

## Evaluation of *Solanum lycopersicum* L. genotypes for high temperature stress tolerance employing temperature induction response technique

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*Solanum lycopersicum* L., an important vegetable crop (Tomato) in most regions of the world, is affected by high temperature stress at various stages of its growth. Likely increase in periodicity of high temperature episodes under climate change conditions would further affect the tomato production. To sustain productivity and yields under climate change situations, there is an urgent need to develop suitable cultivars as an adaptation strategy. For this endeavour, identification of high temperature tolerant lines for crop improvement is a prerequisite. Hence in the present study, temperature induction response (TIR) technique was employed to evaluate thermotolerance in 52 tomato genotypes. Two day old seedlings were subjected to an initial induction treatment i.e., gradual temperature increase from 33-43°C for 3 h followed by 50°C for 3 h as challenging temperature. Wide variability was seen for seedling survival and vigour. In genotypes IIHR-2202, IIHR-2836 and IIHR-2841 seedling survival was as high as 90%, while H-329, H-371, IIHR-2745 and H-335 showed least reduction in growth and also had better seedling vigour index. Of the 52 genotypes, 21 were identified as tolerant, 12 moderately tolerant and 19 susceptible. This TIR technique is simple, quick and less expensive than whole plant screening. Hence, it could be used by plant physiologists and plant breeders for screening seedlings at an early stage in the phenotyping and crop improvement programmes.

**Keywords:** Abiotic stress, Climate change, Genetic variability, Thermotolerance, TIR technique, Tomato

Tomato is an important vegetable crop in India with 19.66 Mt annual production<sup>1</sup>, and India ranks second in world's tomato production. However, tomato producing regions of India are still facing the challenge of variation in temperature during tomato growing seasons similar to the other parts of the world due to climate change. India has been predicted to face 50% loss in vegetable production by the end of this century due to temperature rise<sup>2</sup>. The optimum temperature range for tomato is 25-30°C during day and 16-20°C during night. Temperatures higher than the optimum decrease both plant growth rate and yield drastically<sup>3</sup>. At higher temperatures, the productivity of tomato is far below the average potential yields<sup>4-6</sup>. The adverse effects of high temperature could be seen both at cellular and whole plant level<sup>7</sup>.

Plant stress is a complex phenomenon, and several physiological, morphological, biochemical changes

occur when plants experience stress<sup>8-12</sup>. In tomato, elevated temperature episodes experienced under climate change conditions are known to affect productivity, production and quality. Though, the modifications in farming practices help in adapting to such conditions in the short term, but development of tolerant cultivars is the best adaptation strategy in the long term. Hence, breeding for high temperature tolerant crops is in high demand<sup>13</sup> and in this endeavour, it is essential to fully characterize and identify genetic variation for the high temperature tolerance traits in the available germplasm before using them in a breeding program.

Temperature induction response (TIR) technique is used to identify and characterize high temperature tolerant genotypes for genetic enhancement. Plant breeders use this technique for screening seedlings at an early stage and for advancing generations in accelerating breeding programs. Several studies employing TIR technique have shown that plants develop ability to withstand high temperature when exposed to sublethal followed by severe

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temperatures<sup>14-18</sup>. During the induction process, with gradual rise in temperature, expression of many stress responsive genes occurs and this in turn triggers several physiological and biochemical processes involved in stress tolerance. The genotypes with maximum survivability and growth after recovery period are selected as thermotolerant<sup>16,19,20</sup>. Hence, by optimizing the induction and lethal temperature stress treatments one can identify the intrinsically thermotolerant genotypes<sup>19</sup>.

Prajapati *et al.*<sup>21</sup> studied genotypes exhibited genotypic variability when subjected to induction and challenging temperatures and observed the presence of genetic variability and heritability in diverse genotypes of tomato. According to Rivero *et al.*<sup>22</sup> heat stress in tomato plants occur at 35°C and upon exposure to such high temperatures, plant suffers from both physiological and biochemical damage which ultimately leads to reduction in plant growth and also commercial yield. For improving the yield and yield attributes several varieties of tomato have been evaluated worldwide, both under field and controlled temperature conditions. However, several screening techniques have been used to assess the thermotolerance level and genetic variability. Some of the physiological parameters, such as membrane stability index and chlorophyll bioassay have been considered for screening and evaluating different tomato genotypes under high temperature stress<sup>3</sup> and also some of the specific parameters like net photosynthetic rate and chlorophyll fluorescence have been recorded in five tomato genotypes to evaluate thermotolerance level under heat stress<sup>23</sup>. Similarly, thermotolerance level can be evaluated at seedling stage and temperature induction response (TIR) technique can be employed as an efficient screening technique. It is considered as a better tool to evaluate the genotypes for thermotolerance at seedling stage for screening large number of tomato genotypes<sup>24,25</sup>.

Considering the advantages of this technique for quick screening of germplasm lines, in the present study, we employed TIR technique to evaluate tomato genotypes for high temperature stress tolerance.

## Materials and Methods

### Plant material

The experiment was conducted at the Division of Plant Physiology and Biochemistry, ICAR-Indian Institute of Horticultural Research, Bengaluru during the year 2015-16. Seeds of 52 different tomato

genotypes which included two wild types: IIHR-1940 (Heat tolerant) and IIHR-2101 (Cold tolerant); 30 breeding lines: IIHR-2325, IIHR-2326, IIHR-2327, IIHR-2328, IIHR-2329, IIHR-2330, IIHR-2336, IIHR-2337, IIHR-2339, IIHR-2340, IIHR-2343, IIHR-2346, IIHR-2348, IIHR-2351, IIHR-2353, IIHR-2359, IIHR-2370, IIHR-2382, IIHR-2391 (Advanced breeding lines), IIHR-2190, IIHR-2294 (Drought tolerant lines), IIHR-2202, IIHR-2745, IIHR-2786, IIHR-2852, IIHR-2853, IIHR-2913, IIHR-2914 (Heat tolerant lines) IIHR-2831 (Early blight bacterial wilt resistant) IIHR-2843 (Bacterial wilt resistant); 9 varieties: IIHR-2620, IIHR-2627, Arka Vikas (Heat tolerant varieties); Arka Saurabh (Fresh market), Arka Meghali (Drought tolerant), Arka Alok, Arka Abha (Bacterial wilt resistant); Arka Ahuti, Arka Ashish (Processing lines); and 11 hybrids: IIHR-2836, IIHR-2840, IIHR-2841, Arka Shresta, NS 501, Abhinava, IIHR-329, IIHR-331, IIHR-335, IIHR-369, IIHR-371 (Heat tolerant) were obtained from the Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru. The seeds were surface sterilized by treating with 0.1% bavistin for two min and washed with distilled water for 4-5 times and were soaked overnight and were germinated in Petriplates using germination paper at 30°C with 60% relative humidity (RH). The 2-day old uniform size seedlings (approximately 0.5 cm of shoot length) were used for the study<sup>24</sup>. The seedlings were subjected to temperature treatments under controlled conditions using a programmable growth chamber. The TIR technique involves standardization of challenging temperature and induction temperature. Subsequently, the protocol as specified earlier was employed for screening large number of germplasm for their intrinsic thermotolerance<sup>19</sup>. Twenty five (2-day old) uniform size seedlings were placed from the Petriplate to an aluminium tray on moist germination paper and three trays per genotype were used as replicates.

### Standardization of challenging temperature

In the present study, standardization of challenging and induction temperatures was done using two tomato lines, a hybrid, IIHR-2841 and a germplasm line, IIHR-2325. For standardization of challenging temperature, 2-days old tomato seedlings were directly subjected to different temperatures with specific time periods, such as (i-iii) 49°C for 1, 2 and 3 h, respectively; (vi-vi) 50°C for 1, 2 and 3 h; and

(vii-ix) 52°C for 1, 2 and 3 h, respectively. Subsequently after the exposure, the seedlings were allowed to recover in an incubator at 30°C with 60% RH for 72 h (Fig. 1A). At the end of the recovery period, the number of seedlings that survived was recorded and the percent seedling survival was calculated. To arrive at the challenging temperature, the temperature and the time period combination in which 10-20% seedlings survived was identified as the best challenging temperature.

#### Standardization of induction temperature

In order to determine the optimum induction temperature, seedlings were subjected to two gradual induction temperature treatments of (i) 33-43°C over 3 h and consequently to standardized challenging temperature and (ii) 33-45°C over 3 h and consequently to standardized challenging temperature. The same set of seedlings was allowed to recover in an incubator at 30°C with 60% RH for 72 h (Fig. 1B). At the end of recovery period, percent seedling survival, shoot and root length were recorded for assessing the seedling growth to identify the optimum induction temperature. The seedlings which were maintained at 30°C during the experimental period served as control.

Subsequently, the 52 tomato genotypes were evaluated employing the standardized induction and challenging temperatures. The experiment contained two sets; one set of seedlings exposed to induction and challenging temperatures and another set of

seedlings maintained at 30°C as control. The percent survival was calculated using the formula.

$$\text{Percent seedling survival} = \frac{\text{Number of seedlings survived}}{\text{Total number of seedlings}} \times 100$$

Further, total shoot and root length (cm) of surviving seedlings was recorded and percent reduction in recovery growth (PRIRG) compared to control seedlings was computed using the formula.

$$\text{Percent Reduction in recovery growth} = \frac{(\text{GDR of control seedlings} - \text{GDR of induced seedling})}{\text{GDR of control seedlings}} \times 100$$

where GDR = Growth During Recovery

Seedling vigor index was calculated according to Afrakhteh *et al.*<sup>26</sup> using the formula

$$\text{Seedling vigor index} = \text{Percent germination} \times \text{Seedling length}$$

#### Genetic variability for thermotolerance

The screened genotypes were classified into three different categories, tolerant, moderately tolerant, and susceptible using Normal Z-distribution based on percent seedling survival and percent reduction in recovery growth over control (PRIRG).

#### Statistical analysis

Three biological replicates were taken for all the treatments. The data were analyzed statistically using AGRISTAT software. Significance between control and treatment was compared at  $P > 0.05$ .

## Results and Discussion

Plants overcome abiotic stresses through several physiological, biochemical and morphological mechanisms. Various physiological and biochemical parameters are used at seedling stage for genotypic evaluation under high temperature stress<sup>27</sup>. They are endowed with intrinsic ability to endure high temperatures (basal thermotolerance) and also the ability to acquire thermotolerance<sup>17</sup>. Thus, exposing either to sub lethal high temperatures for a short period<sup>28</sup> or to a gradual increase in temperature to lethal levels<sup>29</sup>, could induce acquired thermotolerance in plants. Hence, in the present study, the TIR protocol for evaluation of tomato was standardized.

#### Standardization of challenging temperature

The challenging temperature was determined by directly exposing the 2-day old tomato seedlings to different high temperature and time durations. The results showed that there was decrease in percent survival as the intensity of temperature increased from

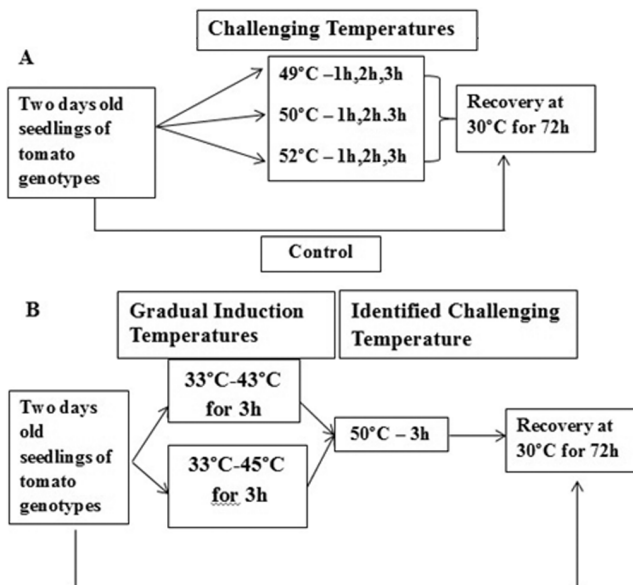


Fig. 1—Steps involved in standardization of challenging and induction temperature

49-52°C (Fig. 2). At 50°C for 3 h, the percent seedling survival was the least and it was 13 and 20% in IIHR-2325 and IIHR-2841, respectively. The temperature treatment which shows least percent seedling survival (10-20%) is considered as the best challenging temperature to assess the thermotolerance<sup>20</sup>. Hence in the present study, the challenging temperature of 50°C for 3 h was considered as the desired challenging temperature. Studies show that the challenging temperatures for individual crops differ, 48°C for 1 h for pea seedlings<sup>16</sup>, 55°C for 2 h for rice seedlings<sup>30</sup>, 55°C for 3 h for groundnut seedlings<sup>7</sup>, 47°C for 3 h for cotton seedlings<sup>20</sup>, 49°C for 2 h for sunflower<sup>17</sup>, 50°C for 30 min for peanut seedlings<sup>31</sup> and 48°C for 2 h for tomato seedlings<sup>25</sup>.

**Standardization of induction temperature**

The optimum induction temperature was standardized in this experiment as 33-43°C for 3 h followed by 50°C for 3 h as challenging temperature based on percent seedling survival and PRIRG over control. Genotype IIHR-2325 showed 47.5 and 86% survival and PRIRG, respectively. Whereas, the genotype IIHR-2841 showed 30 and 67.2% survival and PRIRG, respectively (Fig. 3). Even though the percent survival of seedlings was less, the recovery growth was significantly high in both the genotypes. Hence, the treatment, 33-43°C for 3 h followed by 50°C for 3 h was considered as the best induction treatment for further screening of tomato genotypes. According to the earlier studies on various crops, different induction temperatures were identified. In sunflower, 28-42°C for 2.5 h<sup>12</sup>, 35-45°C for 4 h in groundnut<sup>7</sup>, 38-54°C for 5 h in ragi<sup>32</sup>, and 38-46°C over 3 h in tomato<sup>26</sup>. Thus, the results of our study

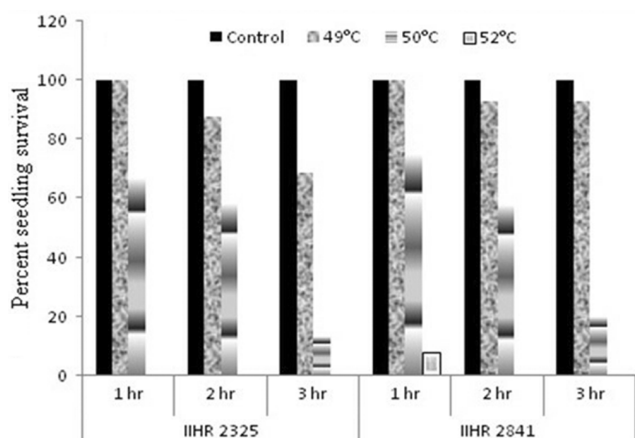


Fig. 2—Standardization of different challenging temperatures and durations on percent seedling survival of tomato

in tomato genotypes also suggest that high temperature tolerance can be increased by exposing the seedlings to gradual increasing temperature i.e., induction treatment before subjecting to challenging temperature. According to Shi *et al.*<sup>33</sup> pre exposure to sublethal treatment followed by harsh lethal treatment is known to improve tolerance to different abiotic stresses at the vegetative stage within and across generations in rice. Under natural conditions, plants experience a gradual increase in stress over a period of time. This gradual increase in temperature results in plants experiencing mild stress before severe stress. Exposure to sublethal temperature followed by severe temperature enhances capability of plants to withstand high temperature stress<sup>14-18</sup>. Acquired tolerance is ubiquitous in plants in response to various types of stresses and has been demonstrated in several species<sup>34-37</sup>. The seedlings which were pre-exposed to the optimum induction temperature exhibited better recovery growth<sup>19</sup>. The induction stress required for optimum expression of stress-response genes also varies among species. Hence, optimizing the induction and challenging temperature treatments helps in identifying genotypes having intrinsic thermotolerance. The standardized TIR protocol employed for further screening of 52 tomato genotypes is shown in Fig. 4.

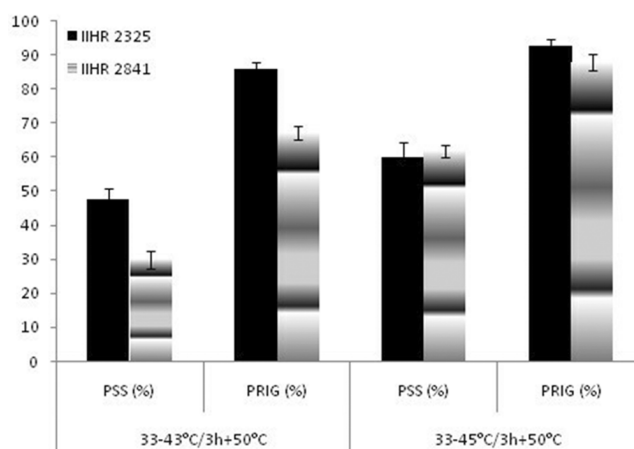


Fig. 3—Standardization of optimum induction temperature based on percent survival of seedlings (PSS) and percent reduction in recovery growth (PRIG)

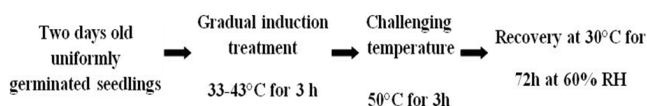


Fig. 4—Temperature Induction Response (TIR) protocol followed for evaluation of 52 tomato genotypes

### Tomato genotypes response to temperature induction treatment

#### *Percent seedling survival*

Adopting the standardized induction and challenging temperature, 52 tomato genotypes were evaluated for high temperature tolerance (Fig. 4). In the present study the percent seedling survival ranged 2.6-90% compared to control (Table 1). Among the wild type genotypes the maximum percent survival was found in IIHR-2101 (66%). Among germplasm lines the maximum percent survival was found in IIHR-2202 (90%), IIHR-2913 (88.33), IIHR-2339 (85%), IIHR-2852 (83.3%) followed by IIHR-2745 (81.67%). The least percent seedling survival was observed in IIHR-2328 (2.6%). The response of tomato varieties also varied significantly with Arka Alok showing the maximum percent survival (83.3%) followed by Arka Ashish (73.3%) and Arka Vikas (69.0%). The least percent survival was recorded in IIHR-2620 (14.67%) and Arka Abha (13.33%). Among 11 hybrids the maximum percent survival was noticed in IIHR-2836 (90%), IIHR-2840 (88.3%) and H-329 (84.5%) and least in NS 501 (45%). Overall, the results revealed that the percent seedling survival of tomato genotypes differed significantly when subjected to induction treatments. Based on percent seedling survival genotypes could be grouped as tolerant and susceptible<sup>16,19,32</sup>. Tomato seedlings exposed to induction temperature and then to severe temperature showed higher survival as compared to seedlings that were directly exposed. The differential adaptive mechanisms among the genotypes could be the reason for differences in thermotolerance<sup>25</sup>.

#### *Shoot and Root length of seedling*

The exposure to induction followed by challenging temperatures significantly affected the seedling growth. The seedlings grown at 30°C (control) had seedling length in the range of 5.65-18.30 cm. Whereas, the seedlings exposed to temperature treatments had seedling length ranging from 0.87-5.46 cm (Table 1). Among wild type tomato genotypes, the maximum seedling length was recorded in IIHR-2101 (1.10). The seedling length varied among the germplasm lines, IIHR-2202 (5.46), IIHR-2343 (4.75), IIHR-2190 (4.67), IIHR-2339 (4.18) and least in IIHR-2328 (0.87). Among the varieties the maximum seedling length was found in Arka Alok (4.62), Arka Ashish (3.76), Arka Saurabh (3.61) and least in Arka Vikas (1.0). Among the hybrids, H-329 (5.2), Abhinava (5.1), H-369 (4.8), H-371 (4.5), H-331 (4.4) showed better seedling growth and the least in NS 501

(1.29). The variability in seedling growth among the genotypes have been reported in ground nut and in pulses<sup>38</sup>.

#### *Percent reduction in recovery growth (PRIRG)*

In the present study the effect of induction temperature on recovery growth among the 52 tomato genotypes showed variable results. The reduction in recovery growth significantly varied from 36.63-91.19% (Table 1). Among the wild type genotypes IIHR-1940 (81.32) showed better reduction in recovery growth. The tomato germplasm lines, IIHR-2745 (40.29), IIHR-2190 (48.15) and IIHR-2202 (48.51) recorded least reduction compared to IIHR-2831 (91.19) which showed maximum PRIRG. Among the varieties, Arka Ashish (53.63) and Arka Alok (61.30) showed better recovery growth compared to IIHR-2627 (88.27). Whereas, among hybrids, H-329 (36.63), H-371 (38.43), H-335 (43.84), H-369 (46.70) followed by Abhinava (49.22) had lower PRIRG and NS 501 (84.67) showed poor recovery growth when exposed to induction treatment. The higher recovery growth of induced seedlings is mainly because of several physiological and biochemical alterations that are involved in response to acclimation as seen in tomato<sup>25</sup>, sunflower<sup>19</sup>, pearl millet<sup>39</sup>, beans<sup>40</sup>, wheat<sup>34</sup>, groundnut<sup>16</sup>, and Rice<sup>41</sup>. Hence, screening method based on recovery growth and percent seedling survival is most appropriate as these parameters signify the greater relevance of the mechanisms involved in acquired thermotolerance upon acclimation which is an indication of tolerance<sup>17</sup>.

#### *Seedling vigour index (SVI)*

The maximum SVI was found in IIHR-2101 under wild type tomato genotypes and among germplasm lines, IIHR-2202, IIHR-2190, IIHR-2339, and IIHR-2852 had maximum seedling vigour. Among the varieties Arka Alok, Arka Ashish and Arka Saurabh had maximum SVI and IIHR-2620 recorded minimum SVI. The hybrids, H-329, Abhinava, H-369, and IIHR-2836 recorded maximum SVI compared to NS 501 which showed least vigour (Table 1). In the present study among the 52 tomato genotypes the response of induction treatment on all growth parameters showed variable results. As a support to this hypothesis, positive correlation was observed between recovery growth in terms of total seedling length and percent seedling survival in the seedling exposed to induction treatments (Fig. 5A). Positive relationship was also observed between seedling vigor index and percent seedling survival of induced

Table 1—Effect of temperature induction on percent seedling survival, shoot and root length (cm), percent reduction in recovery growth (PRIRG), and seedling vigour index on different tomato genotypes

S. No	Genotypes	Percent seed survival		Shoot and Root length (cm) of seedlings		PRIRG	Seedling vigour index	
		I+C	Control	I+C	Control		I+C	Control
1	IIHR-1940	41.33	100	1.06	5.65	81.32	44.00	565.0
2	IIHR-2101	66.67	100	1.10	6.05	81.82	74.17	605.0
3	IIHR-2325	29.33	100	1.15	8.20	86.01	33.29	820.0
4	IIHR-2326	25.33	100	1.14	8.05	85.87	31.20	805.0
5	IIHR-2327	65.33	100	1.12	10.05	88.83	76.40	1005.0
6	IIHR-2328	2.67	100	0.87	7.50	88.67	2.40	750.0
7	IIHR-2329	28.00	100	1.09	7.40	85.24	30.40	740.0
8	IIHR-2330	32.00	100	1.29	6.85	81.12	62.00	685.0
9	IIHR-2336	61.33	100	1.43	9.20	84.42	90.93	920.0
10	IIHR-2337	73.33	100	3.61	8.80	59.03	255.83	880.0
11	IIHR-2339	85.00	100	4.18	11.40	63.34	358.94	1140.0
12	IIHR-2340	63.33	100	3.26	12.30	73.50	301.25	1230.0
13	IIHR-2343	30.00	100	4.75	12.90	66.62	187.50	1290.0
14	IIHR-2346	63.33	100	2.60	13.10	80.14	167.50	1310.0
15	IIHR-2348	70.00	100	3.25	18.30	82.25	225.53	1830.0
16	IIHR-2351	74.90	100	2.76	10.70	74.23	209.02	1070.0
17	IIHR-2353	78.00	100	1.55	8.90	82.58	121.17	890.0
18	IIHR-2359	72.00	100	2.41	8.00	69.86	192.00	800.0
19	IIHR-2370	49.33	100	1.10	7.70	85.71	55.33	770.0
20	IIHR-2382	74.67	100	1.42	11.00	87.12	108.67	1100.0
21	IIHR-2391	41.33	100	1.08	9.20	88.22	42.00	920.0
22	IIHR-2190	78.33	100	4.67	9.00	48.15	371.67	900.0
23	IIHR-2202	90.00	100	5.46	10.60	48.51	481.67	1060.0
24	IIHR-2294	74.33	100	3.54	11.30	68.69	259.96	1130.0
25	IIHR-2745	81.67	100	3.83	6.42	40.29	322.50	642.0
26	IIHR-2786	66.67	100	1.02	8.30	87.68	68.50	830.0
27	IIHR-2831	60.00	100	1.00	11.35	91.19	60.00	1135.0
28	IIHR-2843	58.33	100	1.08	11.05	90.24	63.67	1105.0
29	IIHR-2852	83.33	100	4.07	10.55	61.45	341.85	1055.0
30	IIHR-2853	75.00	100	3.33	11.05	69.83	252.00	1105.0
31	IIHR-2913	88.33	100	3.63	9.70	62.54	323.83	970.0
32	IIHR-2914	26.67	100	1.00	8.65	88.44	40.00	865.0
33	IIHR-2620	14.67	100	1.43	6.25	77.07	20.07	625.0
34	IIHR-2627	26.67	100	1.28	10.90	88.27	35.01	1090.0
35	Arka Vikas	69.00	100	1.07	8.50	87.37	73.99	850.0
36	Arka Ahuti	46.67	100	1.20	6.10	80.35	56.75	610.0
37	Arka Ashish	73.33	100	3.76	8.10	53.63	263.33	810.0
38	Arka Saurabh	46.67	100	3.61	14.10	74.43	169.92	1410.0
39	Arka Meghali	60.00	100	1.43	8.55	83.24	126.28	855.0
40	Arka Alok	83.33	100	4.62	11.95	61.30	393.97	1195.0
41	Arka Abha	13.33	100	1.71	11.95	85.69	37.00	1195.0
42	IIHR-2836	90.00	100	4.18	8.80	52.50	376.17	880.0
43	IIHR-2840	88.33	100	4.13	10.65	61.21	356.83	1065.0
44	IIHR-2841	90.00	100	4.18	12.75	67.22	376.17	1275.0
45	Arka Shresta	74.67	100	2.73	10.20	73.27	199.90	1020.0
46	NS 501	45.00	100	1.29	8.40	84.67	61.06	840.0
47	Abhinava	83.33	100	5.10	10.05	49.22	428.15	1005.0
48	IIHR-329	84.55	100	5.20	8.20	36.63	439.02	820.0
49	IIHR-331	75.00	100	4.37	8.60	49.22	325.00	860.0
50	IIHR-335	76.67	100	4.10	7.30	43.84	311.33	730.0
51	IIHR-369	83.33	100	4.80	9.00	46.70	414.41	900.0
52	IIHR-371	78.33	100	4.49	7.30	38.43	353.12	730.0
	CV		18.48		7.03		8.93	9.22
	LSD @ 0.05		3.34		0.09		10.36	11.61

[I+C = Induction + Challenging]

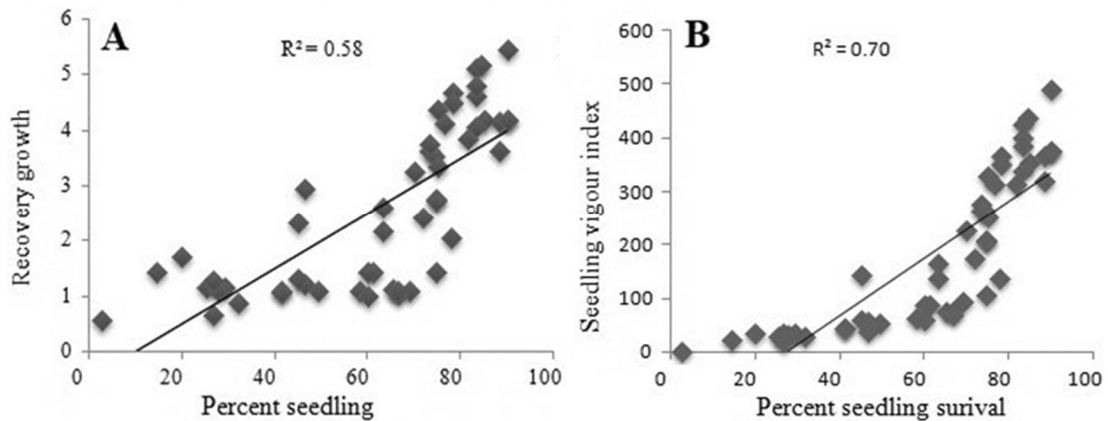


Fig. 5—Relationship between recovery growth and percent seedling survival, seedling vigour index and percent seedling survival in 52 tomato genotypes subjected to induction and challenging temperatures

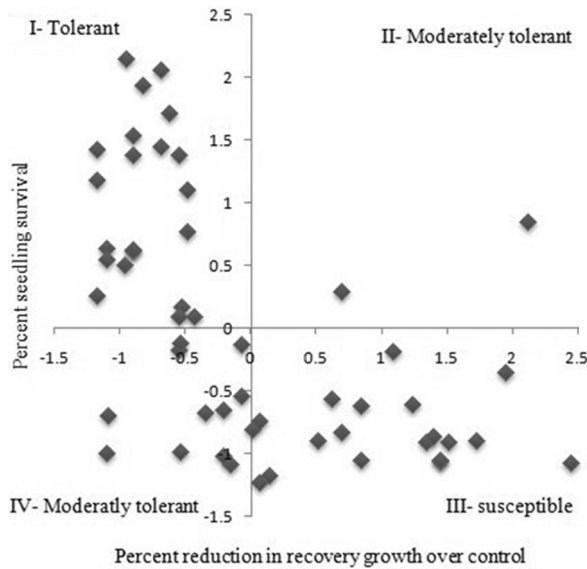


Fig. 6—Genetic variability for high temperature tolerance among 52 tomato genotypes assessed by plotting the Z distribution based on percent seedling survival and percent reduction in recovery growth

seedlings (Fig. 5B) among the 52 tomato genotypes. Thus, indicating that the growth performance after recovery period plays an important role in screening tomato genotypes for thermo tolerance.

**Classification of tomato genotypes**

The genetic variability of 52 tomato genotypes to induction temperature treatment was assessed by normal Z-distribution based on PRIRG and percent seedling survival after recovery period (Fig. 6). The distribution analysis can be employed to cluster the genotypes into different groups like tolerant, moderately tolerant and susceptible (Table 2). The results revealed that among 52 genotypes, 21 genotypes located in Q I were classified as highly

Table 2—Classification of 52 tomato genotypes

Tolerant	Moderately tolerant	Susceptible
IIHR-2337	IIHR-2786	Arka Saurabh
IIHR-2840	IIHR-2382	Arka Ahuti
IIHR-2852	Arks Vikas	IIHR-2620
IIHR-2913	Arka Shresta	IIHR-2330
IIHR-2339	IIHR-2327	IIHR-1940
IIHR-2841	IIHR-2101	IIHR-2391
IIHR-2202	IIHR-2340	IIHR-2370
IIHR-331	IIHR-2343	IIHR-2329
Abhinava	IIHR-2346	IIHR-2326
IIHR-2836	IIHR-2348	IIHR-2325
Arka Ashish	IIHR-2351	IIHR-2328
IIHR-329	IIHR-2353	IIHR-2627
IIHR-371		IIHR-2914
IIHR-2745		NS 501
IIHR-335		Arka Abha
IIHR-369		Arka Meghali
IIHR-2190		IIHR-2336
IIHR-2294		IIHR-2843
IIHR-2359		IIHR-2831
IIHR-2853		
Arka Alok		

tolerant which included 10 germplasm lines, 2 varieties, 9 hybrids. Whereas, QIII consisting of 19 genotypes considered as susceptible included one wild type, 11 germplasm lines, 6 varieties and one hybrid. The remaining 12 genotypes were classified as moderately tolerant as they were located in QII and QIV which included one wild type, 9 germplasm lines, one variety and one hybrid. According to SenthilKumar *et al.*<sup>17</sup> such an analysis provides a reliable method for identifying highly tolerant genotypes with high growth rates during stress and recovery. Based on this analysis, in the present study it has also been possible to select genotypes, H-329 and IIHR-2202 as tolerant and genotypes, IIHR-2620



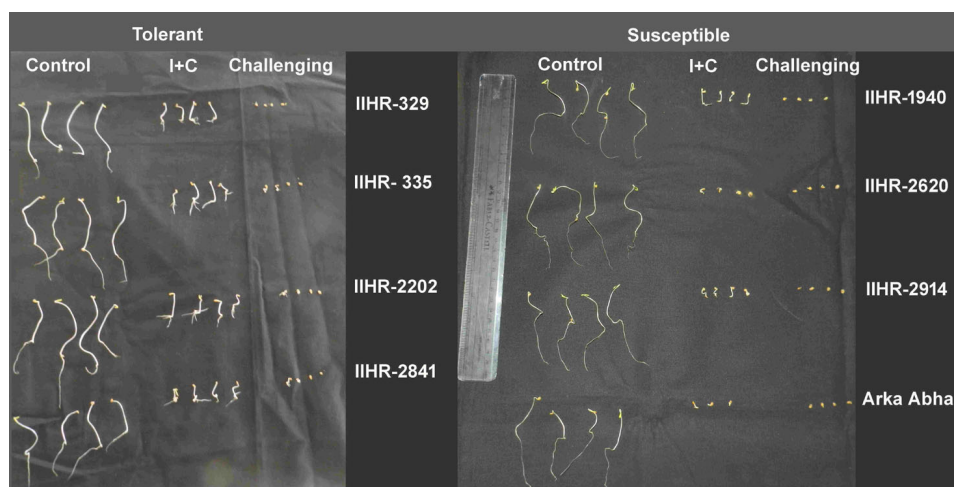


Fig. 7—Response of some of the tolerant and susceptible genotypes of tomato to high temperature stress. [Two days old seedlings were exposed to induction temperature treatment followed by challenging temperature of 33–43°C/3 h+50°C/3 h (I+C) and direct challenging temperature of 50°C/3 h (Challenging). Photograph was taken at the end of recovery period]

as most susceptible. Amongst the tolerant genotypes, H-329 showed substantially high recovery growth and high percent survival. Srikanthbabu *et al.*<sup>16</sup> suggested that while screening a large population, it may be appropriate to screen initially at low stringency temperature stress to choose the tolerant ones. Further, screening of these genotypes at high stringency temperature stress, however, would facilitate in identifying genotypes with higher threshold levels of temperature. Similar classification of tolerant, moderately tolerant and susceptible genotypes was followed in cotton<sup>20</sup>, peas<sup>16</sup>, and rice<sup>41</sup>.

Breeding for heat tolerance is often complicated by the lack of an efficient and easily adaptable screening technique and inadequate information on the availability of genetic variability. Though subjecting tomato seedlings to challenging temperatures caused drastic reduction in seedling survival, the exposure to gradual induction temperature enhanced seedling survival through activation of inherent cellular tolerance mechanisms. This study clearly suggests that the TIR technique is a rapid screening protocol for identifying genetic variability for high temperature stress tolerance in large population of tomato genotypes within a short time period and genotypes selected as tolerant based on TIR at seedling level also showed tolerance at plant level in many crops suggesting that the mechanisms were similar at both heterotrophic and autotrophic phase of the tolerant plant. However there is need to correlate the present results with the whole plant level in future experiments. Hence, this technique would be very

helpful in identifying and characterizing thermo tolerant genotypes for further advancement of the lines developed by different breeding techniques.

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#### Conflict of Interest

The authors declare that they have no conflict of interest.

#### References

- 1 Monthly Report Tomato, May 2019, (Horticulture Statistics Division, Department of Agriculture, cooperation & Farmers Welfare, Ministry of Agriculture & Farmers Welfare, Govt. of India, New Delhi), 2019.
- 2 Ayyogari K, Sidhya P & Pandit MK, Impact of Climate Change on Vegetable Cultivation - A Review. *Int J Agric Environ Biotechnol*, 7 (2014) 145.
- 3 Camejo D, Rodríguez P, Moralez A, Dell Amico JM, Torrecillas A & Alarcon JJ, High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J Plant Physiol*, 162 (2005) 281.
- 4 Maria FM, Giuseppina C, Paolo I, Immacolata M, Stefania G & Rosa AS, Response mechanisms induced by exposure to high temperature in anthers from thermotolerant and thermo-sensitive tomato plants: A proteomic perspective. *PLoS ONE*, 13 (2018) 7. doi.org/10.1371/journal.pone.0201027
- 5 Damodar P, Eva R & Carl-Otto O, Phenotyping from lab to field – tomato lines screened for heat stress using Fv/Fm maintain high fruit yield during thermal stress in the field. *Funct Plant Biol*, 1(2018). doi.org/10.1071/FP17317
- 6 Laxman RH, Sunoj VSJ, Biradar G, Pavithra CB, Dhanyalakshmi KH, Manasa KM, Sadashiva AT &



- Bhatt RM, Growth, reproductive development and yield of tomato (*Solanum lycopersicum* L.) genotypes under mild temperature elevation. *Asian J Bot*, 1 (2018). doi:10.24294/ajb.v1i2.827
- 7 Gangappa E, Ravi K & Veera Kumar GN, Evaluation of groundnut genotypes for temperature tolerance based on temperature induction response (TIR) technique. *Indian J Genet*, 66 (2006) 127.
  - 8 Gnanaraj M, Udayakumar N, Gandhi Rajiv R & Manoharan K, Isolation of gene expression analysis of phospholipase C in response to abiotic stresses from *Vigna Radiata* (L.) Wilczek. *Indian J Exp Biol*, 53 (2015) 335.
  - 9 Lakra N, Tomar PC & Mishra SN, Growth response modulation by putrescine in Indian mustard *Brassica juncea* L. under multiple stress. *Indian J Exp Biol*, 54 (2016) 262.
  - 10 Sharma SB, Sayyed RZ, Sonawane M, Trivedi MH & Thivakaran GA, Neurospora Sp. SR8, a novel phosphate solubiliser from Rhizosphere soil of Sorghum in Kachchh, Gujrat, India. *Indian J Exp Biol*, 54 (2016) 644.
  - 11 Krishnamurthy SL, Sharma PC, Sharma SK, Batra V, Kumar V & Rao LVS, Effect of salinity and use of stress indices of morphological and physiological traits at the seedling stage in rice. *Indian J Exp Biol*, 54 (2016) 843.
  - 12 Charu Lata, Ashwini K, Sharma SK, Jogendra S, Shital S, Pooja, anitha M & Babita R, Tolerance to combined boron and salt stress in wheat varieties; Biochemical and molecular analysis. *Indian J Exp Biol*, 55 (2017) 321.
  - 13 Driedonks N, Rieu I & Vriezen WH, Breeding for plant heat tolerance at vegetative and reproductive stages. *Plant Reprod*, 29 (2016) 67.
  - 14 Ganeshkumar, Krishnaprasad BT, Savitha M, Gopalakrishna R, Kakoli Mukhopadhyay, Ramamohan G & Udayakumar M, Enhanced expression of heat shock proteins in thermotolerant lines of sunflower and their progenies selected on the basis of temperature induction response *Theor Appl Genet*, 99 (1999) 359.
  - 15 Senthil-Kumar M, Udayakumar M & Mohan Raju B, *Development of induction response protocols in tomato and capsicum for improving temperature tolerance using temperature induction response (TIR) technique*. (National seminar on role of plant physiology for sustaining quality and quantity of food production in relation to environment, UAS, Dharwad, India, 5-7 December, 2001).
  - 16 Srikanthbabu V, Ganeshkumar, Krishnaprasad BT, Gopalakrishna R, Savitha M & Udayakumar M, Identification of pea genotypes with enhanced thermotolerance using temperature induction response technique (TIR). *J Plant Physiol*, 159 (2002) 535.
  - 17 Senthil-Kumar M, Srikanthbabu V, Mohan Raju B, Ganeshkumar, Shivaprakash N & Udayakumar M, Screening of inbred lines to develop thermotolerant sunflower hybrid through temperature induction response (TIR) technique: A novel approach by exploiting residual variability. *J Exp Bot*, 54 (2003a) 2569.
  - 18 Senthil-Kumar M, Mohan Raju B, Channakrishnaiah KM & Udayakumar M, *Development of thermotolerant sunflower hybrid: a novel approach based on TIR technique*. Paper presented in National seminar on stress management in oilseeds for attaining self reliance in vegetable oils, Directorate of Oil Seed Research, Hyderabad, India, 28–30 January, 2003b.
  - 19 Kumar G, Krishnaprasad BT, Savitha M, Gopalakrishna R, Mukhopadhyay K, Ramamohan G & Udaya Kumar M, Enhanced expression of heat shock proteins in thermotolerant lines of sunflower and their progenies selected on the basis of temperature induction response (TIR). *Theor Appl Genet*, 99 (1999) 359.
  - 20 Kheir EA, Sheshshayee MS, Prasad TG & Udayakumar M, Temperature induction response as a screening technique for selecting high temperature-tolerant cotton lines. *J Cotton Sci*, 16 (2012) 190.
  - 21 Prajapati S, Tiwari A, Kadwey S & Jamkar T, Genetic Variability, Heritability and Genetic Advance In Tomato (*Solanum lycopersicon* Mill.). *Int J Agric Environ Biotechnol*, 8 (2015) 245.
  - 22 Rivero RM, Ruiz JM, Gario PC, Lopez, Lefebvre LR, Sanchez E & Romero L, Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *Plant Sci*, 160 (2001) 315.
  - 23 Laxman RH, Srinivasa Rao NK, Bhatt RM, Sadashiva AT, John Sunoj VS, Geeta Biradar, Pavithra CB, Manasa KM & Dhanyalakshmi KH, Response of tomato (*Lycopersicon esculentum* Mill.) genotypes to elevated temperature. *J Agrometeorol*, 15 (2013) 38.
  - 24 Senthil-Kumar M & Udayakumar M, Development of thermotolerant tomato (*Lycopersicon esculentum* Mill.) lines: an approach based on mutagenesis. *J. Plant Biol*, 31 (2004) 139.
  - 25 Ankita Chandola, Vijai Pandurangam & Jai Prakash Srivastava, Genotypic Variations in Tomato (*Lycopersicon esculentum* Mill.) for Acquired Thermotolerance to Temperature Induction Response. *Int J Agric Environ Biotechnol*, 9 (2016) 649.
  - 26 Afrakhteh S, Frahmndfar E, Hamidi A & Ramandi HD, Evaluation of Growth Characteristics and Seedling Vigor in Two Cultivars of Soybean dried under different Temperature and Fluidized bed dryer. *Intl J Agri Crop Sci*, 5 (2013) 2537.
  - 27 Gosavi GU, Jadhav AS, Kale AA, Gadakh SR, Pawar BD & Chimote VP, Effect of heat stress on proline, chlorophyll content, heat shock proteins and antioxidant enzyme activities in sorghum (*Sorghum bicolor*) at seedling stage. *Indian J of Biotechnol*, 13 (2014) 356.
  - 28 Sung DY, Kaplan F, Lee KJ & Guy CL, Acquired tolerance to temperature extremes. *Trends Plant Sci*, 8 (2003) 179.
  - 29 Larkindale J & Vierling E, Core genome responses involved in acclimation to high temperature. *Plant Physiol*, 146 (2008) 748.
  - 30 Sudhakar P, Latha P, Ramesh babu P, Sujatha K & Raja Reddy K, Identification of thermotolerant rice genotypes at seedling stage using TIR technique in pursuit of global warming. *Indian J Plant Physiol*, 27 (2012) 185.
  - 31 Selvaraj MG, Burow G, Burke JJ, Belamkar V, Puppala N & Burow MD Heat stress screening of peanut (*Arachis hypogaea* L.) seedlings for acquired thermotolerance. *Plant Growth Regul*, 65 (2011) 83.
  - 32 VenkateshBanu D, Sudhakar P & Reddy YSK, Screening of thermotolerant ragi genotypes at seedling stage using TIR technique. *The Bioscan*, 8 (2013) 1493.
  - 33 Shi W, Lawas LMF, Raju BR & Jagadish SVK, Acquired Thermo-Tolerance and Trans-Generational Heat Stress

- Response at Flowering in Rice. *J Agron Crop Sci*, (2015). DOI: 10.1111/jac.12157.
- 34 Burke JJ, Integration of acquired thermotolerance within the developmental program of seed reserve mobilization. (Ed. JH Cherry; Biochemical and Cellular Mechanisms of Stress Tolerance in Plants. Springer, Berlin), 1994, 191.
- 35 Larkindale J, Hall JD, Knight MR & Vierling E, Heat stress phenotypes of *Arabidopsis* mutants implicate multiple signaling pathways in the acquisition of thermotolerance. *Plant Physiol*, 138 (2005) 882.
- 36 Senthil-Kumar 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 (2006) 111.
- 37 Vierling E, The roles of heat shock proteins in plants. *Ann Rev Plant Physiol Plant Mol Bio*, 42 (1991) 579.
- 38 Partheeban C, Vijayaraghavan H, Sowmyapriya S, Srividhya S & Vijayalakshmi D, Temperature induction response and accumulation of starch granules as indices to identify the thermotolerance of pulses at early growth stages. *Legume Res*, 40 (2017) 655.
- 39 Howarth CJ, Pollock CJ & Peacock JM, Development of laboratory-based methods for assessing seedling thermotolerance in pearl millet. *New Phytol*, 137 (1997) 129.
- 40 Keeler SJ, Boettger CM, Haynes JG, Kuches KA, Johnson MM & Thureen DL, Acquired thermotolerance and expression of the HSP100/ClpB genes of lima bean. *Plant Physiol*, 123 (2000) 1121.
- 41 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 (2015) 368.