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TIR approach and stress tolerance indices to identify donor for high-temperature stress tolerance in pepper (*Capsicum annuum* L.)

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Abstract

Bell pepper or sweet pepper (*Capsicum annuum* L. var. *grossum*) is highly susceptible to high-temperature stress (HT). Hence, search for donor across *C. annuum* for HT tolerance was undertaken by following Temperature Induction Response (TIR) technique. The induction and challenging temperature requirement for TIR screening were standardized in 1 d-old *Capsicum* seedlings. Forty *Capsicum* genotypes were evaluated based on the recovery growth (RG) and per cent reduction in recovery growth (%RRG) of the seedlings. The genotypes Punjab Guchhedar and Ajeet 1 were found to have maximum cellular level tolerance (CLT) to HT with higher RG and lower %RRG compared to the non-induced seedlings and HDC 75 was found to have minimum CLT. In order to confirm the findings, another experiment was conducted under managed stress and control conditions. Absolute yield obtained from both the environments were used to calculate stress tolerance indices such as heat susceptibility index, tolerance index, stress tolerance index, mean productivity, geometric mean productivity and yield stability index. Based on these tolerance indices, Punjab Guchhedar and Ajeet 1 were found to be highly tolerant and HDC 75 as highly susceptible. Further, the combined result of TIR and tolerance indices also gave the same result confirming Punjab Guchhedar and Ajeet 1 can be used as a donor for the future breeding programme aimed at evolving high-temperature-tolerant bell pepper cultivars. The result also confirms the fitness of TIR technique to screen *Capsicum* genotypes for tolerance to HT based on variability in acquired thermotolerance.

Keywords: *Capsicum annuum*, high-temperature stress, pepper, Temperature Induction Response, tolerance indices

Introduction

In India, the total area under pepper is 8041 ha with a production of 67,892 MT (FAO, 2017), in which, bell pepper has a negligible share as it is affected by both the temperature extremes during plant development. In tropical countries like India, high-temperature stress (HT) is a common threat for bell pepper. Hence, commercial growing is seasonal in the tropics and production is concentrated in the cool and dry period. It requires a day and night temperature of 21–25/18°C for optimum growth and production. Above 32°C, it starts showing a reduction in crop growth and yield because of reduced fruit set owing to the abscission of reproductive structures (Erickson and Markhart, 2001). In the present scenario of global climate change, the frequency of high-temperature episodes is likely to increase. Hence, to sustain the production and productivity of bell pepper under such situations, concerted efforts with suitable adaptation strategies are very much required. One such approach calls for rapid identification of donors for HT tolerance in *Capsicum* genus to develop HT-tolerant bell pepper cultivars. Conventionally, it is done by growing the crop in challenging environments and selecting the genotypes giving stable optimum yield in terms of different tolerance indices (Ehlers and Hall, 1998). But, the procedure is time consuming, labour and cost intensive. Therefore, there is a need for a high throughput technique

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to screen the materials in a very early stage, in lesser time with reproducibility and the result of which can be linked to tolerance in the reproductive stage. Many workers have reported about the Temperature Induction Response (TIR) technique based on the principle of acquired cellular level tolerance (CLT) to high temperature in crops such as sunflower, tomato, groundnut and banana (Kumar *et al*., 1999; Senthil-Kumar and Udayakumar, 2004; Gangappa *et al*., 2006; Vidya *et al*., 2017). It proposes that plants can survive and acquire tolerance to lethal stress if they are pre-treated with sublethal stress. Numerous stress-responsive genes get expressed upon exposure to sublethal stress for maintaining metabolic homeostasis during stress or to be able to acclimatize and recover after the stress period (Yan *et al*., 2016; Vidya *et al*., 2017). Further, genetic variability for thermotolerance is seen only upon pre-treatment before lethal stress (Krishnan *et al*., 1989; Uma *et al*., 1995; Jayaprakash *et al*., 1998; Kumar *et al*., 1999; Burke, 2001; Srikanthbabu *et al*., 2002). This sublethal stress range (induction temperature) and the lethal (challenging) temperature is crop specific. Senthil-Kumar and Udayakumar (2004) reported in tomato, the optimum induction temperature at (33°C 1 h + 36°C 1 h + 39°C 1 h + 42°C 1 h), as the seedlings subjected to this treatment maintained 38.5% survival upon challenging to 49°C for 4 h. Hence, this study was taken up (i) to standardize the TIR technique for *Capsicum*, (ii) to confirm the result of TIR study by estimating various tolerance indices and (iii) to identify genotype for HT tolerance in *Capsicum* (bell pepper and chilli).

Materials and method

TIR optimization in Capsicum

This study was undertaken at ICAR – Indian Institute of Horticultural Research, Bengaluru, India (Latitude: 13°7′N and Longitude: 77°29′E; altitude 890 MSL) during 2017–18. Forty diverse genotypes of *Capsicum annuum* (including both hot pepper and sweet pepper) available in IIHR, Bangalore were used for the study. Seeds were allowed to germinate in petri plates lined with moist filter paper under room condition. One-day-old uniformly germinated seedlings were kept in aluminium trays lined with germination paper in a plant growth chamber where the temperature and relative humidity were maintained. For standardization of challenging and induction temperatures, 1-d-old uniform seedlings of Arka Mohini, a susceptible bell pepper cultivar was used. The challenging temperature was standardized by exposing the seedlings to very high temperature at 44, 46, 48, 50 and 52°C temperature for 1 h, 1 h 30 min, 2 h and 3 h duration. After recovery at room temperature at 60% RH for 72 h, the temperature and duration resulting in around 10% seedling survival were considered as the challenging temperature (online Supplementary Table S2). Induction temperature was standardized by placing the seedlings in gradually increasing temperature milder than the challenging temperature for a different duration. As bell pepper shows stress symptoms beyond 32°C, the induction temperatures tried were 33–44°C for 4 h, 33–42°C for 4.5 h and 33–42°C for 4 h followed by challenging temperature. After recovery for 72 h, the temperature range giving a minimum 30% SS was considered as the optimal induction temperature (online Supplementary Table S3).

Using the optimized challenging and induction temperature, 40 pepper genotypes (online Supplementary data) were screened in three replications with 12 seedlings per replication. A total of three sets were maintained, (i) non-induced/control, (ii) challenging /lethal (directly exposed to challenging temperature) and (iii) induced (induced before exposure to challenging temperature). After recovery, observations were recorded on survival per cent (%SS) and per cent reduction in recovery growth (%RRG) of the seedlings compared to the non-induced seedlings as followed by Senthil-Kumar and Udayakumar (2004) in tomato, where per cent seedling survival (%SS) = (No. of seedlings survived after recovery/total No. of seedlings) × 100; recovery growth (RG) = (seedling growth after recovery – initial seedling growth); per cent reduction in recovery growth over control (%RRG) = {(RG of non-induced seedling – RG of induced seedling)/RG of induced seedling} × 100. A correlation was established between %RRG and RG to identify genotypes having highest CLT to HT. Since seedling exposed to stress is at heterotrophic conditions, any variation in survival and recovery can be attributed to intrinsic CLT of the genotype.

Managed stress environment study

To confirm the results of TIR study, another experiment was carried out under managed stress environment in October 2017–February 2018 at ICAR – Indian Institute of Horticultural Research, Bengaluru. All the *Capsicum* genotypes were subjected to HT by erecting a low-cost polytunnel-like structure of 6 feet height and covering the same with PVC sheets at the onset of floral bud initiation. The plants were well maintained inside the tunnel by following the recommended package of practices for growing pepper developed by IIHR, Bengaluru. The data on mean, maximum and minimum temperature (°C) and relative humidity (%) were recorded with Temperature/Humidity data logger (THD-172, MEZARIT, India). A temperature of 42/20°C was recorded as average day/night temperatures in the tunnel during the experimental period. The same set of plants were also maintained under the non-stress

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condition with average day/night temperatures of 32/18°C as a control. All forty genotypes used in the TIR study were grown in two replications with 10 plants per replication in both stress and non-stress conditions. After 30 d of stress imposition, polythene structure was removed and plants were allowed to recover for another 7 d. New buds developed during the recovery periods were removed regularly. After the recovery period, observation on yield was recorded in both stress and non-stress conditions. Stability in yield for the genotypes was estimated by various tolerance indices (given below) which were derived from the yield difference between stress and non-stress environments, such as heat susceptibility index (HSI): HSI = (1–Ys/Yc)/D (Fisher and Maurer, 1978); tolerance index (TOL): TOL = Yc–Ys (Rosielle and Hamblin, 1981); stress tolerance index (STI): STI = (Yc × Ys)/($\bar{Y}c^2$) (Fernandez, 1992); geometric mean productivity (GMP): GMP = $\sqrt{(Yc \times Ys)}$ (Fernandez, 1992); mean productivity (MP): MP = (Yc + Ys)/2 (Rosielle and Hamblin, 1981); yield stability index (YSI) = Ys/Yc (Bouslama and Schapaugh, 1984), where Ys = absolute yield of the genotype under stress condition and Yc = absolute yield of the genotypes under non-stress condition; *D* = average yield of all genotypes under stress condition/average yield of all genotypes under non-stress condition; $\bar{Y}c$ is the yield average of all genotypes under non-stress conditions.

Analysis of variance was performed for yield (under stress and non-stress) and the tolerance indices using the GLM procedure of SAS V 9.3 (SAS Institute Inc., 2012). A correlation was established between Yc, Ys and tolerant indices using Pearson's correlation (two-tailed). The means of yield and the tolerance indices were compared using Fisher's LSD test at 5% probability level. To identify the best HT-tolerant genotypes, a normal distribution graph (*z* distribution) was plotted using RRG obtained from TIR study and the TOL showing maximum significant correlation with yield under stress.

Result

TIR study

Among different temperatures employed in susceptible genotype Arka Mohini, SS recorded after exposure to challenging temperatures showed that the temperature of 48°C for 1.5 h gave 11% SS after recovery and was optimized as challenging temperature for *Capsicum* (online Supplementary data). The treatment of 33–42°C for 4 h with 30.55% SS after recovery (online Supplementary data) was considered as the induction temperature.

All the 40 *Capsicum* genotypes were screened by employing optimized induction temperature of 33–42°C for 4 h followed by challenging temperature of 48°C for 1.5 h. The %SS, RG and %RRG are presented in Table 1. The %SS varied from 40.74 to 100%. Among the 40 genotypes screened, 23 showed 100% survival. RG varied from 0.2 to 1.5 cm and genotypes Punjab Guchhedar, Ajeet 1, Anugraha, Pant C3, K1, LCA 353, LCA 235, LCA 333, Japaneese Long, PC 2062, BCL 5 and Phule Jyoti showed more than 1 cm of RG and genotypes HDC 75 and Arka Mohini showed less than 0.2 cm RG. However, %RRG ranged from 6.2 to 88.6. The genotypes BCL 5 (6.3), Punjab Guchhedar (31.9) and Ajeet 1 (34.9) showed minimum %RRG (less than 35%), whereas the genotypes Solan Bharpur (88.6) and HDC 75 (86.9) recorded a maximum reduction.

To identify the contrasting genotypes, RG after exposure to HT was correlated with %RRG (Fig. 1). Based on which, genotypes Punjab Guchhedar, Ajeet 1, K1, LCA 333, Japaneese Long, PC 2062 and Phule Jyoti exhibited lower %RRG and high RG, out of which, Punjab Guchhedar and Ajeet 1 showed the highest CLT. However, HDC 75, Solan Bharpur, JCA 283, Ajeet 3, Arka Suphal and PBC 535 showed high %RRG along with low RG. Among them, HDC 75 showed the lowest CLT. Genotypes Arka Mohini and BCL 5 were found as outliers as they could not show a perfect correlation between RRG and RG. The rest 25 genotypes were found to show moderate %RRG and RG.

Managed stress environment study

Forty genotypes used in TIR studies were grown under high temperature in the polytunnel. The observations recorded on yield after the recovery period were used for calculation of tolerance indices (Table 2). The HSI ranged from 0.07 to 3.08. Three genotypes namely, Punjab Guchhedar (0.07), Ajeet 1(0.55) and Utkala Yellow (0.76) showed minimum HSI value, and HDC 75 (3.08), LCA 235 (2.90) and LCA 353 (2.86) showed maximum HSI. The TOL value ranged from 2.25 to 169.60 and genotypes Punjab Guchhedar (2.25), Utkala Yellow (14.6) and Arka Basant (15.0) showed minimum TOL values. Genotypes IC 395323(169.6), K1 (148.0) and ICPN 11# 7(143.7) showed maximum TOL value. The MP value varied from 118.28 to 3.75, and genotypes Pant C1 (118.28), ICPN 11# 7 (116.25), K1 (15.6) and Punjab Guchhedar (102.88) showed higher value, and Arka Basant (3.75), HDC 75 (23.65) and Punjab Lal (30.63) showed minimum value. STI value ranged from 0 to 1.13, and genotypes Pant C1 (1.43), Punjab Guchhedar (1.13) and PBC 535 (1.09) showed higher STI and HDC 75, and Arka Basant showed low STI (0). The GMP value varied from 0 to 115.6, and genotypes Pant C1 (115.62), Punjab Guchhedar (102.87) and PBC 535 (100.97) showed highest GMP and genotypes, and HDC 75 and Arka Basant showed minimum GMP (0). YSI varied from 0 to 0.98. Punjab Guchhedar (0.98), Ajeet 1 (0.82) and Utkala Yellow (0.76) gave a higher value. HDC 75 and Arka Basant gave minimum YSI (0). Punjab Guchhedar and Ajeet 1 were found tolerant and HDC 75 was found susceptible for all the tolerant indices.

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Table 1. Observations on survival and recovery growth in TIR study

	Genotype	RG (cm.) Control	RG (cm.) Induced	RRG (%)	SS (%)		Genotype	RG (cm.) Control	RG (cm.) Induced	RRG (%)	SS (%)
1	Pusa Jwala	1.8	0.6	68.1	100.0	21	Solan Bharpur	3.5	0.4	88.6	70.4
2	Arka Lohit	2.3	0.6	75.5	100.0	22	KTPL 19	2.5	0.9	65.7	96.3
3	Arka Abir	3.5	1.0	72.1	100.0	23	Jayanti	3.2	0.8	75.0	100.0
4	Punjab Guchhedar	2.1	1.4	31.9	100.0	24	Hungarian Yellow	2.3	0.8	66.8	96.3
5	Punjab Sadabahar	2.6	0.8	67.7	96.3	25	IC 395322	2.6	0.9	64.4	66.7
6	Byadagi Kaddi	2.7	1.0	65.1	100.0	26	LCA 353	2.0	1.0	48.5	100.0
7	IC 395323	2.2	1.0	54.6	100.0	27	LCA 235	2.8	1.0	63.1	88.9
8	Pant C1	3.1	0.9	72.4	100.0	28	LCA 333	2.2	1.3	40.6	100.0
9	Anugraha	2.0	1.1	45.1	100.0	29	HDC 75	1.3	0.2	86.9	40.7
10	Phule Mukta	3.0	0.8	72.7	100.0	30	JCA 283	2.3	0.4	84.3	81.5
11	Arka Suphal	1.9	0.4	79.4	100.0	31	Japaneese Long	2.3	1.2	47.0	92.6
12	Arka Gourav	2.0	0.8	61.9	92.6	32	PC 2062	2.0	1.2	39.5	100.0
13	Byadagi Dabbi	1.8	0.8	53.4	88.9	33	Ajeet 1	2.4	1.5	34.9	100.0
14	ICPN 11# 7	1.6	0.6	63.8	100.0	34	Ajeet 3	2.5	0.4	82.5	81.5
15	Pant C3	2.5	1.1	56.2	100.0	35	BCL 5	1.5	1.4	6.3	100.0
16	K1	2.2	1.4	38.7	100.0	36	PBC 535	1.5	0.4	76.8	70.4
17	ICPN 11#2	1.5	0.8	49.8	100.0	37	LCA 334	2.3	1.0	57.2	96.3
18	Punjab Surkh	2.4	1.0	60.0	100.0	38	Arka Basant	1.3	0.6	54.0	66.7
19	Punjab Lal	2.9	0.7	74.7	96.3	39	Phule Jyoti	1.8	1.2	35.8	100.0
20	Utkal Yellow	2.6	0.8	68.7	100.0	40	Arka Mohini	0.4	0.2	57.0	48.2

RG, recovery growth; RRG, reduction in recovery growth; SS, seedling survival.

A correlation was established between the yield (under stress and non-stress) and the tolerance indices, and a significant positive correlation ($r = 0.602$) between yield under stress and non-stress condition was observed (Table 3). Yield under stress condition is significantly correlated with all the tolerance indices HSI ($r = 0.985$), TOL ($r = 0.645$), STI ($r = 0.690$), GMP ($r = 0.820$), MP ($r = 0.862$) and YSI ($r = 0.750$) at $P < 0.01$.

In the TIR study, %RRG is the determining factor for CLT, and among the tolerance indices, HSI showed a maximum positive correlation ($r = 0.985$) with yield under stress. A normal distribution graph was plotted by using Z-RRG and Z-HSI to identify the best HT-tolerant line (Fig. 2) which distributed the whole set of genotypes into four different quadrants and identified Punjab Guchhedar and Ajeet 1 as the most tolerant genotypes.

Discussion

Survival and recovery growth

In field condition, plants never get exposed to lethal temperature directly. Temperature increases gradually, and during the process of mild temperature stress exposure, plants acquire thermotolerance for sustaining subsequent lethal temperature as an inherent characteristic. Genetic variability for this thermotolerance between genotypes can be observed only after exposure to induction temperature. Upon exposure to mild stress, various thermotolerant traits such as membrane integrity, cell viability and chlorophyll stability show variation based on which tolerant types can be identified.

Yan *et al.* (2016) generated three cDNA libraries by using mRNA from three samples: control (grown at 24°C), S1 (exposed at 35°C for 30 min) and S2 (exposed at 35°C for 5 h) of spinach leaf. By comparative transcriptome analysis, a number of unique genes in spinach heat response transcript were found. Among these unique differentially expressed genes, some genes were involved in salt stress, organic acid metabolic and carotenoid metabolic through which spinach responded to heat stress. These results were helpful for understanding the molecular adaptation mechanism of spinach during the heat stress.

When suffering from heat stress, plants could activate various stress-responsive signal transduction pathways to generate a series of innate defensive reactions. Calcium signal is important in response to heat stress, and heat stress causes a transient rise of Ca^{2+} levels in many plants. However, selection based on survival, RG and %RRG at seedling stage is

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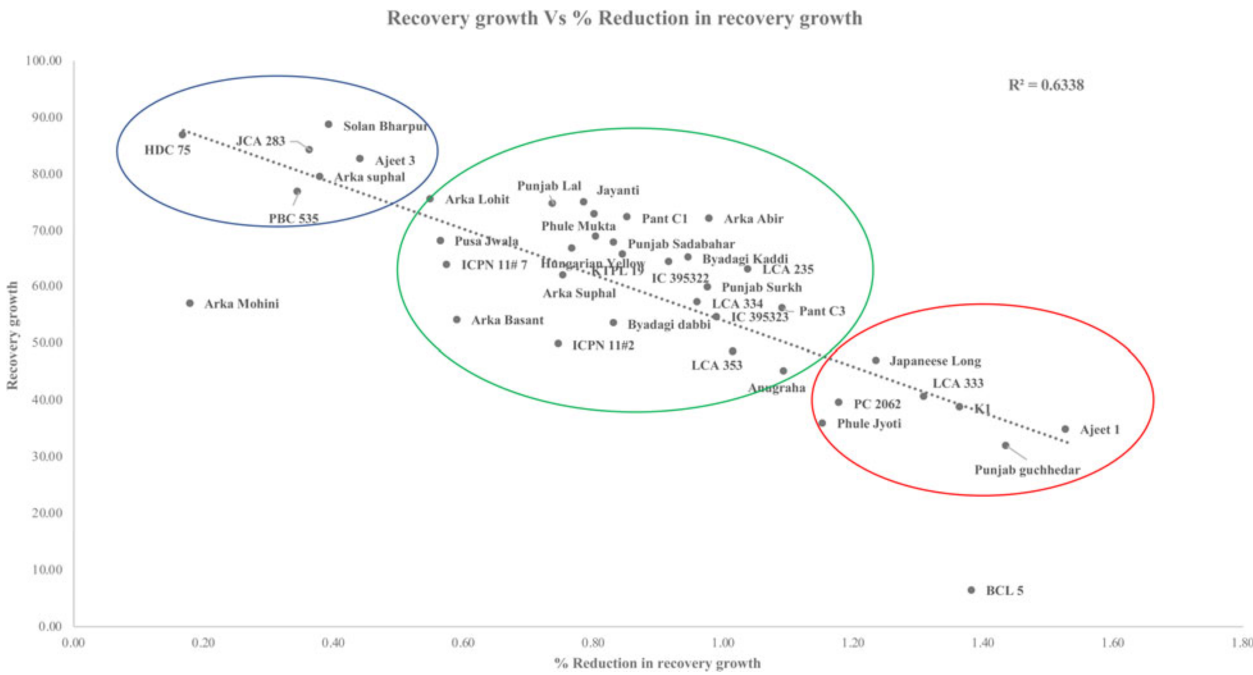

Fig. 1. Correlation between recovery growth (RG) and % reduction in recovery growth (RRG) to identify contrasting genotypes. RG in *X*-axis and %RRG in *Y*-axis. Genotypes in the extreme right of the graph encircled with red oval are with highest CLT; at the extreme left encircled with blue oval are with lowest CLT and in the middle encircled with green oval are with medium CLT.

more convenient as it is a reflection of the sum of variation in intrinsic tolerance brought by different tolerance mechanisms (Senthil-kumar *et al.*, 2006). This is the basis for TIR study and is a non-destructive method of screening a population like segregating, open pollinated, germplasm or mutated population. Venkatesh Babu *et al.* (2013) and Raghavendra *et al.* (2017) also advocated for the use of TIR technique to identify the tolerant and susceptible genotypes based on seedling survivability in ragi and chick pea, respectively.

In the TIR study, the optimization of induction and challenging temperatures is the pre-requisite for a crop species. In *Capsicum* (including bell pepper and chilli), we optimized 33–42°C for 4 h as induction temperature and 48°C for 1 h 30 min as challenging temperature. Senthil-kumar and Udayakumar (2004) have also mentioned in a review paper of using a temperature of 33°C for 1 h followed by 36°C for 1 h and then 39°C for 1 h followed by 42°C for 1 h as induction temperature and 48°C for 1 h 30 min as challenging temperature in bell pepper. In our method, we exposed the seedlings to a continuously increasing temperature instead of putting them in different intermediate temperatures for a fixed period. Similar method for standardization of challenging and induction temperatures has been followed in other crops such as tomato (Senthil-kumar and Udayakumar, 2004), groundnut (Lokesh *et al.*, 2004), cotton (Kheir *et al.*, 2012), ragi (Venkatesh Babu *et al.*, 2013) and banana (Vidya *et al.*, 2017).

Screening *Capsicum* genotypes with the optimized induction and challenging temperatures indicated wide variability in response among the genotypes for survival and RG. Thus, depicting the presence of variability for thermotolerance among the genotypes evaluated. This could be due to the different sub species of *C. annuum* used which vary in their acquired thermotolerance. This phenomenon is an acclimation response and plays an important role in crops such as cotton (Kheir *et al.*, 2012); sunflower (Kumar *et al.*, 1999; Senthil-Kumar *et al.*, 2006); sorghum and pearl millet (Howarth *et al.*, 1997); beans (Keeler *et al.*, 2000); wheat (Burke, 2001) and groundnut (Srikanthbabu *et al.*, 2002), which manifests itself in terms of lower RRG. Out of 40 lines screened, 23 gave 100% survival indicating the effect of induction treatment on improving the survivability even at lethal/challenging temperature. But classifying the lines as tolerant and susceptible merely based on survival may be misleading. Hence, genotypes showing lower RRG coupled with higher RG were considered as the tolerant ones and Punjab Guchhedar and Ajeet 1 were found with the highest CLT to HT. HDC 75 showed the lowest CLT to HT. Since seedling exposed to stress is at heterotrophic conditions, any variation in survival and recovery can be attributed to the intrinsic tolerant ability of the genotype. The use of TIR approach to select thermotolerant lines is appropriate only when there is a sufficient genetic variability in the population to be screened. Hence, TIR is a potential tool not only to identify contrasting genotypes based on thermotolerance but also useful in identifying highly tolerant lines from segregating progenies or from a mutant population. Despite many

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Table 2. Stress tolerance indices derived from yield under stress and non-stress conditions

Sl no.	Genotype	AYC	AYS	HSI	TOL	STI	GMP	MP	YSI	Sl no.	Genotype	AYC	AYS	HSI	TOL	STI	GMP	MP	YSI
1	Pusa Jwala	68.50	11.45	2.56	57.05	0.08	28.00	39.98	0.17	21	Solan Bharpur	92.00	11.00	2.70	81.00	0.11	31.74	51.50	0.12
2	Arka Lohit	130.35	35.90	2.23	94.45	0.50	68.38	83.13	0.28	22	KTPL 19	69.20	37.25	1.43	31.95	0.28	50.73	53.23	0.54
3	Arka Abir	144.00	37.60	2.28	106.40	0.58	72.70	90.80	0.26	23	Jayanti	116.80	35.20	2.16	81.60	0.45	63.58	76.00	0.30
4	Punjab Guchhedar	104.00	101.75	0.07	2.25	1.13	102.87	102.88	0.98	24	Hungarian Yellow	114.90	42.20	1.93	72.70	0.51	69.41	78.55	0.37
5	Punjab Sadabahar	90.60	66.60	0.81	24.00	0.64	77.61	78.60	0.74	25	IC 395322	63.20	24.33	1.91	38.87	0.17	39.13	43.77	0.38
6	Byadagi Kaddi	135.20	56.80	1.79	78.40	0.83	87.63	96.00	0.42	26	LCA 353	101.40	6.75	2.86	94.65	0.07	21.23	54.08	0.07
7	IC 395323	200.20	30.60	2.60	169.60	0.65	76.96	115.40	0.15	27	LCA 235	97.80	5.80	2.90	92.00	0.06	21.48	51.80	0.06
8	Pant C1	143.00	93.55	1.05	49.45	1.43	115.62	118.28	0.66	28	LCA 333	101.40	55.75	1.38	45.65	0.60	74.67	78.58	0.56
9	Anugraha	89.40	33.00	1.94	56.40	0.31	54.27	61.20	0.37	29	HDC 75	47.30	0.00	3.08	47.30	0.00	0.00	23.65	0.00
10	Phule Mukta	77.40	19.60	2.25	57.80	0.16	38.27	48.50	0.27	30	JCA 283	112.60	24.10	2.43	88.50	0.30	51.79	68.35	0.21
11	Arka Suphal	91.60	13.15	2.63	78.45	0.13	34.09	52.38	0.15	31	Japaneese Long	107.45	66.20	1.17	41.25	0.75	83.78	86.83	0.62
12	Arka Gourav	76.20	14.00	2.51	62.20	0.11	32.63	45.10	0.18	32	PC 2062	61.80	16.80	2.24	45.00	0.11	32.22	39.30	0.27
13	Byadagi Dabbi	30.00	5.40	2.58	49.20	0.07	25.46	17.70	0.18	33	Ajeet 1	87.60	70.60	0.55	17.00	0.66	78.51	79.10	0.82
14	ICPN 11# 7	188.10	44.40	2.35	143.70	0.89	91.39	116.25	0.24	34	Ajeet 3	54.90	12.30	2.38	42.60	0.07	25.79	33.60	0.23
15	Pant C3	120.40	42.40	2.00	78.00	0.55	71.44	81.40	0.35	35	BCL 5	57.50	7.90	2.66	49.60	0.05	18.22	32.70	0.13
16	K1	189.60	41.60	2.40	148.00	0.84	88.81	115.60	0.22	36	PBC 535	150.40	68.00	1.69	82.40	1.09	100.97	109.20	0.45
17	ICPN 11#2	113.60	17.00	2.62	96.60	0.21	43.71	65.30	0.15	37	LCA 334	96.40	14.00	2.64	82.40	0.15	36.51	55.20	0.14
18	Punjab Surkh	44.10	19.80	1.76	24.30	0.10	29.07	31.95	0.43	38	Arka Basant	7.50	0.00	3.11	15.00	0.00	0.00	3.75	0.00
19	Punjab Lal	53.75	7.50	2.68	46.25	0.05	17.79	30.63	0.13	39	Phule Jyoti	127.60	24.60	2.48	103.00	0.33	55.60	76.10	0.20
20	Utkal Yellow	59.20	44.60	0.76	14.60	0.28	51.15	51.90	0.76	40	Arka Mohini	58.00	8.40	2.64	49.60	0.05	22.01	33.20	0.14

AYC, average yield under control; AYS, average yield under stress; HSI, heat susceptibility index; TOL, tolerance index; STI, stress tolerance index; GMP, geometric mean productivity; MP, mean productivity; YSI, yield stability index.

Table 3. Correlation among yield (under stress and non-stress conditions) and different tolerant indices

	AYC	AYS	HSI	TOL	STI	GMP	MP
AYC							
AYS	0.602**						
HSI	0.617**	0.985**					
TOL	0.635**	0.645**	0.605**				
STI	0.675**	0.690**	0.717**	0.752**			
GMP	0.546**	0.820**	0.832**	0.460**	0.456**		
MP	0.763**	0.862**	0.875**	0.682**	0.730**	0.705**	
YSI	0.662**	0.750**	0.786**	0.593**	0.687**	0.907**	0.747**

AYC, average yield under control; AYS, average yield under stress; HSI, heat susceptibility index; TOL, tolerance index; STI, stress tolerance index; GMP, geometric mean productivity; MP, mean productivity; YSI, yield stability index.
**Correlation is significant at 0.01 level.

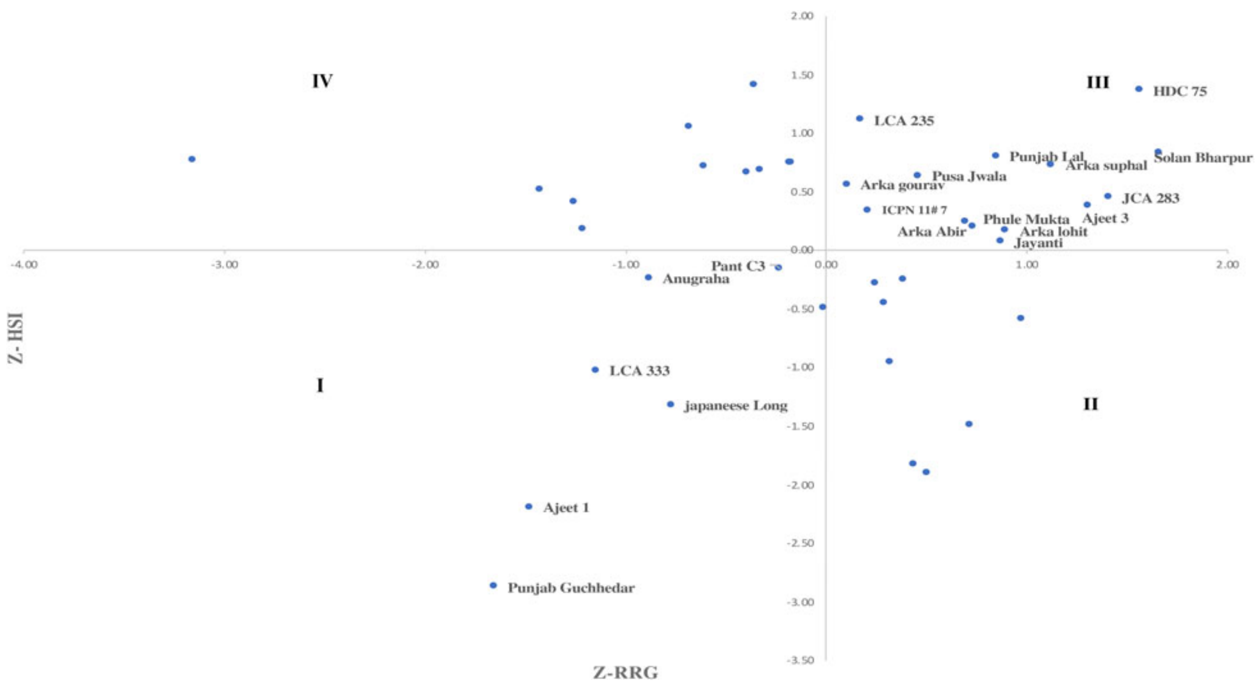

Fig. 2. Normal distribution graph for %RRG and HSI to identify contrasting genotypes. Z-RRG in *X*-axis and Z-HSI in *Y*-axis. Genotypes in quadrant I are tolerant and genotypes in quadrant III are susceptible to HT.

advantages of TIR, it has a limitation of identifying tolerant lines in seedling stage only which may or may not reflect at the reproductive stage later. Hence, it is essential to ensure the thermotolerance of identified lines at the reproductive stage which will justify the application of this screening method (Senthil-kumar and Udayakumar, 2004).

Stress tolerance indices

Many workers have used stress tolerance indices as an established method to identify the most tolerant and susceptible genotypes in different crops (Hassanpanah, 2010; Jamshidi and Javanmard, 2018). To determine the most reliable stress-tolerant indices, correlation between yield (under stress, non-stress) and heat tolerance indices was calculated. A significant positive correlation between yield under both stress and non-stress conditions indicated that high-yielding genotypes can be selected based on both stress and non-stress conditions. Thus, indirect selection for high yield under stress based on yield under normal condition can be done. Kamrani *et al.* (2018) also reported a similar result in durum wheat, where a significant correlation was found between yield under both stress and non-stress conditions. Further, a significant positive correlation

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Table 4. Tolerant and susceptible genotypes based on TIR, tolerance indices and combination of both

Method used	Tolerant genotypes	Susceptible genotypes
TIR	Punjab Guchhedar, Ajeet 1, K1, LCA 333, Japaneese Long, PC 2062, Phule Jyoti	HDC 75, Solan Bharpur, JCA 283, Ajeet 3, Arka Suphal, PBC 535
Low HSI with high absolute yield under stress	Punjab Guchhedar, Ajeet 1	HDC 75
Low TOL with high MP	Punjab Guchhedar, Ajeet 1	HDC 75
High STI and High GMP	Punjab Guchhedar, Pant C1, PBC 535	HDC 75
High YSI	Punjab Guchhedar, Ajeet 1	HDC 75
Combined TIR and stress tolerance indices	Punjab Guchhedar, Ajeet 1, LCA 333, Japaneese Long, Anugraha, Pant C3	HDC 75, Solan Bharpur, JCA 283, Ajeet 3, Arka Suphal, Punjab Lal, Pusa Jwala, LCA 235, Arka Gourav

between the tolerance indices and yield under stress indicated their suitability in identifying the contrasting lines.

Tolerance in terms of stability in yield for the genotype can be estimated by HSI which detects genotypic differences in heat tolerance in terms of quantifying the yield loss under stress situations. Tolerant genotype should have less HSI and high absolute yield under stress condition because only HSI does not provide any information about the yielding ability of the genotype *per se*. Punjab Guchhedar and Ajeet 1 were found tolerant based on low HSI and high absolute yield under stress, and HDC 75 was found the most susceptible one. TOL indicates the tolerance of genotypes under stress based on their yield reduction, but in a reverse correlation and genotypes with low TOL does not mean high yielding under stress. Khodarahmpour and Choukan (2011) in maize and Dorostkar *et al.* (2015) in wheat were also of the same opinion. Hence, genotypes with low TOL coupled with high MP are considered as tolerant ones. Here again, Punjab Guchhedar and Ajeet 1 were found superior based on TOL and MP. STI determines the stability in yield potential of the genotype and GMP indicates the tolerance level under stress conditions. Therefore, selection of genotypes was done based on high STI and high GMP and Punjab Guchhedar and Pant C1 were found superior. YSI is another useful parameter for discriminating genotypes that have higher stability and lower susceptibility to stress conditions. Based on YSI, Punjab Guchhedar and Ajeet 1 are tolerant and HDC 75 and Arka Basant are susceptible.

Moreover, a normal *Z* distribution analysis was done based on %RRG and HSI to identify the best HT-tolerant line as it is considered as an efficient tool to group genotypes into contrasting types. Genotypes falling in first quadrant having negative values for both Z-RRG and Z-HSI namely, Punjab Guchhedar, Ajeet 1, LCA 333, Japaneese Long, Anugraha and Pant C3 were considered to have a high tolerance to HT. However, genotypes falling in quadrant III, HDC 75, Solan Bharpur, JCA 283, Ajeet 3, Arka Suphal, Punjab Lal, Pusa Jwala, LCA 235 and Arka Gourav were considered to be susceptible.

Conclusion

In this investigation using TIR approach, stress tolerance indices and combining both we found that Punjab Guchhedar and Ajeet 1 were consistently found as tolerant and HDC 75 as susceptible for HT stress (Table 4). Hence, they can be used as donor in the future breeding programme. Also, the TIR technique was found as a reliable screening technique for preliminary evaluation of *Capsicum* genotypes for HT tolerance and to assess the genotypic variability in acquired thermotolerance. The main advantage of this technique is its rapidity and reproducibility in screening a large number of genotypes at a very early stage. It also helps the breeder to narrow down to tolerant genotypes in a large set of germplasm and thereby saves the time and energy required for adult plant screening. Further identification of traits conferring tolerance in these identified genotypes can be useful in mapping the gene(s)/QTL(s) which in turn will facilitate breeder in marker-assisted breeding for HT stress.

Supplementary material
The supplementary material for this article can be found at https://doi.org/10.1017/S147926211900042X

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