A Comparative Analysis of Genetic Variability for Acquired Thermo-tolerance in Two Black gram Varieties using ISSR markers

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ABSTRACT:

Two Black gram varieties LBG-806 (resistant variety) and LBG-823 (sensitive variety) were selected for comparative analysis of molecular responses at different temperature treatments (as per TIR protocol). Temperature Induction treatment promote the plant hardening by acquiring thermo-tolerance through several mechanisms. Both the selected cultivars showed varied banding pattern with ISSR-PCR amplification at different treatments. Results of ISSR-PCR amplification of DNA showed higher number of bands in induction treatment than non-induction treatment both in resistant and susceptible varieties. Two extra bands were observed in resistant variety upon induction treatment, one band at 600-700bp for UBC-807 primer and another band at 700bp for UBC-853 primer. Presence of high intensity bands and extra bands in both varieties in induction treatment indicated that they have been acclimated and hardened at the time of induction temperature, as a result protected even at the time of lethal temperature. It is concluded that marker assisted analysis between resistant and susceptible varieties with different treatments as per TIR protocol provided supportive molecular information for relevance of TIR based screening technique.

Key words: TIR, Black gram, SDS-PAGE, ISSR-PCR.

Abbreviations: TIR – Temperature Induction Response, ISSR - Inter Simple Sequence Repeats, PCR – Polymerase Chain Reaction.

I. Introduction:

In recent years, with the recognition of global climate change, studies on climatic impacts in particular food crops come to forefront of scientific community. High temperature stress is one of the agricultural factors that affect plant growth, development and yield. This necessitates the identification of abiotic stress tolerant genotypes (Blum, 1988; Bray, 1997; Ribaut et al., 2002). The phenomenon of adapting to designated severe stress following a mild stress is known as acquired thermo-tolerance (Vierling, 1991).

Blackgram, *Vigna mungo (L.) Hepper*, popularly known as urdbean is most widely consumed for its nutrients, short life cycle and high productivity. It is important to develop suitable varieties of Black gram with adaptation to local agro-climatic conditions for improvement of production (Dash, M., & Shree, D., 2013). TIR (Temperature Induction Response) is a potential tool for screening of thermo-tolerant lines from large populations (Srikanthbabu, V. et al., 2002). Venkateshbabu, D. et al., 2013 in ragi screened genotypes for thermo-tolerant from 100 genotypes using TIR technique.

Temperature changes are sensed through cellular responses due to signal transduction into the cell; facilitate the plant to thrive under heat stress (Hemantaranjan, A. et al., 2014). Genetic diversity is prerequisite in any hybridization programme. Evaluation of genetic diversity would promote the efficient use of genetic variations in the breeding programme (Paterson et al. 1991).

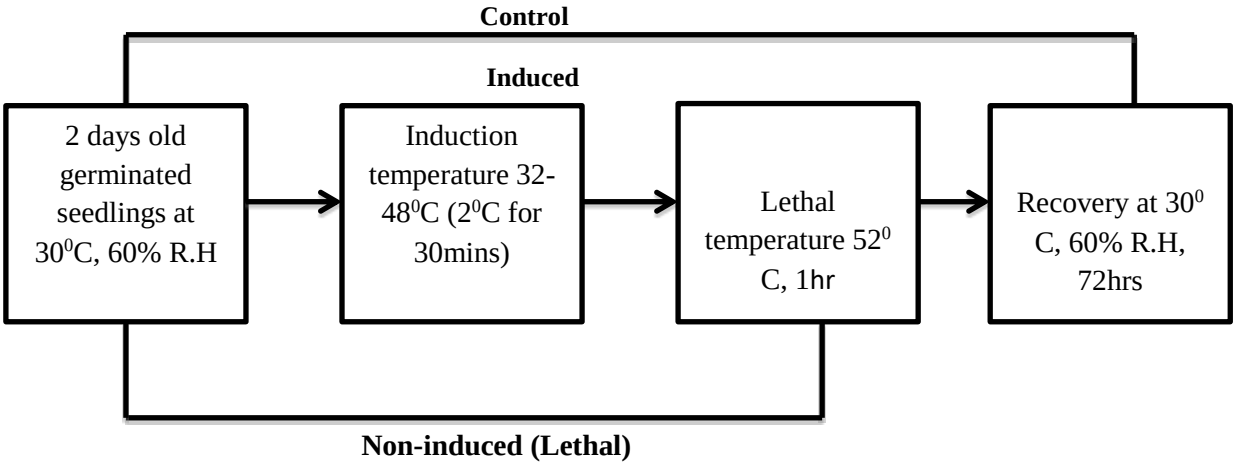
DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann 1983). ISSR markers have been successfully applied for analysis of repeat motifs in green gram (Singh et al. 2013), genetic relationships in the genus Peanut (Raina et al. 2001); Vigna (Ajibade et al. 2000); Oryza (Joshi et al. 2000), varietal identification in black gram (Ranade and Gopalakrishna 2001) and Potato cultivars (Prevost and Wilkinson 1999).
The present study invariably investigates the potentiality of TIR (Temperature Induction Response) technique for screening of temperature tolerant and susceptible variety and identification of genomic level variations in two selected black gram genotypes through ISSR-PCR amplification.

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.

1) **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)

TIR technique involves series of experiments i.e.,, identification of lethal/challenging temperature (by directly exposing the seedlings to severe temperatures), sub-lethal temperatures (by subjecting the seedlings to induction temperature and later on exposing to the identified lethal temperature followed by recovery temperature) and finally screening of thermo-tolerant genotypes from large populations by subjecting aseptically germinated seedlings from each variety to three temperature treatments i.e., control, induced and non-induced.



TIR protocol for identification of thermo-tolerant varieties was reported in pea (Srikanthbabu et al., 2002), sunflower (Sentil kumar et al., 2003), Rice (Vijayalakshmi et al., 2015), tomato (chandola et al., 2016), banana (Vidya et al., 2016), sugarcane (Gomati et al., 2014).

2) Analysis of molecular changes at high temperature stress by ISSR:

a. DNA Extraction;

The plant tissues of both resistant (LBG-806) and susceptible (LBG-823) were collected immediately after treatments (as per TIR protocol) and extracted the DNA by Yoon et al., (1991) with minor modifications.

b. ISSR: Table 1 belongs to ISSR primers, used for testing of amplification of DNA at different treatments. For ISSR Amplification profiles of all treated and controls were compared with each other and bands of DNA fragments scored manually depending on the presence or absence of a particular band.

Table 1: List of ISSR Primers

	Primer name	Sequence	GC content	Tm Value
1	UBC-807	AGA GAG AGA GAG AGA GT	47.1	45.0
2	UBC 812	GAG AGA GAG AGA GAG AC	52.9	44.4
3	UBC-813	CTC TCT CTC TCT CTC TT	47.1	43.5
4	UBC-815	CTC TCT CTC TCT CTC TG	52.9	45.0
5	UBC-816	CAC ACA CAC ACA CAC AT	47.1	51.2
6	UBC-818	CAC ACA CAC ACA CAC AG	52.9	52.1
7	UBC-825	ACA CAC ACA CAC ACA CT	47.1	49.3
8	UBC-831	ATA TAT ATA TAT ATA TYA	5.55	20.2
9	UBC-834	AGA GAG AGA GAG AGA GT	50.0	49.8
10	UBC-841	GAG AGA GAG AGA GAG AC	55.5	45.7
11	UBC-844	CTC TCT CTC TCT CTC TRC	55.5	39.4
12	UBC-853	TCT CTC TCT CTC TCT CRT	50.0	54.0
13	UBC-857	ACA CAC ACA CAC ACA CCG	55.5	57.1
14	UBC-867	GGC GGC GGC GGC GGC GGC	100	58.6
15	UBC-869	GTT GTT GTT GTT GTT GTT	33.3	51.0
16	UBC-874	CCC TCC CTC CCT CCCT	75.0	33.0
17	UBC-876	GAT AGA TAG ACA GAC A	37.5	36.4

18	UBC-878	GGA TGG ATG GAT GGAT	50.0	29.0
19	UBC-879	CTT CAC TTC ACT TCA	40.0	42.2
20	UBC-881	GGG TGG GGT GGG GTG	66.6	66.5

III. RESULTS & DISCUSSION:

ISSR analysis indicated among 20 tested primers (Table 1) 3 primers namely UBC-807, UBC-818 and UBC-853 had shown amplification in both selected varieties.

In control treatment of resistant variety presence of extra band between 300-400bp for primer UBC-807; 400-500bp for UBC-818 and in susceptible variety between 500-600bp for UBC-807; 400-500bp for UBC-853 were observed. In resistant variety band between 500-600bp for UBC-853 was absent whereas it was present in other two treatments.

In induction treatment unique highly intense extra band was observed between 600-700bp for UBC-807 in resistant variety (Table 2 and Plate 1) and a band between 400-500bp for UBC-818; a very dense band at 500bp for UBC-818 were observed in susceptible variety (Table 3 and Plate 2). Band intensities at various positions i.e., between 300-400bp for UBC-807; 400-500bp for UBC-818 were high comparative to other treatments in susceptible variety (Table 3 and Plate 2). Total 3 bands (i.e., b700-800bp; at 500bp for UBC-818 and another band between 600-700bp for primer UBC-853) in resistant variety (Table 2 and Plate 1) and 5 bands (i.e at 300bp; between400-500bp; 800-900bp; at 900bp for primer UBC-807 and betwen 700-800bp for primer UBC-818 in susceptible variety (Table 3 and Plate 2) were absent compared to other treatments.

In Non-induction treatment no unique bands present in both varieties. Total 7 bands (i.e., at 700bp for UBC-807; two bands between 600-700bp, one band between 900-1000bp for primer UBC-818; band between 100-200, 300-400,600bp for primer UBC-853) in resistant variety (Table 2 and Plate 1) and 8 bands (i.e., at 200bp, 500bp, 1000bp for primer UBC-807; band between 500-600, 800-900, at 900bp for primer UBC-818; two bands between 400-500bp for primer UBC-853) in susceptible variety (Table 3 and Plate 2) were absent.

Presence of unique bands, high intense bands and low number of bands absent in induction treatment compared to non-induction treatment proved that induction treatment provided protection in both cultivars and the damage was limited. Functional relevance of stress genes at stress tolerance, needs exposure to acclimation treatment (Sentil Kumar, M et al., 2007). ISSRs have been used for cultivar identification in Black gram (Kanimozhi, M. et al., 2009). 18 Black gram genotypes were analyzed for genetic diversity depending on polymorphism using 25 RAPD and 16 ISSR markers by Souframanien, J and Gopala Krishna, T. (2004).

Many workers used RAPD markers with combination of other markers in different ways. Some instances, assessment of genetic diversity using RAPD and ISSR markers was seen in banana by Laxmanan et al., (2007)., RAPD, ISSR and AFLP markers were used in analysis of genetic diversity among *Lupinus spp*. by Talhinhas. P et al., (2003)., RAPD, ISSR, SAMPL and AFLP markers in detection of genetic polymorphism in *Tribulus terrestris* by Sarwat, M., Das, S., & Srivastava, P. S. (2008), comparison between ISSR polymorphism to RFLP and RAPD in wheat by Nagaoka, T and Ogrihara, Y. (1997)., assessment of genetic diversity using RAPD and ISSR markers in *Trigonella caerulea* Dangi et al., (2004).

1	2	3	4	5	6	7	8	9	M	

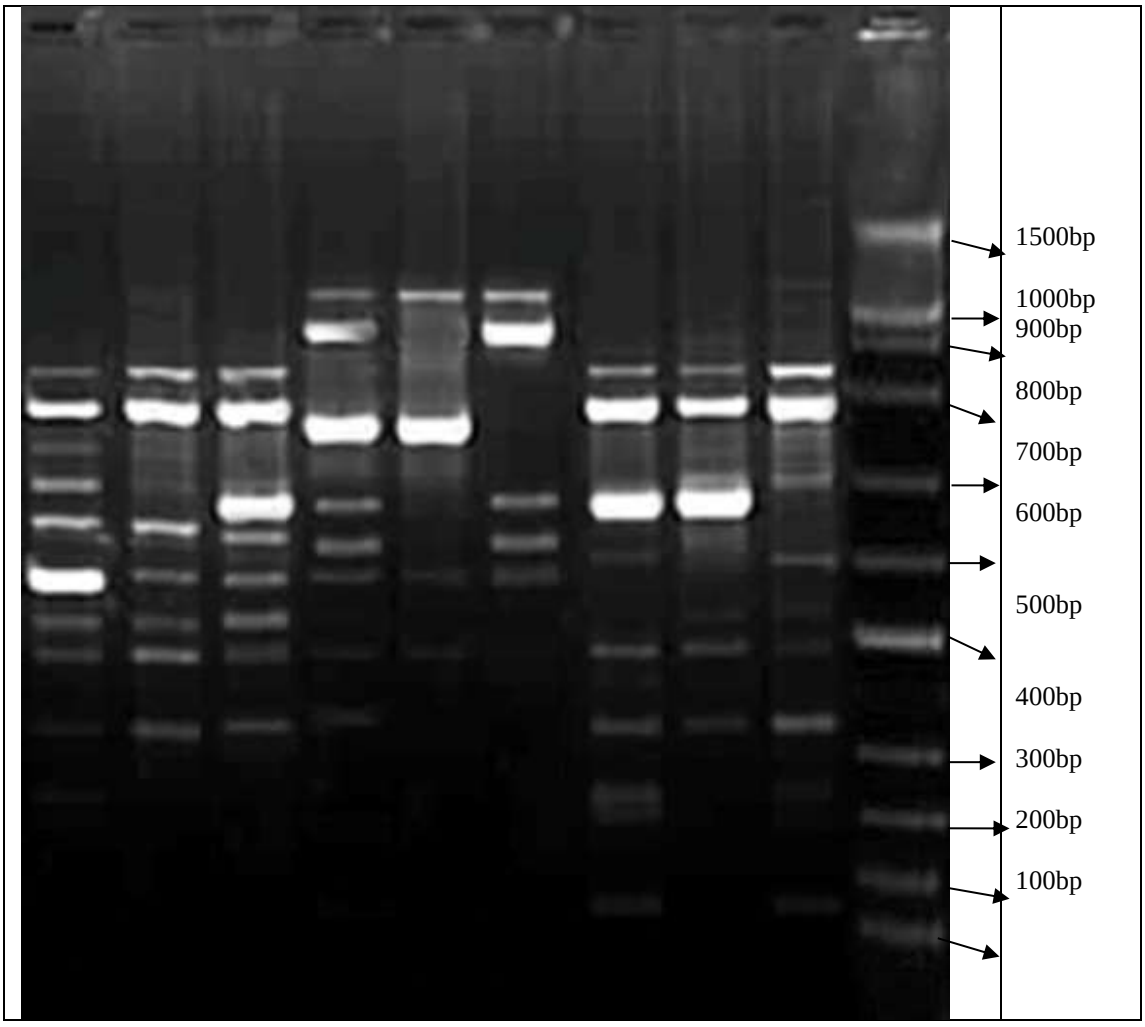


Plate 1: Black gram resistant cultivar (LBG-806) ISSR finger printing agarose gel image. Lane M 100 bp plus marker. Lane 1-UBC- 807 control; Lane 2-UBC- 807 Non-Induced; Lane 3-UBC- 807 Induced; Lane 4- UBC- 818 control; Lane 5-UBC- 818 Non-Induced; Lane 6-UBC- 818 Induced; Lane 7- UBC- 853 control; Lane 8-UBC- 853 Non-Induced; Lane 9-UBC- 853 Induced.

Table 2: Banding pattern in resistant variety (LBG-806) using ISSR-PCR.

Sl.No.	M.(bp)	UBC-807			UBC-818			UBC-853		
		Control (Lane-1)	Non-induced(Lane-2)	Induced (Lane-3)	Contro (Lane-4)	Non-induced(Lane-5)	Induced (Lane-6)	Control (Lane-7)	**Non-induced(Lane-8)**	Induced (Lane-9)
1	1500									
2	1000-1500				++	++	++			
3	900-1000				+++		+++			
4	800-900	+	++	++				+	+	++
5	700-800	+++	+++	+++	+++	+++		+++	+++	+++
6	700	++		+						+
7	600-700			+++	++		++	+++	+++	
		++	++	++	++		++			
8	600	+++	++	++	+	+	+	+		+
9	500-600	++	++	++					+	+
10	500	++	++	++	+	+		++	++	+
11	400-500	+	++	++	+			++	+	++
12	300-400	+						++		+

13	200-300									
14	100-200							+		+

+++ = high intensity band, ++= moderately intense band, + = light intense band. Blank represents no band.

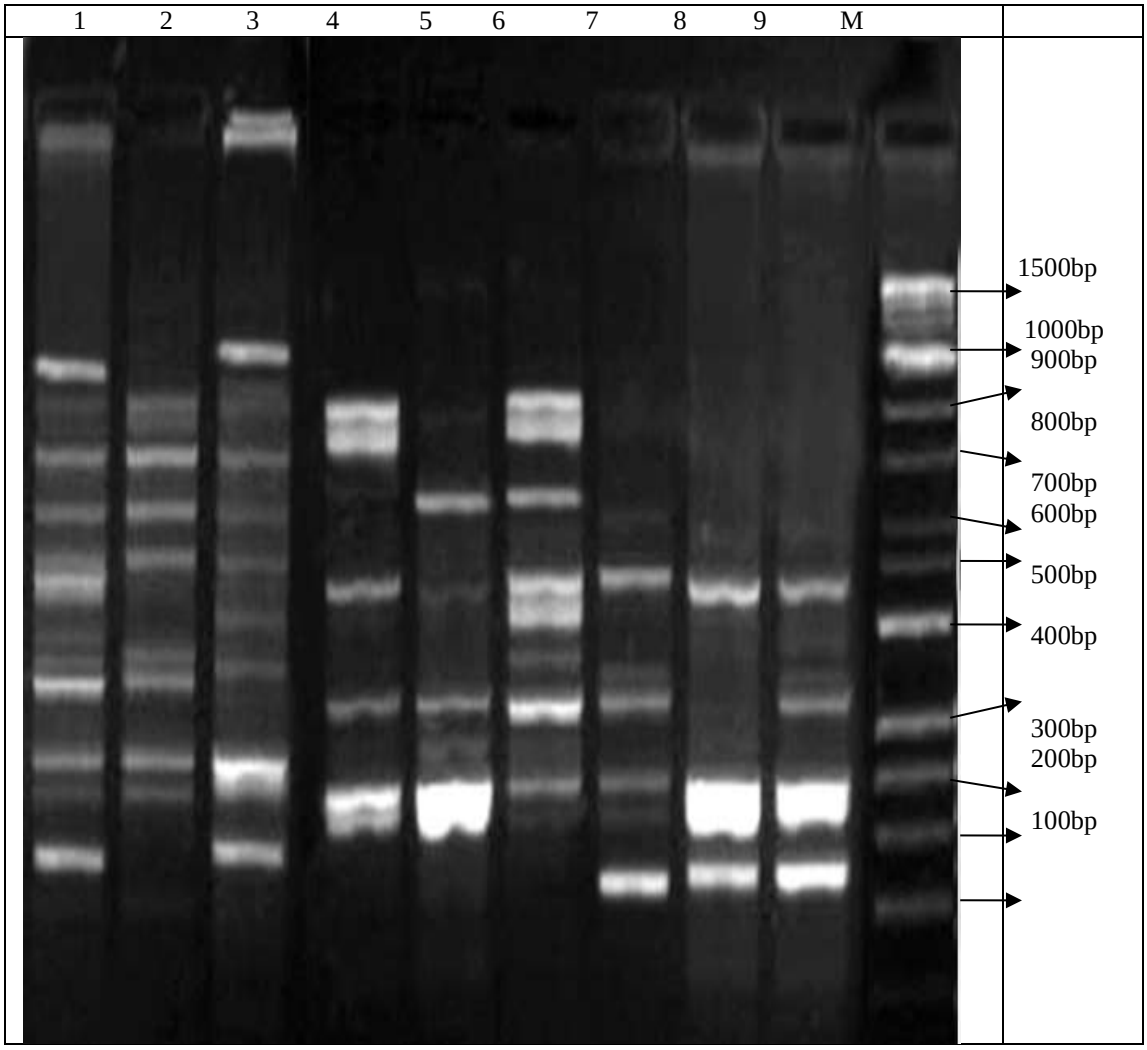


Plate 2: Black gram susceptible cultivar (LBG-823) ISSR finger printing agarose gel image. Lane M 100 bp plus marker. Lane 1-UBC- 807 control; Lane 2-UBC- 807 Non-Induced; Lane 3-UBC- 807 Induced; Lane 4- UBC- 818 control; Lane 5-UBC- 818 Non-Induced; Lane 6-UBC- 818 Induced; Lane 7- UBC- 853 control; Lane 8-UBC- 853 Non-Induced; Lane 9-UBC- 853 Induced.

Table 3: Banding pattern in Susceptible variety (LBG-823) using ISSR-PCR.

Sl.No.	M.(bp)	UBC-807			UBC-818			UBC-853		
		Control (Lane-1)	Non-induced(Lane-2)	Induced (Lane-3)	Contro (Lane-4)	Non-induced(Lane-5)	Induced (Lane-6)	Control (Lane-7)	**Non-induced(Lane-8)**	Induced (Lane-9)
1	1500									
2	1000	+++		+++						
3	900	+	+		+++		+++			
4	800-900	+	+		+++		+++			
5	800	++	++	+						
6	700-800				++	++				

7	700	+	+	+						
8	600	+	+	+						
9	500-600	++			++		+++	++	++	++
10	500	+		+			+++			
11	400-500	+	+				+	+		
		++	++	+				+		+
					++	++	+++	++		++
12	400									
13	300-400	++	++	+++						
14	300	+	++		+++	+++	++	++	+++	+++
15	200	++		++						
16	100-200							+++	+++	+++

+++ = high intensity band, ++= moderately intense band, + = light intense band. Blank represents no band.

IV. CONCLUSION:

ISSR marker assisted assessment coupled with Temperature Induction Response in this research provided basic information of acclimatization during induction treatment. It is the new method for analysis of resistant and susceptible varieties at molecular level. It may be expected that study of variations between resistant and susceptible varieties of black gram with the assistance of ISSR markers can provide precise information for marker assisted selection and breeding.

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