Egypt. J. Bot., **Vol. 58,** No.2, pp. 233 - 247 (2018)

Protective Role of β-sitosterol or Gibberellic Acid to *Lycopersicum Esculentum* Cultivars Under Temperature Stress

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THIS STUDY investigate the efficacy of β-sitosterol ($10^{-3,}$ 10^{-5} and 10^{-7} molar) or gibberellic acid (100ppm) on alleviating harmful effects of temperature stress (10^{o}C and 45^{o}C) on three cultivars of *Lycopersicum esculentum* Mill., mainly Fayrouz, Aziza and N23-48 to the purpose the activities of some antioxidant enzymes, protein patterns and DNA finger print in order to focus on the role of β-sitosterol or gibberellic acid for enhancing plant tolerance against temperature stress. It was found that, temperature stress (10^{o}C and 45^{o}C) decreased the activities of catalase and peroxidase, while β-sitosterol (10^{-3}, 10^{-5} and 10^{-7} molar) or gibberellic acid (100ppm) enhanced these enzymes activity. The electrophoresis studies showed that some new protein bands and DNA finger print were observed probably to increase plant tolerance against temperature stress. These results give a positive indication of the use of β-sitosterol specially 10^{-5} molar or gibberellic acid in field application to ameliorate the toxic effects of temperature on tomato plants.

Keywords: Tomato, Temperature stress, Sitosterol, Gibberellic acid, Enzymes activity, Protein patterns, DNA finger print.

Introduction:

During the normal processes of growth and development, plants are subjected to different types of stress, such as drought, heat, ultraviolet light, air pollution, and pathogen attack .Most plants suffer from both physiological and biochemical damage by exposure to temperatures; higher or lower than optimal for growth. The results of these injuries, which are reflected in most metabolic processes, may be reduced growth capacity of the crops and therefore lower commercial yield (Rivero et al., 2001).

Plants response to temperature stress by change their morphology, anatomy, physiology, biochemical and genetic responses (Camejo et al., 2005; Snider et al., 2010; Chen et al., 2012 and Min et al., 2014). It interrupts the scavenging of ROS by antioxidant system (enzymatic and non-enzymatic) leading to rise of oxidative stress that led to destruction of proteins, cell membranes and DNA (Abdalla, 2011).

The temperature stress causes a decrease in normal protein synthesis accompanied by an accelerated synthesis of new proteins known as heat shock proteins (HSPs). This response of plants is observed when they are exposed to temperatures at least 5°C above their optimal growing temperature. In addition to physiological indicators/markers of heat tolerance, molecular markers are also promising to help tomato screening and breeding phenomena aimed at improving its heat tolerance. The role played by phytohormone signaling in the modulation of DNA repair gene and the resulting effects on plant adaptation to genotoxic stress are poorly investigated (Dona et al., 2013).

Sterols are known to regulate transcriptional and post transcriptional events, which inturn affect lipid synthesis, meiosis, apoptosis, developmental patterning, protein cleavage and protein degradation (Rashad et al., 2009). Gibberellic acid regulates various developmental processes throughout the life cycle of the plant from seed germination through leaf expansion and stem development (Sun & Gubler, 2004).

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DOI: 10.21608/ejbo.2018.2486.1143

The tomato (*Lycopersicum esculentum* Mill.), is an important vegetable crop widely grown all over the world. Tomato is the number nine crop on the lists of food commodities and widely used as a model crop for source–sink studies and stress (Zhou et al., 2016).

The aim of this study was to investigate the influence of β-sitosterol or gibberellic acid on alleviate deleterious effect of temperature stress on tomato plants by detecting biochemical and molecular markers.

Materials and Methods

Experiment preparation

The three cultivars of *Lycopersicom esculentum* Mill (Tomato) that used in this study, Fayrouz F1, Aziza F1 and N23-48 F1 were supplied by the Agricultural Research Center, Ministry of Agricultural, Egypt. According to preliminary experiment, the seeds of the three cultivars were soaked for 10 h in 10^{-3}, 10^{-5} and 10^{-7} molar β-sitosterol, 100ppm gibberellic acid (GA_3) and distilled water (control). Fifty seeds per each (control and treatments) were sown in each germination trays (containing equal amounts of peat moss) at 25±3°C. After 28 days from sowing, initial samples were taken. At the same day the seedlings transferring to three growth chambers at temperature (10, 25, 45 ± 3°C). Forty two days from sowing (as the true leaf fully expanded), vegetative samples from each treatment were collected to determine antioxidant enzymes (catalase and peroxidase) and protein patterns was carried out to the three used varieties with treatments have the best results, in addition DNA finger print of N23-48 tomato cultivar leaves was the best responses under the three temperatures.

Estimation of antioxidant enzymes

The extraction and estimation of the catalase (E.C.1.11.1.6) and peroxidase (E.C.1.11.1.7) was carried out according to Malik & Singh (1980).

Protein patterns

SDS-polyacrylamide gel electrophoresis was performed in 12% acrylamide slab gels following the system of Laemmli (1970) to identify their protein profiles.

Identification of DNA finger print by RAPD-PCR technique

Genomic DNA was extracted from leaves of N23-48 cultivar according to Junhans & Metzlatt (1990) protocol.

Polymerase chain reaction (PCR) conditions and analysis

Reaction of RAPD-PCR was performed according to Williams et al. (1990) using 3 type of 10-mer random primers: (OPA-12 TCGGCGATAG, RAPD5 TTCGACCCAG and RAPD6 AAAGCTGCGG). The PCR reaction was performed in a final volume of 25µl containing: 5µl 5 × PCR buffer, 2.5µl primer, 3µl DNA, 0.5µl Taq polymerase and 14µl sterile distilled water. The PCR was performed with the cycle program of denaturation (one cycle) 94°C for 5 min, 40 cycles 1 min. at 94°C, 30 sec. at 36°C, 1 min. at 72°C and with a final extention of 5 min. at 72°C. The PCR products were detected on 1% agarose gel using ethidium bromide and visualized by gel documentation system (GDS).

Data analysis

The full data of the differently treated groups were statistically analysed and comparison among means was carried out by computer programming method (statgraphic- vers-4-2- Display ANOVA), as described by Scedecore & Cochran (1982).

Analysis of gel electrophoresis

Variation in SDS-PAGE and RAPD-PCR bands were analyzed using computer program Gel Analyzer 3.

Results and Discussion

Changes in antioxidant enzymes

Data in Tables 1 and 2 showed that, the effect of different concentrations of β-sitosterol (10^{-3}, 10^{-5} and 10^{-7} molar) and GA_3 (100ppm) with or without temperature stress (10°C and 45°C) on activities of enzymes catalase and peroxidase, cleared a general significant increase in the used tomato cultivars, compared with untreated plants, during the two stages. However, during vegetative stage as shown in Table 2 of tomato cultivars, a decrease in activities of catalase and peroxidase under temperature stress at 10°C and 45°C as compared with 25°C, was detected.

In accordance with these results, there is a direct link between reactive oxygen species (ROS) scavenging and plant stress tolerance under temperature extremes (Suzuki & Mittler, 2006). Thus, the improvement of temperature stress

tolerance is often related to enhanced activities of enzymes involved in antioxidant systems of plants. Plants exposed to extreme temperatures use several non-enzymatic and enzymatic antioxidants to cope with the harmful effects of oxidative stress; higher activities of antioxidant defense enzymes are correlated with higher stress tolerance. Different plant studies have revealed that enhancing antioxidant defense confers stress tolerance to either high temperature (HT) or low temperature (LT) stress (Huang & Guo, 2005 and Almeselmani et al., 2006).

TABLE 1. Effect of the different concentrations of β-sitosterol or gibberellic acid on different antioxidant enzyme activities (Δ absorbance unit mg/min/g F.W) of the three tomato cultivars shoot at initial stage.

Cultivars	Parameter / Treatment	Antioxidant enzyme	
		Catalase	Peroxidase
Fayrouz	Control	12.26	3.06
	Gibberelins (100ppm)	14.28*	4.02*
	Sitosterol (10^{-3} molar)	*13.73	3.45*
	Sitosterol (10^{-5} molar)	15.19*	4.51*
	Sitosterol (10^{-7} molar)	14.05*	3.99*
Aziza	Control	12.98	3.13
	Gibberelins (100ppm)	13.65*	3.85*
	Sitosterol (10^{-3} molar)	13.77*	4.02*
	Sitosterol (10^{-5} molar)	14.87*	4.35*
	Sitosterol (10^{-7} molar)	13.93*	4.25*
N23-48	Control	13.61	3.34
	Gibberelins (100ppm)	14.58*	3.84*
	Sitosterol (10^{-3} molar)	14.83*	3.92*
	Sitosterol (10^{-5} molar)	15.4*	4.66*
	Sitosterol (10^{-7} molar)	15.21*	4.16*

*= Significant increase or decrease at 0.05 LSD.

TABLE 2. Effect of the different concentrations of β-sitosterol or gibberellic acid on different antioxidant enzyme activities (Δ absorbance unit mg/min/g F.W) of the three tomato cultivars shoot at vegetative stage.

Temperature	Cultivars	Fayrouz		Aziza		N23-48	
	Parameter / Treatment	Catalase	Peroxidase	Catalase	Peroxidase	Catalase	Peroxidase
25 °C	Control	16.52	4.89	15.61	5.32	17.06	4.79
	GA_3 (100ppm)	18.76*	6.12*	15.93*	5.81*	17.41*	5.07*
	Sitosterol (10^{-3} molar)	*16.86	5.33*	16.71*	6.10*	18.08*	5.48*
	Sitosterol (10^{-5}molar)	19.15*	6.28*	18.26*	6.76*	19.86*	6.13*
	Sitosterol (10^{-7} molar)	17.65*	5.71*	17.90*	6.59*	19.33*	5.89*
10 °C	Control	13.95	4.12	14.87	3.75	13.16	3.81
	GA_3 (100ppm)	14.89*	4.78*	16.93*	4.66*	13.36*	4.16
	Sitosterol (10^{-3} molar)	14.12*	4.42*	15.88*	4.37*	15.09*	4.80*
	Sitosterol (10^{-5} molar)	16.09*	5.27*	17.31*	4.91*	15.22*	4.94*
	Sitosterol (10^{-7} molar)	15.81*	5.07*	15.23*	4.09*	14.21*	4.57*
45 °C	Control	15.58	4.39	14.16	2.98	13.65	3.99
	GA_3 (100ppm)	17.63*	5.59*	16.02*	4.67*	14.98*	4.36*
	Sitosterol (10^{-3} molar)	16.56*	5.23*	15.43*	4.23*	16.62*	5.63*
	Sitosterol (10^{-5} molar)	17.93*	5.75*	17.34*	5.07*	16.77*	5.21*
	Sitosterol (10^{-7} molar)	15.87*	4.78*	17.05*	4.96*	15.53*	5.31*

*= Significant increase or decrease at 0.05 LSD.

Hasanuzzaman et al. (2013) reported that, the cellular changes induced by either high temperature or low temperature includes responses those lead to the excess accumulation of toxic compounds, especially reactive oxygen species (ROS). The end result of ROS accumulation is oxidative stress (Mittler, 2002 and Suzuki & Mittler, 2006). There are numerous plant studies which indicate the tolerance to temperature stress in plants is positively correlated with an increase in antioxidants (Hasanuzzaman et al., 2012 and Babu & Devraj, 2008).

The perusal of the recorded data in this study are in record with the results of Djanaguiraman et al. (2010) who observed that high temperature stress decreased antioxidant enzyme activities and increased oxidant production in sorghum. In their study, SOD, CAT and POX activities were decreased in heat stress (22, 15 and 25% lower than control plants) and the greater inhibition of all antioxidant enzymes in heat-stressed plants relative to control plants indicates greater inactivation of all antioxidant enzymes by heat stress. The early effects of thermal stress comprise of structural

alterations in chloroplast protein complexes and reduced activity of enzymes (Ahmad et al., 2010).

In this study, gibberellic acid treatment caused a general significant increase in activities of catalase and peroxidase in both stages of the used tomato cultivars. Gibberellic acid (GA_3) that primarily affect cell enlargement and growth must also coordinately interact with ABA under stress and possibly other stress metabolites, including antioxidants and ROS scavengers (Achard et al., 2006). As a result of ROS production, plant cell has to activate the antioxidant defense system including enzymatic antioxidant to scavenge ROS (Sairam et al., 2002).

It has been found in this investigation that, during the two stages (initial and vegetative) of tomato cultivars, catalase and peroxidase, significantly increased in response to the different pretreatment of β-sitosterol in relation to control values. In this respect, there are reports of further beneficial effects of phytosterols, alone and in combination with other naturally occurring compounds as having possible anti-oxidation activities (Van et al., 2000).

Similarly, it has been observed that BRs (brassinosteroids) application caused an increase in CAT activity in both susceptible and resistant varieties of sorghum seedlings under osmotic stress (Vardhini & Rao, 2003). Furthermore, the activities of antioxidant enzymes such as SOD, APX, CAT, and guaiacol POD were significant in EBL (24-epibrassinolide) treated tomato plants under heat stress (Dhaubhadel et al., 1999) and (Mazorra & Núñez, 2000) reported that, EBL and MH5 (BR analogue) stimulated CAT activity in tomato leaf discs under heat stress. Nevertheless, although BRs and ROS are thought to act as secondary messengers for the induction of antioxidant defences in stressed plants**,** the relationship between BRs and ROS in stress-signal transduction remains unclear (Ogweno et al., 2008).

Change in protein banding patterns of leaves

The best responses while studying the protein patterns of the three tomato cultivars were the ones treated with 100ppm gibberellic acid or 10^{-5} molar β- sitosterol under the three temperature (25°C, 10°C and 45°C), and for that reason they were the ones discussed in this study.

Treatment of Fayrouz cultivar with 100ppm GA_3 (plate1 and Table 3a) led to express 9 protein bands and appearance of new bands at 31.22, 28.96, 27.63 and 23.16kDa and by 10^{-5} molar β- sitosterol, expression of 11 bands with new protein bands (at 50.80, 36.29, 31.22, 28.96 and 27.63) and absence band at 76.85kDa with the two treatments in response to control sample grown under 25°C.

By growing plants under 10°C, no changes were detected in protein patterns between control and treatments with GA_3 and β-sitosterol excepts new band expressed at 49.86kDa in response to treatment by 10^{-5} molar β-sitosterol, total number of bands to both control and 100ppm GA_3were 12 bands but β-sitosterol recorded 13 bands. Concerning treatment at 45°C, increased the protein bands after treatment by 100ppm GA_3 to 13 bands and expression of new protein bands (at 90.60, 50.80, 48.35, 41.01, 31.22, 28.96 and 27.63kDa) and by treatment with β-sitosterol induced increase in the bands to 11 bands and appearance of five new bands (at 50.80, 48.35, 31.22, 28.96 and 27.63kDa) as composed to the control bands (7 bands).

Aziza cultivar leaves exhibited the same bands as in control in response to all treatments under 25°C (Plate 1 and Table 3b). At 10°C, treatment plants with10^{-5} molar β- sitosterol or GA_3 induced decrease the bands to 11 bands as compared with the control which have 13 bands with two unique bands (at 199.50 and 95.26). Application of high temperature (45°C) either alone or plus treatments with 100ppm gibberellic acid or 10^{-5} molar β-sitosterol led to express new protein band, at 83.13kDa.

Concerning N23-48 cultivar grown under 25°C (Plate 1 and Table 3c), the gel indicated that the total number of bands in each treatments 6 bands. Treatment of N23-48 cultivar with 100ppm GA_3 and grown under 10°C led to express of 9 protein bands and appearance of three new bands (at 103.32, 46.60 and 34.10kDa). Meanwhile application of 10^{-5} molar β-sitosterol with 10°C led to separation of 10 protein bands with appearance of 4 new bands (at 151.64 unique, 103.32, 46.60 and 34.16kDa) as compared to the control. On the other hand, under 45°C, treatment of tomato with gibberellic acid expressed 7 bands, with appearance of one new band (at 34.10kDa), whereas application with β-sitosterol led to separation of 9 protein bands with appearance of three new bands (at 66.14, 46.60 and 34.10) and absence of one band in both treatments (GA and β-sitosterol) at 81.21kDa in relation to control.

TABLE 3a. Effect of the different concentrations of β-sitosterol or gibberellic acid on protein banding patterns of Fayrouz cultivar leaves.

Polymorphism	Frequency	Treatments									M.W (kD)	RF
		45° C			10° C			25° C				
		Sito-10^{-5} molar	GA_3 100 ppm	Control	Sito-10^{-5} molar	GA_3 100 ppm	Control	Sito-10^{-5} molar	GA_3 100 ppm	Control		
Monomorphic	1	+	+	+	+	+	+	+	+	+	161.97	0.139
Polymorphic	0.333	-	-	-	+	+	+	-	-	-	105.57	0.321
Monomorphic	1	+	+	+	+	+	+	+	+	+	93.64	0.372
Unique	0.111	-	+	-	-	-	-	-	-	-	90.60	0.386
Polymorphic	0.556	-	-	+	+	+	+	-	-	+	76.85	0.456
Monomorphic	1	+	+	+	+	+	+	+	+	+	64.58	0.530
Polymorphic	0.333	+	+	-	-	-	-	+	-	-	50.80	0.632
Unique	0.111	-	-	-	+	-	-	-	-	-	49.86	0.640
Polymorphic	0.222	+	+	-	-	-	-	-	-	-	48.35	0.653
Monomorphic	1	+	+	+	+	+	+	+	+	+	45.27	0.681
Polymorphic	0.333	-	+	-	+	+	+	-	-	-	41.01	0.723
Unique	0.111	-	-	-	-	-	-	+	-	-	36.29	0.775
Polymorphic	0.667	+	+	-	+	+	+	+	+	-	31.22	0.839
Polymorphic	0.667	+	+	-	+	+	+	+	+	-	28.96	0.871
Polymorphic	0.667	+	+	-	+	+	+	+	+	-	27.63	0.891
Monomorphic	1	+	+	+	+	+	+	+	+	+	24.50	0.942
Unique	0.111	-	-	-	-	-	-	-	+	-	23.16	0.966
Polymorphic	0.889	+	+	+	+	+	+	+	-	+	23.00	0.969
		11	13	7	13	12	12	11	9	7	Total bands	

Sito- = β-Sitosterol

From table of present or absent protein bands showed that :

- Total number of bands =18
- Monomorphic bands = 5, polymorphic = 9 , unique = 4
- Maximum M.W. =161.97, Minimum M.W. =23.00
- Polymorphism (%) = 78.947
- Mean of band frequency = 0.497

TABLE 3b. Effect of the different concentrations of β-sitosterol or gibberellic acid on protein banding patterns of Aziza cultivar leaves.

Polymorphism	Frequency	Treatments									M.W (kD)	RF
		45° C			10° C			25° C				
		Sito-10^{-5} Molar	GA_3 100 ppm	Control	Sito-10^{-5} molar	GA_3 100 ppm	Control	Sito-10^{-5} molar	GA_3 100 ppm	Control		
Unique	0.111	-	-	-	-	-	+	-	-	-	199.50	0.01
Unique	0.111	-	-	-	-	-	+	-	-	-	95.26	0.33
Polymorphic	0.333	+	+	+	-	-	-	-	-	-	83.13	0.389
Monomorphic	1	+	+	+	+	+	+	+	+	+	73.89	0.44
Polymorphic	0.333	-	-	-	+	+	+	-	-	-	65.68	0.491
Monomorphic	1	+	+	+	+	+	+	+	+	+	52.01	0.592
Monomorphic	1	+	+	+	+	+	+	+	+	+	45.17	0.653
Monomorphic	1	+	+	+	+	+	+	+	+	+	41.76	0.687
Monomorphic	1	+	+	+	+	+	+	+	+	+	33.15	0.787
Monomorphic	1	+	+	+	+	+	+	+	+	+	30.36	0.825
Polymorphic	0.333	-	-	-	+	+	+	-	-	-	26.99	0.876
Polymorphic	0.333	-	-	-	+	+	+	-	-	-	24.89	0.911
Monomorphic	1	+	+	+	+	+	+	+	+	+	21.87	0.967
Monomorphic	1	+	+	+	+	+	+	+	+	+	21.08	0.983
		9	9	9	11	11	13	8	8	8	Total bands	

Sito- = β-Sitosterol

From table of present or absent protein bands showed that :

- Total number of bands =14
- Monomorphic bands = 8, polymorphic = 4, unique = 2
- Maximum M.W. =199.50, Minimum M.W. =21.08
- Polymorphism (%) = 50 %
- Mean of band frequency = 0.659

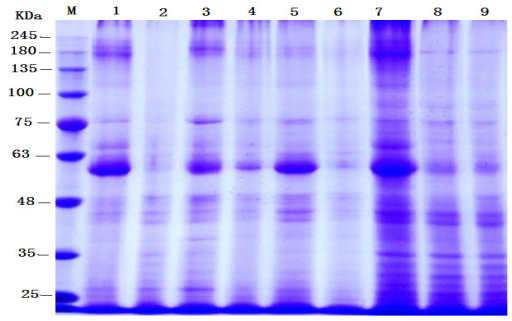
TABLE 3c. Effect of the different concentrations of β-sitosterol or gibberellic acid on protein banding patterns of N23-48 cultivar leaves.

Polymorphism	Frequency	Treatments									M.W (kD)	RF
		45° C			10° C			25° C				
		Sito-10^{-5} molar	GA_3 100 ppm	Control	Sito-10^{-5} molar	GA_3 100 ppm	Control	Sito-10^{-5} molar	GA_3 100 ppm	Control		
Unique	0.111	-	-	-	+	-	-	-	-	-	151.64	0.102
Polymorphic	0.222	-	-	-	+	+	-	-	-	-	103.32	0.274
Polymorphic	0.333	-	-	-	+	+	+	-	-	-	91.19	0.33
Unique	0.111	-	-	+	-	-	-	-	-	-	81.21	0.382
Polymorphic	0.444	+	-	-	+	+	+	-	-	-	66.14	0.474
Polymorphic	0.667	+	+	+	-	-	-	+	+	+	54.11	0.564
Polymorphic	0.667	+	+	+	-	-	-	+	+	+	51.18	0.589
Polymorphic	0.333	+	-	-	+	+	-	-	-	-	46.60	0.631
Polymorphic	0.444	+	+	-	+	+	-	-	-	-	34.10	0.771
Monomorphic	1	+	+	+	+	+	+	+	+	+	30.30	0.824
Monomorphic	1	+	+	+	+	+	+	+	+	+	28.15	0.857
Monomorphic	1	+	+	+	+	+	+	+	+	+	21.60	0.974
Monomorphic	1	+	+	+	+	+	+	+	+	+	21.02	0.988
		9	7	7	10	9	6	6	6	6	Total bands	

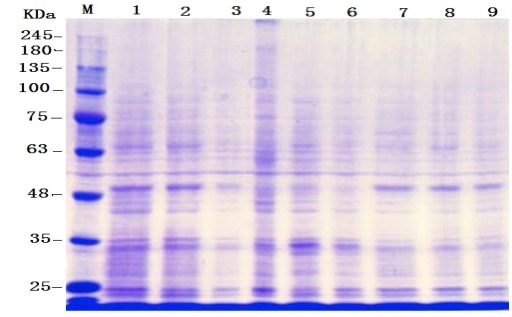
Sito- = β-Sitosterol

From table of present or absent protein bands showed that :

- Total number of bands =13
- Monomorphic bands = 4, polymorphic = 7, unique = 2
- Maximum M.W. =151.64, Minimum M.W. = 21.02
- Polymorphism (%) = 69.23
- Mean of band frequency = 0.564



(Faurouz)



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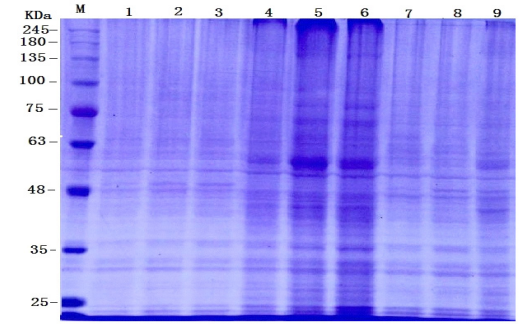

(N23-48)

Plate 1. Effect of the different concentrations of β-sitosterol or gibberellic acid on the protein banding patterns of three tomato cultivars, M = marker (1=control 2=GA_3 3=10-5sito- at 25°C), (4=control 5=GA_3 6=10-5sito- at 10°C) and (7=control 8=GA_3 9=10-5sito- at 25°C).

These results are in agreement with Yang et al. (2006) who found that the pattern of gene expression changed by heat stress, which is the way to improve plant-heat tolerance.

El-Enany et al. (2013) recorded the presence of fifteen protein bands in *Lupinusalbus* leaves grown under high temperature (42˚C) at different time periods with the appearance of few new bands due to heat stress and presence of heat shock protein.

Mahla et al. (2011) stated that, all organisms respond to elevated temperature with the production of a defined set of proteins called heat shocked proteins. These heat shocked proteins (HSPs) protect the cells from detrimental effect of high temperature, and that accumulation of HSPs, leads to increase in thermotolerance. A large number of studies reveal a positive correlation between inductions of HSPs and acquisition of thermal tolerance (Bhattacharjee & Mukherjee, 2006). In wheat seedlings, Joshi et al. (1997) linked the acquired thermo-tolerance with 26kDa plastid localized heat shocked protein. The results are also in accordance with the findings of Mahmoud & Mohamed (2007) who observed the production of HSPs bands of MW 17kDa and 103.7kDa in wheat seedlings exposed to differential temperature stress. Ouebbou & Paulsen (1999) found a new set of heat shocked proteins (17-80kDa) induced within 4 h in wheat when the temperature of incubation was raised to 37ºC.

Dhaubhadel et al. (1999) in a study, suggested that increased stress resistance of 24-epibrassinolide (EBR)-treated seedlings may be due, at least in part, to the increased accumulation of the various HSPs in these seedlings. They suggested that, the higher accumulation of HSPs transcripts during heat stress in EBR-treated seedlings, in addition to higher accumulation of HSPs at later time points, clearly indicates that the treated seedlings are primed towards responding more efficiently to heat stress than are untreated seedlings. In addition, the differences in HSPs accumulation between treated and untreated seedlings were observed several days before the symptoms of damage began to appear.

In current study, Fayrouz and N23-48 tomato cultivars gave the best response by treatments (temperatures and either 100ppm gibberellic acid or 10^{-5} molar β-sitosterol) rather than Aziza cultivar which showed nearly no response. Thus, in Fayrouz and N23-48 cultivars, protein band at 76.85 and 81.21kDa, respectively generally disappeared by all used treatments and these bands could be considered as a negative specific marker for heat stress shock (El- Enany et al. , 2013).

On the other hand, certain new bands expressed generally ranging from 31.22 to 23.00kDa in case of Fayrouz cultivar and from 46.60 to 34.10kDa in case of N23-48. In this concern, the appearance of new bands in heat treated plants should be attributed to expression of heat stress genes.

These results were in harmony with the work of Yamaguchi et al. (1995) and Cherian & Ferreira (2010) who reported that the exposure of plants to heat shock treatment lead to the synthesis of new protein bands with small molecular weight which are called heat shock proteins (HSPs). These changes may attribute to the modifications in gene expression due to heat stress.

In this respect, phytohormones are essential for the ability of plants to adapt to abiotic stresses by mediating a wide range of adaptive responses (Peleg & Blumwald, 2011). They often rapidly alter gene expression by inducing or preventing the degradation of transcriptional regulators via the ubiquitin proteasome system (Santner & Estelle, 2010).

Molecular genetic polymorphism by RAPD-PCR

Although the three tomato cultivars were subjected to the same treatments (temperatures and either 100ppm gibberellic acid or 10^{-3}, 10^{-5} and 10^{-7} molar β-sitosterol), only N23-48 tomato cultivar was the most affected especially while being treated with 100ppm gibberellic acid or 10^{-5} molar β-sitosterol, and that is why this cultivar subjected to these treatments were used while studying the DNA finger print.

Three arbitrary random amplified polymorphic DNA(RAPD) primers (APO-12, RAPD5 and RAPD6) were used to amplify the genotypes of untreated and treated tomato plants subjected to three grades of temperature stress (Plate 2 and Table 4).

A total of 21 amplified DNA fragments ranging in size from 1.9 to 0.35Kbp were realized using the 3 primers, three monomorphic, 13 polymorphic and 5 unique bands were revealed in N23-48 tomato cultivar for the control as well as the sample treated with different treatments. The polymorphic percentage of the 3 primers (APO-12, RAPD5 and RAPD6) was 80, 100 and 71.429% successively. The highest number of RAPD bands was detected for primer RAPD5 (9 bands), while the lowest was scored for OPA-12 (5 bands).

RAPD analysis of N23-48 tomato cultivar revealed the induction of 30 and 36 distinctive bands response to gibberellic acid and β-sitosterol treatment successively of stressed and unstressed plants using the 3 primers as compering 28 bands to untreated. Noteworthy that growth regulators application to both unstressed or variously stressed plants induced the highest number of DNA amplified segments as compared to either control or stressed plants. These criteria can be considered as a positive genetic marker for growth regulators tolerance to stress condition. These results were accommodated with similar studies. In this connection, DNA polymorphisms can become useful markers in general fingerprinting (Kawakami et al., 1999 and Shehata & EL-Khawas, 2003).

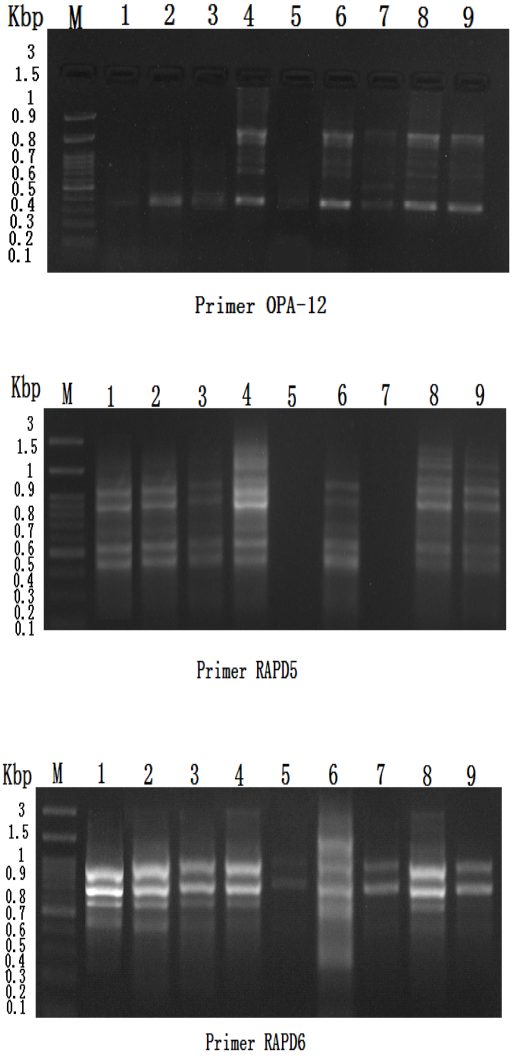

Plate 2. DNA polymorphism of N23-48 tomato cultivar using RAPD primers (OPA-12, RAPD5 and RAPD6), M = marker (1=control 2=GA_3 3=10-5sito- at 25°C), (4=control 5=GA_3 6=10-5sito- at 10°C) and (7=control 8=GA_3 9=10-5sito- at 45°C).

TABLE 4. Analysis of RAPD-PCR of N23-48 tomato cultivar genomic using three RAPD primers (OPA-12, RAPD5 & RAPD6).

Primers name	RF	M.W (Kbp)	Treatments									Polymorphism
			25° C			10° C			45° C			
			Control	GA_3 100 ppm	Sito-10^{-5} molar	Control	GA_3 100 ppm	Sito-10^{-5} molar	Control	GA_3 100 ppm	Sito-10^{-5} molar	
OPA-12	0.43	1.606	-	-	-	+	-	+	+	+	+	Polymorphic
	0.543	0.863	-	-	-	+	-	+	-	+	+	Polymorphic
	0.591	0.662	-	-	-	+	-	+	-	+	+	Polymorphic
	0.66	0.453	-	-	-	-	-	-	+	+	-	Polymorphic
	0.70	0.35	+	+	+	+	+	+	+	+	+	Monomorphic
RAPD5	0.087	1.9	-	-	-	-	-	-	-	+	+	Polymorphic
	0.115	1.669	-	-	-	-	-	-	-	+	+	Polymorphic
	0.137	1.529	-	-	-	+	-	-	-	-	-	Unique
	0.152	1.407	-	-	-	-	-	-	-	+	+	Polymorphic
	0.202	1.116	+	+	+	+	-	+	-	+	+	Polymorphic
	0.251	0.89	+	+	+	+	-	+	-	+	+	Polymorphic
	0.321	0.644	+	+	+	+	-	+	-	+	+	Polymorphic
	0.368	0.518	+	+	+	+	-	+	-	+	+	Polymorphic
	0.425	0.398	+	+	+	+	-	+	-	+	+	Polymorphic
RAPD6	0.102	2.108	-	-	-	+	-	-	-	-	-	Unique
	0.128	1.891	-	-	-	-	-	-	-	+	-	Unique
	0.171	1.579	-	-	-	-	-	-	-	+	-	Unique
	0.235	1.207	-	-	-	-	-	+	-	-	-	Unique
	0.321	0.841	+	+	+	+	+	+	+	+	+	Monomorphic
	0.406	0.589	+	+	+	+	+	+	+	+	+	Monomorphic
	0.472	0.447	+	+	+	+	-	+	-	+	-	Polymorphic

Ganash (2016) stated that, plants sprayed with yeast extract and gibberellic acid showed morphological and/or physiological changes when compared to control samples. These changes might be a result of physiological effect or genetic modifications after using random amplified polymorphic DNA analysis. DNA polymorphisms might be rendered to the loss and/or gain of amplified bands in the treated samples compared with the control. Also, Yang & Quiros (1993) postulated that quantitative changes could be explained on the basis of alterations of some DNA sequences.

Random Amplified Polymorphic DNA analysis is suitable for genotyping, phylogenetic analysis and molecular selection (Gokturk al., 2003; Atak et al., 2004 and Yuzbasioglu et al., 2006). It has been widely used in the phylogenetic analysis of many plants and a general concordance was demonstrated among the results derived from RAPD and other techniques (Naugzemys et al., 2007). The presence or absence of RAPD bands are used to estimate diversity and in measurement of similarity (Choi et al., 2006 and Wang et al., 2009). Appearance of new bands is usually resulting from different structural changes of DNA (breaks, transpositions, deletion etc.) (Danylchenko & Sorochinsky, 2005). Hegazi & Hamideldin (2010) observed that the changes in the DNA bands, where the main changes in the RAPD profiles of the appearance or disappearance of different bands with variation in their intensity. These effects might be due to the structural rearrangements in DNA caused by different types of DNA damages. Khan et al. (2010) reported significant genetic variability in various morphological and physiological traits of wheat under stress conditions.

Conclusion

It is clear from the previous results that soaking of tomato seeds in β-sitosterol especially 10^{-5} molar or gibberellic acid (100ppm) improves the tolerance of three tomato cultivars; especially N23-48 to temperature stress.

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(Received 1/ 1 /2018;
accepted 27/ 2 /2018)

دور كلا من بيتا-سيتوستيرول أو حمض الجبريليك في وقاية اصناف نبات الطماطم (*Lycopersicum esculentum*) المنزرعة تحت الاجهاد الحراري

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تم دراسة فعالية و تأثير تركيزات مختلفة من بيتا-سيتوستيرول (10^{-3}، 10^{-5} و 10^{-7} مولار) أو حمض الجبريليك (100 جزء في المليون) لتلاشي الآثار الضارة على ثلاثة أصناف من نبات الطماطم (*Lycopersicum esculentum*) وهي فيروز، عزيزة و48 -N23 النامية تحت ظروف الإجهاد الحراري (10°م و 45°م) وذلك بدراسة ومتابعة نشاط بعض الإنزيمات المضادة للأكسدة، وأنماط البروتين والبصمة الوراثية من أجل تسليط الضوء على الآليات لتأثير البيتاسيتوستيرول وحمض الجبريلك على نشاط انزيمى الكتاليز والبيروكسيدز فى النباتات المجهده حراريا، حيث اثبتت النتائج انخفاضا فى نشاط انزيمي الكاتاليز والبيروكسيديز تحت الإجهاد الحراري (10°م و 45°م)، في حين أن معاملة النباتات المجهدة و الغير مجهدة بالبيتا-سيتوستيرول (10^{-3}، 10^{-5} و 10^{-7} مولار) أو حمض الجبريليك (100 جزء في المليون) قد عزز من نشاط هذه الإنزيمات. وقد لوحظ من نتائج التفريد الكهربائي للبروتين و البصمة الوراثية زيادة مقاومة النبات للإجهاد الحراري. هذه النتائج تدعم الإستخدام الحقلي للبيتا-سيتوستيرول وبصفة خاصة 10^{-5} مولار أو حمض الجبريليك للتخفيف من الآثار الضارة للإجهاد الحراري على نبات الطماطم.