Journal of Oilseed Brassica, 6 (2): 302-305, July 2015

Short Communication

Heat stress induced changes in protein profile of Indian mustard (*Brassica juncea* L.)

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(Received: 22 December 2014; Revised: 05 June 2015; Accepted: 15 June 2015)

Abstract

The present investigation was undertaken to study the changes in protein pattern of seedlings of Indian mustard (*Brassica juncea* L.) after subjecting to high temperature stress (45±0.5°C) and on revival. Two thermo-tolerant genotypes including BPR-542-6 and NRCDR-02 and two thermo-susceptible genotypes including NPJ-119 and RGN-152 were screened at 45±0.5°C on the basis of time taken to 50% seedling mortality and protein pattern of the seedlings was determined by SDS-PAGE. A major band of 53.12 kDa and minor bands of 100, 89.12, 74.13, 46.76 and 38.9 kDa in thermo-tolerant genotypes and protein bands of molecular weight 25.79 and 30.7 kDa in thermo-susceptible genotypes appeared under high temperature stress which disappeared when the stress was relieved. The newly synthesized proteins might be stress related proteins (heat shock proteins) which may play active role in imparting thermo-tolerance.

Keywords: *Heat shock proteins, heat stress, Indian mustard, SDS-PAGE*

Introduction

Heat stress due to increased temperature is a major problem in agriculture worldwide. It can strike the crop at any time and affects adversely the growth and development. It induces a series of growth and metabolic responses in higher plants. At cellular level, high temperature causes metabolic disturbances, disruption of cellular homeostasis, depletion of respiratory substrates, reduction of photosynthetic activity, denaturation of proteins, inactivation of enzymes and damage to cellular structures (Narwal *et al*., 2007). Plants have evolved a number of adaptive mechanisms that enable them to alleviate the negative effects of heat stress (Wahid *et al*., 2007). One such mechanism is the synthesis of stress protein, particularly heat shock proteins (HSPs) (Wang *et al*., 2014). In general these polypeptides may be grouped into low molecular weight and high molecular weight heat shock protection polypeptides (Koh, 2002). The synthesis of HSP is reversible phenomenon and it has been related to the recovery of protein synthesis after removal of heat stress (Lindquist and Craig, 1998). They have no regular chemical composition and disappear when the stress gets away. These proteins act as molecular chaperons to protect cellular damage against irreversible heat induced denaturation and facilitate refolding of heat induced damaged proteins or form complexes with denatured proteins (Basha *et al*., 2004).

Indian mustard is an important oilseed crop of winter season and its early sowing implies many important advantages. Firstly, early harvest of this crop is desirable to avoid disease infestation, frost and aphid attack that normally coincides with the flowering and pod formation stage. Secondly, shattering of siliquae can be avoided during the time of harvest when crop encounters high temperature. But high temperature prevailing at the sowing time imposes severe limitations on germination of the seed and subsequent seedling establishment and finally causing the reduction in yield. Keeping the importance of global warming and above back ground in view, the present investigation was carried out to study the changes in polypeptide pattern in

thermo- tolerant and thermo-susceptible genotypes of Indian mustard to high temperature and its revival.

Materials and Methods

Seeds of thirty six genotypes of Indian mustard collected from different regions were sown in trays of 40x30 cm having sandy loam soil. Each tray was filled with 7 kg soil which was previously homogenized with enough water to bring the soil to field capacity (150 ml water/kg soil). Each tray filled with soil was uniformly marked into 6 rows and 6 spots in each row. Seedlings were allowed to grow at optimum temperature (25±0.5°C), relative humidity (70%) and 16 hrs light, 8 hrs dark cycle for five days which were taken as control. Screening of these genotypes to high temperature was done by adopting the method of Chhabra *et al.,* (2007). Seedlings were exposed to threshold high temperature (45±0.5°C) and 30% relative humidity continuously. Time taken to 50% mortality was recorded in all the genotypes for screening against high temperature tolerance. Longer the time needed for a genotype to 50% mortality, more a genotype was tolerant to high temperature and vice- versa. After subjecting the seedlings to heat stress, the seedlings were revived by placing the trays at 25±0.5°C for 24 hrs.

SDS-PAGE was carried out for the polypeptide composition by method of Laemmli *et al.* (1970). Two gm seedlings were extracted in 0.062 M Tris-HCl buffer of pH 6.75 containing 10% glycerol, 5% mercaptoethanol and 2% SDS. The extract was centrifuged at 10,000 rpm for 15 min. The supernatant was mixed with equal volume of 2X sample buffer and kept in boiling water bath for five minutes. R_f value of each protein band was calculated as per the method of Laemmli *et al.* (1970).

Results and Discussion

Out of thirty six genotypes screened for high temperature (45±0.5°C) tolerance, two genotypes BPR-542-6 and NRCDR-02 were marked as thermo-tolerant whereas, two genotypes NPJ-119 and RGN-152 were marked as thermo-susceptible one (Table 1). The stressed and revived seedlings of these genotypes were taken for further studies. The protein pattern of the seedlings was determined by SDS-PAGE as shown in Plate 1 and Plate 2, respectively. In susceptible genotypes the molecular weight of polypeptide bands ranged from 20.13 to 74.13 kDa in both the genotypes. Under stressed condition, two major protein bands of 25.79 and 30.70 kDa appeared which disappeared on revival. These newly synthesized proteins might be stress related proteins (heat shock proteins) which may play active role in imparting stress tolerance. In tolerant genotypes, the molecular weight of polypeptide bands ranged from 20.13 to 74.13 kDa in control and revived seedlings while in stressed condition the range was from 20.13 to 100 kDa. A major band of 53.12 kDa and minor bands of molecular weight 100, 89.12, 74.13, 46.76 and 38.90 kDa under stressesd condition appeared in both the tolerant genotypes which disappeared when the stress was relieved. A protein band of 53.12 kDa might play a major role in imparting more tolerance to thermo-tolerant genotypes. The polypeptides of molecular weight 57 and 53.2 kDa have been reported as stress related proteins under salt stress (Jain *et al*.,1993) and drought stress (Phutela *et al*., 2004), respectively in Indian mustard. SDS-PAGE analysis of total extractable soluble proteins from the intact heat shock and salinity stressed

Table1. Screening of Indian mustard genotypes for tolerance to high temperature stress

Group	Time*	Genotypes
I	2:30-3:30	JMM-0702, CS-54, BPR-541-4, NPJ-92, RH-0119, CS-5000-1-1-1-4, NPJ-112, NPJ-113, BPR-541-2, BPR-542-14, BPR-549-2, SKM-531, RH-0116, HUJM-05-05, NPJ-119 and RGN-152.
II	3:31-4:30	BPR-543-2, ONK-1, NPJ-116, BPR-537, BPR-541-3, BPR-540-6, CS-3000-1-1-1-5, RGN-145, RH-0204, BPR-541-5, RGN-73 and RH-0115
III	4:31-5:15	BPR-542-6, NRCDR-02, RB-50, BPR-54-9, RH-0305, NRCDR-601 and NPJ-118

*Time (hrs:min) taken to 50 % seedling mortality

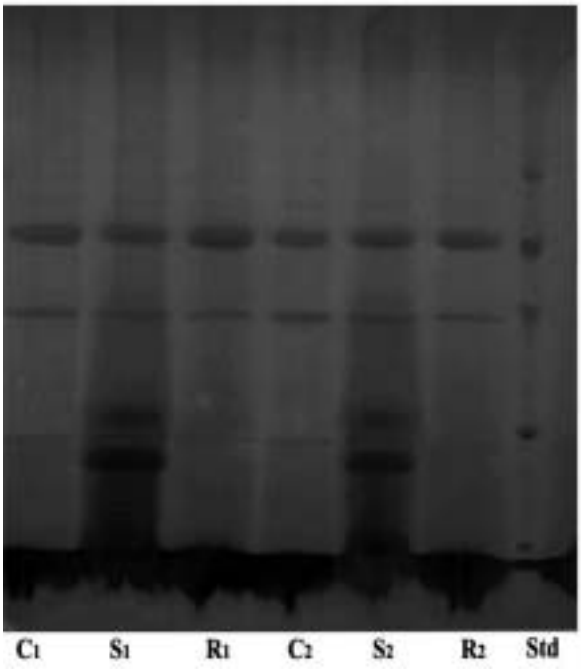

Plate 1: SDS-PAGE polypeptide pattern of thermo-susceptible genotypes of Indian mustard under high temperature stress. C_1 : Control (NPJ-119) ; S_1 : Stressed ; R_1 : Revived ; C_2 : Control (RGN-152) ; S_2 : Stressed ; R_2 : Revived Std : Standard

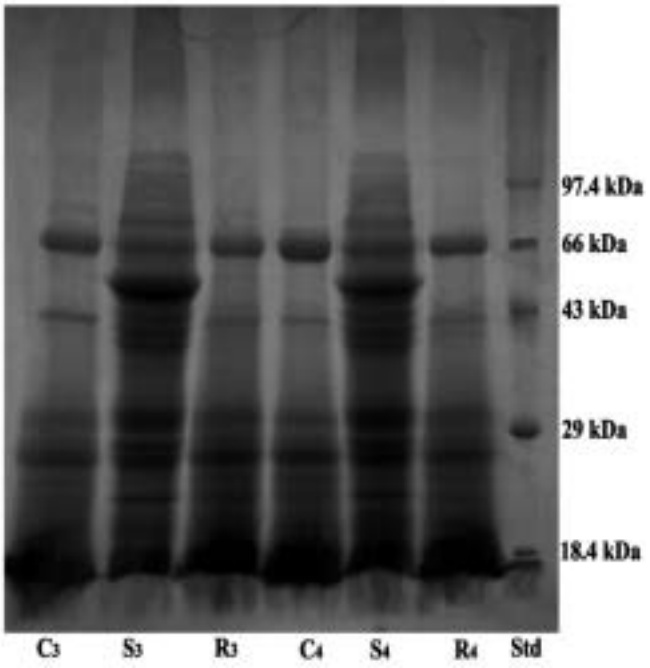

Plate 2: SDS-PAGE polypeptide pattern of thermo-tolerant genotypes of Indian mustard under high temperature stress. C_3 : Control (BPR-542-6) ; S_3 : Stressed ; R_3 : Revived C_4: Control (NRCDR - 2) ; S_4 : Stressed ; R_4 : Revived Std : Standard

Amaranthus seedlings revealed coinducibility of polypeptide bands of 110 and 90 kDa and over expression of polypeptides band of 125 kDa were also noticed under salt and heat stress (Bhattacharjee and Mukherjee, 2006). SDS-PAGE pattern of heat stressed French bean also showed intensification of few proteins bands and appearance of at least five new bands (Nagesh Babu and Devraj, 2008). Mahla *et al*. (2011) reported that following heat shock treatments the heat shock response resulted in the production of several HSPs with different molecular weight in wheat. Heat stress causes sudden changes in the genotypic expression because of an increase in the expression of heat shock transcription factors, HSPs (Kumar *et al*., 2013). The results clearly suggest that heat stress induce the expression of stress associated proteins (SAPs) and accumulation of such SAPs like HSPs has been directly implicated in the induction of thermo-tolerance in plants. These stress induced proteins allow the plants to make biochemical and structural adjustments that enabled them to cope with stress conditions.

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