



IDENTIFICATION AND CHARACTERIZATION OF HEAT SHOCK PROTEINS IN FOUR EGYPTIAN MAIZE INBRED LINES (*Zea mays* L.)

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ABSTRACT

The heat shock protein (HSP) family is one of the proteins universally accumulated under heat stress condition. Four Egyptian maize inbred lines (*Zea mays* L.) were studied to identify heat shock proteins under heat stress at 45°C as protein markers for detecting the thermo-tolerance inbreds. The protein fingerprints of four inbred lines were performed by grain total-soluble protein electrophoretic analysis. The protein electrophoretic analysis showed 18 bands in a distinct pattern of K1 and K7 inbred lines, while 17 bands were present in G342 and Rg59 inbred lines as another distinct pattern indicating different genotypes. The high temperature effect on four maize inbred lines exposed to 45°C for 2 and 4 hours at 14-days old seedlings besides control (25°C) was studied. Several protein bands varied between low and intermediate molecular weights were induced after exposing to heat stress at 45°C. Four bands of heat shock proteins with molecular weights of 82, 22, 17 and 10 kDa appeared in the inbred line K1 after exposing to 45°C for 2 and 4 hours which may be indication of thermo-tolerance. Four and seven bands were enhanced after exposing to high temperature for 4 hours at 45°C which appeared more concentrated in the patterns of K7 and G342 inbred lines, respectively.

INTRODUCTION

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is useful for characterizing, comparing and differentiating corn endosperm proteins in protein isolation studies and ge-

netic investigation (Paulis et al 1975). Ladizinsky and Hymowitz (1979) found that the high stability of the seed protein profile and its additive nature make seed protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants. Chun et al (2001).

Reported that PAGE technology can be used to determine the purity of inbred lines and can provide the basis for controlling fake seeds. Gornstein et al (2004) screened soybean and maize for proteins and their nutritional value. The comparison of the identity and differences between investigated plants was carried out by the obtained SDS-PAGE electrophoretic patterns. Electrophoretic patterns of extracted proteins have shown that the main protein subunits were concentrated between 10 and 50 kDa. Variations were found in major fractions and minor bands as well as in the fine structure. Zeb et al (2006) showed that the SDS-PAGE electrophoretic analysis, together with physiochemical and molecular characteristics, present a good tool to characterize seed storage protein.

Shende (2017) characterized 170 genotypes of rice (*Oryza sativa* L.), based on the total seed protein and analyzed through protein Bradford assay and biochemical characters (sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE). The study revealed that the all genotypes of rice were identified on the basis of total seed protein content. Total of 15 protein bands was observed in SDS-PAGE, eight polymorphic bands were scored.

Cooper and Ho (1983) showed that the pattern of protein synthesis in maize seedlings (*Zea mays* L.) is rapidly altered when the incubation temperature is raised from 25 to 40°C. Shifting the temperature from 25 to 45°C results in a pattern of protein synthesis different from that observed after a shift

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to 40°C. Normal protein synthesis continues, which are produced in small amounts at lower temperatures, show greatly enhanced synthesis at 45°C. These proteins have apparent molecular weights of 83, 81, 68, and 65 kDa. **Schlesinger (1990)** reported that the heat shock protein (HSP) super family is one of the proteins universally accumulated under heat stress condition. **Vierling (1991)** found that sHSPs are divided into six classes based on their sequence alignments, immunological cross reactivity, and cellular compartmentalization. Three classes (I, II, and III) are localized in the cytoplasm or the nucleus and the other three are localized in the chloroplast, the endoplasmic reticulum, and the mitochondria.

Hogan (1990) found that maize plumules responded to heat shock by synthesizing nuclear-encoded HSPs with MW of 108, 89, 84, 76, 73 and 17-29 kDa and at 25°C (control) some proteins were repressed in vivo. **Ristic et al (1991)** using one-dimensional (1-D) SDS PAGE, found a band of HSP of 45 kDa in the leaves of 3-week-old plants of a drought and heat resistance maize (*Zea mays* L.) line, ZPBL1304. A similar HSP band of 45 kDa was lacking in the leaves of a drought and heat sensitive maize line. **Gallagher et al (1993)** performed separation of proteins by SDS-PAGE on 10% polyacrylamide gels for the detection of HSP101 and HSP70 and on 15% polyacrylamide gels to detect HSP 17.8 and HSP 17.6. **Waters et al (1996)** reported that plants which exposed to temperature of 8°C to 10°C above their normal growth temperature (heat stress) synthesize a set of new proteins, called the heat shock proteins (HSPs), they are play a role in protection against heat injury. Also observed that all small-HSPs in plants are encoded by six nuclear gene families, each gene family corresponding to proteins found in distinct cellular compartments like cytosol, chloroplast, endoplasmic reticulum (ER), mitochondria and membranes. Some nuclear-encoded HSPs accumulate in the cytosol at low (27°C) and high (43°C) temperatures, but they accumulate in chloroplast at moderate (37 °C) temperatures.

Giaveno and Ferrero (2003) reported, in temperate regions, that heat stress is one of the most important causes of reduction in yield and dry matter production in many crops, including maize. Also found that some temperate areas of the world behave as sub-tropical due to greenhouse effect and global warming. Therefore, more temperate genotypes show a decrease in yield and dry matter production as consequence of heat stress. **Baniwal et al (2004)** found that high temperature is one of the

major limiting factors that could considerably reduce the yield of crops and impair their wider distribution. Therefore, exploring the complex molecular mechanism of plant response to HS has become a crucial subject of agricultural significance in recent years. Heat shock transcription factors (Hsfs) are the critical components that serve to regulate the expression of genes responsive to HS as transcription factors.

Sanmiya et al (2004) placed the plant HSPs by molecular weight into six groups: HSP100s; HSP90s; HSP70s; HSP60s; and small HSPs (sHSPs) (12–40 kDa) and co-chaperones HSP40 or DNAJ family. **Wahid and Close (2007)** manifested that expression of stress proteins is an important adaptation to crop with environmental stresses. Most of the stress proteins are soluble in water and therefore contribute to stress tolerance presumably via hydration of cellular structures, although heat shock proteins (HSPs) are exclusively implicated in heat-stress response, certain other proteins are also involved. **Timperio et al (2008)** reported that plants respond to various stresses in a similar manner by producing heat shock proteins (HSPs), indicating a similarity in the plant's adaptive mechanisms; in which HSPs protect cells against many stresses. **Muthusamy et al (2017)** observed that small Heat Shock Proteins (sHSPs)/HSP20 are molecular chaperones that protect plants by preventing protein aggregation during abiotic stress conditions, especially heat stress. Due to global climate change, high temperature is emerging as a major threat to wheat productivity. Thus, the identification of HSP20 and analysis of HSP transcriptional regulation under different abiotic stresses in wheat would help in understanding the role of these proteins in abiotic stress tolerance.

The objectives of this study are analysis and fingerprinting of four Egyptian maize inbred lines by total protein electrophoretic analysis of maize embryos and characterization of heat shock-induced proteins for using as molecular markers to improve the heat stress tolerance in Egyptian maize inbred lines.

MATERIALS AND METHODS

MATERIALS

Four Egyptian inbred lines of maize (*Zea mays* L.) with different genetic background, as shown in **Table (1)**, were used in this study. Two inbred lines K1 and line K7 were developed by the plant breed-

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ing group, Genetics and Cytology Dept., National Research Centre, Giza, Egypt. While line K1 was derived after selfing for several years to single cross 10 (introduced from the Maize Research Section, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. In addition, the other two inbred lines G342 and line Rg59 were kindly obtained by the Maize Research Section, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

Table 1. The four maize inbred lines used in the study and their pedigrees

No.	Maize lines	Pedigree
1	K1	Single Cross 10 (S.C.10)
2	K7	2-synthetic variety
3	G342	LocBrod SC12100
4	Rg59	G.S. (Syn.Laposta X 307) X S.C.14

METHODS

Plant materials and seed storage proteins extraction for fingerprinting

Total soluble proteins were extracted from embryos the four maize inbred lines by salt-buffer as follow;

- 50 mM Tris-HCl buffer pH 8
- 200 mM NaCl
- 1% SDS
- 2% β-mercaptoethanol
- Protease inhibitors (1 mM PMSF, 50 μM Leupeptin and 10 mM E-64).

Centrifugation for 10 min at 12,000 rpm and 4°C was carried out and the supernatants containing total soluble proteins were transferred to new eppendorf tubes and kept at deep-freeze until using for electrophoretic analysis. Electrophoresis was carried out and then the gel was stained with Coomassie Brilliant Blue R 250. The de-staining solution containing 300 ml methanol, 50 ml acetic acid and 650 ml distilled water was used to clear the protein bands. They were photographed and scanned by Gel Doc Bio-Rad System (Gel – Pro analyzer V. 3).

Protein extraction from leaves 14 day-old maize seedlings was also performed under similar conditions. Protein extracts were quantified using the Bradford reagent and analyzed by SDS-PAGE (13% polyacrylamide). Extracted proteins were analyzed by one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) which was performed on vertical slabs (20 X 18 X 0.2 cm) according to Laemmli (1970).

Heat stress condition assay by 1D-SDS PAGE

The heat shock proteins were analyzed for the four lines of maize. One gram of leaves of 14 day-old maize seedlings, exposed to heat shock (45°C) for 2 and 4 hours besides control (25°C), were finely ground in a mortar and pestle. 0.2 g ground sample was first added to 1 ml extraction buffer in an eppendorf tube and left in refrigerator overnight, then vortexed for 15 seconds and centrifuged at 12,000 rpm and 4°C for 15 min. The electrophoretic procedure was performed as mentioned above. The protein extraction was maintained at -20°C until further use.

RESULTES AND DISCUSSION

Protein purification and electrophoresis

The embryo total protein electrophoretic patterns for the four maize inbred lines are shown in **Figure (1) and Table (2)**. The electrophoretic patterns showed 18 protein bands in both of K1 and K7 inbred lines in the same loci but different in density and intensity, while the other two lines G342 and Rg59 manifested 17 bands equal in the molecular weights with different density and intensity. The protein band patterns are ranging in molecular weights from 10 to 170 kDa.

The results are in accordance with those of **Gepts (1989)** who reported that, among biochemical techniques, SDS-PAGE is most widely used due to its validity and simplicity for genetic structure of crop germplasm. The SDS-PAGE is a practical reliable method because a seed storage protein is largely independent of environmental fluctuation. **Bean and Lookhart (2000)** showed that SDS-PAGE method has been developed to separating and characterizing cereal storage proteins. **Gorinstein et al (2004)** screened soybean and maize for proteins by SDS-PAGE electrophoretic patterns which found that the main protein subunits were concentrated between 10 and 50 kDa.

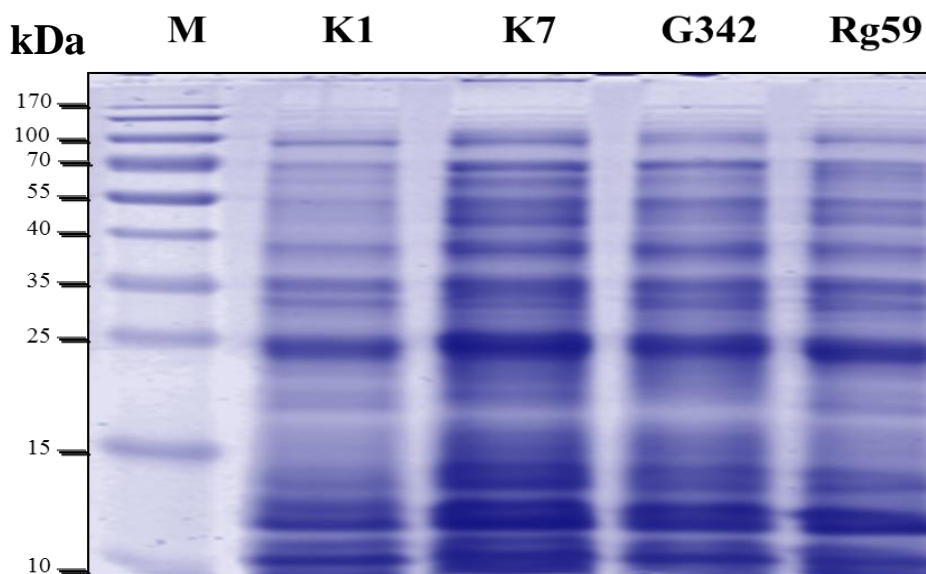


Fig. 1. Profile of total proteins of maize embryos performed by SDS-PAGE analysis for the four inbred lines as indicated at the top of the figure. M: molecular weight in kD of standard proteins

Table 2. SDS-PAGE profile of embryos total proteins of four maize inbred lines, representing band number and molecular weight (MW), where (+) means presence and (-) means absence of band.

Band No.	MW (kDa)	Maize inbred lines			
		K1	K7	G342	Rg59
1	168.2	+	+	+	+
2	158.7	+	+	+	+
3	102.1	+	+	+	+
4	95.6	+	+	+	+
5	70.2	+	+	+	+
6	64.1	+	+	+	+
7	55.2	+	+	+	+
8	49.4	+	+	+	+
9	38.4	+	+	+	+
10	35.0	+	+	+	+
11	30.5	+	+	+	+
12	24.8	+	+	+	+
13	22.0	+	+	-	-
14	20.1	+	+	+	+
15	18.0	+	+	+	+
16	14.3	+	+	+	+
17	13.3	+	+	+	+
18	11.2	+	+	+	+
Total bands		18	18	17	17

Effect of high temperature on protein synthesis

Figure (2) and Table (3) illustrate the effect of heat stress on heat shock protein (HSP) synthesis in two maize inbred lines (K1 and K7) exposed to 25°C and 45°C for two and four hours. The elec-

trophoretic patterns for protein bands gave a total of 16 bands ranged from 200 to 10 kDa. The exposure to heat stress induced the synthesis of several proteins varied between low and high molecular weights where some bands enhanced by heat treatments and detected only at high temperature.

In inbred line k1 some hsp bands were not detectable in the control but appeared after exposing to temperature at 45°C for 2 hours (treatment T1) such as bands of 82 kD and 17 kDa, while after exposing to temperature at 45°C for 4 hours (treatment T2) two another band appeared with 22 and 10 kDa. This finding indicates that this inbred line may be having ability to tolerate high temperature stress. In addition, four bands with molecular weights 38, 37, 31 and 25 kDa in inbred line k7 and one band with 20 kDa in inbred line K1, increased in protein concentration after heat treatment at 45°C for 4 hours. Also, four bands with molecular weights of 200, 38, 37 and 25 kDa appeared more concentrated after exposing to 45°C for 2 hours more than 25°C in inbred line K1.

Figure (3) and Table (4) illustrate the effect of heat stress on heat shock protein (HSP) synthesis in the two maize inbred lines G342 and Rg59 exposed to 25°C and 45°C for two and four hours. The SDS-PAGE revealed a total of 16 bands ranged from 200 to 10 kDa. In inbred line G342, some heat shock protein bands were observed in both control and heat stress at 45°C for 2 hours, but appeared more concentrated after heat stress more than the control. New HSP bands with 22 kD was detected after heat stress of 45°C for two and

four hours and 18 kD after treatment by 45°C for four hours appeared in the inbred line G342. The bands which observed more detection during treatment 2 (45°C for 4 hours) with molecular weights 106, 70, 55, 31, 22, 15 and 10 kDa. On the other hand, the inbred line Rg59 did not show differences between the electrophoretic bands in the two patterns of control and heat treatments except one band of 22 kD was enhanced after heat stress at 45°C for two and four hours as HSP.

These results are in accordance with those of other researchers in their studies on HSPs, which **Lindquist (1986) and Vierling (1991)** found that HSPs ranging in molecular mass from 10 to 200 kDa and are involved in signal transduction during heat stress and protect of functional sites from the adverse effects of high temperature. **Morimoto and Santoro (1998)** indicated that Hsps protect cells from injury and facilitate recovery and survival after a return to normal growth conditions. **Sun et al (2002)** revealed that sHSPs cannot refold non-native proteins, but they can bind to partially folded or denatured substrates proteins, preventing irreversible unfolding or wrong protein aggregation **Nakamoto and Vigh (2007)** concluded that there were some indications that small heat shock proteins play an important role in membrane quality control and thereby potentially contribute to the maintenance of membrane integrity especially under stress conditions.

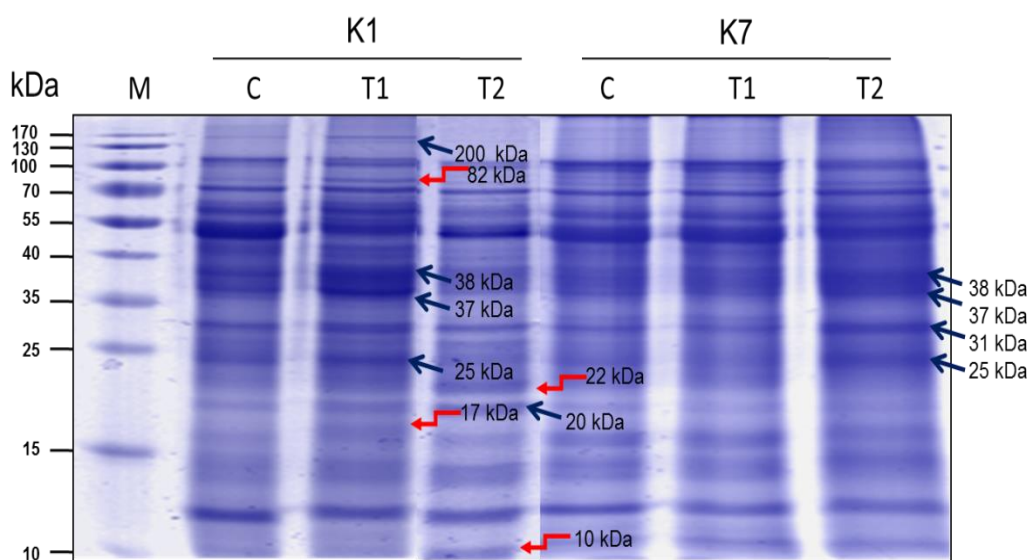


Fig. 2. Coomassie Brilliant Blue-stained SDS-PAGE of total leaf proteins (25µg/lane) extracted from control and heat treated plants of indicated inbred lines. C (control): 25°C, T1: 45°C for 2 hours and T2: 45°C for 4 hours. M: Molecular weight in kD standard proteins. Black arrows indicate bands enhanced in heat treatments. Red arrows indicate bands only present in heat-treated extracts.

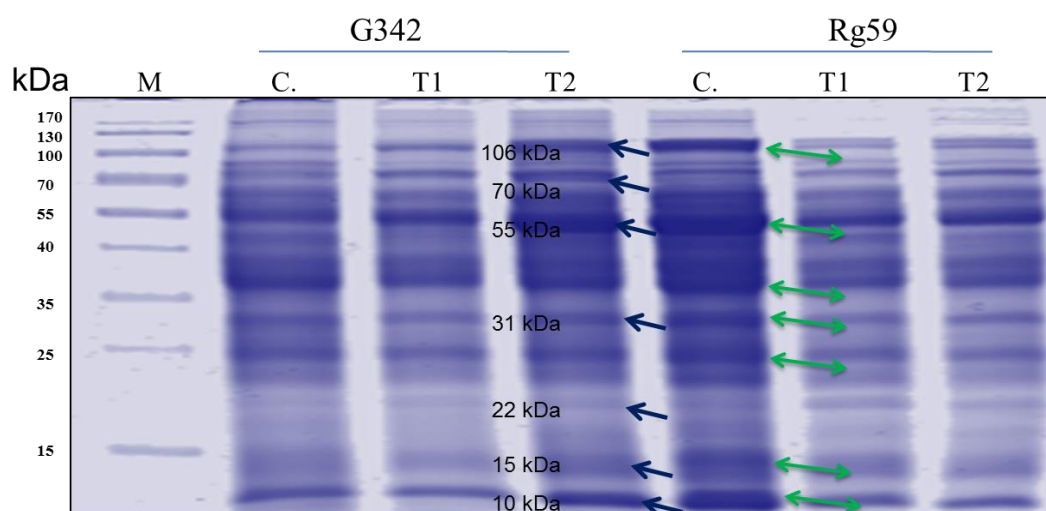


Fig. 3. Coomassie Brilliant Blue-stained SDS-PAGE of total leaf proteins (25µg/lane) extracted from control and heat treated plants of indicated inbred lines. C (control): 25°C, T1: 45°C for 2 hours and T2: 45°C for 4 hours. M: Molecular weight in kD standard proteins. Black arrows indicate bands enhanced in heat treatments. Green arrows indicate bands present in control but not induced by heat-treatment.

Table 3. SDS-PAGE profile for two maize inbred lines after exposing to heat shock. C (control): 25°C, T1 (treatment 1): 45°C for 2 hours and T2 (treatment 2): 45°C for 4 hours. (+) means presence and (-) means absence of band

Band No.	M W (kDa)	Maize inbred lines					
		K1			K7		
		C	T1	T2	C	T1	T2
1	200	+	++	+	+	+	+
2	106	+	+	+	+	+	+
3	82	-	+	-	-	-	-
4	70	+	+	+	+	+	+
5	63	+	+	+	+	+	+
6	55	+	+	+	+	+	+
7	47	+	+	+	+	+	+
8	38	+	++	+	+	+	++
9	37	+	++	+	+	+	++
10	31	+	+	+	+	+	++
11	25	+	++	+	+	+	++
12	22	-	-	+	-	-	-
13	20	+	+	++	+	+	+
14	18	+	+	+	+	+	+
15	17	-	+	-	-	-	-
16	15	+	+	+	+	+	+
17	13	+	+	+	+	+	+
18	10	-	-	+	+	+	+
Total bands		14	16	16	15	15	15

Table 4. SDS-PAGE profile for two maize inbred lines after exposing to heat shock. C (control): 25°C, T1 (treatment 1): 45°C for 2 hours and T2 (treatment 2): 45°C for 4 hours. (+) means presence and (-) means absence of band

Band No.	M W (kDa)	Maize inbred lines					
		G342			Rg59		
		C	T1	T2	C	T1	T2
1	200	+	+	+	+	+	+
2	106	+	+	++	+	+	+
3	82	+	+	+	+	+	+
4	70	+	+	++	+	+	+
5	63	+	+	+	+	+	+
6	55	+	+	++	+	+	+
7	47	+	+	+	+	+	+
8	38	+	+	+	+	+	+
9	37	+	+	+	+	+	+
10	31	+	+	++	+	+	+
11	25	+	+	+	+	+	+
12	23	+	+	+	+	+	+
13	22	-	+	++	-	+	+
14	18	-	-	+	+	+	+
15	15	+	+	++	+	+	+
16	10	+	+	++	+	+	+
Total bands		14	15	16	15	16	16

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