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SCREENING OF THERMOTOLERANT GROUNDNUT GENOTYPES USING TEMPERATURE INDUCTION RESPONSE – A NOVEL APPROACH TO ASSESS GENETIC VARIABILITY

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ABSTRACT
Plants are exposed to different external conditions that affect growth, development, and productivity. Heat stress due to high ambient temperature is a serious threat to crop production worldwide. Groundnut is an important oil and food crop cultivated in arid and semi-arid regions of the world. In the present study, temperature induction response (TIR) was carried out for screening the groundnut genotypes, where the seedlings were exposed to sublethal temperature from 38°C -54°C for 5 hours followed by lethal temperature at 58°C for 3 hours. Among the 20 diverse genotypes screened, 4 showed tolerance to high temperature with mean percent seedling survival of 97.5%, 6 showed moderate tolerance and 10 were identified as temperature susceptible compared with control (100% survival). There was considerable variation among the genotypes screened for acquired thermotolerance. Results suggested that TIR is a simple and powerful technique and can be used to screen large population at seedling stage to identify thermotolerant lines.

KEY WORDS
Groundnut, Temperature induction response, Acquired thermotolerance, Lethal and sublethal temperature.

INTRODUCTION
Global warming has become a serious worldwide threat. Ambient temperatures are rising at a considerable rate as part of the current global climate change. Climate models predict that the global mean temperature will continue this trend, increasing by 1–4°C by the end of the twenty-first century and heat waves are likely to occur more frequently [1,2]. Heat stress is one of the major uncontrollable stresses affecting plant growth, development, and productivity. Unfavorable temperature may significantly affect photosynthesis, respiration, water balance, and membrane stability of leaves. To combat heat stress, plants acquire various defense mechanisms for their survival such as maintaining membrane stability, and scavenging reactive oxygen species by generating antioxidants and stress proteins. Reproductive processes are adversely affected by high temperature, including pollen germination [3].

Peanut, (*Arachis hypogaea* L.) an important oilseed crop, is a major source of edible oil and third most important source of vegetable protein, besides serving as a dietary source of vitamin E and phytosterols. Asia is the major peanut growing region in the world, accounting for 64% of the global production [4]. High-temperature stress affects several crops, including groundnut limiting crop productivity worldwide and the optimum air temperature is between 25 and 30°C. In groundnut, short episodes of high temperature stress significantly affect yield [5]. Plant responses to high temperatures are mediated by both their inherent ability to survive and their ability to acquire thermotolerance. Acquired thermotolerance is the level of protection beyond the inherent thermotolerance, and results from prior exposure to elevated, non-lethal temperatures. Temperature induction response (TIR) has been used as a screening technique to identify thermotolerant lines in many crop plants [6]. The uniqueness of this technique is that the identified thermotolerant seedlings can be established in the field and their progenies can be subsequently screened at higher temperatures through recurrent selection to obtain a highly tolerant genotype. The present study, therefore aimed to screen diverse groundnut genotypes using TIR technique to identify highly tolerant donar genotypes for use in plant breeding programmes.

MATERIALS AND METHODS

Plant material:

The present work was conducted at the Department of Crop Physiology, Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Tirupati, Andhra Pradesh. The Seed materials were procured from Regional Agricultural Research Station, Tirupati, Andhra Pradesh, India. About 2-day old seedlings germinated on filter paper in petri dishes were used for the experiment.

Temperature Induction Response (TIR):

Seeds were surface sterilized by treating with 2 % bavistin solution for 15 minutes and washed with the distilled water for 3-4 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 sublethal temperatures (gradual temperatures increasing from 38ºC-54ºC for 5 hours in the environmental chamber (WGC-450 Programmable Plant Growth Chamber). Later these seedlings were exposed to lethal temperature 58°C for 3 hours (induced). Another set of seedlings were directly exposed to lethal temperatures (non-induced). Induced and non-induced groundnut seedlings were allowed to recover at 30ºC and 60% relative humidity for 48 hours. At the end of the recovery period, the following parameters were recorded and calculated.

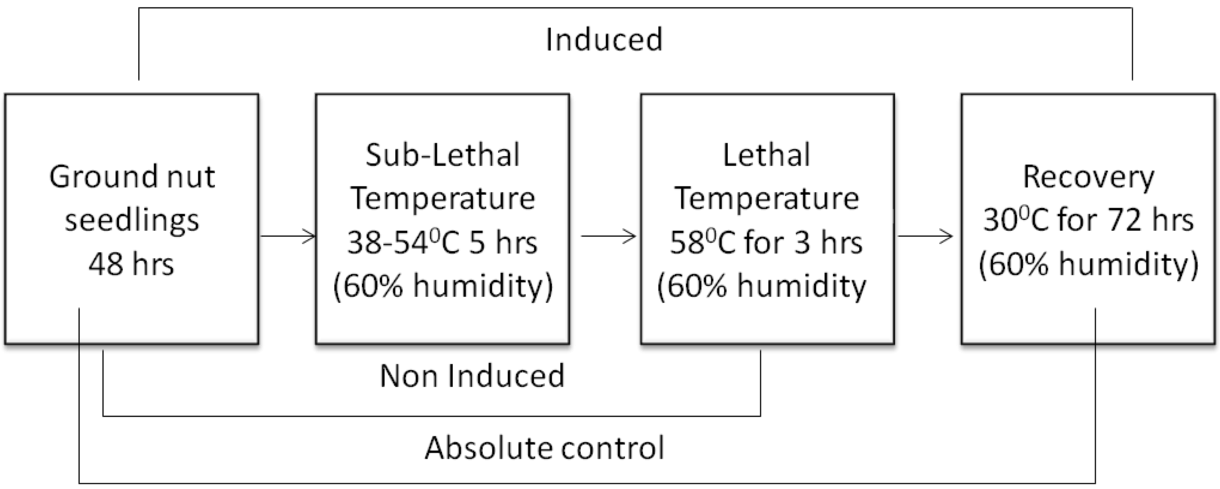
a) % survival of seedlings = (No of seedlings survived at the end of the recovery/ total no of seeds sown) X 100

b) % reduction in root growth =

(root growth of control seedlings- root growth of treated seedling)/= root growth of control seedlings) X 100

c) % reduction in shoot growth = {(shoot growth of control seedlings-shoot growth of treated seedling)/shoot growth of control seedlings}X 100

A lethal temperature of 58ºC for 3 hours and induction treatment from 38-54ºC for five hours using TIR (Thermo Induction Response) was considered as best lethal and induction temperatures for Phenotyping of groundnut seedlings for intrinsic heat tolerance at cellular level standardized by Bharani *et al*. [7] (Flow chart. 1). By using normal Z distribution, the genotypes were classified into susceptible, moderately tolerant and tolerant based on percent survival and percent reduction in recovery growth over absolute control.



Flow chart. 1 Standardized Temperature Induction Response (TIR) Protocol for Groundnut

RESULTS

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 Table 1. The effect of TIR on genotypes revealed variable results. Based on the percent reduction in recovery growth and percent survival after recovery period, the groundnut genotypes were classified into three different categories as tolerant, moderately tolerant and susceptible (Fig.1). The normal z distribution of the genotypes studied showed that along with GN2, GN4, GN5 and GN11 were located in Quadrant I which is classified as highly tolerant, GN15, GN19 and GN20 were grouped in Quadrant III, which is classified as susceptible. The genotypes were classified as moderately tolerant to temperature stresses as they fell in Quadrant II. Similar classification of tolerant, moderately tolerant and susceptible was followed in cotton, rice [8, 9]. The percent survival of seedlings varied from 40 to 100 percent with a mean survival of 91 percent of seedlings compared with control (100%). The percent reduction in root growth varied from 9.58 to 50.0 percent with a mean of 26.54 percent and the percent reduction in shoot growth varied from 6.09 to 62.5 percent with a mean of 30.37 percent over control (Table 1). These results are also in conformity with previous studies, which showed that acclimated plants survive upon exposure to a severe stress, which otherwise could be lethal and is considered to be as thermotolerant [10]. The seedling survival, shoot and root growth were completely affected in the genotypes GN14-GN20 despite of the recovery conditions maintained after exposing to sub lethal to lethal temperature.

S.No	Entries	Germination %	Percent Reduction in root growth	Percent Reduction in shoot growth
1	GN1	100	22.22	16.82
2	GN2	100	10.59	9.45
3	GN3	100	25.39	6.09
4	GN4	100	12.24	13.76
5	GN5	100	9.58	12.57
6	GN6	90	20.98	25.39
7	GN7	90	28.88	19.55
8	GN8	100	40.65	18.06
9	GN9	90	19.83	22.87
10	GN10	100	18.49	22.35
11	GN11	90	9.81	8.67
12	GN12	100	36.72	22.22
13	GN13	90	13.03	57.41
14	GN14	100	28.91	55.56
15	GN15	60	34.94	55.47
16	GN16	40	39.13	46.12
17	GN17	90	50	36.67
18	GN18	80	41.72	44.71
19	GN19	100	36.70	51.31
20	GN20	100	31.04	62.50

Table 1. Screening of thermotolerant groundnut genotypes through TIR technique

DISCUSSION

Among all the genotypes, GN2, GN4, GN5 and GN11 showed the highest thermotolerance in terms of percent seedlings survival and low reduction in root and in shoot growth (Fig. 2). These genotypes are able to survive even when they were exposed to lethal temperatures. Temperature Induction Response (TIR) technique has been developed to identify and select the thermotolerant genotypes. It involves exposing seedlings or plants to induce stress and subsequently challenging with severe temperature and selecting the surviving seedlings at the end of a recovery period. Survival and recovery growth of plants exposed to stress, could be a potential tool to screen for stress tolerance and TIR has been shown in recent studies as a potential tool for empirical assessment for cell survival and recovery growth at seedling or whole plant level [11, 12]. A number of earlier studies shown that TIR technique is the best alternative to evaluate groundnut genotypes for thermotolerance [7, 13, 14]. These results suggest that the TIR technique is the best technique to identify genetic variability in high temperature tolerance in groundnut within a short time period.

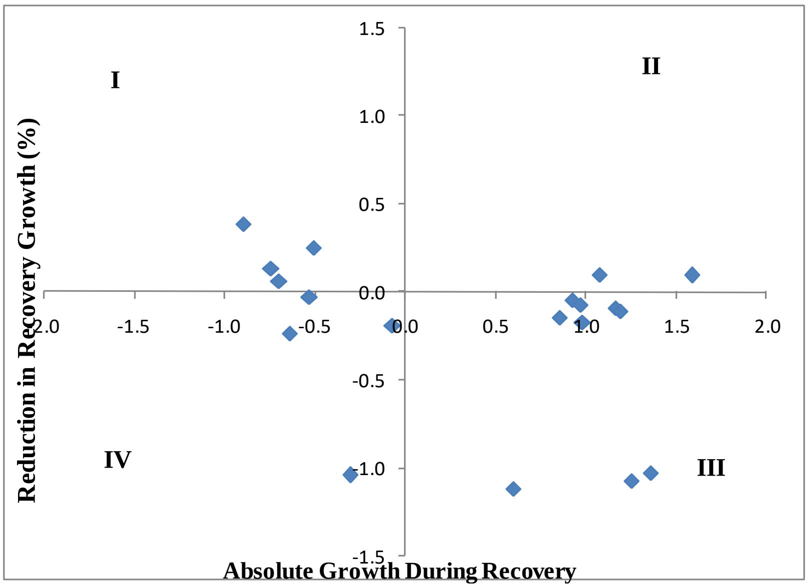


Fig.1 Normal Z-distribution of Groundnut genotypes based on absolute growth after recovery period and percent reduction in recovery growth over control. Quadrant I: heat tolerant genotypes, Quadrant II and IV: moderately heat tolerant genotypes, and Quadrant III: susceptible genotypes. GN2, GN4, GN5, GN11 and GN15, GN18, GN19 are the contrasting tolerant and susceptible genotypes.

Tolerant genotype

Susceptible genotype

Fig. 2 Thermo Induction Response of groundnut seedlings in tolerant and susceptible genotypes

CONCLUSIONS

The present study revealed that TIR is a reliable method to screen groundnut for thermotolerance at seedling stage from a large population. Using this technique, it is feasible to identify thermotolerant lines from diverse lines. The induced seedlings exhibited higher recovery growth at the temperature 38-54°C. Genotypes selected as highly tolerant should be useful in breeding programmes aimed for global warming.

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REFERENCES

[1] IPCC. Summary for policymaker's climate change 2013: The physical science basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Stocker, Qin TF, Plattner D, Tignor G-K, Allen M, Boschung SK, Naue J. Cambridge, United Kingdom and New York, NY, USA, (2013).

[2] Hansen J, Sato M, Hearty P, Ruedy R, Kelley M, Masson-Delmotte V, Russell G, Tselioudis G, Cao J, Rignot E, *et al.* Ice melt, sea level rise and superstorms: evidence from paleoclimate data, climate modeling, and modern observations that 2°C global warming is highly dangerous. Atmos Chem Phys Discuss. 15: 20059–20179, (2015).

[3] Hamidou F, Halilou O, Vadez V. Assessment of groundnut under CombinedHeat and Drought Stress. J Agron Crop Sci. 199: 1–11, (2013).

[4] FAOSTAT, Food and Agricultural Organization of United Nations, (2011). http://www.fao.org/faostat/.

[5] Vara Prasad PV, Craufurd PQ, Kakani VG, Wheeler TR, Boote KJ. Influence of temperature during pre- and post-anthesis stages of floral development on fruit-set and pollen germination in peanut. Aust J Plant Physiol. 28: 233–240, (2001).

[6] Senthilkumar M, Kumar G, Srikanthbabu V, Udayakumar M. Assessment of Variability in acquired thermotolerance: Potential option to study genotypic response and the relevance of stress genes. J Plant Physiol. 164: 111-125, (2007).

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[7] Bharani K, Sudhakar P, Venkatesh babu D. Screening of Thermotolerant Groundnut genotypes at seedling Stage using TIR Technique. Advances in life sciences. 5(9): 3724-3727, (2016).

[8] Kheir EA, Sheshshayee M S, Prasad TG, Udayakumar M. Temperature Induction Response as a Screening Technique Selecting High Temperature-Tolerant Cotton Lines. J Cotton Sci.16: 190–199, (2012).

[9] Vijayalakshmi D, Srividhya S, Vivitha P, Raveendran M. Temperature induction response (TIR) as a rapid screening protocol to dissect the genetic variability in acquired thermotolerance in rice and to identify novel donors for high temperature stress tolerance. Indian J Plant Physiol. 20 (4): 368–374, (2015).

[10] SenthilKumar M, Srikanthbabu V, Mohanraju B, Kumar G, Shivaprakash N, Udayakumar M. Screening of inbred lines to develop a thermotolerant sunflower hybrid using the temperature induction response (TIR) technique: a novel approach by exploiting residual variability. J Exp Bot. 54: 2569–2578, (2003).

[11] Kumar G, Krishnaprasad BT, Savitha M, Gopalakrishna R, Mukhopadhyay K, Ramamohan, G, Udayakumar M. Enhanced expression of heat-shock proteins in thermo-tolerant lines of sunflower and their progenies selected on the basis of temperature-induction response. Theor Appl Genet. 99: 359–367, (1999).

[12] Senthil-Kumar M, Udayakumar M. Development of thermotolerant tomato *(Lycopersicon esculentum Mill.)* lines: an approach based on mutagenesis. J Plant Biol (India). 31: 139–48, (2004).

[13] SenthilKumar M, Kumar G, Srikanthbabu V, Udayakumar M. Assessment of variability in acquired thermotolerance: Potential option to study genotypic re-sponse and the relevance of stress genes. J. Plant Physiol. 164: 111–125, (2006).

[14] Gangappa E, Ravi K, Veera Kumar GN. Evaluation of groundnut *(Arachis hypogaea* L.) genotypes for temperature tolerance based on Temperature Induction Response (TIR) technique. Indian J Genet. 66: 127-130, (2006).

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