature regimes where seedling survival reduction is between 5- 10% should be selected as standardised regime *i.e.,* 38°C-54°C and was considered as standard induction temperature for mungbean seedlings.

Thermo Induction Response (TIR)

Mungbean seeds were surface sterilized by treating with 5 per cent Bavistin solution for 30 minutes and washed with the distilled water for 4-5 times and kept for germination at 30°C and 60 per cent relative humidity in the incubator. After 24 hours, uniform seedlings were selected in each genotype and sown in aluminium trays (50 mm) filled with soil. These trays with seedlings were subjected to sub lethal temperatures (gradual temperatures increasing from 38°C-54°C) for five hours in the environmental chamber (WGC-450

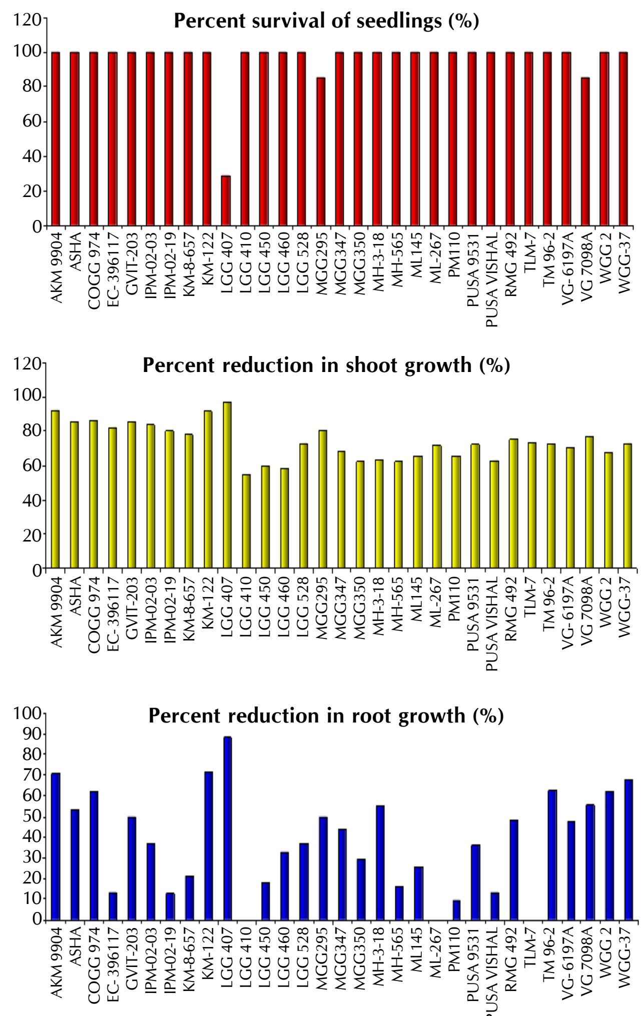

Figure 2 : Mean performance of the thirty one mungbean genotypes for Thermo Induction Response (TIR) characters

Programmable Plant Growth Chamber). Later these seedlings were exposed to lethal temperatures (56°C) for 2 hours (Treated).

A sub lethal(Induction) treatment from 38-54 °C for five hours followed by lethal temperature of 56 °C for 2 hours was standardized as reliable TIR (Thermo Induction Response) protocol for phenotyping of mungbean seedlings for intrinsic heat tolerance at cellular level.

Treated and control mungbean seedlings were allowed to recover at 30°C and 60 per cent relative humidity for 24 hours. The following parameters were recorded from the seedlings.

a) Percent survival of seedlings =

$$\frac{\text{No. of seedlings survived at the end of recovery}}{\text{Total no. of seedlings sown in the tray}} \times 100$$

(b) Percent reduction in root growth =

$$\frac{\text{Growth of control seedlings} - \text{Growth of treated seedlings}}{\text{Growth of control seedlings}} \times 100$$

(c) Percent reduction in shoot growth =

$$\frac{\text{Growth of control seedlings} - \text{Growth of treated seedlings}}{\text{Growth of control seedlings}} \times 100$$

RESULTS AND DISCUSSION

A set of diverse Mungbean germplasm comprising of 31 genotypes were screened for intrinsic tolerance using the standardized Thermo Induction Response (TIR) protocol. The experimental data were recorded and presented in Table 3. (Fig 2).

The genotypes showed high genetic variability for per cent survival of seedlings, per cent reduction in root and shoot growth, respectively. The per cent survival of seedlings varied from 28.57 to 100 per cent with a mean survival of 64.28 per cent of seedlings (Table 4). The per cent reduction in root growth varied from 0 to 88.42 per cent with a mean of 44.21 per cent and the per cent reduction in shoot growth varied from 54.72 to 96.95 per cent with a mean of 75.83 per cent.

Among all the genotypes, LGG 410, ML 267 and TLM-7 showed the highest thermo tolerance in terms of 100 per cent seedlings survival and no reduction in root and low reduction

Sensitive genotype (MGG295) **Tolerant genotype (LGG 410)**

Figure 3: Thermo Induction Response of mungbean seedlings in sensitive and tolerant genotypes

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

Sl.No.	Temperature °C	Percent mortality of mungbean seedlingsafter recovery Duration of temperature		
		1 hour	2 hour	3 hour
1	53	65	86	96
2	55	67	92	100
3	56	74	100	100
4	57	81	100	100
5	58	88	100	100

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

S. No.	Temperature range (induction treatment for 5 hrs)°C	Per cent survival of seedling
1	34-50	45
2	34-52	47
3	35-51	64
4	35-53	78
5	38-54	90
6	38-56	86

Table 3 : Mean performance of mungbean genotypes for Thermo Induction Response (TIR) characters

Genotypes	Percent survival of seedlings(%)	Actual shoot growth in Control	Actual shoot growth in Treated(%)	Percent reduction in shoot growth	Actual root growth in Control	Actual root growth in Treated	Percent reduction in root growth(%)
AKM 9904	100	19	1.5	92.11	3.2	0.93	70.98
ASHA	100	18.14	2.66	85.35	2.71	1.27	53.16
COGG 974	100	19.14	2.64	86.19	4.14	1.56	62.41
EC- 396117	100	17.14	3	82.5	2.93	2.54	13.17
GVIT-203	100	18.29	2.7	85.23	3.86	1.93	50
IPM-02-03	100	19.57	3.14	83.94	2.71	1.71	36.84
IPM-02-19	100	20.14	4	80.14	2.43	2.11	12.94
KM-8-657	100	18.14	4	77.95	2.71	2.14	21.05
KM-122	100	19.29	1.57	91.85	3.43	0.97	71.67
LGG 407	28.57	18.71	0.57	96.95	2.71	0.31	88.42
LGG 410	100	15.14	6.86	54.72	2.6	2.6	0
LGG 450	100	15.57	6.29	59.63	2.14	1.76	18
LGG 460	100	17.43	7.29	58.2	3.4	2.29	32.77
LGG 528	100	17.29	4.71	72.73	3.86	2.43	37.04
MGG295	85.17	16.43	3.21	80.43	2.71	1.36	50
MGG347	100	15.86	5.07	68.02	3.14	1.76	44.09
MGG350	100	17.14	6.43	62.5	3.07	2.17	29.3
MH-3-18	100	15.14	5.57	63.21	3.14	1.41	55
MH-565	100	12.29	4.64	62.21	2.21	1.86	16.13
ML145	100	16.57	5.71	65.52	3.07	2.29	25.58
ML-267	100	18.57	5.29	71.54	2	2	0
PM110	100	18.29	6.34	65.31	3	2.71	9.52
PUSA 9531	100	17.86	4.93	72.4	3.57	2.29	36
PUSA VISHAL	100	18	6.71	62.7	2.14	1.86	13.33
RMG 492	100	17.71	4.36	75.4	4.14	2.14	48.28
TLM-7	100	18.14	4.79	73.62	3.5	3.5	0
TM 96-2	100	18.86	5.21	72.35	5.57	2.07	62.82
VG- 6197A	100	18.86	5.57	70.45	5.71	3	47.5
VG 7098A	85.17	16.71	3.86	76.92	4.57	2.03	55.63
WGG 2	100	14.86	4.81	67.6	4.93	1.86	62.32
WGG-37	100	19.29	5.21	72.96	4.43	1.43	67.74

Table 4: Identification of promising thermo tolerant mungbean genotypes through TIR technique

Tolerance range	Genotype	Per cent reduction in root growth (%)	Per cent reduction in shoot growth (%)	Per cent survival of seedlings (%)
HighlyTolerantGermplasmLines	LGG 410	0	54.72	100
	ML 267	0	71.54	100
	TLM-7	0	73.62	100
HighlySensitiveGermplasmlines	LGG 407	88.42	96.95	28.57
	MGG 295	50.00	80.43	85.17
	VG7098A	55.63	76.92	85.17

Table 5 : Thermo tolerance performance of top ten high yielding mungbean genotypes

Sl. No.	Genotype	Grain yield / plant (g)	% survival of seedlings	% reduction in root growth	% reduction in shoot growth
1	LGG 410	6.06	100	0	54.72
2	ML 267	9.58	100	0	71.54
3	TLM-7	6.48	100	0	73.62
4	PM 110	10.40	100	9.52	65.31
5	PUSA VISHAL	5.32	100	13.33	62.70
6	LGG 450	5.20	100	18.00	59.63
7	MH 565	7.45	100	16.13	62.21
8	ML 145	7.69	100	25.58	65.52
9	IPM-02-19	13.23	100	12.94	80.14
10	EC 396117	6.53	100	13.17	82.50

in shoot growth (Fig 3). These varieties are able to survive even when they were exposed to lethal temperatures. These results are also in conformity with several studies, which showed that acclimated plants survive upon exposure to a severe stress, which otherwise could be lethal and is considered to be as thermo tolerant (Senthil Kumar *et al.,* 2003). The seedling survival, shoot and root growth were completely affected in the genotypes LGG 407, MGG 295 and VG- 7098A despite of the recovery conditions maintained after exposing to sub lethal to lethal temperature.

Genotypes like LGG 410, ML 267 and TLM-7 which showed 100 per cent survival of seedlings, 0 per cent reduction in root growth and 54.72 to 73.62 per cent reduction in shoot growth were selected for further field evaluation of genotypes under imposed moisture stress conditions.

The TIR response of top ten genotypes revealed that LGG 410 showed the lowest percent reduction in root (0.0%) and shoot growth (54.72 %) with 100% of survival of seedlings followed by ML 267 and TLM-7 (Table 5). Hence, these can be used as a potential donor parents for obtaining thermo tolerant lines.

The technique of exposing young seedlings to sub lethal and lethal temperatures in order to identify the thermo tolerant lines has been validated in many crop species such as Wheat by Ravindra *et al.,* 2014, Ragi by Venkatesh Babu *et al.,* 2013, Rice by Renuka *et al.,* 2013and Sudhakar *et al.,* 2012, Cotton by Ehab Abou Kheir *et al.,* 2012, Ground nut by Gangappa *et al.,* 2006, Sunflower by Senthil Kumar *et al.,* 2003 and Pea by Venkatachalayya *et al.,* 2001.This novel temperature induction response technique has been demonstrated to reveal genetic variability in intrinsic stress tolerance at cellular level (Narayana Swamy, 2010 in rice). The present study also revealed that the TIR technique could be used in mungbean crop for identification of thermo tolerant genotypes. The identified genotypes *viz.,* LGG 410, ML 267 and TLM-7 were found to possess high level of thermo tolerance.

In conclusion, the above results suggest that the TIR technique is a powerful and constructive technique to identify genetic variability in high temperature tolerance in mungbean within short period of time and is suitable for screening a large number of genotypes. The identified genotypes LGG 410, ML 267 and TLM-7 could be used as donars for developing drought and high temperature tolerant mungbean genotypes.

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