

Review Article

Temperature Induction Response: A Rapid Screening Technique for Thermotolerance in Plants

Abstract

Global warming is a never-ending disastrous threat to the world and the existence of life. For many years, researchers have been cautioning about the disastrous results on the climate if the world touches average temperatures of 1.5°C above the pre-industrial levels. The high-carbon pathway, the worst of the situations, would see global temperatures increase by more than 4°C by the end of the century (IPCC, 2022). High-temperature stress affects the growth and development of crops and eventually lowers yields to significant levels. Thermotolerance is a complex trait, and along with agronomic practices, there is a need for the identification and characterization of genotypes for heat tolerance, which is a prerequisite for crop improvement. Temperature Induction Response (TIR) is a high-throughput means successfully employed for assessing, identifying, and screening crop plants for thermotolerance in different crop species. TIR is based on the principle of the LD₅₀ concept and acquired thermotolerance, which is crop-specific in nature. Hence, this review focuses on the relevance, methodology, standardization, mechanism, utilisation and significance of the TIR technique for crop improvement in different crop species to combat heat stress.

Keywords: Temperature Induction Response, Heat Stress, Thermotolerance.

Introduction

According to the recent reports of the World Research Institute (2022), global temperatures have climbed by 1.1°C so far, and we are already experiencing natural disasters such as forest fires, floods, hurricanes and other events. The recent reports of the IPCC (2022) warn that the world is set to reach the 1.5°C level within the next two decades. The world has never seen temperature rises of more than 2.5°C over a short time for more than 3 million years, and recently, 2020 is the second warmest year recorded. According to NOAA (2022) recent temperature data, The average global land and ocean surface temperature for January–December 2021 was 0.84°C above the 20th-century average of 14.0°C, and this ranks as the fifth-warmest September–November period in the 143-year record. In the current scenarios studied by the IPCC (2022), there is a more than 50% chance that the 1.5 degrees C target will be reached or crossed between 2021 and 2040 (with an estimate of early 2030s). Under a

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higher greenhouse gas emissions scenario, the world gets the 1.5°C threshold temperature even more quickly (2018-2037).

Environment exhibits various abiotic stresses on plants, among high temperature is one of the major ones. The effect of increasing temperature trends has no exception on agriculture. The influence of higher temperature rests on the crop's optimum temperature for growth and development. If the temperature exceeds the crop's optimum temperature, it will weaken the growth and development of crop plants, resulting in a loss of yield and quality of agricultural produce (Waqas *et al.*, 2021). A temperature increase of 2.2°C degrees by 2050 would cut global GDP by 20%. (Oxford Economics High Emission Scenario 2022). Warming up to 5°C by 2100 will lead to economic obliteration, consistent with mass extinction thresholds. (Song *et al.*, 2021). Heat waves are baking crops, causing severe damage by altering the activity of powerful antioxidant enzymes, membrane damage, lipid peroxidation and protein synthesis (Kumari *et al.*, 2022). Heat stress limits crop growth, leading to oxidative stress, an increase in the production of reactive oxygen species, and a considerable decrease in the yield and quality of agricultural produce (Devasirvatham *et al.*, 2018; Moore *et al.*, 2021)

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In the Indian situation, according to the latest reports, wheat and mustard were got affected in several districts of Uttar Pradesh. Heat waves caused the reduction of wheat yield by 15 to 20% in Gonda, 21 to 11% in Kushinagar and Baghpat, 9 to 21% in Gorakhpur, and 32 to 34% in Jhansi. In the Gorakhpur and Kushinagar districts of Uttar Pradesh, mustard cowpea pea yields were reduced by 14 to 18% and 9 to 11%, respectively. In the same way, several NICRA villages in Rajasthan experienced heat waves and recorded yield losses of up to 4 to 5 q/ha in wheat and 2 to 3 q/ha in mustard compared to normal (Bal *et al.*, 2022). Each degree-Celsius rise in worldwide mean temperature would decrease the global yields of wheat by 6.0%, rice by 3.2%, maize by 7.4%, and Soybean by 3.1%. (Zhao *et al.*, 2017) Hence, crop improvement is needed to cope with the high-temperature trends.

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The TIR method is developed from the widely accepted principle of Lethal Dose₅₀ (LD₅₀), proposed by Trevan in 1921 for the biological standardization of insulin, toxins and drugs. Still, nowadays, it is widely used in clinical research and toxicology to determine the toxicity of drugs, pesticides and fungicides (Pillai *et al.*, 2021). The temperature induction response (TIR) technique is a robust and widely recognised empirical and non-destructive method for rapidly assessing the heat tolerance in crop plants at the seedling stage (Raghavendra *et al.*, 2017). Any stress develops gradually, and the plants are typically exposed to sublethal stress, also called induction stress, before being exposed to severe stress, also called lethal stress, which activates the acquired thermotolerance mechanism in plants to

cope with heat stress. The temperature at which hundred per cent or the total mortality of seedlings was found is called the lethal temperature. The induction temperature could be the nonlethal low temperature for a specific duration or a gradual increase (Senthil-Kumar *et al.*, 2003). The period provided to seedlings after exposure to temperature treatment during which plants recover growth occurs. These temperature factors are crop-specific; hence, determining these parameters is the key point in TIR. (Babu and Raghavendra 2017). The rate of thermotolerance is determined in terms of the Per cent survival of seedlings, per cent reduction in root growth, shoot growth and per cent reduction in total growth (Root + Shoot) of the seedlings (Rekha *et al.*, 2016; Mishra *et al.*, 2019) as compared to non-induced seedlings or absolute control (another set of seedlings grown at room temperature without exposure to any temperature is taken as absolute control may be taken for comparison). Based on this TIR technique, genetic variability for cellular level tolerance has been developed in many crop species like Rice (Vijayalakshmi *et al.*, 2015; Harihar *et al.*, 2014), Cotton (Kheir *et al.*, 2012), Soybean (Ange *et al.*, 2016), Millets (Sowmyapriya *et al.*, 2018; Bheemesh *et al.*, 2018; P Bhavana *et al.*, 2018,) maize (Dar *et al.*, 2016), groundnut (Gangappa *et al.*, 2006), tomato (Chandola *et al.*, 2018), sugarcane (Gomathi *et al.*, 2014), etc.

Temperature Induction Response (TIR)

Principle

Initially, the seedlings are exposed to sub-lethal temperature (induction temperature), following which the seed is exposed to lethal temperature and allowed for retrieval. This retrieval growth is determined as an amount of tolerance to extreme temperature stress (Raghavendra *et al.*, 2017).

Recovery period

The period that is provided to seedlings after exposure to temperature treatment during which plant recovery growth takes place

Lethal temperature:

The temperature at which hundred per cent or the total mortality of seedlings was found.

Induction temperature (Sub-lethal temperature)

The induction temperature could be the nonlethal low temperature for a specific duration or a gradual increase.

Standardisation of TIR for any specific crop

TIR will be standardised based on the LD₅₀ concept, and challenging temperature levels can be done at both the seed and seedling stage

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Seedling growth: The viable seeds of any crop were soaked in water for 10-12 hours (depending on the crop), allowed to be imbibed in a glass beaker, and later allowed to germinate in germination papers. The uniform seedlings were selected for further process in replicates.

Determination of lethal temperature

Step 2: The 1-3 days old seedlings were exposed to graded temperatures right from their minimum temperature to maximum temperature, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60degreesCelsius for the duration of 1, 2 and 3 hours respectively.

Step 3: Such treated seedlings should be subjected to recovery growth at room temperature or 30-32°C for 24-72 hours, depending upon the crop. The critical temperature level and duration of exposed temperature will be finalised based on the per cent recoverability of seedlings. Recovery growth will be determined as an amount of tolerance to severe temperature stress. After recovery growth, the temperature at which more than ninety per cent of seedling's mortality was seen is called a lethal temperature and is also known as a challenging temperature.

Step-4: Determination of optimum sub-lethal or induction temperature

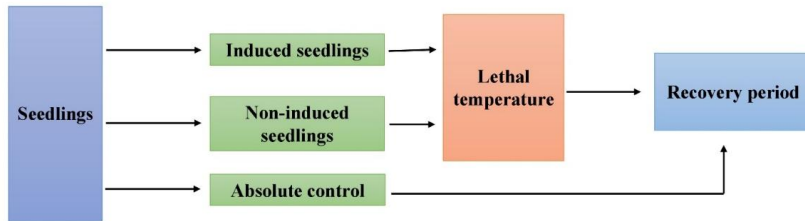
Seedlings were exposed to a gradual increase in temperature for a specific period. These temperature regimes and periods are diverse from crop to crop and need to be standardised.

The seedlings were subject to gradually increasing temperatures, i.e., 32-40°C, 32-42°C, 32-44°C, 32-46°C, 32-48°C, and 32-50°C for a known duration. After this induction treatment, seedlings were exposed to standardised lethal temperature and then allowed to recover at room temperature and sixty per cent relative humidity for 24-72h based on the crop species. The temperature regimes and durations are varied to arrive at the optimum induction protocol. The optimum sub-lethal temperatures were reached based on the per cent survival of seedlings. The temperature range at which the highest per cent of seedlings survival rate was seen is considered as the optimum induction temperature.

TIR procedure: Exposure to standardised optimum induction temperature followed by exposure to standardised lethal temperature and measurement of recovery growth as a tolerance after exposure to recovery period by using below growth parameters.

The details of the TIR technique standardised in different crops are given in the table 2.

Figure 1: Generalised Temperature Induction Response (TIR) Protocol used for thermotolerance screening.



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The list of parameters used to determine recovery growth as a means of tolerance (Rekha *et al.*, 2016):

Percent survival of seedlings:

$$\frac{\text{Number of seedlings survived at the end of recovery}}{\text{total number of seedlings sown}} \times 100$$

Per cent reduction in root growth:

$$\frac{\text{Root growth of actual control seedlings} - \text{Root growth of treated seedlings}}{\text{Root growth of actual control seedlings}} \times 100$$

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Per cent reduction in shoot growth:

$$\frac{\text{Shoot growth of actual control seedlings} - \text{Shoot growth of treated seedlings}}{\text{Shoot growth of actual control seedlings}} \times 100$$

Per cent reduction in total growth (Root + Shoot) of seedlings:

$$\frac{\text{Total growth of actual control seedlings} - \text{Total growth of treated seedlings}}{\text{Total growth of actual control seedlings}} \times 100$$

Table 1: Standardised Temperature Induction Response (TIR) technique in different crops

Serial number	Crop	Induction temperature	Challenging temperature or Lethal temperature	Recovery conditions	Author and year	The age of the seedlings used	Variety/genotype
1	Maize (<i>Zea mays</i> L.)	30-45°C for 5 hours	50° C for 3 hours	30°C and 60% RH for 72 hours	Dar <i>et al.</i> , 2016	Two days old seedlings	GM-6
2	Sorghum (<i>Sorghum bicolor</i> L.)	38-52°C for 4.5 hours and	56°C for 3 hours	30°C and 60% RH for 48 hours	Babu and Raghavendra, 2017	3 days old seedlings	NA
3	Mungbean (<i>Vigna radiata</i> L.)	38- 54°C for 5 hours	56°C for 2 hours	30°C and 60 % RH for 24 hours.	Rekha <i>et al.</i> , 2016	1day old seedlings	NA
4	Rice (<i>Oryza sativa</i>)	38-48°C for 3 h	54°C for 3 h	30°C and 60 % RH for 3 days.	Vijayalakshmi <i>et al.</i> , 2015	7 days old seedlings	Improved White Ponnivand CO 51

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5	Rice (<i>Oryza sativa</i> L.)	36-44°C for 5 h	52°C for 3h	30°C and 60% RH for 72 h.	Harihar <i>et al.</i> , 2014	3 days old seedlings	White Ponni
5	Pea (<i>Pisum sativum</i> L.)	33°C for 1 h, 37°C for 1h and 40°C for 2 h	48°C for 1h	30°C and 60% RH for 72h	Srikanthbabue <i>t al.</i> , 2002	2 days old seedlings	NA
6	chickpea (<i>Cicer arietinum</i> L.)	38-48°C for 4.5h	50°C for 3h	30°C and 60% RH for 3h	Raghavendra <i>et al.</i> , 2017	2 days old seedlings	NA
7	Groundnut (<i>Arachis hypogea</i> L.)	35°C for 1 h, 40°C for 1h and 45°C for 2 h	55°C for 3h	30°C and 60%RH for 3h	Gangappaet <i>al.</i> , 2006)	2 days old seedlings	NA
8	Sugarcane (<i>Saccharum officinarum</i> L.)	40°C for 10 h and	48°C for 20 h and	24 hours at room temperature	Gomathi <i>et al.</i> , 2014)	30 days old settlings	Co 86032
9	Banana (<i>Musa sp.</i>)	30-42°C for 2.5h	55°C for 2.5h	30°C and 60% RH for 10 days	Vidya <i>et al.</i> , 2017	5-6 weeks old seedlings	Grand Naine

10	Soybean (<i>Glycine max</i> L.)	34-42°C for 3 h	48°C for 3h	30°C and 60% RH for 72 h	Ange <i>et al.</i> , 2016	3 days old seedlings	Cosoy3
11	Sunflower (<i>Helianthus annuus</i> L.)	28-42°C for 2.5h	49°C for 2 h.	30°C and 60% RH for 72 h.	Senthil-Kumar <i>et al.</i> , 2003	2 days old seedlings	KBSH-1 hybrid and CMS 234
12	Cotton (<i>Gossypium hirsutum</i> L.)	28-40°C for 4h	47°C for 3 h,	30°C and 60% relative humidity (RH) for 48 h	Kheir <i>et al.</i> , 2012	1 to 1.5 cm radicle length seedlings	Sahana
13	Finger millet (<i>Eleusine coracana</i> L.)	37-54°C for 5h	58°C for 2.5h	30°C and 60% relative humidity (RH) for 48 h	Bhavana <i>et al.</i> , 2018	24h old seedlings	NA
14	Foxtail millet (<i>Setariaitalica</i> L.)	38-58°C for 5h	59°C for 2h	30°C and 60% relative humidity (RH) for 48 h	Bheemesh <i>et al.</i> , 2018	24h old seedlings	NA

15	Black gram (<i>Vigna mungo</i> L.)	32-48°C (with 2°C rise for 30mins for each temperature)	52°C for 1h	30°C and 60% RH for 72h	Sujatha <i>et al.</i> , 2018	2 days old seedlings	PU-31(c)
16	Minor millets*	46-54°C for 3h	56°C for 3h.	30°C and 60% RH for 72 h	Sowmyapriya <i>et al.</i> , 2018	7 days old seedlings	NA
17	Tomato (<i>Lycopersicon esculentum</i> Mill.)	38 °C for 1h, 42°C for 1h and 46°C for 1h	48°C for 2h	34°C and 60% RH for 72h	Chandola <i>et al.</i> , 2018	NA	NA
18	Capsicum (<i>Capsicum annuum</i> L.)	of 33–42°C for 4 h with	48°C for 1.5 h gave	Room temperature and 60% RH for 72h	Mishra <i>et al.</i> , 2019	1 day old seedlings	Punjab Guchhedar and Ajeet

*Minor millets: *Setaria italica* (Thenai CO6), *Echinochloa colona* (Kudiraivalli CO2), *Paspalum scrobiculatum* (Varagu CO3), *Panicum miliaceum* (Panivaragu CO5), *Panicum sumatrense* (Samai CO4). h- Hours, NA- Not Applicable and RH- Relative Humidity.

Table 2: Thermotolerant genotypes identified by Temperature Induction Response (TIR) technique in different crops

Serial number	Crop	Tolerant	Moderately tolerant	Susceptible	Author
1	Groundnut (<i>Arachis hypogaea</i> L.)	DH-991, TG-37 F, K-134, K-1240, TNAU-325, TNAU- 326, CO-3, JL-24	DH-992, TG-36, B K- 1238, TNAU-269, JNAU-406, TMV-10, GPBD-4, ICGS-76, Somnath and Tirupathi local	TVG-9563, ICGV-8659012, JSSP-15, JSSP-16 J-54, AK-159, VG-9711, VRIGN-5 13, TNAU-359, 14 COGN-5, RG-369, K-1257 and TNAU-281	Gangappaet <i>al.</i> , 2006)
2	Tomato (<i>Lycopersicon esculentum</i> Mill.)	NDTVR-60, DT- 2, PMS-1	Kashi sahrad, Punjab sharad, Angurlata and Kashi anupam	Feb-4 , Azad T-5, TLC-1, CO-3, Kashi vishesh, EC-520061, Shalimar-2, GT H- 88-7-4, FLA-7171, Kashi amrit, Hisar anmol Selection-7, Swarn lalima, B-S-31- 3, Flawery, NF-315, B-S-2-5, DT-10, BT- 120, VR-20, B-S-18-7 and T-local	Chandola <i>et al.</i> , 2018
3	Rice (<i>Oryza sativa</i>)	N22, Apo and Norungan	Ponni and CO51	Anaikomban and FR13A	Vijayalakshmi <i>et al.</i> , 2015

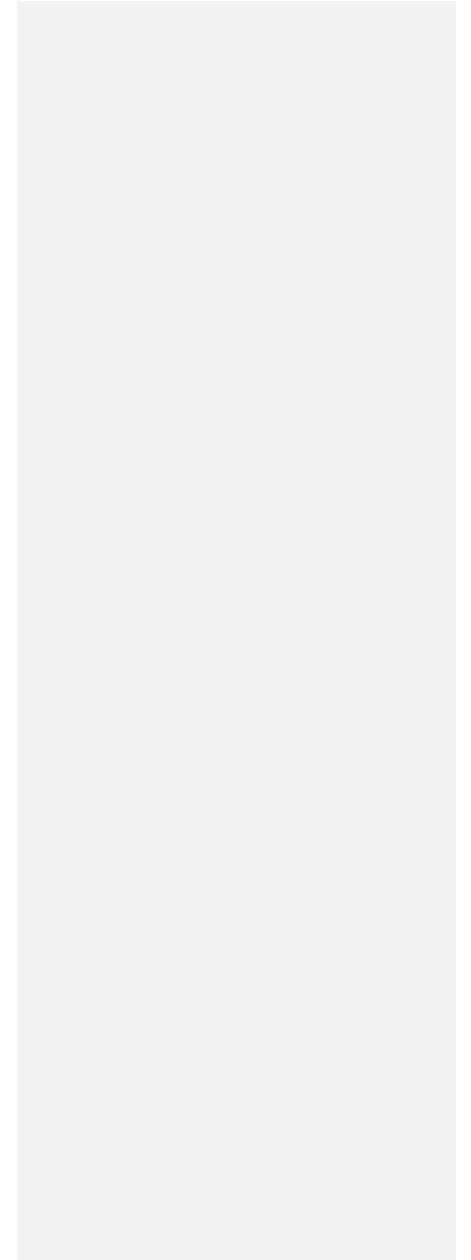
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4	Mungbean (<i>Vigna radiata</i> L.)	LGG 410, ML 267 and TLM-7		LGG 407 MGG 295 and VG7098A	Rekha <i>et al.</i> , 2016
5	Pea (<i>Pisum sativum</i> L.)	Acc.695, Acc.623 and Acc.765	NA	Acc.476, Bonneville and FC-2	Srikanthbabu <i>et al.</i> , 2002
6	Banana (<i>Musa sp.</i>)	Grand Naine and Rasbale	NA	Red Banana and Kunnan	Vidya <i>et al.</i> , 2017
7	Soybean (<i>Glycine max</i> L.)	ADT1 and CoSoy1	NA	MACS450	Ange <i>et al.</i> , 2016
8	Cotton (<i>Gossypium hirsutum</i> L.)	<i>G. hirsutum</i> - H-28	NA	<i>G. barbadense</i> - B-4	Kheir <i>et al.</i> , 2012
9	Finger millet (<i>Eleusine coracana</i> L.)	BR-36, TNAU-1214, GPU-28, VR-900, KOPN-933 and PPR-2885	NA	WWN – 25, IC – 382797, Srichaithanya and IC – 306421	Bhavana <i>et al.</i> , 2018

10	Foxtail millet (<i>Setaria italica</i> L.)	SiA 3580, SiA 3604, SiA 3618, SiA 3623 and SiA 3625	NA	SiA 3555, SiA 3563, SiA 3569 and SiA 3572	Bheemesh <i>et al.</i> , 2018
11	Black gram (<i>Vigna mungo</i> L.)	LBG-806 and LBG-808	NA	LBG- 823, PU 31 and LBG-45	Sujatha <i>et al.</i> , 2018
4	Capsicum (<i>Capsicum annum</i> L.)	Punjab Guchhedar, Ajeet 1, K1, LCA 333 Japanese Long, PC 2062, Phule Jyoti	NA	HDC 75, Solan Bharpur, JCA 283, Ajeet 3, Arka Suphal, PBC 535	Mishra <i>et al.</i> , 2019
12	Minor millets*	Samai (CO4) and Thenai (CO6)	NA	NA	Sowmyapriya <i>et al.</i> , 2018
13	chickpea(<i>Cicer arietinum</i> L.)	NBeG-528, NBeG- 458, NBeG-511, NBeG-177, NBeG-747, NBeG-732 and VIHAR	NA	NA	Raghavendra <i>et al.</i> , 2017

*Minor millets: *Panicum sumatrense* (Samai CO4) and *Setaria italica* (Thenai CO6), NA: Not Applicable

UNDER PEER REVIEW



Physiological and molecular basis of the temperature induction response (TIR) in crop plants

The study conducted on rice showed that the latent capacity of a genotype to withstand heat stress is its capacity to sustain cellular membrane integrity immediately after exposure to a lethal temperature. The antioxidant enzymes superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT), which are intricate in the scavenging of reactive oxygen species, were found to be significantly higher under TIR treatment. In sugarcane seedlings, a considerably higher activity of SOD was noticed in induced (30.0 units g⁻¹ fr.wt.min⁻¹) compared to non-induced (15.75 units g⁻¹ min⁻¹) and control (12.5 units g⁻¹ fr.wt. min⁻¹), respectively (Gomathi *et al.*, 2014). The same trend was also reported in other crops: rice Vijayalakshmi *et al.*, 2015; Soybean (Ange *et al.*, 2016) and Sugarcane (Gangappa *et al.*, 2006).

The tolerant genes like Heat Shock Proteins (HSPs) and heat shock transcription factors (HSFs) were expressed significantly high under TIR treatment, which facilitated the plants to endure higher temperatures. The Heat Shock Proteins were substantially synthesised during induction stress, which facilitated in promotion of physiological and biochemical processes essential for the adaptation to lethal stress (Vidya *et al.*, 2017). SDS-PAGE protein profiling of the induced tomato seedlings showed considerably higher expression of heat shock proteins and was persistent even after the recovery period of three days. These HSPs facilitate the physiological and biochemical processes crucial for the adaptation to high-temperature stress. This thermotolerance appeared to be solely due to overexpression of the sHSP24.4 gene (Chandola *et al.*, 2018). The accumulation of heat-inducible protein (Hsp 90, Hsp70) and dehydrins (27 kDa) in induced seedlings and calli play an essential role in cell protection from heat stress damage (Gangappa *et al.*, 2006). Expression of a low and a high molecular weight HSP was analysed upon TIR treatment in Pea, the tolerant genotype Acc.623 displayed higher expression of hsp70 and hsp18.1 transcripts and higher gathering of HSP104 and HSP90 proteins compared to the susceptible genotype Acc.476 (Srikanthbabu *et al.*, 2002).

Heat stress, the most extensively considered facet, is the boosted expression of heat shock proteins (Hsps). Synthesis and localisation of Hsps have been revealed to initiate many biochemical and physiological processes, such as the maintenance of chaperoning proteins and membrane stability (ul-Haq *et al.*, 2019). The expression of Hsps is primarily controlled by the heat-dependent activation of the heat shock transcription factors (HSFs) (Berka *et al.*, 2022). There is strong evidence that the proteins and genes responsive to stress are mainly expressed during sub-lethal stress, leading to metabolic changes that enable plants to withstand subsequent severe stress (Gangappa *et al.*, 2006). The Hsp70, under normal conditions,

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enables the folding of newly synthesised proteins, preventing undesirable interactions, but under heat stress, Hsp70 protects heat-labile proteins from denaturation. (Khan *et al.*, 2021). The induced seedlings of Sunflower showed a higher level of expression of HSP 90, which was high at the end of the induction treatment. At the plant level, the higher molecular weight HSP 104 was also expressed relatively high in induced plants. The HSF, which is 55 kDa, has shown substantially higher levels of expression upon induction as compared to non-induced plants (SenthilKumar *et al.*, 2003). The induced seedlings (125.1 $\mu\text{g g}^{-1}$) and calli (142.5 $\mu\text{g g}^{-1}$) of the sugarcane showed significantly higher accumulation of the phenolic compounds. Since the synthesis of phenolics takes place in cells, it is thought that soluble phenolics are ROS scavengers. The proline and glycine betaine acts as an osmolyte and protects against cellular damage caused by high temperature. The proline was significantly higher in induced seedlings of the sugarcane (68.6% over control) compared to non-induced seedlings (37.6% over control), and the induced seedlings had higher glycine betaine accumulation (34.50 $\mu\text{g g}^{-1}$ with 41.50% increase over control) than non-induced seedlings (Gomathi *et al.*, 2014). The thermotolerance genotypes identified by TIR technique are grouped into several categories in different crops are listed in the table 2.

Conclusion and Future Prospectus

Plants have developed various ways to adapt to high-temperature stress, physiologically, biochemically, and molecular strategies. The TIR technique was a dependable screening technique for the primary evaluation of heat tolerance genotypes and assessing the genotypic variability in acquired thermotolerance. TIR triggers tolerance mechanisms and makes the plant adopt high-temperature stress. Genetic makeup and variability among different crop species confer the tolerance to heat stress. The main benefit of this technique is its reproducibility and quickness in screening a vast population of genotypes at the seedling stage. It also benefits the breeder to narrow down tolerant genotypes in a big population, saving the energy and time required for screening. TIR is a potential technology for screening tolerant genotypes from a diverse germplasm group. Later, these tolerant lines can be utilised for further documentation of traits conferring heat tolerance in these identified genotypes and can also be used for mapping the QTLs or genes, which will help breeders in marker-assisted selection for heat stress and other improvement programs.

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