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Genotypic Variations in Tomato (*Lycopersicon Esculentum* Mill.) for Acquired Thermotolerance to Temperature Induction Response

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Abstract

Thirty genotypes of tomato (Lycopersicon esculentum Mill.) were evaluated for acquired thermotolerance in seedlings based on temperature induction response (TIR) technique. The study showed that all the genotypes exhibited better growth and survival after temperature induction treatment. Out of thirty on exposure to direct challenging temperature nineteen genotypes were found to be susceptible, five moderately tolerant, three tolerant and four dead whereas after induction five were found to be susceptible, eight were found to be moderately tolerant and seventeen genotypes found to be tolerant with better seedling survival percentages. Growth during recovery was also found to be increased in maximum number of genotypes under induction treatment. Genotype GT showed 25.3% of survival when exposed directly to challenging temperature whereas it showed 100% of survival after induction treatment. The GDR of genotype EC-520061 was found to be maximum (4.15 cm) but with lower survival percentage (27.6%) after challenging treatment. SDS-PAGE leaf protein profiling confirmed the presence of additional protein bands as a result of induction. Hence, TIR serves to be a better tool to identify tolerant and susceptible genotypes for acquired thermotolerance even at seedling stage.

Highlights

- Temperature induction response technique was used to screen thirty genotypes of Lycopersicon esculentum Mill.
- Growth and survival of all the genotypes was found to be better after induction treatment.
- SDS-PAGE protein profiling of two selected genotypes was also performed.

Keywords: acquired thermotolerance, heat stress, protein profile, TIR technique, tomato

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely consumed and popular vegetable throughout the world because of its acceptable flavour, nutritive value, short lifecycle and high productivity. There has been a gradual increase in the area under cultivation while production has been fluctuating due to weather related factors of which high temperature stress is one of the most important factor that results in yield loss. Therefore, there is a need to screen out efficient genotypes that can perform better under high temperature. Temperature Induction Response (TIR) has proved to be an efficient technique to increase thermotolerance by induction at sub-lethal temperatures (Srikanthbabu *et al*. 2002, Senthil-Kumar *et al*. 2003, Gangappa *et al*. 2006, Selvaraj *et al*. 2011, Kheir *et al*. 2012). The best characterized aspect of acquired thermotolerance is production of heat shock proteins (HSPs) (Vierling 1991, Burke 2001). Studies in different plant species demonstrated that upon acclimation there is significant increase in HSPs (HSP 18.1, HSP 70, HSP 90 and HSP 104)

both in seedlings as well as in plants that conferred thermotolerance to seedlings (Uma *et al.* 1995, Srikanthbabu *et al*. 2002, Senthil-Kumar *et al.* 2003, Zhou *et al.* 2016). It has also been reported that small HSPs (HSP 17.9 & 18.1) were upregulated upon induction in pea (Senthil-Kumar *et al.* 2003).

Materials and Methods

Plant material

Seeds of thirty different genotypes of tomato namely Azad T-5, CO-3, Selection-7, Flawery, Punjab Sharad, Angurlata, Shalimar-2, FLA-7171, KashiVishesh, T-Local, Kashi Anupam, NF-315, Swarn Lalima, NDTVR-60, Kashi Sharad, Kashi Amrit, B-S-31-3, B-S-2-5, EC-520061, B-S-18-7, BT-120, TLC-1, H-88-7-4, VR-20, GT, FEB-4, DT-10, DT-2, PMS-1 and Hisar Anmol were obtained from Indian Institute of Vegetable Research (IIVR), Varanasi, India. Ten seeds of each genotype with 3 replicates were germinated in petriplates using germination paper at 34ºC. 5 days old seedlings were used to study the responses. The experiment was conducted under controlled conditions in growth chamber (Narang, NSW-193). Standard statistical methods were used to analyse the data using SAS software and the design used was FCRD.

Temperature Induction Response (TIR) technique

TIR technique is a suitable laboratory procedure for efficient screening of genotypes which follows the principle of 'acquired thermotolerance'. It involves exposure of seedlings to a range of gradually increasing temperatures above ambient for specific time periods before being exposed to severe challenging temperature as given below:

Challenging temperature: The seedlings were first subjected to the following different challenging temperatures for specific time periods as (i) 48ºC for 1 hr, (ii) 48ºC for 2 hrs, (iii) 50ºC for 1 hr and (iv) 50ºC for 2 hrs to standardize the challenging temperature and time period combination which caused 90% seedling mortality (In other words 10% of seedlings to survive).

Induction temperature: The seedlings were subjected to the following different induction temperature ranges for specific time periods that resulted in 4ºC increase after every 1-2 hour(s) as (i) 36ºC (1 hr) - 40ºC (1 hr) - 44ºC (1 hr), (ii) 36ºC (2 hrs) - 40ºC (2hrs) - 44ºC (2 hrs), (iii) 38ºC (1 hr) - 42ºC (1 hr) - 46ºC (1 hr) and (iv) 38ºC (2 hrs) - 42ºC (2 hrs) - 46ºC (2 hrs). These induction ranges were studied along with standardized challenging temperature for selecting maximum TIR based on per cent seedling survival.

Categorization of genotypes: The experiment was divided into 3 sets; Control (Co), Induced (In) and Challenging (Ch). Ten seedlings of each genotype were grown for every set in three replications. The control set (Co) seedlings were continuously grown at 34ºC. The induced set (In) of seedlings were exposed to standardized temperature induction range (Section 2.2.2) and then subjected to standardized challenging temperature (Section 2.2.1). Seedlings of the challenging set were exposed directly to challenging temperature without induction. The induced and challenging sets were then allowed to recover at 34ºC for a period of 3 days (Fig. 1) and the following observations were recorded:

Survival Percentage: The survival percentage was calculated using the given formula.

$$\text{Survival Percentage} = \frac{\text{Number of seedlings survived}}{\text{Total number of seedlings}} \times 100$$

The genotypes that exhibited 71–100% survival percentage were categorized as tolerant, 51-70% survival percentage as moderately tolerant and 0-50% survival percentage as susceptible.

Growth During Recovery (GDR) was calculated by comparing seedling growth (length, cm) before and after recovery using the formula

GDR= Growth after recovery - Growth before recovery

SDS-PAGE (Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis) **Protein Profiling**

Two genotypes, one which was found to be tolerant with and without induction namely NDTVR-60 and one which was susceptible without induction but become tolerant after induction namely GT were selected for leaf protein profiling by SDS-PAGE. Nurseries of these genotypes were raised in trays and were transplanted after twenty-five days of sowing in pots of size 15×15 cm. The nursery and transplanted seedlings were grown at 34ºC in

plant growth chamber with optimum light (10/14 photoperiod) and with 85% relative humidity. They were divided into 3 sets; Control (Co), Induced (In) and Challenging (Ch) in 3 replications. The control set (Co) seedlings were continuously grown at 34ºC. Induced set of the seedlings were given induction treatment 30 DAT (Days after Transplanting) as given in Fig. 1 and the challenging set of seedlings were directly exposed to challenging temperature. The induced and challenging sets were then placed for recovery for 3 days at 34ºC. Total soluble protein content of uppermost fully expanded leaves was determined by following the method of Bradford (1976) and equal quantity of protein was loaded for profiling by SDS-PAGE (Laemmli 1970).

Results and Discussion

Challenging temperature

Depending on the survival percentage after recovery 48ºC for 2 hrs was selected as standardized challenging temperature. It resulted in 90% seedling mortality. Gangappa *et al.* (2006) reported 55ºC for 3 hrs in groundnut seedlings with 80% reduction in growth, 47ºC for 3 hrs in cotton seedlings with 99.6% of growth reduction (Kheir *et al.* 2012), 49ºC for 2 hrs and 90% reduction in growth was observed in sunflower (Senthil-Kumar *et al.* 2003) and Selvaraj *et al.* (2011) treated the seedlings with 50ºC for 30 minutes as the lethal temperature in peanut.

Induction temperature

Depending upon the survival percentages recorded after recovery the induction range of 38°C (1 hr) - 42°C (1 hr) - 46°C (1 hr) with 48°C (2 hrs) as challenging temperature was found to be the best range for maximum TIR. According to earlier studies on different crop different induction temperatures were identified like in cotton 28 to 40°C over 4 hrs (Kheir *et al.* 2012), 28°C to 42°C for 2.5 hrs in sunflower (Senthil-Kumar *et al.* 2003), 35°C to 45°C for 4 hrs in groundnut (Gangappa *et al.* 2006) and 38ºC to 40°C in peanut (Selvaraj *et al.* 2011).

Thus, results of our experiment clearly demonstrate that tolerance to high temperature can be induced in seedlings by prior exposure to gradual increasing non-lethal temperature in otherwise susceptible genotypes. Shi *et al.* (2015) reported that in rice, pre-exposure to sublethal treatment followed by harsh lethal treatment is known to improve tolerance of different abiotic stresses at the vegetative stage within and across generations. The main cause in all the systems behind the tolerance by induction that have been studied to date, is the synthesis of HSPs that strongly correlates with the enhanced tolerance or acquired thermotolerance against a subsequent otherwise lethal heat stress (Lindquist and Craig 1988). The synthesis and localization of HSPs trigger several important physiological and biochemical parameters (Chen *et al.* 1990), including the maintenance of membrane stability (Kader *et al.* 1991) and chaperoning of the proteins (Sanchez and Lindquist 1990, Vierling and Nguyen 1992) and these changes facilitate the maintenance of cellular function under stress. Therefore, only the seedlings which were pre-exposed to the optimum temperature-induction exhibited better recovery growth (Kumar *et al.* 1999).

Categorization of genotypes

Survival percentage: The seedling survival percentage without and with induction treatment is presented in Table 1. Prajapati *et al.* (2015) reported the presence of genetic variability and heritability after screening 39 diverse genotypes of tomato. The studied genotypes exhibited genotypic variability when subjected to induction and challenging temperatures. The genotypes were categorized based on the percentage of seedlings survived without induction i.e. challenging temperature (Table 2 (a)) and with induction (Table 2 (b)). In the present investigation it was found that among thirty genotypes NDTVR-60 was found to have maximum survival percentage and was highly significant when exposed to direct challenging temperature. Though, its survival percentage was found to increase after induction treatment but was found to be significantly reduced when compared to GT, Swarnlalima and DT-2. The genotype GT shows the maximum survival percentage under induction treatment and was highly significant amongst all the genotypes but it was also found to have very low survival percentage when directly exposed to challenging temperature. Hence, the importance of TIR technique for acquired thermotolerance can be observed from above data and can be more clearly noted from categorization where it was found that only three genotypes

exhibited tolerance under challenging temperature whereas seventeen genotypes were found to be tolerant under induction. Similarly, the number of moderately tolerant genotypes became just double from four (under challenging) to eight (under induction) and the number of genotypes showing susceptibility was greatly reduced from twenty three (under challenging) to five (under induction) amongst which four genotypes was found dead after challenging treatment whereas under induction treatment every genotype was found to be survived. The work was found to be in accordance with the earlier works reported by several authors on acquired thermotolerance that exposure to high temperature can be induced in seedlings by prior exposure to gradual increasing non-lethal temperature in otherwise susceptible genotypes but with certain exceptions in different crop plants under heat stress (Uma *et al.* 1995, Jayaprakash *et al.* 1998, Kumar *et al.* 1999, Burke *et al.* 2000, Burke 2001, Srikanthbabu *et al.* 2002, Senthil-Kumar *et al.* 2003, 2007).

Growth during recovery: The length of tomato seedlings after induction and challenging treatment i.e. before recovery and after recovery were recorded (data not mentioned here). The difference between the length of seedlings before and after recovery was calculated as growth during recovery (GDR) and is present in Table 1. In the present investigation it was observed that among thirty varieties of tomato, twenty genotypes showed increase in growth under induction treatment over challenging. Six genotypes were found to behave differently by exhibiting decrease in growth under induction over control. Genotype GT with maximum survival after induction was found to show 76.6% growth over control after induction and 69.29% of growth over control after challenging. Many earlier studies have also demonstrated that seedlings exposed to a sub-lethal temperature prior to challenge with severe temperature have better recovery growth than those seedlings exposed directly to severe temperature (Kumar *et al.* 1999, Srikanthbabu *et al.* 2002). In sunflower Kumar *et al.* (1999) demonstrated that the induced seedlings exhibited a higher recovery growth compared to the non-induced and also accumulated higher levels of a few low and high-molecular weight HSPs such as HSP 18.1, HSP 90 and HSP 104.

SDS-PAGE Protein Profiling

The leaf protein profile by SDS-PAGE of 2 selected genotypes, which had maximum survival percentage under induction (GT) and challenging (NDTVR-60) is given in Plate 1. Comparison of leaf protein profiles of control, after induction and after challenging treatments was made. NDTVR-60 possessed inherent tolerance whereas GT exhibited acquired thermotolerance. The differences were observed in terms of the band intensities. In case of NDTVR-60 the proteins that were induced, probably the heat shock proteins by induction treatment were found to be present even in challenging treatment which is evident from the number of bands and band intensities of leaf protein (Lanes 3 and 4) in comparison to control (Lane 2). During induction treatment in NDTVR-60 proteins bands of sizes around 18 kDa and 20 kDa were found to be intense when compared to control (Lane 2 and 3). They may correspond to HSPs 17.9 and 18.1 which were upregulated upon induction as observed in pea (Srikanthbabu *et al.* 2002) and there are considerable amount of evidences indicating that heat shock proteins (HSPs) are key components in the molecular machinery activated in response to high temperature (Hong and Vierling 2000, Kotak *et al.* 2007, Larkindale and Vierling 2008, Scharf *et al.* 2012, Murthy *et al.* 2016). In the case of genotype GT it was observed that the intensity of protein bands are much higher in leaves after induction in comparison to control (Lanes 5 and 6) which clearly support the results of TIR after which GT was observed to be shifted from susceptible to tolerant range whereas the intensity of protein bands of leaves after direct challenging was observed to be lesser in comparison to induction treatment but higher than control (Lanes 3 and 4). In *L. esculentum* it had observed that there are genotypic differences that occur during recovery from heat shock. In case of NDTVR-60 the proteins that were induced, probably the heat shock proteins by induction treatment were found to be persistent even after the recovery period of 3 days which is evident from the band intensities of leaf protein (Lanes 6 and 7). Mahesh *et al.* (2013) isolated small HSP24.4 (MasHSP24.4) cDNA from wild banana (*Musa accuminata*) and introduced it into the cultivated tomato. The gene was expressed in tomato under 45°C, showed significantly better growth performance in the recovery phase following

the stress. This thermotolerance appeared to be solely due to over expression of the sHSP24.4 gene.

In GT genotype protein bands of 18 kDa and 20 kDa are found to be induced during induction treatment but not in leaves exposed to challenging temperature (as per the intensity).These changes may be the cause for thermotolerance in NDTVR-60 and temperature susceptibility when directly exposed to higher temperature in GT genotypes. Hu *et al.* (2010) also observed that introduction of sHSP 17.7 gene from carrot to potato was shown to enhance thermotolerance by affecting cellular membrane stability.

The protein corresponding to 50 kDa is probably the rubisco large subunit whose intensity is higher due to induction and which is reduced under direct challenging in genotype GT. This is in contrast to the observation in NDTVR-60 where it has been found that the protein band corresponding to 50 kDa is more intense in both, immediately after induction and after direct challenging treatments. Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) is the major enzyme assimilating CO2 into in the plants.

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Table 1. Survival Percent and Growth During Recovery (GDR) of 30 genotypes of tomato.

S.No.	Genotype	Survival percent (%)#			GDR (cm)##		
		CO	IN	CH	CO	IN	CH
1.	Angurlata	100 (99.97)	64.3 (53.31)	46.0 (42.71)	2.85	1.38 (2.2)	1.35
2.	Azad T-5	100 (99.97)	91.0 (72.54)	44.0 (41.55)	8.20*	2.28 (-0.9)	2.30
3.	B-S-2-5	100 (99.97)	10.3 (18.72)	00.0 (0.03)	2.25	0.04 (-3275.0)	1.35
4.	B-S-18-7	100 (99.97)	47.9 (43.80)	10.6 (19.00)	2.74	0.20 (-)	(-)
5.	B-S-31-3	100 (99.97)	34.7 (36.09)	16.0 (23.58)	2.50	0.95 (91.6)	0.08
6.	BT-120	100 (99.97)	10.0 (18.43)	00.0 (0.03)	6.97	0.09 (-)	(-)
7.	CO-3	100 (99.97)	72.3 (58.24)	38.3 (38.23)	3.95	1.13 (-123.0)	2.52
8.	DT-2	100 (99.97)	98.3 (82.51)	87.0 (68.87)	6.33	2.80 (5.7)	2.64
9.	DT-10	100 (99.97)	82.3 (65.12)	10.0 (18.43)	3.76	1.76 (86.9)	0.23
10.	EC-520061	100 (99.97)	75.3 (60.20)	27.6 (31.69)	1.81	1.93 (-145.1)	4.73*
11.	Feb-04	100 (99.97)	95.6 (77.89)	44.0 (41.55)	5.33	2.90 (43.8)	1.63
12.	FLA-7171	100 (99.97)	83.6 (66.11)	20.3 (26.78)	5.48	1.77 (-127.7)	4.03
13.	Flawery	100 (99.97)	51.0 (45.57)	15.3 (23.03)	8.50*	4.74 (48.5)*	2.44
14.	GT	100 (99.97)	100 (99.97)*	25.3 (30.20)	3.42	2.62 (9.5)	2.37
15.	Hisar anmol	100 (99.97)	60.0 (50.77)	19.8 (26.42)	5.47	3.03 (38.9)	1.85
16.	H-88-7-4	100 (99.97)	74.3 (59.54)	24.3 (29.53)	5.86	2.84 (32.4)	1.92
17.	Kashi amrit	100 (99.97)	62.0 (51.94)	20.0 (26.57)	5.24	2.03 (-63.1)	3.31
18.	Kashi anupam	100 (99.97)	91.0 (72.54)	60.0 (50.77)	2.26	3.50 (72.0)	0.98
19.	Kashi sharad	100 (99.97)	67.6 (55.30)	52.0 (46.15)	2.76	1.88 (36.2)	1.20
20.	Kashi vishesh	100 (99.97)	53.6 (47.06)	29.6 (32.96)	3.92	2.56 (80.5)	0.50
21.	NDTVR-60	100 (99.97)	96.0 (78.46)	88.6 (70.27)*	3.60	1.82 (58.2)	0.76

22.	NF-315	100 (99.97)	82.0 (64.90)	14.0 (21.97)	8.20*	2.50 (53.2)	1.17
23.	PMS-1	100 (99.97)	81.0 (64.16)	71.0 (57.42)	3.54	3.38 (54.7)	1.53
24.	Punjab sharad	100 (99.97)	55.6 (48.22)	47.0 (43.28)	6.67	2.67 (58.1)	1.12
25.	Selection-7	100 (99.97)	54.4 (47.52)	18.0 (25.10)	6.93	1.73 (76.9)	0.40
26.	Shalimar-2	100 (99.97)	96.6 (79.37)	26.0 (30.66)	4.62	2.22 (95.5)	0.10
27.	Swarnlalima	100 (99.97)	98.3 (82.51)	18.0 (25.10)	3.42	2.19 (19.6)	1.76
28.	TLC-1	100 (99.97)	90.6 (72.15)	41.3 (39.99)	2.92	2.01 (54.7)	0.91
29.	T-Local	100 (99.97)	12.0 (20.27)	00.0 (0.03)	2.40	0.54 (-)	(-)
30.	VR-20	100 (99.97)	96.6 (79.37)	00.0 (0.03)	3.34	2.79 (-)	(-)
	LSD ≤ 0.05	0	1.77	1.21	0.66	0.57	0.28
	SE(m)±	10.54	8.38	0.0026	0.205	0.110	0.122

* Significant at P≤0.05

The value in parenthesis indicate the arsine transformation of survival percentages

##The value in parenthesis indicate the percent increase/decrease in growth under induction over challenging

(-) Indicates that the plant was dead under challenging

Table 2(a): Classification of tomato genotypes based on the survival percentages of non-induced seedlings exposed to challenging temperature

Tolerant (71-100%)	Moderately tolerant (51-70%)	Susceptible (0-50%)
NDTVR-60 (88.6)	Kashi sahrad (52.0)	Feb-4 (44.0)
DT-2 (87.0)	Punjab sharad (47.0)	Azad T-5 (44.0)
PMS-1 (71.0)	Angurlata (46.0)	TLC-1 (41.3)
	Kashi anupam (60.0)	CO-3 (38.3)
		Kashi vishesh (29.6)
		EC-520061 (27.6)
		Shalimar-2 (26.0)
		GT (25.3)
		H-88-7-4 (24.3)
		FLA-7171 (20.3)
		Kashi amrit (20.0)
		Hisar anmol (19.8)
		Selection-7 (18.0)
		Swarn lalima (18.0)
		B-S-31-3 (16.0)
		Flawery (15.3)
		NF-315 (14.0)
		B-S-2-5 (10.6)
		DT-10 (10.0)
		BT-120 (0.0)
		VR-20 (0.0)
		B-S-18-7 (0.0)
		T-local (0.0))

* The values in parenthesis indicate the percentage of seedlings survived.

Table 2(b): Classification of tomato genotypes based on the survival percentages of induced seedlings exposed to challenging temperature

Tolerant (71-100%)*	Moderately tolerant (51-70%)	Susceptible (0-50%)
GT (100.0)	Kashi sharad (67.6)	B-S-2-5 (47.9)
DT-2 (98.3)	Angurlata (64.3)	B-S-31-3 (34.7)
Swarn lalima (98.3)	Kashi amrit (62.0)	T-local (12.0)
Shalimar-2 (96.6)	Hisar anmol (60.0)	B-S-18-7 (10.3)
VR 20 (96.6)	Punjab sharad (55.6)	BT-120 (10.0)
NDTVR-60 (96.0)	Selection-7 (54.4)	
Feb-4 (95.6)	Kashi vishesh (53.2)	
Azad T-5 (91.0)	Flawery (51.0)	
Kashi anupam (91.0)		
TLC-1 (90.6)		
FLA-7171 (83.6)		
DT-10 (82.3)		
NF-315 (82.0)		
PMS-1 (81.0)		
EC-520061 (75.3)		
H-88-7-4 (74.3)		
CO-3 (72.3)		

* The values in parenthesis indicate the percentage of seedlings survived.

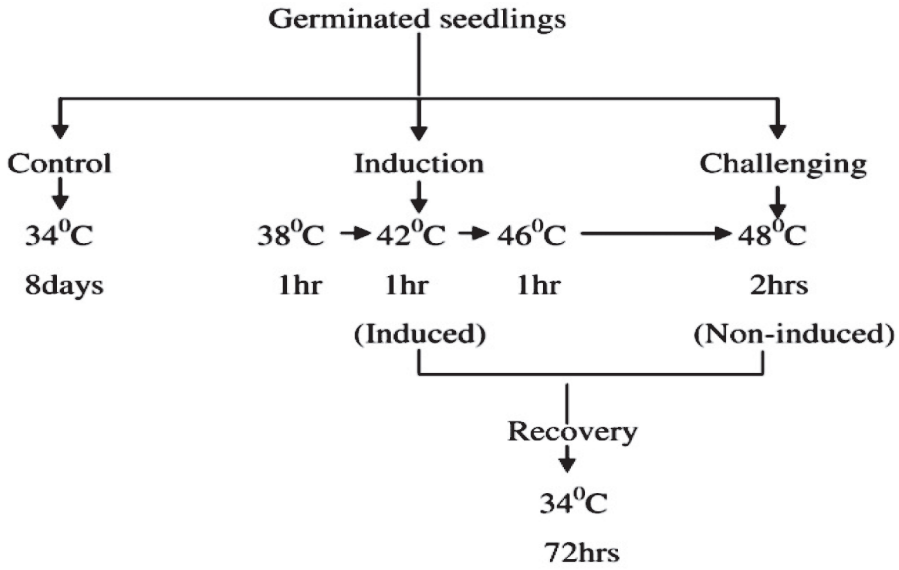
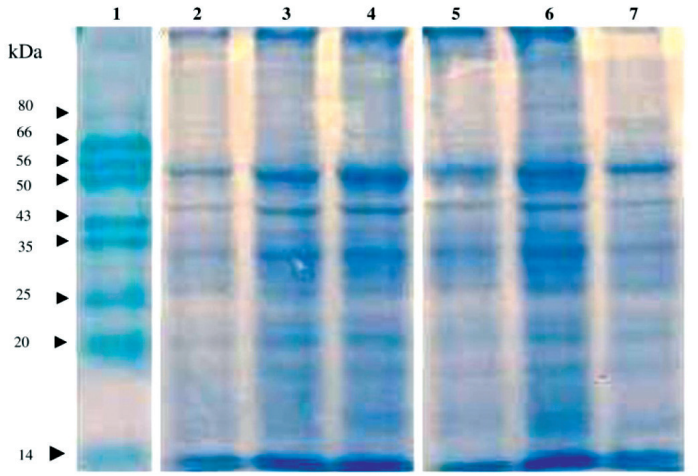


Fig. 1: Line diagram for TIR technique



Lane 1: Mid range marker (14-80 kDa), BioLit-SRL; Lane 2: NDTVR-60 control; Lane 3: NDTVR-60 after induction; Lane 4: NDTVR-60 after challenging; Lane 5: GT control; Lane 6: GT after induction; Lane 7: GT after challenging

Plate 1. SDS-PAGE leaf protein profilling of GT and NDTVR-60 genotype of tomato.

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