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# Evaluation of groundnut (*Arachis hypogaea* L.) genotypes for temperature tolerance based on Temperature Induction Response (TIR) technique

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**Abstarct**

**Thirty-two selected groundnut (*Arachis hypogaea* L.) genotypes were evaluated for temperature tolerance employing a new technique called Temperature Induction Response (TIR) technique, where two days old seedlings were exposed to gradual increase of temperature from 35° to 55°C for a specific time and finally subjected to the lethal temperature. The genotypes showing maximum survivability at 55°C and growth were considered to be temperature tolerant and those, which showed less or no survivability and recovery growth were considered to be temperature susceptible. The results of this study revealed a significant genetic variability among the genotypes for temperature tolerance. By using this technique, genotypes like K-134, K-1240, TNAU-325, JL-24 were identified as temperature tolerant and AK-159, VG-9711, TNAU-284 and JSSP-15 as temperature susceptible genotypes.**

**Key words:** Groundnut, Temperature Induction Response, lethal temperature

## Introduction

Prevalence of high temperature is the major limitation for the cultivation of crops in tropical conditions. The effect of high temperature can be seen at cellular level and at whole plant level affecting growth, reproduction and productivity of crop plants. Moisture stress coupled with high temperature is known to adversely affect the growth and development in groundnut, ultimately resulting in low pod yield. To increase the productivity and to stabilize production in the ever-changing environment, development of genotypes that are capable to survive better under abiotic stresses is essential.

Screening of groundnut genotypes for high temperature stresses in natural conditions, which are highly variable is very difficult. The best alternative therefore is to develop suitable laboratory procedures for screening. The earlier workers [1-6] have developed optimum protocols on Temperature Induction Response (TIR) techniques wherein the genetic variability for temperature/stress response can be examined in crop plants by assessing the survival and recovery growth rates after exposing the germinated seedlings to lethal temperature stress. In the present study an attempt was made to evaluate selected groundnut lines for attributes related to temperature tolerance based on Temperature Induction Response (TIR) technique.

## Materials and methods

Thirty-three groundnut genotypes were evaluated for temperature tolerance based on Temperature Induction Response (TIR) technique. The principle assumption behind this technique is that a genotype will withstand lethal temperature stress by maximum expression of stress-induced genes. Some of the studies conducted at the Department of Crop Physiology, GKVK campus, University of Agricultural Sciences, Bangalore [1, 3-6] and elsewhere have shown that the genetic variability and difference in the expression of stress responsive genes for stress tolerance is seen only upon prior induction at sub-lethal stress. The genotype in which there is enhanced expression of stress responsive genes (quantitative) or unique stress responsive gene (qualitative) will survive better under severe stress conditions. Therefore, selection for stress tolerance should be done upon optimum induction.

*Temperature Induction Response (TIR) technique*: A screening protocol was developed wherein groundnut seedlings were exposed to gradual induction temperatures (sub lethal stress) and later they were exposed to lethal temperature. About 5-10 per cent of seedlings that survive at this level of stress are considered as highly tolerant because they recover after being exposed to a very severe lethal stress. During the gradual induction of stress several stress responsive proteins are expressed which in turn trigger several physiological and biochemical parameters, which confers stress tolerance.

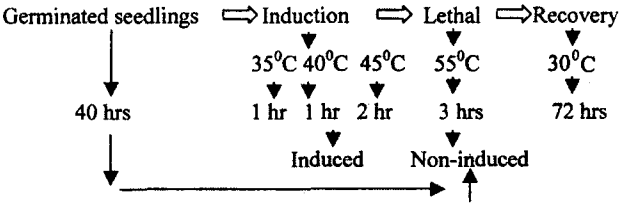
*Induction protocol, induced and non-induced seedlings*: Induction protocol is the sequence of temperature treatments during which the seedlings are

exposed to an optimum sub lethal stress (induction) to severe stress (lethal) and then the seedlings are kept for three days (recovery) at room temperature before recording observations. Such seedlings are referred to as induced seedlings. The seedlings that are directly exposed to lethal temperature (55°C) are referred as non-induced seedlings.

*Lethal temperature*: The temperature, which causes more than 80 per cent reduction in growth in the non-induced seedlings, is the lethal temperature. To determine the lethal temperature for groundnut seedlings were subjected to lethal temperature. The lethal temperature for groundnut was optimized at 55°C for three hours.

*Induction temperature*: The induction temperature could be a non-lethal low temperature for a specific duration or a gradual increase in temperature of known duration. In the present study, based on the earlier work done in this area, induction temperature was optimized by exposing the groundnut seedlings to variable temperatures like 35°C for 1 hr, 40°C for 1 hr and 45°C for 2 hrs. After this treatment the seedlings were exposed to the lethal temperature. Uniform freshly dehydrated seeds were soaked for 2 hrs and later spread in a petri dish with 15 ml of distilled water and were allowed to germinate for 40 hrs in an incubator at 30°C and 50% relative humidity. Later the germinated seedlings were used for different experiments as shown below.

An example of one induction treatment is as given below:




The phenotypically uniform seedlings from each genotypes were transferred to three different sets of petri plates for further studies on (i) induction temperature (ii) direct exposure to lethal temperature and (iii) control. Initial root and shoot length were recorded before subjecting the seedlings to different treatments. The induced and non-induced seedlings were then transferred to a lethal temperature (55°C for 3 hrs). After exposure to the lethal temperature the seedlings were maintained/allowed for recovery at 30°C for 72 hrs. One set of control seedling set was maintained at 30°C all through the experiment to serve as the control.

*Recording of observations*: Observations were recorded on shoot length and root length in all the three sets at the end of recovery period. Optimum induction response was assessed at the end of the induction period (based on maximum recovery growth after the seedlings were subjected to induction stress followed by lethal stress. As the rate of germination is genotype specific, the seedling radical length would vary among the genotypes. Therefore, to arrive at induction response, the difference in growth before subjecting to induction and after the recovery growth period was determined in this system. In addition, the following parameters were computed to assess the induction response.

*Growth During Recovery (GDR)*:

GDR = Growth at the end of the recovery - Growth at the end of the induction.

*Percent reduction over absolute control in recovery growth*:

$$\frac{\text{GDR of control} - \text{GDR of induced}}{\text{GDR of control}} \times 100$$

*Percent increase in growth of induced over non-induced seedlings*:

$$\frac{\text{GDR of induced seedlings}}{\text{GDR of non-induced seedlings}} \times 100$$

*Quantification of total soluble protein*: Total soluble proteins were estimated using a simple dye binding method suggested by Bradford, using bovine serum albumin as the standard [7].

## Results and discussion

*Assessment for thermo tolerance in groundnut genotypes*: The differential response of genotypes to induction stress is considered as the basis for the observed genetic variability at lethal stress. The results on the genetic variability for temperature tolerance of 32 groundnut genotypes of the study are given in Table 1. The data revealed a significant difference among the groundnut genotypes for seedling length two days after germination. Further, subjecting the same set of genotypes to temperature induction treatment followed by recovery for three days has again resulted in a significant difference among the genotypes for seedling growth. Based on this analysis the groundnut genotypes were classified into three categories *viz.*, resistant, moderately resistant and susceptible genotypes (Table 2).

Based on the percent reduction in recovery growth (after exposing to induction temperature) over absolute control, a few genotypes like K-134 (8%), K-1240 (10%), JL-24 (22.25%) and TNAU-325 (20.7%) were identified as highly tolerant types for temperature stress. The

**Table 1.** Genetic variability for temperature tolerance among 32 genotypes of groundnut

| Sl. No. | Genotypes | Length of seedling (cm) during treatment (2 days old) (A) | Length of seedling (cm) 3 days after treatment (recovery) (B) | Growth during recovery (B-A) | Growth of control during recovery period | Per cent reduction over control |
|---|---|---|---|---|---|---|
| 1 | DH-991 | 2.13 | 5.67 | 3.54 | 5.05 | 29.90 |
| 2 | DH-992 | 2.03 | 5.74 | 3.71 | 6.85 | 45.80 |
| 3 | TG-37 D | 2.00 | 4.70 | 2.70 | 9.40 | 71.20 |
| 4 | TG-36 B | 2.11 | 4.20 | 2.09 | 3.40 | 38.50 |
| 5 | TG-37 F | 2.42 | 5.06 | 2.64 | 3.70 | 28.60 |
| 6 | TVG-9363 | 2.13 | 4.15 | 2.02 | 4.24 | 52 30 |
| 7 | ICGV-86590 | 2.94 | 5.17 | 2.23 | 5.96 | 62.50 |
| 8 | JSSP-15 | 3.00 | 3.85 | 0.85 | 5.40 | 84.20 |
| 9 | JSSP-16 | 1.56 | 3.31 | 1.75 | 4.14 | 57 50 |
| 10 | JSSP-17 | 2.45 | 4.60 | 2.15 | 6.20 | 65.30 |
| 11 | K-134 | 1.14 | 3.56 | 2.42 | 4.45 | 8.00 |
| 12 | K-1238 | 2.80 | 5.18 | 2.38 | 3.86 | 38.40 |
| 13 | K-1240 | 3.17 | 5.16 | 1.99 | 1.56 | 10.00 |
| 14 | TNAU-325 | 2.75 | 5.57 | 4.82 | 3.70 | 20.70 |
| 15 | TNAU-326 | 2.90 | 5.50 | 1.60 | 2.35 | 28.50 |
| 16 | TNAU-269 | 1.37 | 3.70 | 2.33 | 3.85 | 37.80 |
| 17 | TNAU-406 | 2.11 | 4.30 | 2.00 | 3.71 | 38.00 |
| 18 | TNAU-359 | 1.68 | 2.48 | 0.80 | 2.54 | 68.50 |
| 19 | CO-3 | 1.80 | 3.60 | 1.80 | 2.92 | 30.00 |
| 20 | TMV-10 | 1.50 | 3.30 | 1.80 | 1.12 | 35.00 |
| 21 | J-54 | 1.79 | 4.05 | 2.26 | 5.41 | 58.14 |
| 22 | JL-24 | 2.00 | 4.41 | 2.41 | 3.51 | 22.25 |
| 23 | AK-159 | 2.75 | 1.56 | 0.50 | 5.22 | 90.00 |
| 24 | VG-9711 | 1.66 | 2.76 | 1.10 | 3.70 | 70.20 |
| 25 | RG-369 | 2.40 | 4.45 | 2.05 | 4.85 | 67.20 |
| 26 | K-1257 | 1.46 | 3.36 | 1.90 | 4.99 | 65.00 |
| 27 | TNAU-281 | 2.53 | 4.10 | 1.57 | 6.53 | 75.90 |
| 28 | VRIGN-5 | 1.80 | 4.00 | 1.80 | 1.34 | 45.00 |
| 29 | GPBD-4 | 2.65 | 4.10 | 2.50 | 3.50 | 37.00 |
| 30 | ICGS-76 | 2.53 | 3.70 | 1.20 | 2.80 | 35.00 |
| 31 | Somanath | 2.13 | 4.10 | 2.00 | 2.66 | 50.00 |
| 32 | Tirupathi local | 2.12 | 3.90 | 2.06 | 1.34 | 37.50 |
| CD at 5% | | 1.82 | | | 2.41 | |
| CV (%) | | 46.38 | | | 34.65 | |

genotypes AK-159 (90%), JSSP-15 (84.2%) and VG-9711 (70.2%) were identified as highly susceptible ones. The genotypes, which showed tolerance to temperature stress in lab, also performed well in water stress condition in the field in terms of production of more number of pods per plant (Table 3).

Any morphological or physiological adaptation of genotypes is a consequence of gene expression; and the gene product brings about the required metabolic changes for adaptation. In the present study, despite the exposure of different genotypes to optimum induction temperatures, the recovery growth differed amongst the genotypes. Variation in the stress adaptive mechanisms among the genotypes could be the reason for observed differences for thermo tolerance. However, it is well known that, the stress responsive genes are many and diverse.

**Table 2.** Grouping of 32 groundnut genotypes into different categories based on their recovery growth of root and shoot (percent reduction in growth over control)

| Sl. No. | Susceptible 50-90% | Moderately tolerant 30-50% | Tolerant 8-30% |
|---|---|---|---|
| 1 | TVG-9563 | DH-992 | DH-991 |
| 2 | ICGV-86590 | TG-36 B | TG-37 F |
| 3 | JSSP-15 | K-1238 | K-134 |
| 4 | JSSP-16 | TNAU-269 | K-1240 |
| 5 | JSSP-16 | JNAU-406 | TNAU-325 |
| 6 | J-54 | TMV-10 | TNAU-326 |
| 7 | AK-159 | GPBD-4 | CO-3 |
| 8 | VG-9711 | ICGS-76 | JL-24 |
| 9 | RG-369 | Somnath | |
| 10 | K-1257 | Tirupathi local | |
| 11 | TNAU-281 | | |
| 12 | VRIGN-5 | | |
| 13 | TNAU-359 | | |
| 14 | COGN-5 | | |

*Quantitative assessment of the protein content*: To understand the biochemical basis of genetic variability in stress responses, the amount of total protein content among two temperature tolerant (TNAU-325 and K-134) and one susceptible groundnut genotype (AK-159) were quantified and is presented in Table-4. The data revealed that, the total protein content between the induced ones and its corresponding controls ranged from 3.39 to 3.90 mg/g. Further, there was not much difference among the tolerant and susceptible genotypes for total protein content. However, it is assumed to be differing qualitatively as reported by earlier workers [8-9]. At the molecular level, one of the most extensively characterized stress responses in higher plants is the synthesis of stress shock proteins (SSP's). These proteins are synthesized under a variety of stresses such as high temperature [10, 11]; desiccation [12, 13] and salinity [14-16]. Many of these proteins are known to protect the cell against the adverse effect of stress. The relevance of these stress proteins has been well characterized in several studies [17, 18]. These proteins are synthesized when the genotype is exposed to a mild-lethal level of stress often, referred to as an induction stress. The ability of induced systems to tolerate several levels of stress signifies the importance of stress proteins [17-19].

*Is it possible to use TIR technique to identify tolerant genotypes in groundnut?*: This approach has practical significance because the major lacuna for breeding for stress tolerance has been lack of suitable field environmental condition and screening techniques

**Table 3.** Mean of different characters in groundnut genotypes under water stress condition

| Genotypes | Plant height (cm) | Root length (cm) | Shoot-root ratio | No. of primary branch/ plant | No. of secon-dary branch/ plant | No of pods/ plant | Bio-mass per plant (g) |
|---|---|---|---|---|---|---|---|
| CO-3 | 43.6 | 11.8 | 3.69 | 8.2 | 0.0 | 13.0 | 120 |
| TMV-10 | 44.6 | 12.6 | 3.53 | 9.0 | 3.6 | 16.0 | 130 |
| VRGN-5 | 36.2 | 14.8 | 2.44 | 11.0 | 5.4 | 14.0 | 100 |
| COGN-4 | 36.5 | 14.0 | 2.6 | 6.0 | 2.0 | 9.0 | 130 |
| TNAU-359 | 27.5 | 11.5 | 2.69 | 5.0 | 0.0 | 12.0 | 75 |
| TNAU-406 | 47.5 | 13.5 | 3.4 | 6.5 | 3.0 | 13.0 | 110 |
| TNAU-269 | 38.1 | 14.5 | 2.62 | 5.0 | 2.0 | 11.0 | 90 |
| TNAU-325 | 42.0 | 14.5 | 2.89 | 6.0 | 2.0 | 17.0 | 80 |
| TNAU-326 | 47.0 | 15.5 | 3.03 | 6.0 | 3.0 | 15.0 | 115 |
| TNAU-281 | 50.5 | 14.0 | 3.6 | 6.0 | 2.0 | 10.0 | 105 |
| K-134 | 46.0 | 13.8 | 3.33 | 6.0 | 4.0 | 23.0 | 130 |
| K-1238 | 54.5 | 15.6 | 3.49 | 6.0 | 2.0 | 18.0 | 140 |
| K-1240 | 48.5 | 14.5 | 3.34 | 5.5 | 1.5 | 18.0 | 105 |
| K-1257 | 30.0 | 13.8 | 2.17 | 5.0 | 0.0 | 15.0 | 95 |
| JSSP-15 | 35.0 | 13.5 | 2.59 | 6.0 | 3.0 | 9.0 | 80 |
| JSSP-16 | 28.0 | 13.5 | 2.07 | 6.0 | 2.0 | 7.0 | 80 |
| JSSP-17 | 40.8 | 15.6 | 2.61 | 6.0 | 2.0 | 11.0 | 95 |
| TG-37-D | 32.0 | 12.0 | 2.66 | 6.0 | 0.0 | 13.0 | 95 |
| TG-37-F | 25.0 | 10.5 | 2.38 | 5.0 | 0.0 | 8.0 | 80 |
| TG-3613 | 32.5 | 10.0 | 3.25 | 5.0 | 0.0 | 18.0 | 120 |
| TVG-9363 | 43.0 | 12.5 | 3.44 | 6.0 | 0.0 | 13.0 | 115 |
| RG-369 | 34.5 | 14.0 | 2.46 | 6.5 | 2.0 | 22.0 | 100 |
| ICGV-86590 | 37.0 | 14.0 | 2.64 | 5.0 | 0.0 | 13.0 | 150 |
| DH-991 | 38.5 | 14.0 | 2.75 | 5.0 | 0.0 | 18.5 | 160 |
| DH-992 | 33.5 | 14.5 | 2.31 | 5.0 | 1.0 | 23.0 | 130 |
| AK-159 | 40.0 | 16.8 | 2.3 | 6.0 | 3.0 | 14.0 | 110 |
| GPBD-4 | 41.0 | 13.5 | 3.03 | 6.0 | 0.0 | 12.0 | 100 |
| KADIRI-1 | 37.5 | 16.5 | 2.27 | 6.5 | 0.0 | 9.0 | 125 |
| Somnath | 38.5 | 13.0 | 2.96 | 4.0 | 1.0 | 18.0 | 100 |
| JL-24 | 32.5 | 12.2 | 2.68 | 4.5 | 1.0 | 15.0 | 115 |
| J-54 | 46.75 | 16.5 | 2.83 | 5.0 | 1.0 | 13.0 | 110 |
| Tirupathi local | 40.5 | 15.8 | 2.6 | 7.0 | 3.0 | 11.0 | 125 |
| ICGS-76 | 29.0 | 13.0 | 2.23 | 6.5 | 3.5 | 10.0 | 110 |
| VG-9711 | 41.5 | 13.0 | 3.19 | 6.0 | 0.0 | 19.0 | 135 |

**Table 4.** Quantitative assessment of total protein content in selected groundnut genotypes

| Genotype | Total protein content (mg/g) |
|---|---|
| TNAU-325 (control) | 3.39 |
| TNAU-325 (Induced) | 3.51 |
| K-134 (control) | 3.90 |
| K-134 (induced) | 3.45 |
| AK-159 (control) | 3.63 |
| AK-159 (induced) | 3.57 |

to evaluate germplasm lines, populations, segregating population etc. Hence, we propose that this approach can be adopted for identifying tolerant lines for segregating populations.

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