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Changes in Protein Expression in Response to Heat Shock and Heat Stress in Three Tomato (Solanum lycopersicum) Landraces from Sudan

Aida Ali Elsharief and Eltayeb, E. A. Ahmed
College of Sciences and Arts- Khafji, University of Hafr Al Batin, Saudi Arabia
aidaelsharief@yahoo.com
Faculty of Agriculture, University of Khartoum, Khartoum, Sudan
eltayebahmed23@yahoo.com

ABSTRACT

This work was carried out to study types of proteins induced and degraded in response to heat shock treatment and at flowering stage after growth under heat stress, in three landraces of tomato *(Solanum lycopersicum*) from Sudan "HSD 0977, Toktuk and Abu-Zarif " in relation to a heat sensitive commercial genotype "Strain B". SDS PAGE method was applied for determination of proteins.

Heat shock and heat stress caused degradation of some constitutive proteins and induced some HSPs, but to different extents in the studied genotypes which may indicate difference in heat tolerance. At flowering stage HSD 0977 showed the highest response against the effect of heat stress followed by Strain B this was expressed by the induction of the largest number of heat shock proteins. Toktuk and Abu-Zarif were nearly similar in their response to heat stress by induction of only three types of sHSPs which may reflect their higher thermotolerance compared to the two remaining genotypes.

Indexing terms/Keywords

Tomato, Solanum lycopersicum , Heat shock proteins, Heat stress, SDS PAGE.

Academic Discipline And Sub-Disciplines

Biology

SUBJECT CLASSIFICATION

Molecular Biology

MATERIALS AND METHODS

This work was carried out in the experimental green house of the Agricultural Genetic Engineering Research Institute (AGERI), Egypt.

Three landraces of tomato *(Solanum Lycopersicum*) grown by traditional farmers in different sites of Sudan were used; HSD 977, a type of cherry tomato from the Blue Nile State, Toktuk, from North Darfur characterized by its large fruits and Abu Zarif, a landrace with large fruits grown in Wad Ramly, North to Khartoum. , in addition to Strain B, a heat sensitive commercial cultivar grown during winter

Tomato seeds of the four genotypes were sown in fiber trays containing a mixture of soil constitute of peat moss and vermiculite. Trays were kept inside the green house at 30 ^{o}C and 80% relative humidity and irrigation was carried out at interval of two days. Two weeks after germination, single seedlings were transferred to 15cm diameter pots. The fungicide topsin (1g/l) was added mixed with irrigation water. Seedlings were fertilized with N.P.K. (1g/l) and irrigation continued at interval of two days. Temperature was raised to 37 / 23^{o}C day and night respectively. At the age of five weeks the heat shock treatment was applied; Plants were irrigated at the day before the treatment. Half the number of plants from each genotype was transferred to a growth chamber to carry out the heat shock treatment and the other half was left as a control. Temperature inside the growth chamber was raised from 37 ^{o}C to 48 ^{o}C within one hour and kept at 48 ^{o}C for 4 hours (Vierling, 1991). Two plants were taken randomly from each genotype for heat shock and control plants to carry out proteomic analysis.The remaining heat shock plants were returned to the green house with the control plants to continue growth at 45/29 ^{o}C (Average day and night temperatures during summer in Sudan).

Proteins were separated with Sodium Dodecycle Sulfate Polyacryle Amide Gel Electrophoresis (SDS-PAGE) according to the method of Laemmli (1970) with some modifications.200 mg of young leaves were grind into a fine powder using liquid nitrogen. Protein samples were diluted 1:2 in 2x Laemmli sample buffer, boiled in water bath for 5 minutes and kept at -20 ^{0}C. 20 ml of 12% separating gel was prepared and poured in the Bio-Rad protein larger unit and let to get firmed. 10 ml of 5% stacking gel were poured on top of the separating gel. The unit was fixed to electeophoresis apparatus and 15µl of each protein sample was loaded in each well and a protein marker was loaded in the first well. After electrophoresis gel was stained with Commassie blue 0.2%, examined under light box and photographed. Bands were read using Gel-Documentation system 2000. The software used was Diversity Data Base (Bio-Rad). Analysis was carried out in two replicas. The same proteomics analysis was done at flowering stage.

INTRODUCTION

Heat stress due to increased temperature is an agricultural problem in many areas in the world including Sudan. Due to the expected climate changes of a few degrees especially temperature, cold weather crops are expected to decline by about 15% in the next fifty years (Lane and Jarvis, 2007).

Plants are capable of adapting to a wide range of temperatures by reprogramming their transcriptome, proteome, and metabolome and even by activating cell death mechanisms leading to organ abortion or entire plant death (Qi, *et al*, 2011).

The plant in an unfavorable environment could face two situations; lethal stress where the plant may ultimately die due to the increased senescent activities, and sub-lethal stress where certain adaptive changes may occur leading to survival of the plant. These adaptations could be at the molecular level involving changes in gene expression and synthesis of stress proteins and at the biochemical level involving changes in biochemical pathways which may bring about the physiological response and finally the whole plant response (Grover *et.al,* 2001).

Expression of stress proteins is an important adaptation to cope with environmental stresses. Most of the stress proteins are soluble in water and therefore, contribute to stress tolerance via hydration of cellular structures (Wahid and Close, 2007). Increased production of heat shock proteins (HSPs) occurs when plants experience either sudden or gradual increase in temperature (Nakamato and Hiyama, 1999). In higher plants HSPs are usually induced under heat shock at any stage of growth (Vierling, 1991). In the absence of stress several HSPs were found to assume important cellular functions such as aid in folding, protein translocation through membranes and the control of degradation (Bukau and Harwich, 1998).

Tolerance to heat is characterized by a lesser effect on essential processes such as photosynthesis and by consistent increases of transcripts involved in the biosynthesis of protective components. As photosynthesis and reproductive development are the most sensitive physiological processes to stress (Prasad, *et al*, 2008) Reproductive processes in tomato were more sensitive to high temperature than the vegetative ones and more affected by heat shock treatment (Abdelmageed, *et al*., 2003). Tolerance conferred by heat shock proteins results in improved physiological phenomena (Schőfll *et al,* 1999). Such type of tolerance makes plant growth and development possible under heat stress. Tremendous variations in heat tolerance exist within and between species, providing opportunities to improve crop heat stress tolerance through genetic means (Ehlers and Hall, 1998).

Heat stress is known to switch the pattern of gene expression inducing the HSP compliment and inhibiting many genes expressed under normal temperatures (Yost and Linquist, 1988). Heat stress is responsible for the up-regulation of several heat inducible genes; commonly referred as "heat shock genes" (HSGs) which encode HSPs and these active products are very much necessary for plant's survival under fatal heat stress. High temperature induced constitutive expression of most of these proteins protect intracellular proteins from denaturation and preserve their stability and function through protein folding; thus it acts as chaperones (Chang, *et al*, 2007) Due to their thermotolerant nature, the expression of HSP can be induced by heat treatment in the presence of conserved heat shock elements (HSEs) in the promoter region of HSGs, which triggering transcription in response to heat. These *cis*-acting elements (HSE) consist of the palindromic nucleotide sequence (5-AGAANNTTCT-3) that serve as recognizing as well as binding site for heat shock transcription factors or simply heat shock factors (HSFs) Heat shock factor binding recruits other transcriptional components, resulting in gene expression within minutes in increased temperature. Since all HSGs contain HSE conserved sequence, over expression of HSF gene intern turned on almost all HSGs and consequently provides protection against heat stress (Nover, *et al*, 2001). Protein denaturation occurs under high temperatures because decreased cellular volume increases the likelihood of degradative molecular interactions. Heat shock proteins maintain and repair companion protein structure and target incorrectly aggregated and non-native proteins for degradation and removal from cells (Reis, *et al*, 2012). The small heat shock proteins are a group of proteins ranging in size between 14-42 KDa. In plants they are produced in response to heat stress (Scharf, *et al.,* 2001).

Genotypic differences in thermotolerance exist in many plant species. Genotypes which thrive under relatively high temperatures are expected to have a high degree of thermotolerance possibly conferred by stress proteins. In Sudan very high temperatures are predicted to have a general negative effect on tomato landraces growth and development, leading to catastrophic loss of crop productivity. However, the landraces under study may furnish a source of thermotolerance that could be utilized to develop that trait in more productive and less thermotolerant cultivars.

This study aimed to study the expression of stress proteins produced as a component of thermotolerance , after heat shock treatment and at flowering stage of growth under heat stress.

RESULTS

Heat shock treatment:

Figure 1 and table 1 exhibited different types of heat shock proteins induced and degraded in tomato seedlings as a result of heat shock treatment. In HSD 0977 two new types of proteins with high molecular weight (244 and 204 KDa) were formed in addition to nine types of lower molecular weight in a range of 99 to 24 KDa. Proteins which had been degraded as a result of heat shock treatment were nine types in a range of 63 to 20KDa. The degraded proteins included three types; 53, 24 and 22 KDa which were common to all genotypes. In Toktuk heat shock led to induction of two proteins with high molecular weight (242 and 206 KDa) and a variety of proteins of lower molecular weight, ranging between 164 and 37 KDa including the three previously mentioned common proteins (53, 24, and 22 KDa). Abu Zarif was the least respond to heat shock treatment, this was reflected in the induction of only one high molecular weight protein of 303 KDa and extra six proteins of lower molecular weight ranging between 80 and 60 KDa. Lost proteins were only the three common

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proteins. Strain B was the most affected by heat shock; one protein of high molecular weight (251 KDa) was formed in addition to 12 types of proteins ranging between 190 and 20 KDa. Proteins degraded due to heat shock were ten types of lower molecular weight ranging from 74 to 22 KDa including the three common proteins

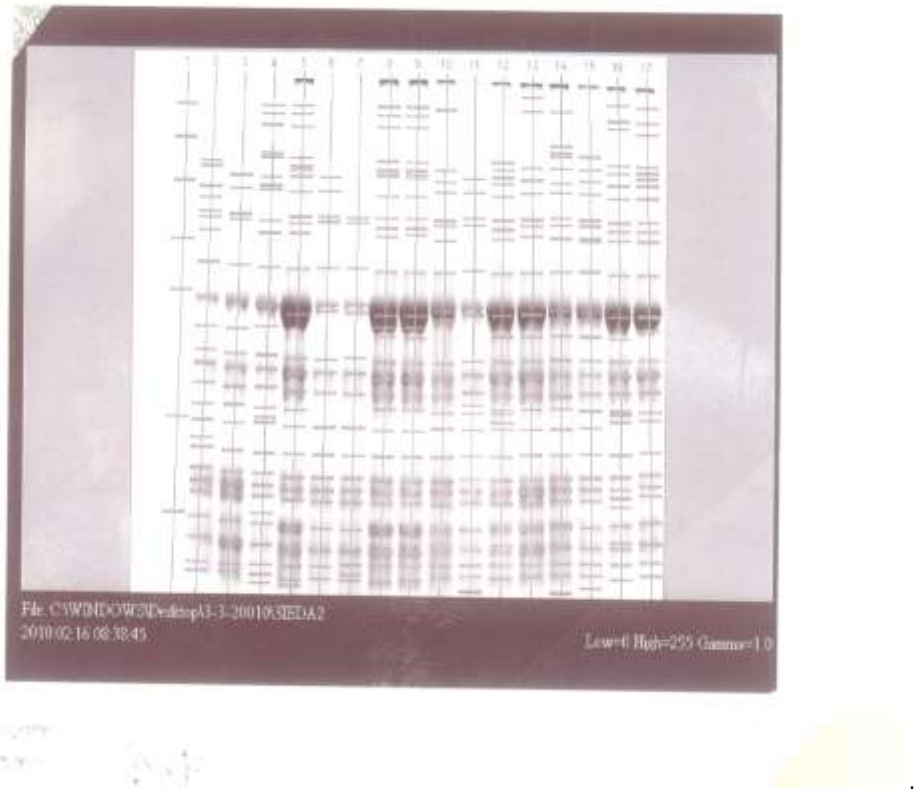

Figure 1. Profiles of cytoplasmic protein in leaves of the four genotypes of tomato (*Solanum lycopersicum*) after heat shock treatment

Table 1. Molecular weight (KDa) for induced (I) and degraded (D) protein bands in the four genotypes after heat shock treatment.

HSD 977		Toktuk		Abu Zarif		StrainB	
I	D	I	D	I	D	I	D
244	63	242	53	303	53	251	74
204	53	206	24	80	24	190	62
99	48	164	22	74	22	151	53
90	38	111		68		104	42
80	27	104		67		100	40
62	24	100		62		49	33
51	22	97		60		41	30
44	21	89				36	28
42	20	74				30	24
29		49				26	22
24		47				22	
		37				21	
						20	

Heat stress:

Figure 2 and table 2 show induction and degradation of proteins in tomato seedling grown under heat stress after heat shock treatment. The largest number of proteins (Eight types) was induced in HSD 0977 compared to the other genotypes most of which were of the lower molecular weight and five types of proteins were degraded. Three types of proteins were induced In Toktuk and four types in Abu Zarif, while six proteins were degraded in Toktuk and five from Abu Zarif mos t of which from the low molecular weight proteins. An 18 KDa protein was a common protein that has been degraded in the three landraces and 20 KDa was a common degraded protein in Toktuk and Abu Zarif. Strain B showed induction of new seven low molecular weight proteins ranging between 75 to 15 KDa and degradation of three low molecular weight proteins.

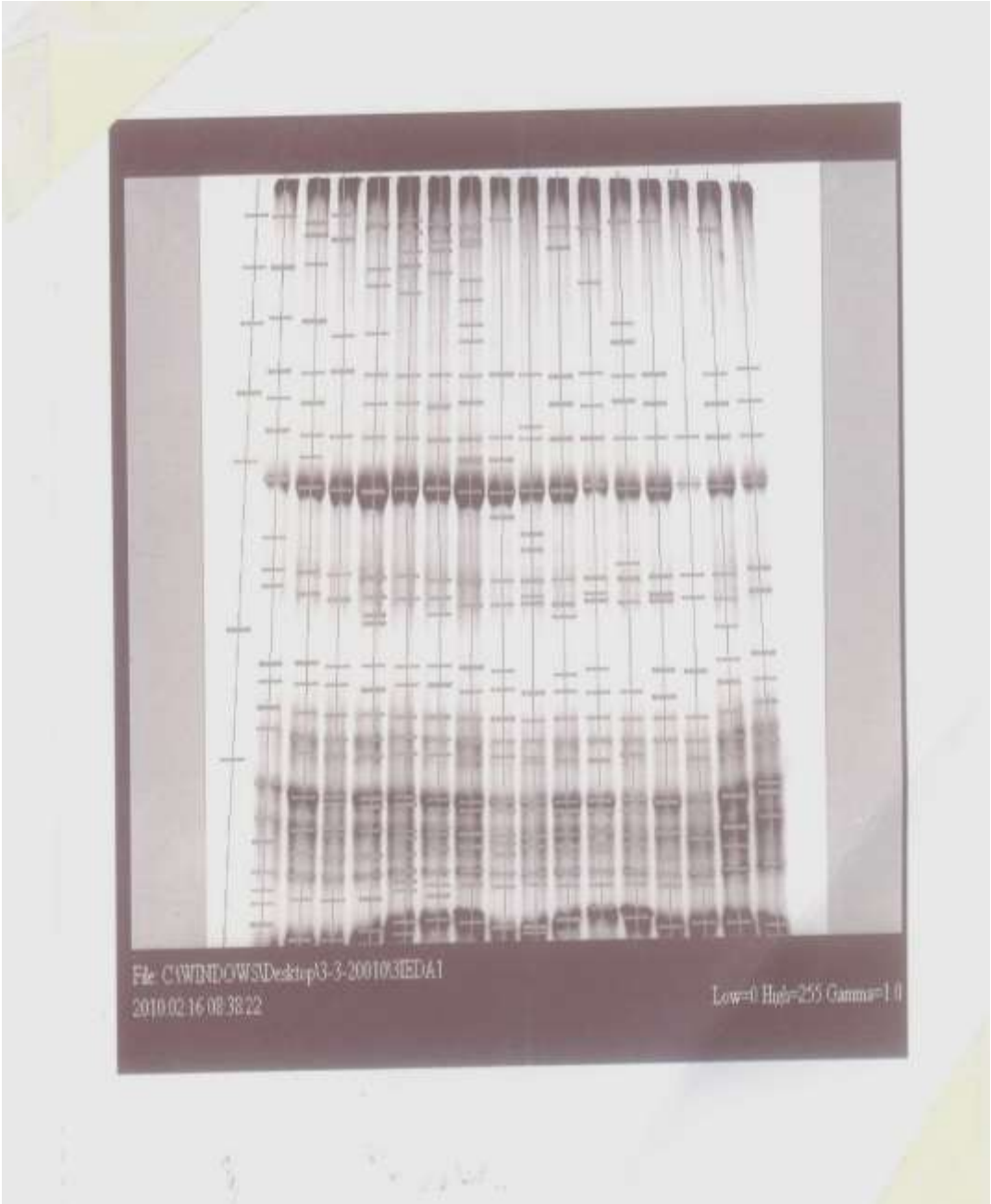

Figure 2. Profiles of cytoplasmic protein in leaves of the four genotypes of tomato (Solanum lycopersicum) at flowering stage

Table 2. Molecular weight (KDa) for induced (I) and degraded (D) protein bands in the four genotypes at flowering stage under heat stress

HSD 977		Toktuk		Abu Zarif		Strain B	
I	D	I	D	I	D	I	D
132	205	54	152	215	155	75	37
40	131	22	50	91	36	67	28
38	95	16	20	50	20	40	22
20	55		19	16	18	33	
19	18		18		17	23	
18			17			18	
17						15	
15							

Discussion

Heat shock:

Induction of heat shock proteins after heat shock treatment was interpreted by Schőfll *et al.* (1999) who reported that the acquisition of thermotolerance normally results from prior exposure to a conditioning pre-treatment which can be short but sub-lethal high temperature that can protect cells and organisms from a subsequent heat stress. Limited number of high molecular weight proteins was induced by heat shock treatment, in a range of 241-303 KDa. More studies may need to explain their relationship to thermotolerance. Heat shock proteins, 90 and 100 KDa groups were induced in most genotypes. This result agreed with Queitsh *et al.* (2000) who reported the important role of HSP 101 in acquired thermotlerance in *Arabidopsis* and maize, and Krishna and Gloor (2001) who stated that the members of HSP 90 in cytosol, mitochondria and chloroplast in *Arabidopsis* were reported to be heat induced. This result is also in line with Hong and Vierling (2000) and Neuwald *et al.* (1999) who showed that HSP 100 group is critically required for resolubilizing protein aggregates formed due to heat stress. One member of the HSP 60 group was induced in HSD 0977 and a number of HSPs 70 group were induced in Toktuk and Abu-Zarif. This agreed with Preczewski *et al.* (2000) who found that chloroplast HSP 60 and cytosol HSP 70 in tomato are involved in determining photosynthetic thermotolerance. It was also confirmed by Vierling (1991) who reported that both chloroplast HSP 60 and cytosol HSP 70 are expressed molecular chaperones although the specific function they fulfill in plants is unknown. The occurrence of HSPs 60 and 70 KDa groups in strain B prior to exposure to heat shock may be interpreted as that strain B is an imported cultivar and the normal conditions in Sudan may represents relatively stress conditions and then, group 60 and 70 may be developed normally in this genotype for thermoprotection. When plant subjected to more heat stress by heat shock treatment, these proteins may not be enough for thermoprotection and a large number of small heat shock proteins are induced to protect against severe heat stress.

The induction of 68 KDa HSP in Abu-Zarif after heat shock treatment may confirm the report by Neumann *et al.* (1993) that HSP 68 KDa is constitutively expressed but their synthesis increased during heat stress in tomato. In terms of the induction of small heat shock proteins (sHSPs) after heat shock treatment, three to four types of sHSPs ranging in molecular weight between 20-49 KDa were induced in Toktuk and Abu- Zarif while 8 types were induced in strain B. Similar results were reported by Giese and Vierling (2000) who found that under heat stress conditions, a rapid reorganization of the sHSPs was observed, which facilitates association with denatured proteins. The result can be interpreted by Yost and Lindquist (1988) who reported that heat stress is known to switch the patterns of gene expression inducing the heat shock compliment and inhibiting many genes expressed under normal temperatures. Five of the sHSPs induced by strain B were of molecular weight 20-30 KDa including 21 and 22 sHSPs. Neta –Sharir *et al.* (2005) described the role of sHSP 21 in tomato as protecting phostosystem II from oxidative damage. On the other hand, Polenta *et al.* (2007) concluded that the accumulation of proteins of mass 15-30 KDa in tomato seems to be an important component of thermoprotection and that proteins around 21 KDa are of interest in the response to heat stress. Similarly Nover and Scharf (1984) studied protein extracts of tomato under heat stress and found 21 and 22 KDa proteins.

Heckathorn *et al.* (1998) mentioned that the chloroplast sHSPs 24 KDa protects photosystem II and the oxygen evolving complex at high temperature and therefore is involved in photosynthetic thermoprotection. This was confirmed by the induction of that type of protein in HSD 0977 after heat shock treatment.

In general strain B showed the highest response against heat stress resulting from heat shock treatment which was manifested by induction of the largest number of HSPs most of which were small heat shock proteins. This may reflect the higher heat sensitivity compared to the other genotypes.

Three types of HSPs and 6 types of heat shock proteins of groups 70, 90 and 100 KDa groups were induced in Toktuk after heat shock treatment, which had been regarded as components of thermotolerance (Vierling, 1991). No sHSPs were

induced by Abu–Zarif which respond in a different way to heat shock by induction of 60 and 70 KDa groups of HSPs which seems to be enough for thermoprotection in this genotype.

Degradation of proteins as a result of heat shock occurred in all genotypes. 53, 24 and 22 KDa proteins were degraded in the four genotypes due to heat shock. The three proteins were the only proteins degraded in Toktuk and Abu -Zarif which may suggest that the genes suppressed as a result of heat shock were similar in the two genotypes.

Heat stress

Follow up of change in the induction of proteins between heat shock and control plants at flowering stage revealed that most proteins induced at this stage were small heat shock proteins. High temperature increases the level of transpiration and hence less water is available for reproductive growth (Nover *et al*., 1989). On the other hand Lin *et al.* (2016) reported that high day temperatures were deleterious to tomato when flowers were visible. So, the induction of small heat shock proteins (sHSPs) at this stage may be protective, at this heat sensitive stage.

At flowering stage HSD 0977 showed the highest response against the effect of heat stress followed by strain B this was expressed by the induction of the largest number of heat shock proteins. This phenomenon is perhaps an ada ptation to protect the reproductive processes as it has been reported by Wahid and Close (2007) that expression of stress proteins is an important adaptation to cope with environmental stresses. Furthermore strain B retained two types of HSPs related to group 60 and group 70 KDa that have been lost after heat shock treatment . These proteins are involved in several cellular processes (Bukau *et al.*, 2006) and the expression of HSP 60 may indicate an essential role in plant growth and development. Zabaleta *et al.* (1994) indicated that the absence of HSP 60 in tobacco resulted in delay in flowering. Toktuk and Abu-Zarif were nearly similar in their response to heat stress by induction of limited number of HSPs which may reflect their higher thermotolerance compared to the two remaining genotypes.

In general heat shock and heat stress caused degradation of some constitutive proteins and induced some HSPs, but to different extent in the studied genotypes which may indicate difference in heat tolerance.

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