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Phenotypic Characterization of Groundnut (*Arachis hypogaea* L.) Genotypes Based on Shoot and Root Mass Indices Using Temperature Induction Response (TIR) Approach

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is a vital global crop cultivated across diverse agro-climatic regions. However, the prevalence of high-temperature stress due to climate change poses a significant challenge to groundnut production. This study employed the Temperature Induction Response (TIR) technique to assess the heat tolerance of 36 advanced groundnut breeding lines. The TIR method involved subjecting seedlings to sub-lethal and lethal temperatures, followed by recovery, and evaluating shoot and root mass indices. The results revealed significant genetic diversity in heat tolerance. Some of the genotypes exhibited greater resilience to heat stress. Genotypes ICGVs 16598, 16594, and 06040 were identified as heat-tolerant, while ICGVs 181023, 181013, and 181031 were found to be heat-susceptible. These findings underscore the importance of genetic variability in crop resilience and highlight the potential of the TIR technique as a reliable and cost-effective tool for assessing heat stress tolerance at the seedling stage. Further research into the underlying mechanisms of heat tolerance in these genotypes offers promising avenues for enhancing crop resilience in the face of climate change.

Keywords: *Groundnut, Shoot and root mass indices and TIR*

Groundnut (*Arachis hypogaea* L.) commonly known as peanut, is a versatile legume crop of significant agricultural importance worldwide. Belonging to the genus *Arachis* and the family Leguminosae, cultivated groundnut is an allotetraploid with a genome of AABB type, derived from its ancestral species *A. duranensis* (AA genome) and *A. ipaensis* (BB genome) (Bertioli *et al*., 2019). It is cultivated across diverse agro-climatic regions, including arid and semi-arid areas, where it serves as a crucial source of income and livelihood for millions of farmers. With cultivation in about 113 countries, groundnut plays a crucial role in the global economy. In 2021, the worldwide production of groundnut was about 53.9 million tons, covering an area of 32.7 million hectares, with an average productivity of 1.6 tons per hectare (FAOSTAT, 2021). Over the past decade, there has been a steady increase in both the area and production of groundnut, with China leading in production with 18.3 million tons, followed by India with 10.2 million tons (FAOSTAT, 2021). The nutritional value of groundnut is highly appreciated, containing essential minerals, vitamins, antioxidants, and bioactive compounds such as resveratrol, tocopherol, and arginine. Their consumption is associated with potential health benefits, including reducing the risk of inflammation, diabetes, and cancer (Arya *et al*., 2016). Peanut and its derived products, particularly the confectionary varieties, are highly popular and in demand globally (Variath and Janila, 2017).

Despite its adaptability to various environments, groundnut production in arid and semi-arid regions faces numerous challenges, primarily due to the prevalence of biotic factors such as diseases, pests, and weeds, along with abiotic factors including drought, heat, salinity, and metal toxicity, collectively exert significant pressure on groundnut cultivation and hinder its potential for achieving optimal yields. Among these, high temperature stress emerges as a major concern, as the frequency and intensity of heatwaves continue to rise with ongoing climate change. High temperatures can affect the rate of development and duration of various developmental stages in plants

(Gangappa *et al*., 2006). Prolonged exposure to high temperatures during critical growth stages disrupts physiological processes, alters metabolic pathways, and impairs overall plant health. While groundnut exhibits optimal growth and productivity within a temperature range of 25°C to 30°C, temperatures exceed 35°C (heat stress) have detrimental effects on yield and overall biomass (Vara Prasad *et al*., 2003). Heat stress during germination stage reduce seedling establishment, while elevated temperatures during flowering lead to a decline in pollination and fertilization rates, resulting in flower abortion and poor pod set (Ruan *et al*., 2010).

In the face of rising climate challenges, identifying and developing heat-tolerant groundnut cultivars has become crucial for sustaining agricultural productivity. Also, heat tolerance is a complex trait and controlled by multiple genes in plants. Plants can acquire basal tolerance to survive high temperatures and can withstand when exposed to sub-lethal temperatures known as acquired thermotolerance (Zhang *et al*., 2005). Heat tolerance in groundnut varies among different genotypes, and it is influenced by a combination of genetic, physiological, and biochemical factors. There is need to exploit genetic diversity and identify key traits associated with heat tolerance to develop climate-resilient groundnut varieties capable of thriving under high-temperature conditions. To identify genetic variability among groundnut genotypes at seedling stage, the Temperature Induction Response (TIR) technique is a powerful and reliable tool. It involves identifying tolerant seedlings that can be established in the field, and their progenies are screened under high-temperature stress through recurrent selection, resulting in development of tolerant genotypes (Kokkanti *et al*., 2019). Thus the present study was planned to screen the groundnut genotypes for thermo tolerance using T/R techniques for identifying the heat tolerant genotypes for exploitation in breeding programmes.

MATERIAL AND METHODS

Plant material

The study was conducted with thirty-six (36) advanced breeding lines of groundnut, including two heat tolerant checks (ICGVs 13249 and 16553) and two heat susceptible checks (ICGVs 16516 and 16690) (Table 1). The selected advanced breeding lines represent various traits, such as short duration and bold, high oleic acid content, drought tolerant, high oil content, high Fe and Zn content and phosphorous efficiency. These genotypes were subjected to TIR screening experiment to evaluate the genotype response for heat tolerance.

Screening for heat tolerance using Temperature Induction Response (TIR) technique

The Temperature Induction Response (TIR) technique works on the principle of subjecting plantlets to sub-lethal temperatures followed by exposure to lethal temperatures and subsequently assessing their growth and recovery. By exposing seedlings to both lethal and sub-lethal temperatures, enables the detection of genotypes with a greater ability to withstand and recover from heat stress. This comprehensive assessment offers valuable insights into the genetic basis of heat tolerance and assists the breeders to select the most suitable genotypes for further breeding programs in high temperature environments. The present study was conducted at Central Instrumentation Cell, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad and the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Hyderabad.

The TIR experiment was conducted in a WGC-450 programmable plant growth chamber under controlled conditions during the seedling stage. Seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 30 s and washed thrice with distilled water. They were allowed to germinate in petri plates for 48 h at 30°C. Later, the uniformly germinated seedlings were selected and sown in aluminum trays filled with a mixture of sterilized sand and vermicompost in the ratio of 2:1. Seeds were sown in three replications and each replication consisted of 10 seedlings. The seedlings were then subjected to high temperatures in two different treatments. The induced treatment involves exposing the seedlings to sub-lethal temperatures of 38°C to 54°C @ 0.5°C rise in temperature for every 10 min for 5 h, followed by exposure to lethal temperatures of 58°C for 3 h and then recovering them in a greenhouse at 30°C with 60% relative humidity for 72 h. In contrast, in non-induced treatment, the seedlings were directly exposed to lethal temperatures of 58°C for 3 h, followed by the same recovery process, as the induced treatment. A control set was also maintained, where the seedlings were kept in a greenhouse at 30°C with 60% relative humidity for 72 h (Kokkanti *et al.*, 2019) (Fig. 1). After the recovery period, observations such as fresh weight and dry weight of the seedlings were recorded. Fresh weight of the seedlings (g) was measured immediately using a precision balance. Later, place the freshly weighed seedlings in a drying oven at a predetermined temperature (70°C) for a period of 48 h, to ensure complete drying. After drying, weighed the seedlings again to determine their dry weight (g). The following observations were recorded after recording the fresh weight and dry weight of genotypes:

a) Shoot mass ratio (SMR) $= \frac{\text{Shoot dry weight}}{\text{Total dry weight}}$

b) Root mass ratio (RMR) $= \frac{\text{Root dry weight}}{\text{Total dry weight}}$

c) Root to shoot ratio (RSR) $= \frac{\text{Root dry weight}}{\text{Shoot dry weight}}$

Statistical Analysis

The data recorded in the TIR experiment was sujected to statistical analysis. For the TIR recorded traits, analysis of variance (ANOVA) was conducted using SPSS software. The variability parameters were estimated using R studio software (https://posit.co/products/open-source/rstudio). Correlation analysis was performed to determine the degree of association between the recorded traits using SPSS software (https://www.ibm.com/products/spss-statistics).

RESULTS AND DISCUSSION

In the context of induced treatment, a notable increase in shoot mass ratio (SMR) was observed, ranging from 0.21 to 0.50. Conversely, the non-induced treatment exhibited a reduced SMR, with a range of 0.01 to 0.37. On the other hand, the control treatment showed SMR values ranging from 0.31 to 0.50. These results suggest that induced genotypes have a greater capacity to withstand heat stress (Fig. 2a).

Genetic diversity in terms of root mass ratio (RMR) was assessed across induced, non-induced and control treatments. Within the induced treatment, RMR values ranged from 0.10 to 0.30, contrasting with the non-induced treatment where RMR values ranged from 0.02 to 0.24, and the control treatment where it ranged from 0.19 to 0.43 (Fig. 2b). Regarding the root to shoot ratio (RSR), the induced treatment displayed values ranging from 0.17 to 1.17, while the non-induced treatment exhibited variations from 0.03 to 0.99, and the control treatment ranged from 0.35 to 2.25 (Fig. 2c). Notably, the seedlings in the induced treatment showed higher mass ratios for both shoot and root growth compared to the non-induced treatment seedlings. This indicates the effect of high temperatures on shoot and root biomass. Additionally, the reduction in the root to shoot ratio in the non-induced treatment compared to the induced treatment indicates the effect of severe heat stress on root growth (Giri *et al.*, 2017).

Individual ANOVA analysis revealed significant variations ($p<0.05$) among genotypes for each of the traits, SMR, RMR and RSR. Furthermore, the treatment effect showed significant differences ($p<0.05$) in all three traits, SMR, RMR and RSR. In contrast, the effect of replication showed non-significant differences ($p>0.05$) across the traits, including SMR, RMR and RSR (Table 2). These findings align with previous studies highlighting the importance of genotype response in determining heat tolerant genotypes for traits such as SMR, RMR and RSR. Batool *et al.* (2021) reported similar significant differences among the genotypes and also heat treatments for the traits such as shoot dry weight, root dry weight and shoot/root ratio.

Through correlation analysis, significant positive correlations were observed between the root mass ratio (RMR) of the induced treatment and the root to shoot ratio (RSR) of the induced treatment (r=0.55**). Furthermore, the RMR of the non-induced treatment displayed significant positive correlations with the RSR of both the non-induced treatment (r=0.78**) and the control treatment (r=0.34*). Likewise, the RMR of the control treatment exhibited a significant positive correlation with the RSR of the control treatment (r=0.49**). Conversely, the shoot mass ratio (SMR) of the control treatment exhibited significant negative correlations with the RMR of the non-induced treatment (r=-0.35*) and the RSR of the control treatment (r=-0.72**). Furthermore, the SMR of the non-induced treatment displayed a significant negative correlation with the RSR of the control treatment (r=-0.44**) (Fig. 3).

Identification of heat tolerant genotypes

The identification of heat tolerant and heat susceptible genotypes based on their response to heat stress conditions is important in the context of crop resilience and climate change adaptation. In this study, we employed Temperature Induction Response (TIR) technique to evaluate groundnut genotypes, focusing on shoot and root mass indices as indicative traits. The results yielded critical insights into the varying degrees of heat tolerance among the tested genotypes, giving the way for a discussion on the implications of these findings.

The genotypes ICGVs 16598, 16594, and 06040 were identified as heat tolerant and the genotypes ICGVs 181023, 181013 and 181031 were identified as heat susceptible. Notably, the identified heat tolerant genotypes exhibited higher shoot and root dry weights under induced and non-induced heat stress conditions (Table 3). The consistent performance of heat tolerant genotypes across all treatments under heat stress conditions indicate their potential to be utilized as breeding candidates for the development of heat-resistant groundnut varieties. Conversely, the heat susceptible genotypes exhibited clear sensitivity to heat stress across all treatments, indicating the need for focused research to elucidate the factors contributing to their susceptibility. The susceptibility of these genotypes underscores the complexity of heat stress responses and the importance of genetic variability within crop species. A study by Singh *et al.,* (2017) reported after screening the maize inbred lines in TIR experiment and found that the identified tolerant inbred lines recorded high shoot and root dry weights compared to other genotypes. Similar results were also reported in heat tolerant genotypes of maize by Batool *et al.* (2021).

In conclusion, the objective of the study was to evaluate the heat tolerance of groundnut genotypes using shoot and root mass indices through TIR technique. The proposed method using fresh and dry weights of seedlings provides a reliable and cost-effective approach for screening heat stress tolerance at the seedling stage. The results from the experiment provided valuable insights into the variability of heat tolerance among the genotypes. Furthermore, the utilization of physiological traits such as shoot mass ratio (SMR), root mass ratio (RMR) and root to shoot ratio (RSR) emphasized the importance of selecting adaptable genotypes. Hence, the TIR technique and mass ratios can serve as an initial screening tool for assessing the performance under high temperature conditions. Furthermore, investigating the underlying mechanisms governing the heat tolerance of these genotypes is a promising avenue for future research. Understanding the genetic, physiological, and molecular factors that enable these genotypes to thrive under heat stress can inform the development of

p resilience in a

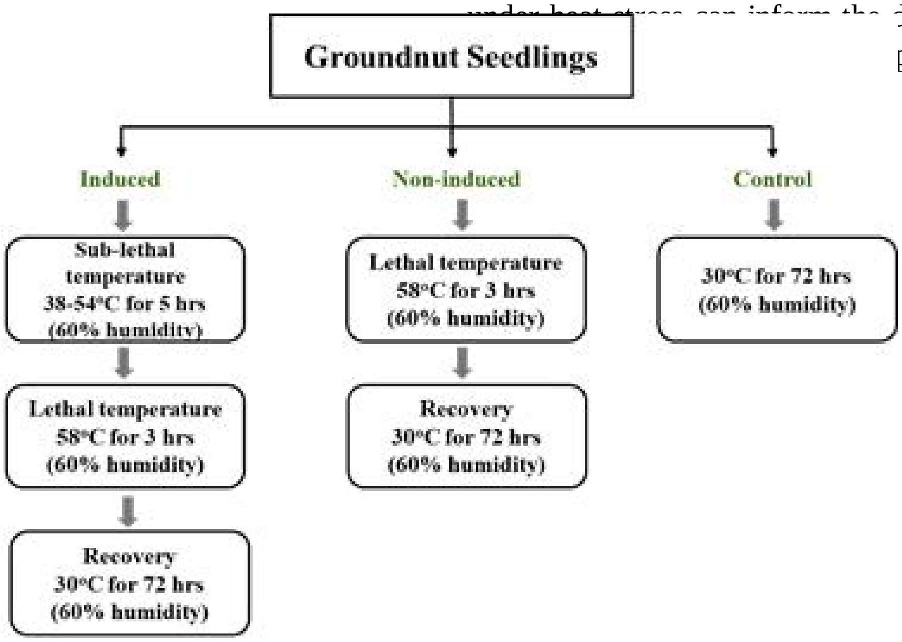
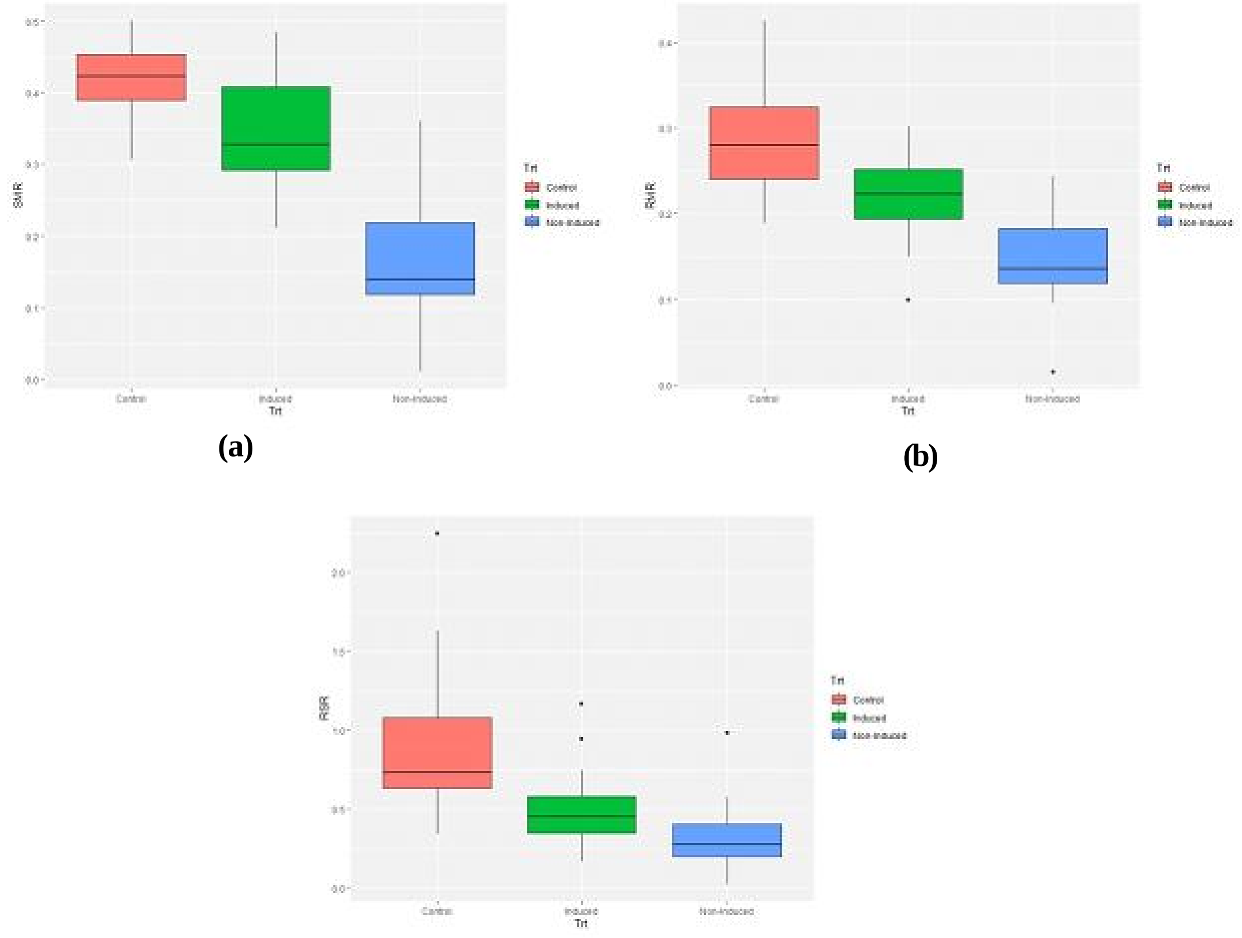


Fig. 1. Temperature Induction Response (TIR) protocol to screen thirty-six groundnut genotypes for heat tolerance at seedling stage



(a)

(b)

(c)

Fig. 2. The box-plot representation of a) Shoot mass ratio (SMR), b) Root mass ratio (RMR) and c) Root to shoot ratio (RSR).

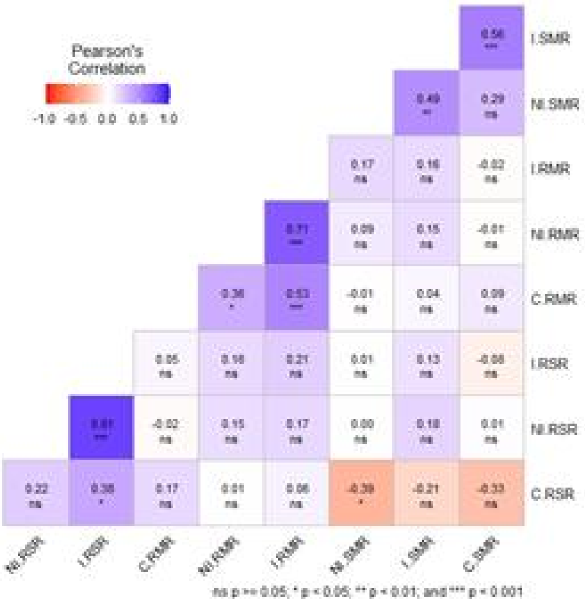


Fig. 3. Correlation analysis of recorded traits among thirty-six groundnut genotypes screened under TIR experiment. Note: * p< 0.05; ** p<0.01; *** p<0.001; ns non-significant.

Table 1. List of thirty-six advanced breeding lines of groundnut (*Arachis hypogaea* L.) screened for heat tolerance using TIR technique under controlled conditions.

Entry No.	Designation	Trait
1	ICGV 16593	Short Duration + Bold
2	ICGV 16594	Short Duration + Bold
3	ICGV 16598	Short Duration + Bold
4	ICGV 16599	Short Duration + Bold
5	ICGV 16606	Short Duration + Bold
6	ICGV 181030	High Oleic Acid
7	ICGV 181031	High Oleic Acid
8	ICGV 16679	High Oleic Acid
9	ICGV 171051	High Oleic Acid
10	ICGV 16688	High Oleic Acid
11	ICGV 181013	High Oleic Acid
12	ICGV 181076	High Oleic Acid
13	ICGV 181009	High Oleic Acid
14	ICGV 181023	High Oleic Acid
15	ICGV 181008	High Oleic Acid
16	ICGV 181064	High Oleic Acid
17	ICGV 15083	High Oleic Acid
18	ICGV 13189	Marker assisted backcross line (MABC)
19	ICGV 14421	Marker assisted backcross line (MABC)
20	ICGV 13207	Marker assisted backcross line (MABC)
21	ICGV 13280	Drought Tolerant
22	ICGV 13254	Drought Tolerant
23	ICGV 13312	Drought Tolerant
24	ICGV 13277	Drought Tolerant
25	ICGV 10315	Drought Tolerant
26	ICGV 01260	Drought Tolerant
27	ICGV 10365	Drought Tolerant
28	ICGV 07222	Drought Tolerant
29	ICGV 06040	High Iron & Zinc
30	ICGV 03042	High Oil Content
31	ICGV 06146	Phosphorous Efficient
32	ICGV 10143	International Medium Duration Line
33	ICGV 13249	Drought Tolerant
34	ICGV 16553	High Oil Content
35	ICGV 16516	High Oil Content
36	ICGV 16690	High Oleic Acid

Table 2: Analysis of variance (ANOVA) for the recorded traits among thirty-six groundnut geno types in TIR experiment.

Source of Variation	df	Shoot Mass Ratio (SMR)	Root Mass Ratio (RMR)	Root to Shoot Ratio (RSR)
Entry	35	1.50*	1.39*	0.81*
Replication	2	14.71 ns	4.01 ns	4.38 ns
Treatment	2	22.72***	19.47***	22.87***

Note: * $p < 0.05$; ** $p<0.01$; *** $p<0.001$; ns non-significant.

Table 3: List of identified heat tolerant and heat susceptible genotypes based on the recorded traits in TIR experiment.

S. No.	Designation	Shoot Mass Ratio (SMR)			Root Mass Ratio (RMR)			Root to Shoot Ratio (RSR)		
		Induced	Non-induced	Control	Induced	Non-induced	Control	Induced	Non-induced	Control
1	ICGV 16598	0.5	0.37	0.49	0.28	0.23	0.36	0.75	0.57	0.64
2	ICGV 16594	0.48	0.19	0.5	0.27	0.17	0.33	0.95	0.57	1.11
3	ICGV 06040	0.25	0.13	0.48	0.3	0.24	0.43	0.2	0.03	0.35
4	ICGV 181023	0.22	0.08	0.4	0.16	0.11	0.26	0.28	0.16	0.71
5	ICGV 181013	0.28	0.08	0.46	0.1	0.02	0.21	0.22	0.15	0.5
6	ICGV 181031	0.24	0.14	0.33	0.21	0.12	0.32	0.17	0.11	0.81

LITERATURE CITED

Arya S S, Salve A R and Chauhan S 2016. Peanuts as functional food: a review. *Journal of Food Science and Technology* 53:31-41.

Batool A, Abbas G, Hasnain Z, Perveen N and Akhtar M N 2020. Comparative response of seedlings of heat tolerant and sensitive maize genotypes regarding early growth traits against heat stress. *Pakistan Journal of Agricultural Research* 34:329-336.

Bertioli D J, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, Leal-Bertioli S C, Ren L, Farmer A D, Pandey M K and Samoluk S S 2019. The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. *Nature Genetics* 51:877-884.

FAOSTAT 2021. Peanut (groundnuts excluding shelled) production in 2021. Food and Agricultural Organization of the United Nations, Statistics Division. Retrieved March 24, 2023.

Gangappa E, Ravi K and Veera Kumar G N 2006. Evaluation of groundnut (*Arachis hypogaea* L.) genotypes for temperature tolerance based on Temperature Induction Response (TIR) technique. *Indian Journal of Genetics and Plant Breeding* 66:127-130.

Giri A, Heckathorn S, Mishra S and Krause C 2017. Heat stress decreases levels of nutrient-uptake and-assimilation proteins in tomato roots. *Plants* 6:6.

Kokkanti R R, Hindu V, Latha P, Vasanthi R P, Sudhakar P and Usha R 2019. Assessment of genetic variability and molecular characterization of heat stress tolerant genes in *Arachis hypogaea* L. through qRT-PCR. *Biocatalysis and Agricultural Biotechnology* 20:101242.

Portner H O, Roberts D C, Adams H, Adler C, Aldunce P, Ali E, Begum R A, Betts R, Kerr R B, Biesbroek R and Birkmann J 2022. Climate change 2022: impacts, adaptation and vulnerability. IPCC.

Ruan Y L, Jin Y, Yang Y J, Li G J and Boyer J S 2010. Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. *Molecular Plant* 3:942–955.

Singh A, Kuchanur P H and Ravikumar R L 2017. Identification of heat tolerant maize inbred lines using TIR technique and its association with field tolerance. *The Bioscan* 12:2053-2058.

Variath M T and Janila P 2017 Economic and academic importance of peanut. *The Peanut Genome* pp.7-26.

Zhang Y, Mian M A R, Chekhoviskiy K, So S, Kupfer D, Lai H and Roe B A 2005. Differential gene expression in Festuca under heat stress conditions. *Journal of Experimental Botany* 56: 897-907.

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