SCREENING OF BLACK GRAM [*VIGNA MUNGO* (L.) HEPPER] GENOTYPES WITH ACQUIRED THERMO-TOLERANCE USING TEMPERATURE INDUCTION RESPONSE (TIR) TECHNIQUE

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Abstract: High temperature stress in plants reduces crop yield because it negatively affects several plant physiological processes, including photosynthesis, respiration, growth, development and partitioning. Pulses are more sensitive to high temperature stress at reproductive stage. Blackgram is an important source of protein and widely used in daily diet. It has much economic value as about 70% of worlds black gram production comes from India. (Wikipedia). Hence it necessitates the identification and improvement of stress tolerant varieties of black gram with adaptation to local conditions. TIR is a potential tool for identification of heat tolerant varieties from the large population. This technique involves standardization of lethal and sub lethal temperatures based on percent growth reduction and percent survival at the end of recovery. PU-31(c) is used for standardization of lethal and sub lethal temperatures. The induction temperature range was standardized as 32 to 48 °C (with 2°C rise, at 30mins for each temperature) and followed by lethal temperature at 52 °C for 1 hour. A total of 14 blackgram genotypes were screened and evaluated for thermo tolerance, using standardized optimum induction and challenging temperature. Black gram seedlings exposed to induction temperature prior to challenging temperature exhibited higher recovery growth compared to seedlings which were exposed directly to challenging temperature. Based on root length and shoot length of induced seedlings over control seedlings, the cellular level tolerance in terms of least reduction in growth and highest survival percentage was calculated. Among the 14 genotypes LBG-823, PU 31 (c), and LBG-45 has shown more susceptibility to heat stress, where as LBG-806, and LBG-808 has shown highest intrinsic heat tolerance and therefore this can be explored as donor source in breeding program aimed for global warming.

(***Key words***: TIR, thermo-tolerance, lethal temperature)

Introduction:

Black gram [*Vigna mungo* (L.) Hepper] is a tropical leguminous plant, which belongs to the asiatic Vigna species along with V. radiata, V. trilobata, V. aconitifolia, and V. glaberecence. It has high nutritive value and consists of high content of proteins, vitamins, and minerals. *V. mungo* forms one of the important constituents in the dietary practices of the local communities. It is cultivated as short duration fallow crop after rice cultivation in India (Manasi Dash,and Dhara Shree, 2013). India ranks first in the world in terms of pulses production (25.5% of total world's production) (FAOSTAT, 2014).But pulses are very sensitive to drought, water logging and high temperature. Recently, high temperature is implicated as a major limiting factor for yield decline particularly when flowering and anthesis coincides with temperature rise (Onoriode

Coast *et al*., 2014). Development of suitable varieties and genotypes ofblack gram with adaptation to local agro-climatic conditions is an important factor for its increase and improved production (Manasi Dash,and Dhara Shree, 2013).

Temperature induction response is an efficient screening technique to identify thermo-tolerant lines from large populations. This approach involves identification of lethal and sub lethal temperatures prior to screening of genotypes for intrinsic heat tolerance. The main principle in induction response technique is to initially expose seedlings to a less severe temperature before they are challenged with severe temperature and subsequently recovery growth is measured. The seedling growth and recovery growth is considered as criteria to arrive at optimum acclimatization stress levels (Senthil Kumar *et al*., 2006). Thermo-protection on exposure to acclimatization treatment was also observed not only in seedlings but also in mature plant level (Vijayalakshmi *et al*., 2015). They also reported that acclimated plants showed significantly higher leaf recovery growth compared to plants that were directly exposed to challenging temperature. The phenomenon of adapting to designated severe stress following a mild stress is known as acquired thermo-tolerance (Vierling, 1991). Similar protocol for identification of heat tolerant varieties was reported in Pea (Srikanthbabu *et al*., 2002), Sunflower (Sentil kumar *et al*., 2003), Rice (Vijayalakshmi *et al*., 2015; P.Bhoominathan *et al*., 2014; Renukadevi *et al*., 2013), Ragi (B.Sujatha *et al*., 2018; Venkateshbabu *et al*., 2013), tomato (Chandola *et al*., 2016), banana (S.M. Vidya *et al*., 2016), sugarcane (Gomati *et al*., 2014). Therefore, evaluating the relative performance of blackgram genotypes for high temperature tolerance using TIR technique is the main objective.

Materials and Methods:

Temperature Induction Response (TIR) technique involves series of events like, aseptic germination of seeds, identificationof challenging temperature (lethal temperature) and induction temperature and subjecting all the germplasm to TIR procedure in order to assay heat tolerant lines. All these experiment was conducted at the Department of Botany, Andhra University, in Visakhapatnam. Fourteen black gram genotypes were used for the study and screened for thermo-tolerance, of which were collected from

Agricultural (Millets) Research Station, Vizianagaram. PU-31 (c) was used for standardization of this technique. Three replications of 20 seedlings were examined for each experiment.

Aseptic germination of seeds:

Black gram seeds were aseptically germinated by treating them with 5% Bavistin (carbendizum) along with few drops of tween 20 for 1hr followed by soaking them in sterile distilled water overnight. Such sterile soaked seeds were germinated in sterilized germination medium containing soil + vermicompost + vermiculite in 2:1:1 ratio under ambient conditions.

Identification of lethal temperature (Challenging temperature):

To identify the lethal temperature for Temperature Induction Response Technique, aseptically germinated two day old black gram seedlings PU-31(c) were exposed to different challenging temperatures such as 50, 51, 52 and 53 °C for varying durations i.e., 1hr, 2hrs and 3hrs at each temperature respectively, in the temperature controlled heat chamber. After the heat treatment, the seedlings were allowed to recover at 30 °C with 60% relative humidity for 72hrs in the same chamber.

At the end of recovery period, reduction in growth and percentage of mortality of the seedlings was measured to arrive at the lethal temperature. The minimum temperature at which 90% reduction in growth and 90% mortality of seedlings occurs was taken as lethal or challenging temperature. Results were tabulated (table 1) to identify lethal temperature by the following formula

$$\text{Percentage mortality of seedlings} = \frac{\text{No. Of seedlings died after recovery}}{\text{Total no. Of seedlings sown in the tray}} \times 100$$

Standardization of optimum induction temperature for TIR:

For identification and standardization of induction temperature, aseptically germinated two days old black gram seedlings, PU 31 (c) were exposed to different temperature ranges viz., 28-44 ^{0}C, 30-46^{0}C, 32-48 ^{0}C and 34-50 ^{0}C with gradually increment at the rate of 2 °C per 30mins time interval, following which they were transferred to a standardized lethal temperature i.e., 52 °C for 1hr. Subsequently seedlings are

allowed to recover at 30 °C at 60% relative humidity for 72hrs. After recovery, observations on seedling survival percent, and reduction in growth (shoot length and root length) were measured (table 2) for assessing the seedling growth.

Temperature induction response (TIR):

Aseptically germinated two days old uniform seedlings from each genotype were experimented in three sets (in triplicate for each set). One set of seedlings were exposed to identified lethal temperature (non induced) i.e., 52 °C for 1hr, without prior induction. Another set of seedlings were subjected to standardized induction temperature 32-48 °C (rise in 2 °C for 30mins) followed by lethal temperature i.e., 52 °C for 1hr, subsequently kept for recovery at 30 °C and 60% R.H for 72hrs. And the remaining set which were maintained at 30 °C and 60% Relative humidity through out the experiment, were taken as absolute control.

Measurement of cellular level tolerance using TIR:

Using standardized optimum induction and lethal temperature, the cellular level tolerance was observed in 14 genotypes of black gram. Based on root length and shoot length of induced over control seedlings, the cellular level tolerance in terms of reduction in growth and survival percentage was calculated.

$$\text{(a) Percentage survival of seedlings} = \frac{\text{No. Of seedlings survived after recovery}}{\text{Total no. Of seedlings sown in the tray}} \times 100$$

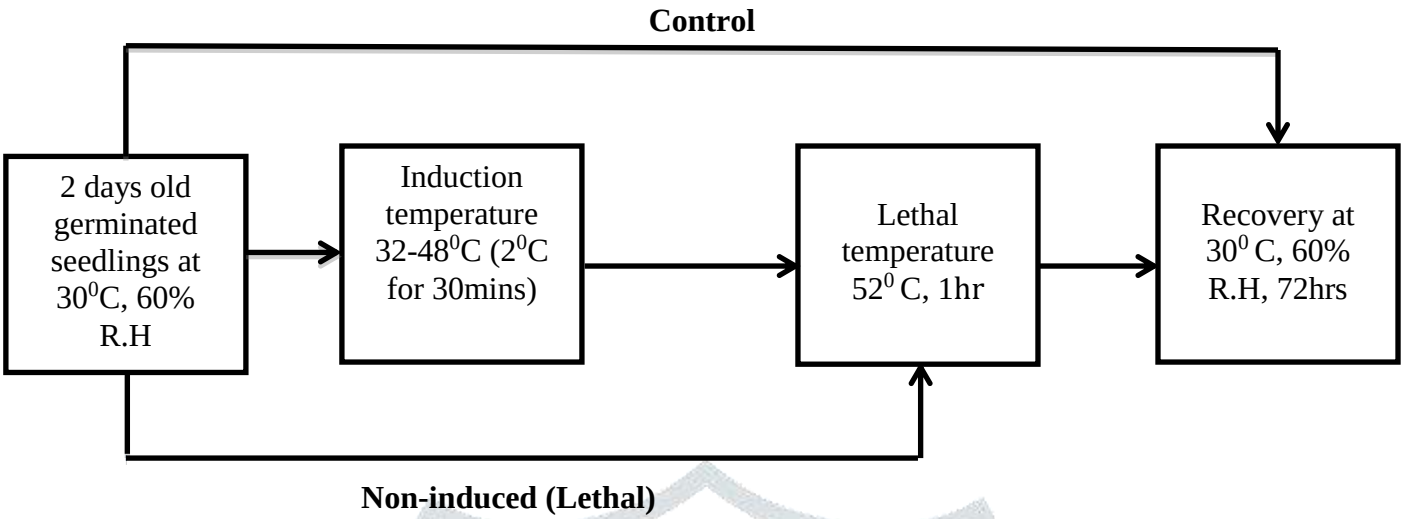
(b) Percent reduction in root growth:

$$\text{Percentage reduction in root growth} = \frac{\text{Actual root growth of Control} - \text{Actual root growth of induced}}{\text{Actual root growth of control seedling}} \times 100$$

(c) Percent reduction in shoot growth:

$$\text{Percentage reduction of Shoot growth} = \frac{\text{Actual shoot growth of Control} - \text{Actual shoot growth of induced}}{\text{Actual shoot growth of control seedling}} \times 100$$

Temperature induction response:



RESULTS & DISCUSSION:

Identification of lethal temperature:

For identification of lethal temperature, 2 days old Black gram seedlings PU-31(C) were directly exposed to different temperature regimes (50,51,52 & 53 ^{0}C) for varying durations (1,2, & 3hrs) with subsequent recovery temperature at 30 ^{0}C, 60% R.H for 72 hrs.In the (table 1) percent mortality & reduction in growth were recorded. The lethal temperature was identified as 52^{0}C for 1hr, where 90% mortality & 90% reduction in growth was observed.

Observations revealed that as the temperature increase with same duration the percent mortality & reduction in growth were increased. For instance, for 1hr duration percent mortality was 50,65,90 & 100 to 50, 51,52 & 53^{0}C and percent reduction in growth was 0,60,90,100 to 50,51,52 & 53^{0}C respectively.Increase in temperature or increase in duration affects the percent mortality & reduction in growth to increase. The lethal temperature for previous findings, in Rice 54^{0}C, 3hrs (Vijayalaxmi *et.al*., 2015), Ragi 57^{0}C, 2hrs (Venkateshbabu *et.al*., 2013), Ground nut 55^{0}C, 3hrs (Gangappa et.al., 2006), Pea 48^{0}C, 1 hr (Srikanthbabu et.al., 2002), in cotton 47^{0}C ,3 hrs(Ehab Abou Kheir et al., 2012), were recorded.

Table 1: percent reduction in growth and mortality of black gram seedlings at different lethal temperatures

Temperature (^{0} C)	Reduction in growth (1hr)	% Mortality	Reduction in growth (2hr)	% Mortality	Reduction in growth (3hr)	% Mortality
50	50±0.77	0±0.00	69±0.98	64±0.53	77±0.44	75±0.98

51	65±0.38	60±0.44	72±0.45	76±0.81	83±1.16	80±0.77
52	90±0.91*	90±1.51*	100±0.00	100±0.00	100±0.00	100±0.00
53	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Mean±SD followed by * in a row are statistically significant p≤0.01 and p≤0.05.

Standardization of induction (sub-lethal) temperature:

Optimum sub lethal temperature range was assessed by subjecting 2 days old Black gram seedlings PU-31(C) to different induction cycles & subsequently exposing them to standardized lethal temperature (52^0C, 1hr) followed by recovery temperature at 30^0C, 60% RH for 72 hrs.Among the induction cycles 32-48^0C (rise in 2^0C for 30mins) was identified as optimum induction range with high percent survival (70%) & low reduction in growth (60%) over absolute control. experimental data was showed in (table 2).

Table 2: Percent survival and reduction in growth at different induction cycles

Temperature range (2 ^{0} C rise for 30mins)	% Reduction in growth	% Survival after recovery
28 – 44^0 C	89±0.78	64±1.04
30 – 46^0 C	84±0.91	66±1.28
32 – 48 ^{0} C	60±0.45*	70±0.94*
34 – 50^0 C	90±0.88	64±0.68

Mean±SD followed by * in a row are statistically significant p≤0.01 and p≤0.05.

Induction temperature for different crops, like Soyabean 34-42^0C for 3hrs @ 2^0C per hr by Uwimana. Marie Ange *et.al*., 2016, Rice (38-48^0C for 3hrs @0.05^0C min^{-1}) by Vijayalakshmi*et.al*., 2015 and(36-44^0C for 3 hrs) by Sapna Harihar et al., 2014, Groundnut (35-45^0C for 3hrs @ 5^0C per hr) Gangappa *et.al*., 2006 has been standardized. It has been observed that genetic variability is only seen upon induction treatment prior to severe heat stress (Krishnan *et.al*., 1989 ; Uma *et.al*., 1995 ; Jaya Prakash *et.al.*, 1998; Kumar *et.al*., 1999; SrikanthBabu *et.al*., 2002).Hence it is the prerequisite to expose the Black gram seedlings to induction temperature before being exposed to lethal temperature.

Temperature Induction Response (TIR):

Following standardization of lethal & sub-lethal induction temperatures the thermo-tolerance of 14 Black gram genotypes has been evaluated by using Temperature Induction Response (TIR) technique.

Aseptically germinated 2 days old seedlings from each genotypes were exposed to induction temperature range 32-48^0C (by 2^0C rise for 30mins) & later subjected to lethal temperatures of 52^0C for 1hr

followed by recovery temperature 30^0C & 60% R.H for 72hrs. After recovery growth percent survival, percent reduction of root growth, shoot growth,total length of plant was calculated over control and results were tabulated in (Tables 3,4,5&6) respectively.

Table 3: Percent survival of induced and non-induced after TIR

Variety	% Survival after recovery (non induced)	% Survival after recovery (induced)
LBG 808	92±1.58	100±0.00
LBG 806	94±0.98	100±0.00
LBG 811	0±0.00	86±1.28
LBG 796	30±0.52	66±0.98
LBG 823	20±0.24	64±1.04
LBG 827	30±0.72	80±1.48
GBG 12	40±0.68	84±1.32
GBG 45	0±0.00	68±1.18
GBG 47	0±0.00	72±0.86
LBG 752	60±1.02	90±1.68
PU 31(c)	10±0.38	70±0.76
PBG 32-1	50±0.88	92±0.94
PBG 32-2	10±0.42	46±0.82
Local variety	70±1.06	98±1.22

Mean±SD followed by * in a row are statistically significant p≤0.01 and p≤0.05.

Table 4: Percent reduction in root growth of induced and non-induced over control after TIR

Variety	Control	Induced		Non induced	
	Root length (cm)	Root length (cm)	% Reduction in root length over control	Root length (cm)	% Reduction in root length over control
LBG 808	2.56±0.24	4.13±0.81	- 61.32±2.10	0.60±0.08	76.56±2.89
LBG 806	2.20±0.81	3.33±0.55	- 51.36±0.98	1.00±0.15	54.54±1.66*
LBG 811	3.73±0.09	4.26±0.93	- 14.09±0.88	0.30±0.02	91.95±3.23
LBG 796	3.26±0.61	3.00±0.36	07.97±0.76	0.40±0.07	87.34±3.06
LBG 823	3.90±0.55	1.50±0.11	61.53±1.01	0.70±0.10	75.86±2.44
LBG 827	3.10±0.42	3.00±0.64	01.29±0.05	0.90±0.17	70.96±2.13
GBG 12	3.40±0.89	3.06±0.42	10.00±0.69	0.30±0.03	91.17±3.65
GBG 45	2.80±0.32	2.30±0.71	17.85±1.05	0.70±0.09	75.00±3.11
GBG 47	3.10±0.88	3.10±0.54	00.00±0.00	0.20±0.01	93.54±2.89
LBG 752 (c)	3.76±0.91	2.80±0.32	24.32±0.99	0.30±0.02	92.02±2.02
PU 31 (c)	3.90±0.76	1.31±0.09	66.41±1.20	0.10±0.01	97.43±3.78
PBG 32-1	3.60±0.09	2.80±0.14	22.22±1.05	0.40±0.05	88.76±3.12
PBG 32-2	3.80±0.65	2.56±0.26	34.21±1.10	1.40±0.56	63.15±1.98
Local variety	4.30±0.95	2.14±0.05	51.16±2.24	0.30±0.08	93.02±2.95

Mean±SD followed by * in a row are statistically significant p≤0.01 and p≤0.05.

Table 5: Percent reduction in shoot growth of induced and non-induced over control after TIR

Variety	Control	Induced		Non induced	
	Shoot length (cm)	Shoot length (cm)	% Reduction in shoot length	Shoot length (cm)	% Reduction in shoot length

			over control		over control
LBG 808	10.93±0.78	07.77±0.54	28.91±1.08	01.00±0.11	90.85±3.11
LBG 806	09.70±0.55	07.43±0.85	23.40±0.98*	01.65±0.19	82.98±2.42*
LBG 811	10.20±0.96	07.80±0.62	23.52±0.99*	01.20±0.22	87.62±2.60
LBG 796	10.20±1.01	06.06±0.73	40.58±2.05	0.90±0.08	91.17±3.77
LBG 823	11.30±0.99	04.20±0.21	62.83±2.47	01.40±0.10	90.78±2.89
LBG 827	10.40±0.67	05.76±0.33	44.61±1.72	01.10±0.21	89.42±1.98
GBG 12	09.13±0.71	06.93±0.43	24.06±0.97	01.50±0.32	83.57±1.50
GBG 45	10.20±1.11	04.85±0.26	52.45±1.42	01.00±0.09	90.85±2.79
GBG 47	09.30±1.03	06.20±0.56	33.33±1.09	01.00±0.12	89.24±2.56
LBG 752 (c)	10.00±1.22	06.90±0.81	31.05±0.98	0.90±0.30	91.38±3.06
PU 31 (c)	10.10±1.35	04.20±0.12	58.41±3.05	1.40±0.03	87.21±3.22
PBG 32-1	10.60±0.82	06.90±0.44	34.90±0.77	01.50±0.16	85.84±2.41
PBG 32-2	10.60±0.97	07.00±0.91	33.96±0.81	01.50±0.09	85.84±3.06
Local variety	08.60±0.66	05.40±0.25	37.20±1.01	01.70±0.23	80.23±1.88*

Mean±SD followed by * in a row are statistically significant p≤0.01 and p≤0.05.

Table 6: Calculating Variations in thermo-tolerance by measuring total plant length of induced and non induced over control after TIR

Variety	Control	Induced		Non induced	
	Total plant length (cm)	Total plant length (cm)	% Reduction in Total plant length (cm) over control	Total plant length (cm)	% Reduction in Total plant length (cm) over control
LBG 808	13.49±1.09	11.90±0.66	11.78±0.56*	01.60±0.22	88.13±2.01
LBG 806	11.90±0.89	10.76±0.75	09.57±0.15*	02.65±0.35	77.73±2.31*
LBG 811	13.93±0.77	12.06±1.00	13.42±0.43*	01.50±0.18	88.83±1.88
LBG 796	13.46±0.81	09.06±0.47	28.67±0.60	01.50±0.21	88.85±2.50
LBG 823	15.20±1.11	05.70±0.22	62.50±0.58*	01.40±0.09	90.78±2.21
LBG 827	13.50±1.02	08.76±0.71	35.11±0.33	02.00±0.42	85.15±3.01
GBG 12	12.53±0.87	09.99±0.89	20.27±0.78	01.80±0.38	89.41±1.89
GBG 45	13.00±0.92	07.15±0.91	45.00±2.01	01.70±0.22	86.92±2.66
GBG 47	12.40±0.69	09.30±0.74	25.00±1.05	01.20±0.08	90.32±3.09
LBG 752 (c)	13.70±1.31	09.70±0.29	29.19±1.23	01.40±0.10	89.78±2.20
PU 31 (c)	14.00±1.82	05.51±0.30	60.64±2.44*	01.40±0.05	90.02±3.11
PBG 32-1	14.20±0.99	09.70±0.88	31.69±0.82	01.90±0.15	86.61±2.56
PBG 32-2	14.40±0.90	09.50±1.01	34.02±0.94	02.90±0.36	79.86±2.21
Local variety	12.90±0.50	07.50±0.55	41.86±1.09	02.00±0.20	84.49±1.90

Mean±SD followed by * in a row are statistically significant p≤0.01 and p≤0.05.

Black gram seedlings exposed to induction temperature prior to challenging temperature showed significant increase in recovery growth & percent survival compared to seedlings, which are directly exposed to challenging temperature. Recovery growth in non-induced ranges from 0-94 where as in induced it is 46- 100%. For root percent reduction over control in induced is highest in LBG 823 & PU-31(c) (61.53; 66.41), negative reduction seen in LBG 808, LBG 806, LBG 811 (-61.32; -51.36; -14.06) respectively.For

shoot, percent reduction in induced over control is highest in LBG 823, LBG 45 & PU-31 (c) (62.83; 52.45; 58.41), lowest in LBG 806, LBG 811 & GBG 12 (23.40; 23.52; 24.06) respectively. Percent reduction of total plant growth over control is highest in LBG 823 (62.5%) & lowest in LBG 806 (9.57). Hence, LBG 823 is considered as susceptible with highest reduction growth (62.5%) & least percent survival (64%), LBG 806 is considered as resistant with least reduction growth 9.57% & highest percent survival (100%).

Differential expression of stress responsive mechanisms explains the variations of stress responses in plants. Seedlings exposed to induction followed by challenging temperature maintained cell viability than the directly exposed seedlings (Sentilkumar., 2004). Upon acclimation during induction, reactive oxygen species in the form of enzymes superoxide dismutase (SOD), Peroxidase (POX) & Catalase (CAT) activities increased in Rice (Vijayalakshmi et.al., 2015).Enhanced expression of Hsps during induction period confers plants to withstand to the severe high temperature stress in Pea (SriKanth Babu *et.al*., 2002).

CONCLUSION:

Plants adopt morphological, anatomical, physiological, biochemical & molecular strategies for severe abiotic conditions. Induction temperature (TIR) elevates these mechanisms & makes the plant acclimatize to heat stress.

Differential expression due to genetic variability confers the plants resistance to severe heat stress. Hence, TIR is a potential tool for screening of resistant genotypes from large germplasm.

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