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Expression Level of Heat Shock Protein in Blackgram under Elevated Heat Stress

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Abstract

The study was conducted at Department of Crop Management, Faculty center for Agricultural Education and Research, Ramakrishna Mission Vivekananda Educational and Research Institute, Coimbatore, Tamil Nadu, India (Latitude 11° 13'N, Longitude 76° 94' E) from March, 2022 to June, 2022. Proteins play an indispensable role in bodybuilding. Thus, it is necessary to add the protein-rich food in daily diet. Black gram, one of the pulses, is being rich in proteins, which is about 20–25%. Heat is one of major important abiotic stress that affect the growth physiology and ultimately reduces the crop productivity. Heat stress majorly affects the reproductive phase and its leads decline in the yield. Plants can able to develop adaptive response to even mildest heat stress at morphological, physiological and biochemical levels. The main objective of this study was to evaluate the blackgram seedlings under high temperature to identify the expression level of heat shock protein for temperature stress tolerance. Heat tolerant plants develops the rapid phenological and biochemical changes which favors higher production. So, this study was carried out to evaluate the response pattern of blackgram under elevated temperature stress imposed on seedling stage in different genotypes. Six blackgram genotypes VBG-06-002, VBG-06-005, VBG-06-010, VBG-07-001, VBG-10-008 and COBG-759 were taken for this experiment. The protein profile as evidenced by SDS page analysis indicated distinct expression of 40 KDa protein in genotypes VBG-07-001, VBG-06-010, VBN-6 and COBG-11-02 not expressed in VBG-06-002 and COBG-759. This expression of protein maybe an indicative feature of thermo tolerance.

Keywords: Blackgram, heat shock protein, heat stress, thermotolerance

1. Introduction

Pulses are cultivated throughout the world as either major or minor crops for the nutrition and livelihood of millions of people (Kumar et al., 2019). Pulses are called as "Poor man's Protein" because it contains 25% of protein by weight (Martin et al., 2022). The green and dry plant parts of pulses are used as feed for livestock production systems (Ali and Gupta, 2012). Black gram is thermosensitive crop (Banerjee et al., 2021). Abiotic stresses are responsible for reduction of yield more than 50% directly worldwide (Khan and Shahwar, 2020). Abiotic stresses at the latter stages are considered more serious as they affect productivity to the huge extent (Gaur et al., 2014). The short episode of temperature rise will affect the reproductive and pod setting (Nadeem et al., 2018). According to IPCC (AR6) reports, there will be a rise in temperature up to 1.5°C before 2040 itself. FAO at the UN climate change conference (COP20, Peru) reports that the climate change is vulnerably affecting the livelihood and food security. The impact of climate change on chemical and physical processes in soils, with nutrient uptake from them, had been reviewed comprehensively (Pilbeam, 2015). The short episode of temperature rise will affect the reproductive and pod setting of blackgram (Bains et al., 2015). WHO recommendation for pulse is 80 g day^{-1}, but availability of per capita consumption is 41.9 g day^{-1} (John et al., 2021). High temperature stress affects the morphological and reproductive phases (Kaur et al., 2015, Goswami et al., 2021). The impacts of stress vary with its intensity, period and growth phase (Gurumurthy et al., 2019). Thus, aberrant weather conditions and global warming are expected to threaten pulses productivity in the near future as soaring temperatures will lead to production of poor biomass, reduction in days to flowering, poor fertilization rate, and seed formation (Kumar et al., 2016, Sita et al., 2017, Sehgal et al., 2017). Heat Stress is the temperature rise beyond a threshold level for a period of time that cause irreversible damage in plant growth and development

(Wahid et al., 2007). High temperature often occurs with the combination of solar irradiance, moisture stress and cyclone which can worsen plant damage even in well-watered plants (Hall, 1992). High temperature stress affects initial growth, morphology, reproductive phase, pollen viablility, pollen germination, starch granules and hilum (Partheeban et al., 2017, Partheeban et al., 2020). High temperature stress adversely affects the yield (Campbell et al., 1992) because of injury to reproductive structures (Hall, 1992), hastening in the proportion of plant growth (Gan et al., 2004), and reduction in the growth and development of reproductive organs (Angadi et al., 2000). Moreover, high temperature stress occurs during reproductive development phase causes negatively impact on Pollen germination, pollen viability and fertilization, floral bud development (Prasad et al., 2011) and seed filling (Boote et al., 2005). Heat shocks cause severe damages to the plants by alteration in the protein synthesis, major enzymes inactivation and damages to the membranes (Mittler, 2002, Khan and Shahwar, 2020, Ajila and Prasada Rao, 2009). Expressions of Heat Shock Genes (HSG) are due to heat stresses, which then encode Heat Shock Proteins (HSP). Heat Shock Factors bind to the specific binding sites of Heat Shock Elements (HSE) in HSG promoters, including HSPs expression on heat treatment. HSPs act as chaperones and protect intracellular proteins against denaturation, maintain structural stability through protein folding (Hasanuzzaman et al., 2013, Rodriguez et al., 2005). To meet the demands of increasing population, there is a need to identify the thermotolerance genotypes. This present study was conducted to assess the thermotolerance mechanism of genotypes at genotypic level protein expression under elevated temperature.

2. Materials and Methods

2.1. Plant material and site of study

Six blackgram genotypes, namely VBG-06-002, VBG-06-005, VBG-06-010, VBG-07-001, VBG-10-008 and COBG-759 were characterized at Department of Crop Management, Faculty centre for Agricultural Education and Research, Ramakrishna Mission Vivekananda Educational and Research Institute, Coimbatore, Tamil Nadu, India (Latitude 11° 13'N, Longitude 76°94' E) under heat stress conditions during March to June, 2022.

2.2. Growing conditions

The experiment was conducted in completely randomized block design having five replications. The blackgram seeds were soaked in water for 6 h and then drained the water (Raja et al., 2019). Uniform size seeds without any damage were placed in the petriplate for germination (Floss et al., 2013). Three days old uniform height seedlings were exposed to different induction temperature such as 36°C to 46°C at the rate of increase 2°C h^{-1} (Partheeban et al., 2017). After completion induction temperature, the seedling was exposed to lethal temperature of 50°C for 3 h. The same seedlings should be kept under 30°C for 72 h (Partheeban et al., 2017). The seedlings were exposed to 30°C throughout the experiment was considered as absolute control.

2.3. Analysis of protein profile in the induced and control seedlings

Immediately after the temperature induction response, the control and heat induced seedlings plant sample was collected and frozen in liquid nitrogen for SDS-PAGE analysis (Ghafoor et al., 2002). Total protein was extracted from the seedlings of six blackgram genotypes (VBG-06-002, VBG-06-005, VBG-06-010, VBG-07-001, VBG-10-008 and COBG-759). Total soluble protein was extracted from induced and control seedlings by rapid homogenization in Tris buffer (pH 7.8) containing 0.1 M Tris, 0.02 M sodium sulfite, 5 mM β-mercapta ethanol, 1 mM phenyl methyl sulfonyl fluoride (PMSF) and 4% poly vinyl poly pyrrolidone (PVPP). 0.5 g of tissue sample was ground in 1:2 (W/V) ratio of extraction buffer under cold conditions. Homogenate was centrifuged at 12,000 rpm for 15 m by using high speed centrifuge. Supernatant was collected as crude protein extract and the total soluble protein was estimated by Bradford (1976) dye-binding method using bovine serum albumin as the standard.

Qualitative analysis of stress responsive proteins was analysed using Sodium Dodecyl Sulphate (SDS) poly acrylamide gel electrophoresis (PAGE) following Laemmli's method (1970). The gel was submerged in staining solution and kept overnight (12–16 h) at room temperature. Destaining was carried out by placing the gel in destaining solution (50% methanol, 10% glacial acetic acid) in water until clear bands are visible. After complete destaining, the banding pattern was analyzed.

3. Results and Discussion

3.1. Analysis of protein profile in the induced and control seedlings

The electrophoretic pattern of the protein bands of the tolerant and susceptible genotypes is shown in image (Figure 1, Table 1). The banding pattern of the control seedlings was similar to that of the induced seedlings, but it was observed that in the induced seedlings intensity of the protein band

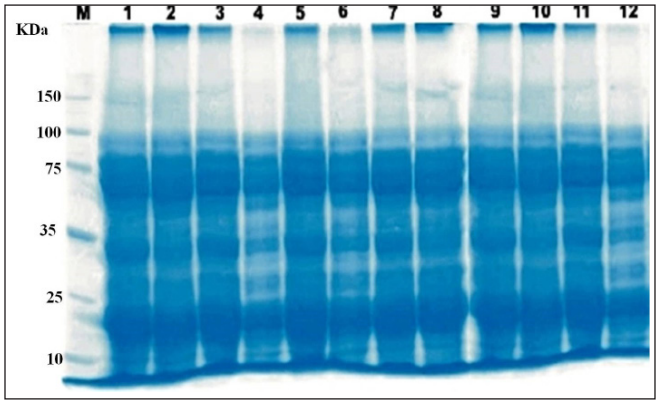

Figure 1: Expression level of heat shock protein in blackgram seedlings by SDS - PAGE analysis

Table 1: Details of SDS-Page analysis

Sl. No.	Sample information
M	Marker
Sample 1	VBG-07-001 - Induced seedlings
Sample 2	VBG-07-001 - Control seedlings
Sample 3	VBG-06-010 - Induced seedlings
Sample 4	VBG-06-010 - Control seedlings
Sample 5	VBN-6 - Induced seedlings
Sample 6	VBN-6 - Control seedlings
Sample 7	COBG-11-02 - Induced seedlings
Sample 8	COBG-11-02 - Control seedlings
Sample 9	COBG-11-03 - Induced seedlings
Sample 10	COBG-11-03 - Control seedlings
Sample 11	VBG-08-003 - Induced seedlings
Sample 12	VBG-08-003 - Control seedlings

was more when compared to the control seedlings. Protein bands were observed at 100, 75, 35, 25 and 10 KDa. SDS-PAGE analysis of the protein profile of different genotypes under induced and control conditions in the seedling stage revealed two major proteins, one with a higher molecular weight (75 KDa) and another with a low molecular weight (25 KDa). These two proteins were present under both the conditions (Figure 1). The intensity of the proteins was found to be varying in the control and induced samples and also among the genotypes. In the induced seedlings the expression of both high molecular weight and low molecular weight proteins was more intense in VBG-07-001, VBG-06-010 and COBG-11-02 as observed in the protein banding. In the control seedlings, the intensity of both the proteins was less in VBG-07-001, VBG-06-010 and VBN-6.

The protein profile as evidenced by SDS page analysis indicated distinct expression of 40 KDa protein in tolerant genotypes VBG-07-001, VBG-06-010, VBN-6 and COBG-11-02 which is not expressed in the other genotypes. Probably, the association of 40 KDa protein band would be indicative feature of thermo tolerance. Reports are available on accumulation of specific protein due to heat stress. Bansod and Malode (2012) demonstrated accumulation of specific protein accumulation in Vigna mungo. Tourinho-dos-Santos et al. (1994) reported similar results of the specific protein accumulation.

4. Conclusion

This induction technique helps the plants to produce heat shock protein slowly in early stages of the crop growth and also can able withstand in the critical situation. The expression of heat shock protein may be a useful parameter for selecting tolerant genotypes. The protein expression level at 40 KDa was an indicative feature for identify the thermotolerance genotype at seedling level. This present study paves a way to identify a powerful tool to screen large number of genotypes for thermotolerance.

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