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Assessment of Genetic Variability for Acquired Thermo-Tolerance in Two Black gram Varieties Using RAPD Markers

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Key Points:

- Two Black gram varieties (Tolerant, Susceptible) were selected as per the TIR protocol.
- DNA extracted from both the cultivars after the temperature treatments.
- Extracts were amplified by using RAPD Primers to know the acclimatization with varying temperature treatments.

ABSTRACT

Two Black gram varieties LBG-806 (tolerant variety) and LBG-823 (susceptible variety) were selected for comparative analysis of molecular responses at different temperature treatments (as per TIR protocol). In T.I.R technique, during induction treatment plants acquire thermo-tolerance through several mechanisms. Both the selected cultivars showed varied banding pattern with RAPD-PCR amplification at different temperature treatments. Results of RAPD-PCR amplification, in susceptible variety upon induction treatment showed three extra bands i.e., for OPC-5 primer, a band below 100bp, a band at 200bp, a band between 400-500bp sizes. Presence of high intensity bands and extra bands in both varieties in induction treatment indicated that they have been acclimatized and hardened at the time of induction temperature. It is concluded that this marker assisted analysis provided supportive molecular information for relevance of TIR based screening technique.

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

Abbreviations: TIR – Temperature Induction Response, RAPD - Randomly Amplified Polymorphic DNA, PCR – Polymerase Chain Reaction.

I. Introduction:

The increasing threat of climatological extremes including very high temperatures might lead to catastrophic loss of crop productivity and result in wide spread famine (Bita and Gerats, 2013). Heat stress imposes challenges for legume crops and has deleterious effects on the morphology, physiology, and reproductive growth of plants (Lobell and Asner, 2003). Black gram, *Vigna mungo (L.) Hepper*, popularly known as urd bean is most widely consumed for its nutrients.

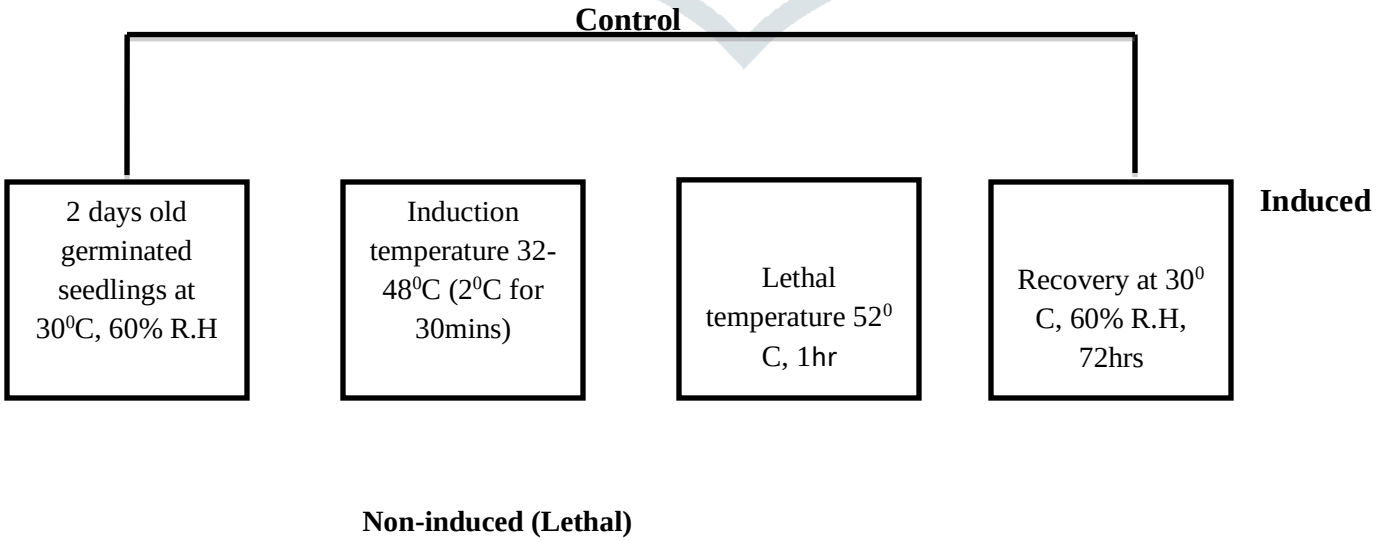
The phenomenon of adapting to designated severe stress following a mild stress is known as acquired thermo-tolerance (Vierling, 1991). TIR (Temperature Induction Response) is a potential tool for screening of thermo-tolerant lines from large populations (Srikanthbabu, V. et al., 2002).

At the molecular level, alterations in the expression of genes in connection with heat stress involved in the protection from high temperature stress (Chinnusamy *et al.,* 2007). DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann 1983). Genetic diversity is a prerequisite in any hybridization program. Evaluation of genetic diversity would promote the efficient use of genetic variations in the breeding program (Paterson *et al.,* 1991).

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 RAPD – PCR.

II. MATERIALS AND METHODS:

1) **PLANT SAMPLINGS:** Black gram temperature tolerant 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 and susceptible genotypes from large populations by subjecting aseptically germinated seedlings from each variety to three temperature treatments i.e. control, induced and non-induced.



2) Analysis of molecular changes at high temperature stress by RAPD

a. DNA Extraction The plant tissues of both tolerant (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. RAPD: Table 1 belongs to RAPD primers, used for testing of DNA amplification at different treatments. RAPD 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.

S.No		
1	OPH-4	GGAAGTCGCC
2	OPD-6	GGGAATTCGG
3	OPD-8	GTGTGCCCCA
4	OPH-19	CTGACCAGCC
5	OPK-19	CACAGGCGGA
6	OPH-20	GGGAGACATC
7	ADG-4	CCCGCCGTTG
8	OPK-4	CCGCCCAAAC
9	OPB-1	GTTTCGCTCC
10	OPA-02	TGCCGAGCTG
11	**OPA-04**	**AATCGGGCTG**
12	**OPA 11**	**CAATCGCCGT**
13	OPA 14	TCTGTGCTGG
14	OPC-05	GATGACCGCC
15	**OPC-09**	**CTCACCGTCC**
16	OPC-14	TGCGTGCTTG
17	**OPC-16**	**CACACTCCAG**
18	OPC-20	ACTTCGCCAC
19	**OPE-17**	**CTACTGCCGT**
20	OPF-02	GAGGATCCCT

Table 1: List of RAPD Primers

III. RESULTS & DISCUSSION:

b. RAPD:

RAPD analysis indicated that among 20 tested primers (Table 1) 3 primers namely OPA-11, OPC-5 AND OPF-2 had shown amplification for tolerant variety. And 2 primers namely OPC-5 AND OPF-2 showed amplification for susceptible variety. In this research for the first time RAPD banding pattern was tabulated according to their intensity or absence.

In control treatment of tolerant variety (Table 2 and Figure 1), unique amplification for primer OPA-11 at 600-700bp and for OPF-2 at 700-800bp was observed. In susceptible variety (Table 3 and Figure 2) amplification for primer OPF-2 at 200,400,500bp was noticed in control. In Induction treatment of susceptible variety (Table 3 and Figure 2) 3 extra bands were observed for OPC-5 i.e., below 100bp, 200bp, 400-500bp size. In non-induction treatment, primer OPC-5 showed unique band at 600-700bp in tolerant variety.

In tolerant variety (Table 2 and Figure 1) total number of bands absent in induction treatment was at 400-500bp size for primer OPF-2, whereas in non-induction treatment it was five (i.e. for primer OPA-11 at 600bp size, for primer OPC-5 at 300-400,500-600,700,800-900bp size). In susceptible variety (Table 3 and Figure 2), total number of bands absent in induction treatment were zero, whereas in non-induction treatment 5 bands (i.e., for primer OPC-5 at less than 100bp, 500bp, 500-600bp, 600-700bp size and for primer OPF-2 at 300bp size) were absent compared to the other treatments.

The absence of normal bands may be related to the events such as DNA damage (such as single and double strand breaks, modified bases, oxidized bases, bulky adducts, DNA protein cross links) or point mutations and/or complex chromosomal rearrangements induced by genotoxins (De Wolf. H. et al., 2004). This damage is high at non-induced treatment as it was directly exposed to lethal temperatures lead to loss of primer binding site. Use of RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity have been used in several plant species, i.e., in barley cultivars (Fernandez, M.E. et al., 2002), ash gourd (Verma, V.K. et al., 2007), ground nut (Dwivedi, S.L. et al., 2001), mung bean (Lakhanpaul, S et al., 2000), chickpea (Sonnante, G et al., 1997).

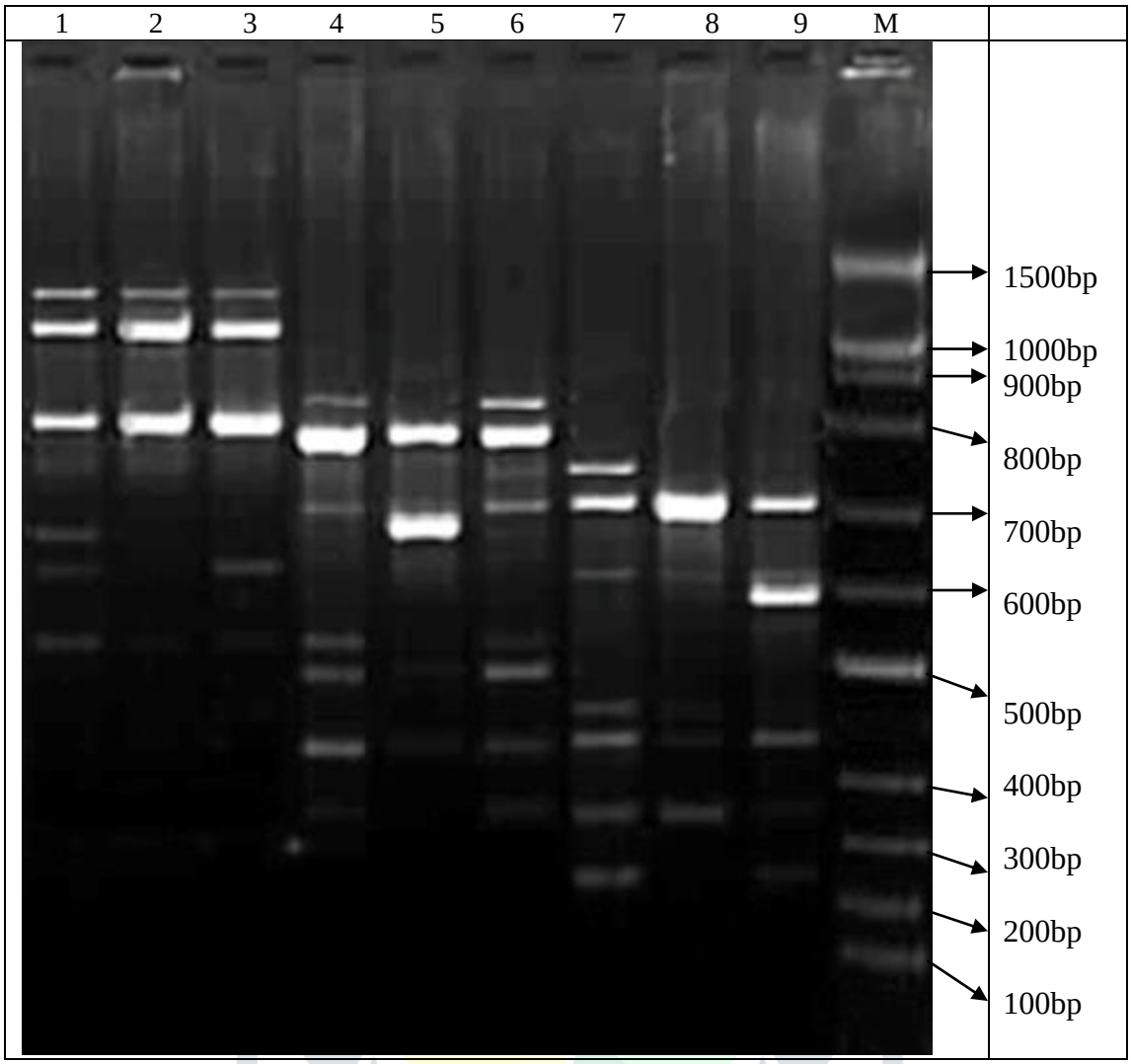


Figure 1: Black gram tolerant cultivar(LBG-806) RAPD finger printing agarose gel image. Lane M 100 bp plus marker. Lane 1- OPA- 11control; Lane 2- OPA- 11 Non-Induced; Lane 3- OPA- 11 induced; Lane 4- OPC-5 control; Lane 5-- OPC-5 Non-Induced; Lane 6-- OPC-5 Induced. Lane 7- OPF-2 control; Lane 8-- OPF-2 Non-Induced; Lane 9-- OPF-2 Induced.

Table 2: Banding pattern in Tolerant variety (LBG-806) using RAPD-PCR.

Sl.No.	M.(bp)	OPA-11			OPC-5			OPF-2		
		Control (Lane-1)	Non-induced (Lane-2)	Induced (Lane-3)	Control (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	1000	++	+++	+++						
4	900									
5	800-900				+		++			
6	800	+++	+++	+++	+++	+++	+++			
7	700-800							++		
8	700				++		++	++	+++	++
9	600-700	++				+++				
10	600	++		++				++	+	+++
11	500-600	++	+	+	++		+			
12	500				++	+	++			
13	400-500							++	+	
14	400-500 lower				++	+	++	++	+	++
15	300-400				+		+	++	++	+
16	300									
17	200-300								++	+

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

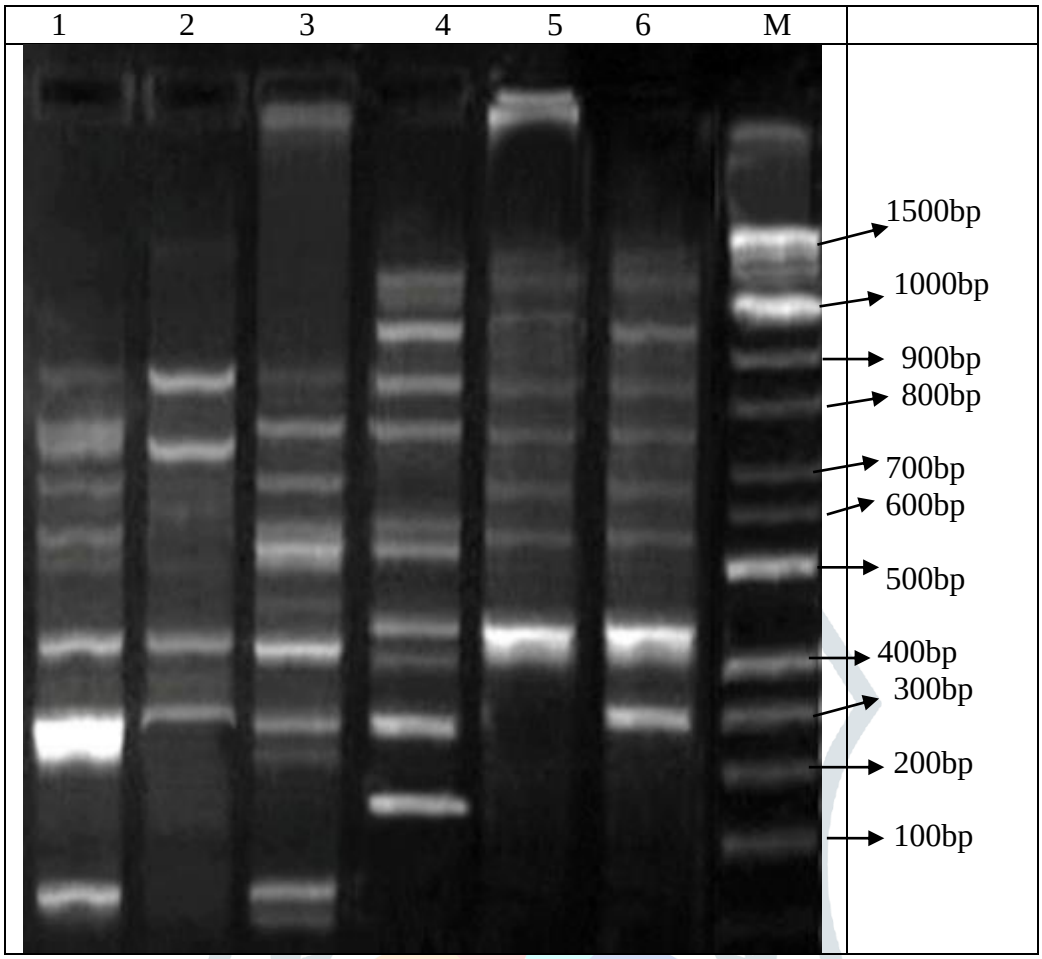


Figure 2: Black gram susceptible variety (LBG-823) RAPD finger printing agarose gel image. Lane M 100 bp plus marker. Lane 1- OPC-5 control; Lane 2-- OPC-5 Non-Induced; Lane 3-- OPC-5 Induced. Lane 4- OPF-2 control; Lane 5-- OPF-2 Non-Induced; Lane 6-- OPF-2 Induced.

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

Sl.No.	M.(bp)	OPC-5			OPF-2		
		Control (Lane-1)	Non-induced (Lane-2)	Induced (Lane-3)	Control (Lane-4)	Non-induced (Lane-5)	Induced (Lane-6)
1	1500						
2	1000-1500				++	+	+
3	900-1000				++	+	+
4	800- 900	+	++	+	++	+	+
5	700-800	++	++	++	++	+	+
6	700						
7	600-700	+		++		+	+
8	500-600	+		+	+	+	+

9	500	+		++	++		
10	400-500			+	++	+++	+++
11	400	++	++	++	+		
12	300	+++	++	++	++		++
13	200			+			
14	100-200				++		
15	BELOW 100	++		++			
16	Far below 100			++			

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

IV. CONCLUSION:

RAPD 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 tolerant and susceptible varieties at molecular level. It may be expected that study of variations between tolerant and susceptible varieties of black gram with the assistance of RAPD markers can provide precise information for marker assisted selection and breeding.

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