Comparative Analysis of Protein and SDS-PAGE of two Black gram genotypes under TIR (Temperature Induction Response) Technique

Prof. B. Sujatha[1], Y. Vijaya Bharathi[2] & P. Sirisha[3]
[1]Head of the Department Botany, Andhra University, Visakhapatnam, Andhra Pradesh (state), India
[2]Lecturer in Botany, Government Degree College, Salur, Vizianagaram (Dist), Andhra Pradesh (state), India
[3]Jr. Lecturer in Botany, Government Junior College, Gurla, Vizianagaram (Dist), Andhra Pradesh (state), India

Received: February 13, 2019 Accepted: March 20, 2019

ABSTRACT: *To understand the genotypic variability of two Black gram genotypes LBG-806 (resistant variety) and LBG-823 (sensitive variety) to acquired thermo-tolerance under different temperature treatments (TIR technique), we analyzed total protein content and protein banding profile at SDS-PAGE. During induction treatment resistant variety exhibited enhanced total protein content (35.65%), whereas susceptible variety showed reduction (2.5%) over control. In Non-induction treatment both resistant and susceptible varieties showed reduction in protein content i.e. 80.90% and 75.26% respectively. In SDS-PAGE profiling, resistant variety showed presence of additional protein band (45kDa and 65kDa) and appearance of very thick bands between 10kDa and 21kDa; 30kDa and 45kDa in induction treatment. This study highlights the importance of induction treatment for developing thermo-tolerance through the expression of heat related proteins.*

Key Words: *Black gram, TIR, thermo-tolerance, SDS-PAGE.*

I. INTRODUCTION:

Black gram (*Vigna mungo)* is one of the most highly prized pulses of India. It is valued not only for its dense mixture of nutrients but also its easily cultivable nature, but high temperature during its growing season is one of the major factors that limit its productivity. To alleviate this threat, it is necessary to screen thermo-tolerant lines that can be used as new source of donors in breeding program. Tolerance limits vary between genotypes, but are also subject to acclimation (Weis et al.,1988). TIR (Temperature Induction Response) is a potential and versatile one for identifying highly thermo-tolerant genotype from a large population (Srikantbabu et al., 2002)

Due to induction treatment through TIR technique, *Gossypium hirsutum (H-28*) exhibited increased cell viability and protein synthesis capacity (Kheir Ehab Abou et al., 2012). In sugarcane, induced settlings and calli showed higher soluble protein content than non-induced (Gomati et al., 2014). Wheat plants respond to high temperature stress by the synthesis of an assortment of heat shock proteins that have been correlated with an acquired thermal tolerance or lethal temperatures (Krishnan, M., 1989).

Results in sunflowers hybrids indicated acclimation was mainly due to enhanced expression of HSP 104 and HSP 90 in resistant hybrid IHM 318 (Sentil-Kumar et al., 2007). HSP104 mutant in Saccharomyces cerevisiae failed to acquire thermotolerance at pre-heat treatment and died at subsequent high temperature (Yolands Sanchez and Susan L Lindquist, 1990). Studies on Arabidopsis revealed HSP 101 plays a pivotal role in heat tolerance (Queitscha et al. 2000).Studies in different plant species indicated that upon acclimation there is significant increase in HSPs such as HSP 18.1, HSP 70, HSP 90 AND HSP 104 both in seedlings as well as in plants that conferred thermotolerance.(Uma et al., 1995, Srikanthbabu et al., 2002, Senthil-Kumar et al., 2003).

In response to heat stress, plants synthesize a variety of HSPs which was observed from SDS-PAGE (Geetika pant et al., 2013). In tomato, inherent tolerant variety NDTVR-60 showed intense protein bands around 18kDa and 20kDa in SDS-PAGE protein profiling, GT variety exhibited acquired tolerance by the presence of protein bands at the same molecular weight only upon induction treatment but not in challenging treatment (Chandola et al., 2016).

The present study, an attempt was made to evaluate genetic variability of stress tolerance of two selected Black gram genotypes under temperature treatments (TIR protocol), using key physiological trait total protein content and its banding profile at SDS-PAGE.

II. MATERIALS AND METHODS:

All the experiments were conducted at the Department of Botany, Andhra University, in Visakhapatnam. Black gram genotypes were collected from Agricultural Research Station, Vizianagaram.

TIR Protocol:

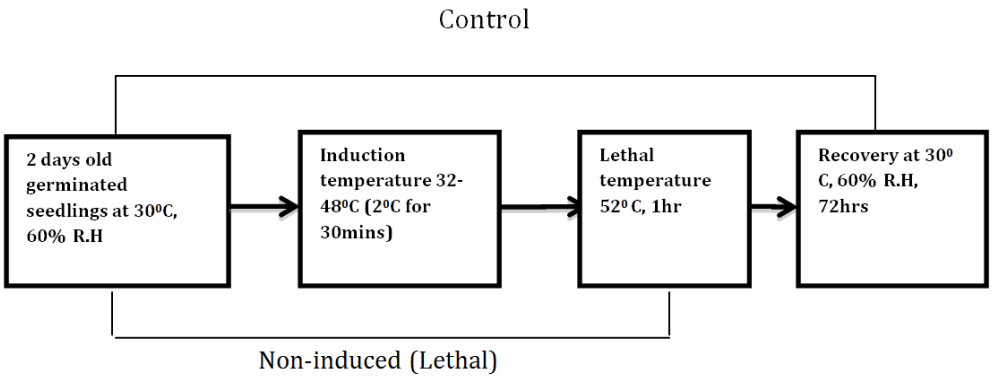

Fig: 1 Standardized temperature induction response (TIR) protocol for Black gram

Standardization of lethal temperature includes exposing the seedlings at different varying temperatures with varying duration. The temperature at where seedling mortality or reduction in growth was high, taken as lethal/challenging temperature. These seedlings were called as Non-induced.

In the next step, seedlings were exposed to different induction temperatures with gradual increment of temperature followed by identified lethal temperature and subsequently to the recovery temperature. The induction range at where the seedlings % survival high/low reduction growth was taken as sub-lethal temperature. These seedlings were called as Induced seedlings.

Finally aseptically germinated 2 days old Black gram seedlings from each genotype were subjected to 3 different treatments i.e., control, induced and non-induced for screening of thermo-tolerant and susceptible varieties

This technique was used to screen thermo-tolerant lines of rice (Vijayalakshmi et al., 2015; Sapna Harihar et al., 2014; Renuka Devi et al., 2013), ragi (Sujatha et al., 2018; Venkatesh Babu et al., 2013), groundnut (Gangappa et al., 2006).

PLANT SAMPLINGS: Black gram temperature resistant variety LBG-806 and susceptible variety LBG-823 were selected based on the previous study through TIR technique (Sujatha et al., 2018).

1. TOTAL PROTEIN CONTENT :

Protein from the plant tissue of different treatments (as per TIR protocol) control, induced, non-induced of resistant (LBG-806) and susceptible(LBG-823) varieties were extracted immediately after the treatment and subsequent days by the method Grimplet et al., 2009 and estimated by the method Lowry et al.,1951. Standard deviation values has been correlated with one way ANOVA in order to signify the results statistically.

2. SDS-PAGE (Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis) Protein Profiling:

Proteins from plant tissue of both varieities were extracted immediately after different treatments as per TIR protocol by the method Grimplet et al., 2009. SDS-PAGE was developed for protein profiling by adopting the method Laemmli U.K. (1970) with minor modifications. LBG-806 (resistant variety) was loaded in lane 1, 2 and 3 i.e. control, induced and non-induced and LBG-823 (susceptible variety) in lane 4, 5 and 6 i.e. control, induced and non-induced respectively.

III. RESULTS & DISCUSSION:

1. TOTAL PROTEIN CONTENT:

In control treatment for both selected varieties, total protein content was gradually increased with increasing days. Total protein content in induction treatment of resistant variety (Fig 2) was decreased from 0th to 1st day (i.e. 7.83%) and after that gradual increase was noticed day by day. In susceptible variety (Fig 3) upon induction treatment protein content was raised only 10.04% to the 1st day, but significant increase i.e. 79.51% was noticed from 1st to 2nd day. After that 2.82% increase from 2nd to 3rd day was observed.

In non-induction treatment protein content of resistant variety (Fig. 2) was decreased from 0th to 1st day (i.e. 13.92%) and gradual raise was observed with increasing days. Whereas in susceptible variety (Fig. 3) gradual increase was observed from 0th to 2nd day, but there was a minor decrease i.e. 0.85% at 3rd day. In

non-induction treatment overall reduction was 7.59% in resistant variety (Fig 2), and 14. 92% improvement in susceptible variety (Fig 3) from 0th day to 3rd day.

When we compare protein content of seedlings of different treatments with control, in resistant variety (Fig 2) in the 0th day it showed 38.17% increment in induced treatment and 78.61% reduction in non-induced treatment. This increase was statistically significant at P value 0.05. In susceptible variety (Fig 3) there was 49.32% and 77.26% reduction in induced and non-induction treatments over control respectively. At the 3rd day of resistant variety (Fig 2) there was 35.65% increment in induced treatment and 80.90% reduction in non-induced treatment. But in susceptible variety (Fig 3) 2.5% and 75.26% reduction in induced and non-induced treatments respectively.

Hsps produced were localized in organelles fractions like nucleus, mitochondria and ribosomes during pretreatment followed by incubation provide thermal protection to subsequent severe temperatures was reported in soybean by Chu-Yung Lin et al., (1984). A review of Bavita Asthir., (2015) about protective mechanisms of heat stress responses stated that heat-shock responses were characterized by repression of normal cellular protein synthesis and induction of heat shock protein synthesis takes place. The co-operative mechanism between Hsp/chaperon play a vital role in cellular homeostasis under plant stress conditions.(Wangxia Wang et al., 2004). Protection of structural proteins, enzymes and membranes and expression of heat shock proteins (HSPs) are some of the biochemical processes that can impart thermo-tolerance.

Due to potential expression of stress related proteins during induction cycle is important to screen and identify heat-tolerant genotypes. . Heat injuries can be mitigated in the induction period by the expression of heat related proteins. So that results in this study suggested that genetic variations in resistant and susceptible plants is due to differential expression of proteins during induction period. And also induced plant of resistant variety can withstand at lethal temperatures successfully than non induced due to thermal stability was developed and as well as structural and functional cell integrity was protected during induction period by the expression of HSPs.

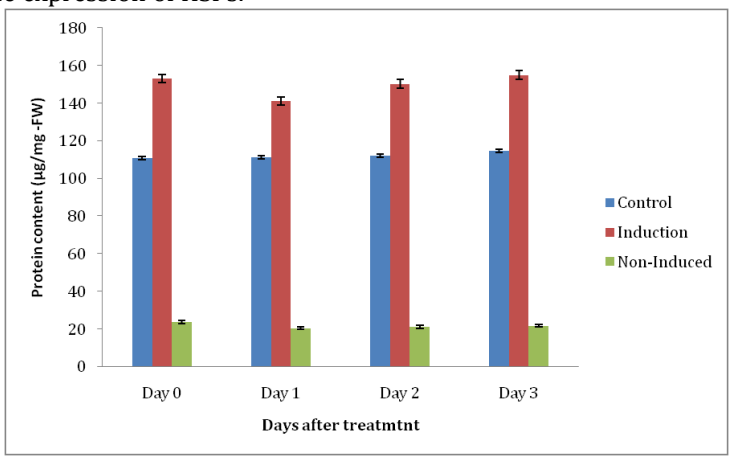

Figure 2: Total protein content of Resistant variety (LBG-806)

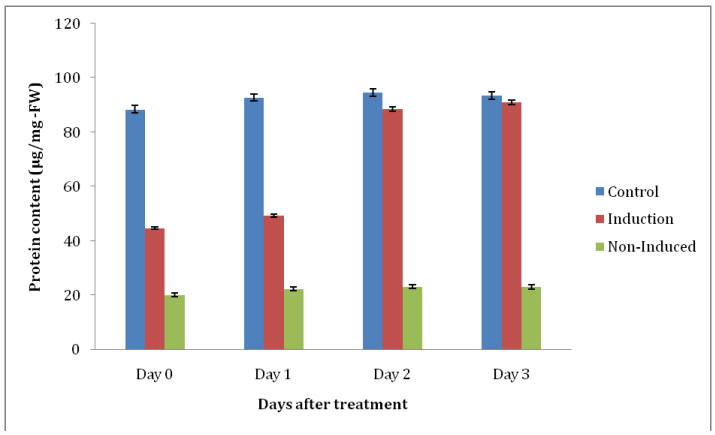

Figure 3: Total protein content of susceptible variety (LBG-823)

2. SDS-PAGE (Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis) Protein Profiling.:

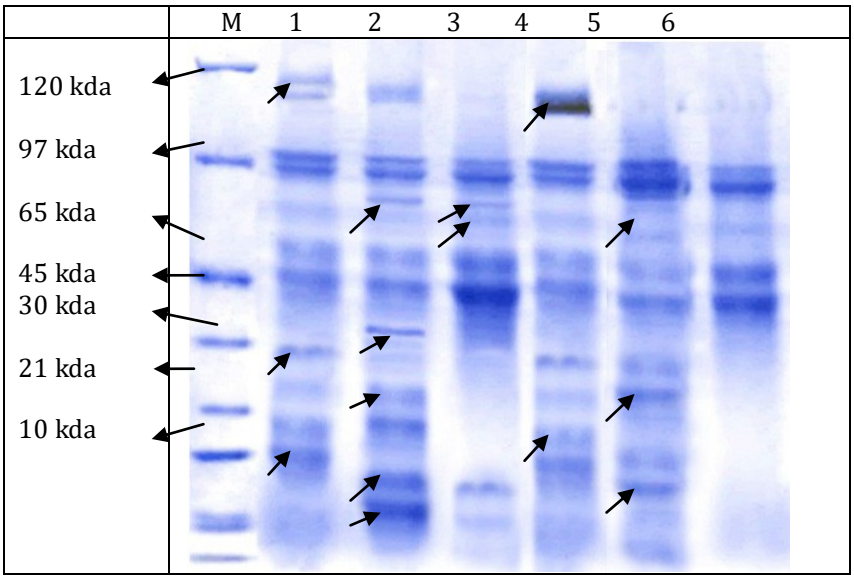

Plate 1: SDS-PAGE Protein profile M=Marker Black gram resistant variety LBG-806 was loaded in line 1=control, 2= induced, 3= non-induced, susceptible variety LBG-823 was loaded in line 4=control, 5=induced, 6= non-induced.

In SDS-PAGE protein profiling (Plate 1) resistant variety, 10kDa band was prominent at lane 2 where it was not clear in lane 1 and 3. Band between 10 to 21kDa was dense in lane 2 and light in lane 3 and it was absent in lane 1. 21kDa band was absent in lane 2&3 where it was present in lane 1. A band between 30 and 45kDa was thick and prominent in lane 2 and it was very light in lane 1 and absent in lane 3. Band between 45kDa and 65kDa was only present in lane 2 when compared with lane 1 and 3. 30kDa band was present in lane 1 &2, absent in lane 3. A band between 65 and 97kDa was present in lane 2 and 3 but not in lane 1. Another second band between 65kDa and 97kDa was present only in lane3 but not in 1&2. A band equal to 120kDa was missing in both lane 2&3 treatments where it was present in lane 1 only. A band below 120kDa was present in lane1 and 2 but absent in lane 3.

In susceptible variety, many bands were similar between lane 4 and lane 5, but an extra band was present between 10kDa and 21kDa; 65kDa and 97kDa in lane 5. Band between 30 and 45Kda was conspicuous in lane 5 than lane 4. A conspicuous 120kDa band was missing in lane 5 which was present in lane 4. There were no extra bands noticed in lane 6.

In resistant variety, presence of unique bands in lane 2 (between 45 and 65; 65 and 97kDa) compared to lane 1&3 might be due to enhanced expression of heat protective genes during induction period, promoted the production of HSPs and enzymatic antioxidants for adaptation of plant to high temperature.

Gradual raise in temperatures in induction treatment eventually associated with stress related proteins to contribute the plant for the maintenance of homeostasis at lethal temperatures. These proteins may act as

1.Enzymatic antioxidants to defend the cell from free radical.

2. HSPs which maintain cell membrane integrity and prevention of protein aggregation and molecular chaperons.

3. Compatible solutes that may induce hormonal changes for plant adaptation to severe temperature.

Total number of bands in lane 3 (non-induced) were very less when compared with lane 1 & 2 (control and induced respectively in resistant variety. There was much decrease in number of bands in lane 6 (non-induced) when compared with lane 4 (control) and lane 5(induced) in susceptible variety. Absence of protein bands in both varieties in non-induction treatment might be due to protein denaturation or DNA damage/mutation for HSP gene under high temperature stress.

In SDS-PAGE protein profile of Mulberry leaf metabolism Chaitanya, K. V et al., (2001) noticed additional proteins (68 and 85kDa) under heat stress compared to control. Significant improvement of total Protein content in the seedlings of induction treatment than control and non-induced was correlated with increase in number of protein bands in SDS-PAGE. The hot 1 mutants for HSP 101 gene of Arabidopsis thaliana unable to acquisition of tolerance to high temperature stress (Hong and Vierling 2000).

IV. CONCLUSION

Our results indicated that significant genetic variation was observed between two genotypes during induction and non-induction treatments. LBG-806 showed vigorous tolerance than LBG-823 upon induction treatment that which can be used in breeding programe.

Acknowledgement:

We are very much thankful to Dr. T.S.S.K. Patro, Sr. Scientist ARS, Vizianagaram, A.P. for providing research material.

REFERENCES:

1. Bavita Asthir (2015). Protective mechanisms of heat tolerance in crop plants. *Journal of Plant Interactions. 10(1): 202-210.*
2. Chaitanya, K. V., Sundar, D., Ramachandra Reddy, A (2001). Mulberry Leaf Metabolism under High Temperature Stress. *Biologia Plantarum. Vol. 44, Issue3, pp 379-384.*
3. Chandola, Ankita, Pandurangam, Vijai and Srivastava, Jai Prakash (2016). Genotypic variations in tomato(Lycopersicon esculetum Mill.) for acquired thermotolerance to temperature induction response. Internat.J.Agric.,Environ.&Biotechnol.,9(4):649-657.
4. CHU-YUNG LIN, ROBERTS, J.K., and JOE L. KEY (1984). Acquisition Of thermotolerance in Soybean Seedlings. SYNTHESIS AND ACCUMULATION OF HEAT SHOCK PROTEINS AND THEIR CELLULAR LOCALIZATION *Plant Physiol. 74, 152-160.*
5. Gangappa,E., Ravi,K. and Kumar,N.Veera(2006). Evaluation of groundnut(Arachis hypogea L.) genotypes for temperature tolerance based on temperature induction response (TIR) technique. Indian J. Genetics,66(2): 127.
6. Geetaika Pant., Hemalatha, S., Arjunan, S., Sudhakar, M. and Sibi, G (2013). Effect of heat stress in synthesis of heat shock protein and antioxidative enzyme response in *Trigonella foenum-graceum* L. *Journal of Plant Sciences* 1(4): 51-56.
7. Gomathi,R., Siyamala,S., Vasantha,S. and Suganya,A. (2014). Optimization of temperature conditions for screening thermotolerance in sugarcane through temperature induction response (TIR) technique. Intenat. J. Sci.,3:5-18.
8. Grimplet, J., Wheatley, M.D., Jouira, H. B, Deluc, L. G., Cramer, G.R., Cushman, J. C. (2009). Proteomic and selected metabolite analysis of grape berry tissues under well-watered and water-deficit stress conditions. *Proteomics 9(9), 2503-2528.*
9. Hong, S.W. & Vierling, E. Mutants of Arabidopsis thaliana defective in the acquisition of tolerance to high temperature stress. Proc. Natl. Acad. Sci. USA 97, 4392-4397
10. Kheir Ehab Abou, Sheshshayee, M.S., Prasad, T.G. and Udayakumar, M. (2012). Temperature induction response as a screening technique for selecting high temperature-tolerant cotton lines. J. Cotton Sci., 16: 190-199.
11. Krishnan, M., Henry T. Nguyen. And John J. Burke (1989). Heat Shock Protein Synthesis and Thermal Tolerance in Wheat. *Plant Physiol. 90, 140-145.*
12. Laemmli,U.K. (1970). Cleavage of structural protein during the assembly of the head of the bacteriophase T_4. *Nature 227: 680-685.*
13. Lowry, O.O., N.J. Rosebrough, A.L. Farr and R.L. Randall (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem*., 193:265-275.
14. Queitscha, C., Hongb, S., Vierlingb E and Lindquista, Susan (2000). Heat shock protein 101 plays a crucial role in thermotolerance in Arabidopsis. *Plant cell. 12(4): 457-60.*
15. Renuka Devi, K., Siva Shankar, A. and Sudhakar, P. (2013). Screening of rice genotypes for thermotolerance by TIR technique. Internat. J. Appl. Biol. & Pharmaceu. Technol., 4(4):505-21
16. Sapna Harihar, Srividya S., Vijayalakshmi, C. and Bhoomiathan, P.(2014). Temperature induction response – A physiological approach to identify thermotolerant genotypes in rice. Internat. J. Agric.Sci.,10:230-232.
17. Sentil-kumar, M., Srikanthbabu, V., Mohan Raju, B., Ganeshkumar, Shivapraksah, N. and Udayakumar, M. (2003). Screening of inbred lines to develop thermotolerant sunflower hybrid using the temperature induction response (TIR) technique: a novel approach by exploiting residual variability. J. Experimental Bot., 54(392): 2569-2578.
18. Senthil-Kumar, M., Kumar, G., Srikanthbabu, V. and Udayakumar, M. 2007. Assessment of variability in acquired thermotolerance: Potential option to study genotypic response and the relevance of stress genes. Journal of Plant Physiology 164: 111-125.
19. Srikanthbabu, V., Kumar, G., Krishnaprasad, B.T., Gopalakrishna, R., Savitha, M. and Udaya Kumar, M. (2002). Identification of pea genotypes with enhanced thermotolerance using temperature induction response technique (TIR). Journal of Plant Physiology 159: 535-545.
20. Sujatha, B., Bharathi, Y. V. and Sirisha, P (2018). Screening of Black Gram [*Vigna mungo (L.)* Hepper] Genotypes with Acquired Thermo-tolerance using Temperature Induction Response (TIR) Technique. *JETIR.*, Vol. 5 (12):

588-598
21. Sujatha, B., Sirisha, P., Bharathi Y.V., (2018). Screening of acquired thermotolerant ragi (*Elusina coracana(L) Gaertn*) genotypes using T.I.R. technique. Internat. J. Plant Sci., 13(1):165-170.
22. Uma, S., Prasad, T.G. and Udayakumar, M. 1995. Genetic variability in recovery growth and synthesis of stress proteins in response to polyethylene glycol and salt stress in Finger millet. Annals of Botany 76: 43-49.
23. Venkatesh Babu, D., Sudhakar, P. and Reddy, Y. Sharat Kumar (2013). Screening of thermotolerant Ragi genotypes at seedling stage using TIR technique. *Bioscan*, 8(4): 1493-1495.
24. Vijayalakshmi, D., Srividya, S., Vivitha, P. and Raveendran, M. (2015). Temperature induction response (TIR) as a rapid screening protocol to dissect the genetic variability in acquired thermotolerance in rice and to identify noval donors for high temperature stress tolerance. Indian. J. Plant Physiol., 20(4):368-374.
25. Wangxia Wang, Basia Vinocur, Oded Shoseyov and Arie Altman (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *TRENDS in Plant Science vol.9. No. 5.*
26. Weis E and Berry JA (1988). Plants and high temperature stress. *Symp Soc Exp Bio. 42: 329-46.*
27. Yolanda Sanchez and Susan L Lindquist, (1990). HSP104 required for induced thermotolerance. *Science 248(4959), 1112-1115.*