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Research Article
Priming Tomato Cultivars in β-sitosterol or Gibberellic Acid Improves Tolerance for Temperature Stress

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Abstract

Background and Objective: Tomato is an important vegetable crop all over the world. Extreme temperatures affect the growth, yield and quality of plant production. This study was conducted with an aim to investigate the impact of presoaking of seeds for 10 h in 10^{-3}, 10^{-5} and 10^{-7} M β-sitosterol and 100 ppm gibberellic acid in addition to temperature on three tomato cultivars (*Lycopersicon esculentum* Mill); Fayrouz, Aziza and N23-48 on growth, leaf anatomy and ultrastructure to show whether temperature can be offset by the application of β-sitosterol or gibberellin. **Materials and Methods:** After 28 days from sowing, plants were transferred to growth chambers at three temperature levels (10 and 45±3°C) as low and high, respectively, comparing to tomato grown at 25°C (control), after 42 days from sowing, sampling takes place. **Results:** The low temperature alone decreased growth parameters, leaf thickness, upper and lower epidermis while palisade and spongy layer increased. Although spongy layer increased markedly by high temperature a decreased in growth parameter, palisade layer, leaf thickness and upper and lower epidermis was detected. Sitosterol and gibberellin treatments in addition to, temperature caused a general significant increase in the determined measurements especially the number and area of leave and the thickness of cell wall epidermis. These results may provide support for the field application of sitosterol and gibberellin to alleviate the harmful effects of temperature on tomato plants. **Conclusion:** It is evident from the above results that, the resistance of the three cultivars of tomato plant to temperature stress (high and low) was more or less improved by priming the seeds in 100 ppm gibberellic acid or β-sitosterol specially in response to 10-5 M. Thus, these plant growth regulators could be used, as safe compounds to improve the resistance of the used tomato cultivars to temperature stress.

Key words: Tomato Fayrouz, Aziza, N23-48, temperature stress, sitosterol, gibberellin, growth, leaf anatomy and ultrastructure

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is an important agricultural crop, not only for the economic importance, but also for the nutritional value. Among all vegetables and fruits, tomato is rich by antioxidants such as lycopene that preserves cells of plants from oxidants which have been related to cancer as well as minerals and vitamins. Each fresh tomato fruit (135 g) has 47% vitamin C, 22% vitamin A and 25 calories energy[1].

Tomato crops are developed in vast sorts of environments with diverse climatic in the universe from the tropical areas to some degrees of the Arctic Circle. The biggest tomato producing nations involve China, USA, India, Turkey, Egypt, Italy, Spain, Brazil, Iran, Mexico, Canada, Greece and Russia[2]. The total world production has grown from 119.5-164.0 million tonnes during the past decade[3].

Atmospheric concentrations of greenhouse gases such as CO_2, CH_4 and N_2O have increased dramatically since the beginning of the industrial revolution due to fossil fuel combustion, deforestation and land development; together, these probably led to a rise in ground-level air temperatures at an unprecedented rate over the past three decades[4]. Moreover, the global mean temperature will continue to rise at a rapid rate and our climate is likely to warm by 1.1-6.4°C within the next century[5]. Most plant species only grow in a certain temperature range, thus, some are likely to adapt to warmer temperatures by changing their growth and development or by shifting their ranges, provided that the optimum temperatures are not exceeded. Some species may fail to adapt to this global change and may even become extinct if the air temperature is too high[6,7]. Many studies have investigated plant responses to global warming at community level while few studies were performed at the individual level or focus on sub-individual level such as responses of leaves to increase in temperature[7,8]. Because leaf is the key organ performing photosynthesis and transpiration, its development which varies with environmental factors, is an important determinant of total plant productivity[9]. In addition, leaves can be used as indicators of plant community responses to global warming, because their responses are not only the basis of changes at the community level, but they are among those organs that show visible impacts that can express phenotypically plastic responses to growth temperature[10-12].

Temperature is one of the most crucial environmental factors determining plant growth and development[13]. Temperature has a significant influence on many aspects of growth and development in tomato (*Lycopersicon esculentum* Mill.). The optimum temperature for tomato production is 21-25°C with an average monthly minimum temperature >18°C and a monthly maximum temperature of 27°C[14,15]. Fruit set is optimal between 18 and 20°C[16].

High temperature stress (HT) is defined as the rise in temperature beyond a critical threshold for a period of time sufficient to cause irreversible damage to plant growth and development[17]. In tomato plants high temperatures (≥35°C) have a negative effect on cell metabolic activity, growth and photosynthesis[18], changes in plant morphology, anatomy and physiology that are manifested from the whole-plant to the cellular or subcellular levels[19]. Low Temperature (LT) or cold stress is another major environmental factor that often affects plant growth and crop productivity and leads to substantial crop losses[20,21]. Chilling stress results from temperatures cool enough to produce injury without forming ice crystals in plant tissues, whereas freezing stress results in ice formation within plant tissues. The LT may affect several aspects of crop growth viz., survival, cell division, photosynthesis, water transport, growth and finally crop yield[22].

Sitosterol is a phytosterol and a structural component of the lipid core of cell membranes and is the precursor of numerous secondary metabolites, including plant steroid hormones, or as carriers in acyl, sugar and protein transport[23]. Sterols play an important role in plant development including cell expansion, vascular differentiation, etiolation and reproductive development[24]. Sitosterol involved in the regulatory function of plant development, affected gene expression involved in cell expansion and cell division, vascular differentiation and other diverse developmental programs[25]. Sitosterol is known to influence permeability and fluidity characteristics of the plasma membrane and other organellar membranes in the plant[26]. A number of studies have provided evidence that fluctuation in the sitosterol ratio plays a role in response to biotic and abiotic stresses[27]. Kumar *et al.*[28] and Abu-Muriefah[29] indicated the role of phytosterols in providing tolerance to stress. In addition gibberellins (GAs) are phytohormones that are essential for many processes of plant development, such as seed germination, stem elongation, leaf expansion, flowering and seed development[30]. Gibberellic acid (GA) accumulates rapidly when plants are exposed to both biotic[31] and abiotic stresses[32]. There are some reports that described gibberellins have the protective role in plant adaptation to abiotic stresses and detoxification of heavy metals[33-35].

The objective of this study was to investigate the effect of sitosterol or gibberellins on counteracting harmful response of three tomato cultivars (Farouz, Azize and N23-48) plants grown under temperature stress (TS) by following up growth, anatomy and ultrastructure leaves in order to highlight the

possible mechanisms by which β-sitosterol and gibberellic acid increases plant stress tolerance.

MATERIALS AND METHODS

Experiment preparation: The three cultivars of *Lycopersicon 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, *Lycopersicon esculentum* Mill., seeds were soaked for 10 h in 10^{-3}, 10^{-5} and 10^{-7} M β-sitosterol, 100 ppm gibberellic acid 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 before taken, transferring the seedlings to three growth chambers at temperature (10, 25, 45±3°C). Forty two days from sowing (as the true leaf fully expanded), samples from each treatment were collected to determine growth parameters (10 samples were taken) and relative water contents (triplicate samples were analyzed). In addition one sample only was taken for leaf structure (anatomy and ultrastructure).

Relative water contents: Based on the method described by Ritchie *et al.*[36] and Pardossi *et al.*[37].

Light and electron microscopy: Small sample of the fully expanded true leaf of the control and treated plants were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 6.8, at 0-4°C for 4 h. After washing in the buffer and post-fixation with 1% osmium tetraoxide they were dehydrated in the graded ethanol series and embedded in Epon-Spurr resin[38]. Semi-thin sections (1 μm) were cut with a glass knife on ultramicrotome (Ultracut, Reichert-Jung, Germany), semi-thin sections were stained with 1% toluidine blue and documented with light microscope equipped with camera (Digtal camera for microscope DCM510 5 M pixels CMOS ship, Germany). Stained section were examined with a JEM _ JEOL 2100/Japan Transmission Electron Microscope at the Electron Microscopy Unit/Mansoura University.

Analysis of cell structure: The size of cells and other organism were analysed using the public domain ImageJ software package http://rsb.info.nih.gov/ij/.

Statistical analysis: The effects of the temperature and treatment were tested by one-way analysis of variance (ANOVA). Means were compared between the treatments by least significant difference (LSD)[39] at the 0±05.

RESULTS AND DISCUSSION

Changes in vegetative growth: The obtained results in Table 1 showed that, at initial stage, a general significant increase was detected in tomato growth parameters, shoot length (cm), number of leaves per plant, total leaf area (cm^2 $plant^{-1}$), shoot fresh and dry weight (g) and relative water content with exception of the non-significant increase in number of leaves per plant of three tomato cultivars in response to all used treatments as compared to control.

Effect of temperature stress: During vegetative stage (42 DFS) the estimated growth parameters of tomato cultivars

Table 1: Effect of the used treatments on growth parameters for the three cultivars of *Lycopersicon esculentum* Mill., shoot at initial stage

		Growth parameters					
Cultivars	Treatments	Shoot length (cm)	No. of leaves per plant	Leaves area per plant (cm^2)	Shoot fresh weight (g $plant^{-1}$)	Shoot dry weight (g $plant^{-1}$)	Relative water content (%)
Fayrouz	Control	17.66	3	21.16	0.74	0.07*	71.70
	Gibberellin (100 ppm)	20.1*	3.33	23.12*	1.17*	0.12*	78.48*
	Sitosterol (10^{-3})	19.5*	3	26.9*	0.77	0.08	75.08*
	Sitosterol (10^{-5})	21.83*	3	29.47*	1.05*	0.1*	78.49*
	Sitosterol (10^{-7})	20.3*	3	27.0*	0.94*	0.09*	77.25*
Azize	Control	16.5	3	12.35	0.52	0.04	70.72
	Gibberellin (100 ppm)	19.56*	3.66	25.23*	0.79*	0.06*	74.84*
	Sitosterol (10^{-3})	18.23*	3	17.5*	0.84*	0.07*	73.50*
	Sitosterol (10^{-5})	19.9*	3.33	20.56*	0.99*	0.08*	75.44*
	Sitosterol (10^{-7})	17.93*	3.33	18.6*	0.95*	0.08*	74.05*
N23-48	Control	15.46	3	17.53	0.54	0.03	71.82
	Gibberellin (100 ppm)	17.13*	3	28.92*	0.81*	0.07*	77.71*
	Sitosterol (10^{-3})	16.9*	3.66	31.6*	0.83*	0.06*	75.62*
	Sitosterol (10^{-5})	18.3*	3.66	33.39*	0.98*	0.08*	77.02*
	Sitosterol (10^{-7})	16.5*	3.66	29.0*	0.92*	0.07*	76.25*

*Significant increase or decrease at 0.05 LSD

were decreased in response to 10 and 45°C (temperature stress 'TS') as compared to 25°C values (Table 2). The most negative effect of TS was observed at 45 and 10°C by the reduction of relative water content and leave area/plant, respectively.

The inhibitory effects of (TS) on growth of tomato plants reported in this study were may probably due to decreased of water absorption and alters cell division and cell elongation rates which affect the leaf size and weight[22]. Exposure of plants to severe heat stress decreased the stem growth resulting in decreased plant height[40]. According to Angadi *et al.*[41], temperatures below 10°C result in slower and reduced growth and premature stem elongation in *Brassica napus*, *Brassica rapa* and *Raphanus sativus*. It is well reported that plants at their seedling stage are very much sensitive to cold stress[22].

Effect of gibberellic acid: Effect of gibberellic acid with TS on the three used cultivars led to a significant increase in the all estimated growth parameters (shooth length (cm), number of leaves/plant, total leaf area (cm^2 $plant^{-1}$) and shoot fresh and dry weight (g)) and relative water content as compared to untreated values (Table 2).

The application of gibberellic acid led to a significant increase in growth parameters. These effects might be due to the role of gibberellin in improving vegetative growth characteristics since GAs are plant hormones that participate in the regulation of many growth developmental processes in plants[42]. The GA3 treatments (100 ppm) improved the growth criteria[43]. The role of GA in regulating plant growth in response to stress came from the observation that growth restraint on exposure to several forms of abiotic stress is at least in part mediated by DELLA proteins[44]. In *Arabidopsis thaliana* seedlings, exposure to salinity triggered a reduction in endogenous bioactive GAs[45,44], which coincided with DELLA accumulation[45].

Effect of β-sitosterol: Concerning the effect of different β-sitosterol concentrations at vegetative stage under the used TS (Table 2) showed that a general significant increase in the estimated growth parameters.

Similar results were obtained by El-Wahed *et al.*[24], working on wheat, who found that both sitosterol and spermidine caused stimulation of vegetative growth characteristics (shoot length, leaf area, plant fresh and dry weights) and net assimilation rate and vascular bundles differentiation of wheat. This increase in growth parameters is probably caused by increasing the efficiency of water uptake and utilization, enhancing cell division and/or cell enlargement, resulting in longer shoots and increasing leaf area which, consequently, increased the dry matter of shoots, presumably, as a result of larger surface area available for anabolic activities[46].

In this connection Thussagunpanit *et al.*[47] concluded that the increase in the shoot and root fresh weights under heat stress might be explained by the greater water uptake to those organs after 24-epibrassinolide (EBR) or 7,8-dihydro-8a-20-hydroxyecdysone (DHECD) application. Moreover, the EBR and DHECD treatments increased the leaf area before the exposure of plants to high temperature and maintained a higher leaf area under heat stress. An increase in the leaf area after brassinosteroids (BR) application has been reported in pigeon pea[48], tomato[49] and wheat[50].

Changes in leaf anatomy

Effect of temperature stress: The leaf section of plants treated with (TS) showed decreased in thickness of both leaf and lower epidermis of all tomato cultivars compared to 25°C, while upper epidermis was increase and decrease of Azize and N23-48, respectively, but in Fayrouz it showed increase and decrease after treatment with 10°C and 45°C, respectively (Table 3).

As regards the effect of (TS) on palisade and spongy mesophyll layer of tomato cultivars and as compared to the untreated values, palisade layer records a remarkable height with treated by 10°C (182.38 µm) and decreased as treated with 45°C (28.20 µm) while 79.79 µm at 25°C of N23-48 cultivar (Table 3), whereas the other cultivars, increased in Azize of both treatments but in Fayrouz cultivar these parameters increased by treatment with 10°C and decreased with 45°C. Meanwhile, the spongy layer increased in thickness after treated with 10°C and decreased at 45°C as compared to growing in 25°C of Fayrouz and conversely of N23-48, while decreased; in response to temperatures of Azize cultivar (Table 3).

Xu *et al.*[51] reported that leaf thickness and mesophyll cell size were found to decrease with warmer temperature. Lower growth temperature also indicates that increased leaf size is mainly due to cell expansion (increased size) rather than cell division (more cells), as has been observed previously. Similar anatomical changes have been observed during plant acclimation to high light[52-54]. Growth of plant leaves *Spinacia oleracea* L. cv Savoy at low temperature resulted in a twofold increase in leaf thickness from about 290 um for 16°C to 567 um for 5°C[55].

Effect of gibberellic acid: The application of 100 ppm gibberellic acid caused anatomical changes in all anatomical measured parameters of tomato cultivars as compared to control values without gibberellic acid (Table 3). The data presented in Table 3 revealed a general significant

Table 2: Effect of treatments on growth parameters for the three cultivars of *Lycopersicon esculentum* Mill., plant at vegetative stage

		Cultivar					
		Fayrouz		Azize		N23-48	
Temperature (°C)	Treatments	Shoot length (cm)	No. of leaves per plant	Shoot length (cm)	No. of leaves per plant	Shoot length (cm)	No. of leaves per plant
25	Control	30	4	23.7	4	31.2	4
	Gibberellin (100 ppm)	33.0*	5*	29.5*	4	34.0*	5*
	Sitosterol (10^{-3})	32.2*	5*	28.5*	4	32.5*	4.33
	Sitosterol (10^{-5})	36.5*	5.33*	30.3*	4	35.2*	5*
	Sitosterol (10^{-7})	32.4*	5*	27.2*	4	33.8*	4.33
10	Control	22.9	4	18.2	4	18.9	3
	Gibberellin (100 ppm)	24.19*	4	21.6*	4	20.6*	4*
	Sitosterol (10^{-3})	24.16*	4	20.53*	4	20.36*	4*
	Sitosterol (10^{-5})	25.0*	4	21.1*	4	22.8*	4*
	Sitosterol (10^{-7})	24.26*	4	20.83*	4	20.23*	4*
45	Control	27.2	4	23.2	4	28.2	3
	Gibberellin (100 ppm)	29.3*	4	26.5*	5*	33.6*	4*
	Sitosterol (10^{-3})	28.5*	4	30.25*	4.33	31.3*	4*
	Sitosterol (10^{-5})	31.0*	4	33.6*	5*	34.0*	4*
	Sitosterol (10^{-7})	29.1*	4	31.06*	4.66	32.5*	4*

		Cultivar					
		Fayrouz		Azize		N23-48	
Temperature (°C)	Treatments	Leaves area per plant (cm^2)	Fresh weight (g $plant^{-1}$)	Leaves area per plant (cm^2)	Fresh weight (g $plant^{-1}$)	Leaves area per plant (cm^2)	Fresh weight (g $plant^{-1}$)
25	Control	22.98	2.10	17.17	1.23	40.59	2.78
	Gibberellin (100 ppm)	27.71*	2.47*	29.93*	1.62*	52.81*	3.06*
	Sitosterol (10^{-3})	28.32*	2.21*	27.30*	1.44*	56.0*	3.05*
	Sitosterol (10^{-5})	32.66*	3.30*	31.60*	1.80*	59.97*	4.35*
	Sitosterol (10^{-7})	28.33*	2.29*	28.30*	1.53*	57.28*	3.29*
10	Control	21.36	1.11	15.6	0.65	18.37	0.71
	Gibberellin (100 ppm)	25.0*	1.50*	26.0*	1.01*	28.37*	0.80
	Sitosterol (10^{-3})	27.2*	1.41*	20.5*	0.98*	29.22*	1.01*
	Sitosterol (10^{-5})	30.6*	1.51*	25.2*	1.1*	33.07*	1.67*
	Sitosterol (10^{-7})	27.5*	1.47*	21.9*	1.0*	30.19*	1.24*
45	Control	21.99	1.12	16.33	0.86	31.03	1.34
	Gibberellin (100 ppm)	26.12*	2.0*	28.54*	1.38*	42.52*	1.75*
	Sitosterol (10^{-3})	27.16*	1.44*	26.8*	1.31*	40.82*	1.48*
	Sitosterol (10^{-5})	31.91*	1.80*	30.43*	2.14*	42.85*	1.68*
	Sitosterol (10^{-7})	27.6*	1.62*	28.11*	1.50*	41.7*	1.52*

		Cultivar					
		Fayrouz		Azize		N23-48	
Temperature °C)	Treatments	Dry weight (g $plant^{-1}$)	Relative water content (%)	Dry weight (g $plant^{-1}$)	Relative water content (%)	Dry (g $plant^{-1}$)	Relative water content (%)
25	Control	0.21	75.76	0.197	72.44	0.28	70.57
	Gibberellin (100 ppm)	0.30*	86.14*	0.27*	82.52*	0.39*	77.92*
	Sitosterol (10^{-3})	0.28*	81.47*	0.25*	80.89*	0.364*	74.38*
	Sitosterol (10^{-5})	0.46*	85.82*	0.30*	83.19*	0.47*	76.92*
	Sitosterol (10^{-7})	0.29*	84.79*	0.26*	82.20*	0.38*	75.14*
10	Control	0.10	67.81	0.05	70.11	0.05	70.98
	Gibberellin (100 ppm)	0.15*	76.32*	0.08*	78.18*	0.08*	78.66*
	Sitosterol (10^{-3})	0.119	72.60*	0.08	73.85*	0.07*	77.44*
	Sitosterol (10^{-5})	0.13*	75.10*	0.09*	75.86*	0.13*	80.75*
	Sitosterol (10^{-7})	0.122*	73.70*	0.09*	74.17*	0.09*	78.23*
45	Control	0.11	60.84	0.07	59.61	0.13	31.03
	Gibberellin (100 ppm)	0.17*	67.27*	0.156*	68.50*	0.17*	42.52*
	Sitosterol (10^{-3})	0.16*	63.60*	0.151*	65.70*	0.15*	38.30*
	Sitosterol (10^{-5})	0.21*	66.35*	0.308*	67.47*	0.20*	42.85*
	Sitosterol (10^{-7})	0.18*	65.54*	0.260*	64.58*	0.16*	40.40*

*Significant increase or decrease at 0.05 LSD

Table 3: Effect of the used treatments on thickness of epidermis and mesophyll layers (μm) for the three cultivars of *Lycopersicon esculentum* Mill., plant at vegetative stage

		Cultivar					
		Fayrouz		Azize		N23-48	
Temperature (°C)	Treatments	Leaf thickness	Leaf thickness	Leaf thickness	Upper epidermis	Upper epidermis	Upper epidermis
25	Control	236.96	20.45	273.78	20.45	271.30	22.64
	Gibberellin (100 ppm)	287.94*	27.84*	264.94*	27.84*	163.42*	17.51*
	Sitosterol (10^{-5})	263.83*	32.92*	275.39	32.92*	174.83*	19.04*
10	Control	212.65	27.20	214.71	27.20	252.66	22.20
	Gibberellin (100 ppm)	287.03*	29.93*	266.23*	29.93*	352.51*	26.81*
	Sitosterol (10^{-5})	294.51*	42.19*	407.72*	42.19*	442.19*	38.37*
45	Control	208.68	23.77	221.90	23.77	191.91	15.59
	Gibberellin (100 ppm)	302.01*	29.19*	224.77*	29.19*	267.15*	21.22*
	Sitosterol (10^{-5})	366.07*	22.86	214.59*	22.86	240.06*	33.66*
		Cultivar					
		Fayrouz		Azize		N23-48	
Temperature (°C)	Treatments	Lower epidermis (μm)	Palisade layer (μm)	Lower epidermis (μm)	Palisade layer (μm)	Lower epidermis (μm)	Palisade layer (μm)
25	Control	22.64	77.95	29.60	101.73	26.01	79.79
	Gibberellin (100 ppm)	23.99	94.98*	26.44*	69.92*	16.40*	60.04*
	Sitosterol (10^{-5})	20.25*	94.89*	25.07*	96.35*	21.32*	68.53*
10	Control	17.51	104.29	19.38	115.66	11.97	182.38
	Gibberellin (100 ppm)	21.35*	96.50*	28.48*	105.27*	15.88*	130.69*
	Sitosterol (10^{-5})	19.10*	120.85*	30.21*	145.34*	39.37*	145.24*
45°C	Control	19.12	73.65	19.73	107.74	12.37	28.20
	Gibberellin (100 ppm)	24.72*	115.84*	17.33*	97.36*	20.49*	93.37*
	Sitosterol (10^{-5})	48.65*	121.83*	20.53	76.52*	22.86*	83.90*
		Cultivar					
		Fayrouz		Azize		N23-48	
Temperature (°C)	Treatments	Spongy layer (μm)		Spongy layer (μm)		Spongy layer (μm)	
25	Control	102.25		140.09		122.48	
	Gibberellin (100 ppm)	135.88*		125.78*		57.69*	
	Sitosterol (10^{-5})	102.51		107.72*		73.46*	
10	Control	129.58		123.89		73.44	
	Gibberellin (100 ppm)	123.04*		102.67		182.82*	
	Sitosterol (10^{-5})	125.65*		159.71*		146.24*	
45	Control	89.91		71.93		130.26	
	Gibberellin (100 ppm)	138.71*		85.47*		125.06*	
	Sitosterol (10^{-5})	150.57*		88.52*		107.67*	

*Significant increase or decrease at 0.05 LSD

increase in leaf thickness, lower epidermis thickness, palisade and spongy layers at 25°C and leaf thickness and lower epidermis thickness at 10°C; the all anatomical parameters measured at 45°C of Fayrouz cultivar by gibberellic acid application, whereas, Azize cultivar showed significant increase in upper epidermis thickness at 25°C; leaf thickness, lower and upper epidermis thickness at 10°C; leaf thickness, upper epidermis thickness and spongy layer at 45°C by gibberellic acid application. In addition, significant increase in leaf thickness, upper and lower epidermis thickness under 10 and 45°C and spongy layer at 10°C and palisade layer at 45°C of N23-48 cultivar were observed.

On the other hand, the other anatomical parameters of three tomato cultivars were significant decrease per treatments as compared to control. Thickness of cell walls in epidermis (outer and inner walls) of tomato showed a general significant increment in response to treatments at low and high temperature where as compared to control in all cultivars (Table 4).

The GA_3 increased the epidermis cell width and length, These anatomical changes indicate that salt stress on the stems of radish may be reduced by growth regulators[56]. Foliar application of GA_3 caused the changes in the anatomical structure of date palm leaf (*Phoenix dactylifera* L.) enhanced thickness of the cuticle as compared to the control[57].

Table 4: Effect of the used treatments on thickness of cell walls in epidermis (μm) for the three cultivars of *Lycopersicon esculentum* Mill., leave at vegetative stage

		Cultivar					
		Fayrouz		Azize		N23-48	
Temperature (°C)	Treatments	Outer walls	Linner walls	Outer walls	Linner walls	Outer walls	Linner walls
Upper epidermis							
25	Control	1.54	0.81	1.80	0.824	1.41	0.60
	Gibberellin (100 ppm)	2.46*	1.28*	3.15*	0.96*	1.34*	0.61
	Sitosterol (10^{-5})	2.25*	0.98*	2.40*	1.92*	2.94*	1.47*
10	Control	2.12	1.24	3.70	1.70	1.84	0.84
	Gibberellin (100 ppm)	2.47*	1.31	3.48*	1.56*	3.79*	1.13*
	Sitosterol (10^{-5})	3.42*	1.59*	4.21*	3.82*	3.00*	1.51*
45	Control	3.13	1.02	1.69	0.85	2.66	1.01
	Gibberellin (100 ppm)	4.43*	1.51*	4.70*	1.62*	2.78	0.96
	Sitosterol (10^{-5})	4.11*	1.42*	4.45*	1.87*	4.35*	1.86*
		Cultivar					
		Fayrouz		Azize		N23-48	
Temperature (°C)	Treatments	Outer walls	Linner walls	Outer walls	Linner walls	Outer walls	Linner walls
Lower epidermis							
25	Control	1.32	0.68	1.41	0.93	1.73	0.69
	Gibberellin (100 ppm)	2.01*	0.74*	1.49*	0.97*	1.82	0.68
	Sitosterol (10^{-5})	2.07*	0.97*	3.00*	1.08*	2.06*	1.26*
10	Control	1.76	0.98	2.18	1.43	1.67	0.71
	Gibberellin (100 ppm)	2.22*	1.17*	3.11*	2.03*	2.21*	0.95*
	Sitosterol	3.28*	1.59*	3.33*	2.35*	2.36*	0.82
45	Control	1.92	0.83	1.41	0.65	2.00	1.27
	Gibberellin (100 ppm)	3.86*	1.66*	3.63*	1.41*	2.88*	1.27
	Sitosterol (10^{-5})	5.41*	2.15*	2.96*	2.63*	4.29*	2.44*

*Significant increase or decrease at 0.05 LSD

Effect of β-sitosterol: Treatment of tomato cultivars with 10^{-5} M β-sitosterol caused a general significantly increase in (leaf thickness and palisade and spongy layers under 25°C; leaf thickness, lower epidermis thickness and palisade layer under 10°C; all anatomical parameters measured under 45°C of Fayrouz cultivar), in (leaf thickness and upper epidermis thickness under 25°C; all measured anatomical parameters under 10°C; lower epidermis thickness and spongy layer at 45°C of Azize cultivar) and in (leaf thickness, upper and lower epidermis thickness under 10 and 45°C and spongy layer at 10°C and palisade layer at 45°C of N23-48 cultivar) compared with control values (Table 3).

Moreover, this treatment caused a general significant decrease in (upper and lower epidermis thickness at 25°C; upper epidermis thickness and spongy layer at 10°C of Fayrouz cultivar), in (lower epidermis thickness, palisade and spongy layers at 25°C; leaf thickness, upper epidermis thickness and palisade layer at 45°C of Azize cultivar) and in (all measured anatomical parameters at 25°C; palisade layer at 10°C; spongy layer at 45°C of N23-48 cultivar) as shown Table 3.

Thickness of cell walls (outer and linner walls) in epidermis of tomato showed a general significant increment in response to 10^{-5} M β-sitosterol at low and high temperature as compared to control values in all cultivars (Table 4).

Stigmasterol increased the thickness of epidermis, cortex, vascular cylinder and palisade and spongy tissues of soybean plant[58]. Ali *et al.*[59] studied the effects of sitgmasterol treatments at the concentrations of 100 and 150 ppm when foliarly sprayed twice to rice plants at leaf tube and tillering stages. Such favourable effects resulted in increasing leaf thickness, upper and lower epidermal layers, mesophyll tissue and dimensions of both main and smaller leaf vascular bundles. El-Wahed *et al.*[24] concluded that, sitosterol had stimulatory effect in increasing thickness of either the upper epidermal layer or mesophyll tissue layer and whole leaf thickness. Sitosterol increased stem diameter/cross section, thickness of the ground tissue and diameter of the pith cavity. The response could be due to the growth promoting effect of brassinosteroid (BR) on cell elongation especially on meristematic tissue.

Nassar *et al.*[60] found that foliar application with stigmasterol at concentration of 90 ppm increased the diameter of the main stem, at its median portion, of flax cv Sakha-1 by 17.3% more than that of the control. The increase which was observed in stem diameter, due to foliar

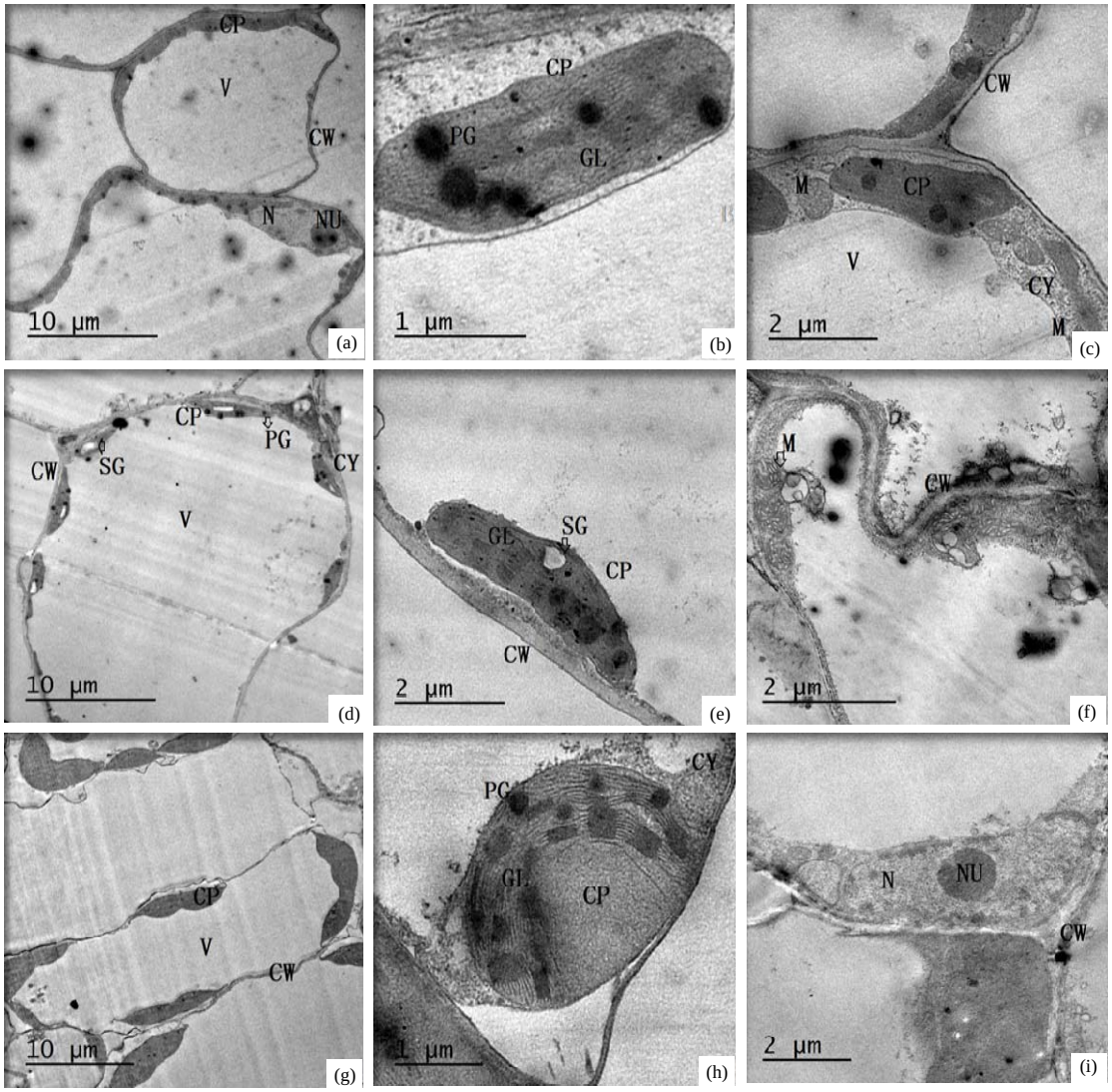

Fig. 1(a-i): Ultrastructure of mesophyll cell of tomato leaf grown at 25°C (a-c) Control, (d-f) 100 ppm gibberelliic acid and (g-i) 10^{-5} M β-sitosterol. V: Vacuole, CW: Cell wall, CP: Chloroplast, N: Nucleus, NU: Nucleolus, CY: Cytoplasm, M: Mitochondria, SG: Starch granules, PG: Plastoglobules, GL: Grana lamella

application with 90 ppm stigmasterol, could be attributed mainly to the prominent increase in all included tissues. The thickness of epidermis, cortex, fiberous region, secondary phloem and xylem tissue as well as diameter of the pith were 5.6, 47.1, 20.2, 14.1, 30.0 and 8.1% more than those of the control, respectively. Moreover, number of fiberous bundles/cross section were increased in treated plants by 9.4% more than those of untreated ones. The present findings are generally in accordance with those reported by Ali *et al.*[59] using 100 or 150 ppm stigmasterol on rice plants as well as by Nassar[58] using 100 ppm stigmasterol on soybean plants and by Helal and Gomaa[61] using 80 ppm stigmasterol on Egyptian lupine plants. They recorded favourable anatomical changes in stem anatomy due to the effect of stigmasterol which induced prominent increases in most of included tissues for investigated species.

Change in ultrastructure of the leaves

Effect of temperature stress: Measurements results of Transmission Electron Microscope (TEM) in mature tomato leaves of N23-48 cultivar grown under TS showed a general increase in total volume of cytoplasm, chloroplast, mitochondria and thickness of cell wall (Table 5), chloroplasts were bigger and almost spherical with large quantity of starch granules, plastoglobules and grana lamella loosened in the leaves under 10°C as compared with 25°C (Fig. 1, 2). While in the alteration, leaves of the plant that grown under 45°C

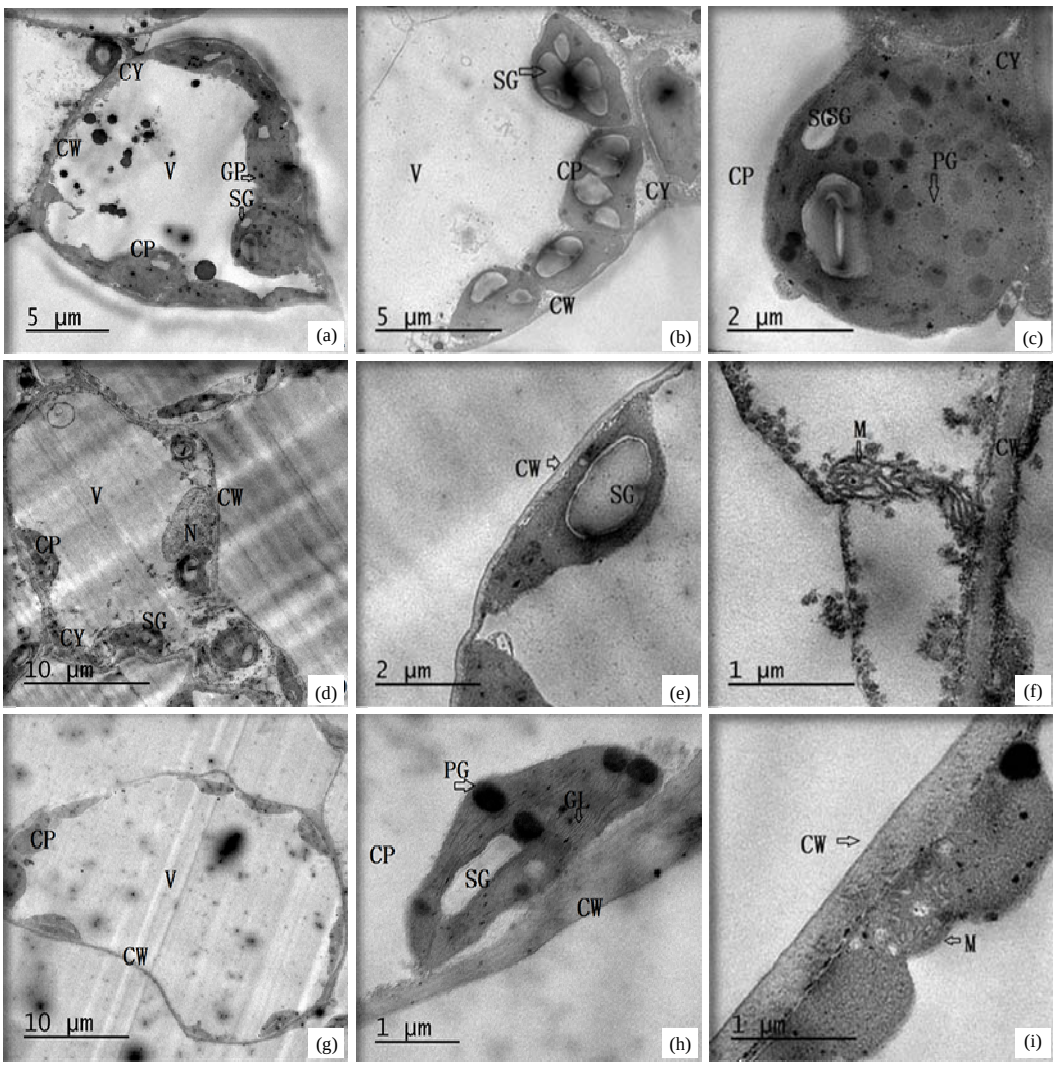

Fig. 2(a-i): Ultrastructure of mesophyll cell of tomato leaf grown at 10°C (a-c) Control, (d-f) 100 ppm gibberellic acid and (g-i) 10^{-5} M β-sitosterol. V: Vacuole, CW: Cell wall, CP: Chloroplast, N: Nucleus, NU: Nucleolus, CY: Cytoplasm, M: Mitochondria, SG: Starch granules, PG: Plastoglobules, GL: Grana lamella

(Fig. 3), increased number and total volume of chloroplast with large starch granules and loose structure of grana lamellae (Fig. 3). Whereas cell volume and cell wall as well as vacuole were decreased as compared with leaves of those grown at 25°C (Table 5). High temperature caused an anatomical changes; include reduced size and damaged cells[62,17]. High temperature considerably affects anatomical structures not only at the tissue and cellular levels but also at the sub-cellular level. At the sub-cellular level, main modifications refer to the shape of chloroplasts, swelling of stromal lamellae[62].

Recently, Gielwanowska *et al.*[63] reported that, the ultrastructural organization of organelles determines the response of cells and entire plants to abiotic stress. Metabolic disruptions induced by environmental factors are manifested in the ultrastructure of cell organelles. Cellular components have varied tolerance to low temperature, dehydration and excessive light exposure. Chloroplasts are the most sensitive organelles, whereas cell nuclei, mitochondria and peroxisomes are characterized by greatest stability[64].

Zhang *et al.*[65] reported that, the microstructure of leaves and ultrastructure of chloroplasts were examined in tomato (*Lycopersicon esculentum* L.) plants treated with elevated temperature. Plants were exposed to 35°C for 30 days after florescence. The plants grown continuously under 25°C served as controls. The damage of chloroplast

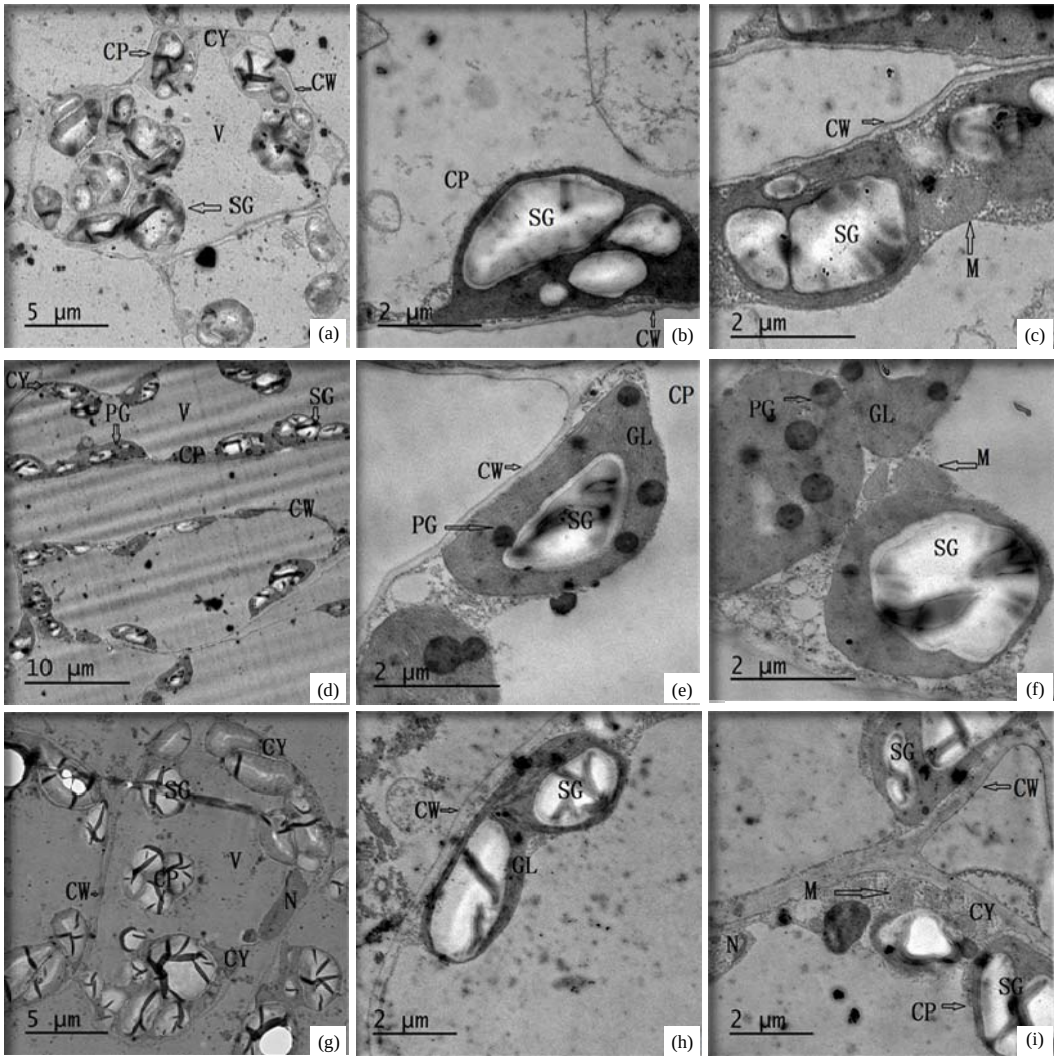

Fig. 3(a-i): Ultrastructure of mesophyll cell of tomato leaf grown at 45°C (a-c) Control (d-f) 100 ppm gibberellic acid and (g-i) 10^{-5} M β-sitosterol. V: Vacuole, CW: Cell wall, CP: Chloroplast, N: Nucleus, NU: Nucleolus, CY: Cytoplasm, M: Mitochondria, SG: Starch granules, PG: Plastoglobules, GL: Grana lamella

Table 5: Means of cellular and sub-cellular volume of N23-48 cultivar leaves with treatments as well as control samples

								Chloroplast			Starch/Chloroplast		Mitochondria		
Temperature (°C)	Treatments	Cell volume	Cell wall thickness	Cytoplasm volume	Vacuole	Nucleus volume	Nucleolus volume	No.	Volume	Total volume	No.	Volume	No.	Volume	Total volume
25	Control	195.92	0.12	32.930	162.19	8.563	0.836	5	5.704	28.52	-	-	3	0.292	0.876
	GA (100 ppm)	337.6*	0.28*	112.92*	224.7*	11.09*	1.416	10*	6.65*	66.5*	1	0.223	2	0.278*	0.556*
	Sito (10^{-5})	252.6*	0.14*	99.95*	152.6*	6.674	0.807	12*	5.98*	71.7*	-	-	2	0.183*	0.366*
10	Control	219.33	0.153	96.426	122.9	2.400	-	7	7.84	54.88	3	1.64	3	0.347	1.04
	GA (100 ppm)	361.0*	0.27*	152.03*	209.0*	17.54*	-	7	8.89*	62.2*	3	1.62	1*	0.384*	0.384*
	Sito (10^{-5})	595.3*	0.36*	124.83*	470.5*	12.80*	-	10*	5.348	53.5	1*	0.396*	1*	0.367*	0.367*
45	Control	199.85	0.09	140.72	59.132	10.015	-	9	6.82	61.38	3	2.51	1	0.783	0.783
	GA (100 ppm)	301.9*	0.13*	91.691	210.2*	8.369*	-	11*	5.405	59.45	1*	2.144	2	0.283*	0.566*
	Sito (10^{-5})	204.1*	0.25*	110.408	93.77*	5.809*	-	12*	9.53*	114.3*	1*	2.44	2	0.239*	0.487*

*Significant increase or decrease at 0.05 LSD

membrane occurred earlier and was more serious in the plants under elevated temperature. At the same time, the thylakoids were loosely distributed with lesser grana, but the number of lipid droplets increased in chloroplasts. The number of starch grains in chloroplasts increased first and then decreased.

Effect of gibberellin: The cells of leaves treated with 100 ppm gibberellic acid under 25°C showed significantly increases in all measurements except number and total volume of mitochondria which significantly decreased (Table 5) and chloroplasts with few and small starch as compared with untreated value (Fig. 1).

After treatments of N23-48 cultivar with GA and LT significant increases were recorded in volume of cells, cytoplasm, vacuole, nucleus, chloroplast, mitochondria and cell wall become more thicker (Fig. 2), as compared with untreated value (Table 5), these treatment also obtained paddle shape of mitochondria and cristae were noticed (Fig. 2). On the other hand, chloroplasts in leaves treated with GA and HT had few starch grains, plastoglobules and the lamellar structure was shown to be more clear as compared with untreated (Fig. 3). Moreover, the volume of cell, vacuole, thick of cell wall and chloroplast number were increased significantly, but other measurements (cytoplasm, nucleus, nucleolus, number and total volume of mitochondria) were significantly decreased as compared with untreated value (Table 5).

In this respect GA plays a critical role in controlling and coordinating cell division, cell expansion and chloroplast biogenesis through influencing the DELLA protein family in both dicot and monocot plant species[66]. The application of GA_3 in combination with calcium chloride caused reduced membrane damage[67]. Gibberllic acid causes the increase of cell division and increase of elastic properties of the cell wall[68]. Gibberellic acid application induced ultrastructural changes in chloroplasts of Marchantia polymorpha. Starch grains disappeared and membrane development was accompanied by an increase in granal thickness and length until dense, parallel arrays of thylakoids extended throughout the plastid[69].

Effect of sitosterol: Table 5 shows the application of β-sitosterol alone led to a significant increase in volume of cell, cytoplasm, number and total volume of chloroplasts while volume of vacuole, nucleus, nucleolus, number and volume of mitochondria were significantly decreased as compared to the control at 25°C. The β-sitosterol treated in addition to heat show that ultrastructure measurements significantly varied as result of the 10^{-5} β-sitosterol with TS application. At 10°C, general significant increase in ultrastructure measurements; the chloroplast contained small starch grains and the thylakoid structure was clear (Fig. 2) and cell wall was thicker than that of untreated value. Whereas, the plant grown under 45°C showed changes in ultrastructure with 10^{-5} of β-sitosterol treatment (Fig. 3), such as significant increase in cell volume, the chloroplast larger and more, arrangement of grana lamella was clear and parallel, starch content is lower, mitochondria smaller, the cell wall was thicker and vacuole is bigger than the untreated value.

In this connection the effect of brassinosteroids BR on barley leaf cell ultrastructure was examined under salt stress. Leaf segments were pre-incubated in either BR solution or water and then incubated in 0.5 M NaCl solution in the presence or absence of BR. The BR had no effect on the leaf cell ultrastructure under normal conditions. However, damages imposed by salt stress on nuclei and chloroplasts were significantly reduced by BR treatment[70].

In support, like the impact of brassinosteroids, sitosterol included in the administrative capacity of plant improvement, influenced quality expression required in cell augmentation and cell divisio[25]. Moreover, brassinosteroid as all steroidal compounds promoted cell wall formation and resulted in hyperpolarization of cell membranes and accelerated growth cycle[71]. Kumar *et al.*[28] reported that sitosterol may have a role in abiotic stress tolerance by enhancing membrane stability.

CONCLUSION

It could be concluded from the above recorded data that, the used growth substances (GA_3 or sitosterol) especially 100 ppm and 10^{-5} M, respectively, partially enhance the resistance of the three used tomato cultivars (Fayrouz, Azize and N23-48) to temperature stress (growing the plant under 10 or 45°C) in addition to improving the vegetative growth of these used tomato cultivars grown under stress condition and control one that grown under 25°C. The obtained resistance; in response to using these safe growth substances could be due to the changes in the cell wall and plastids structure and other factors that reflect on more healthy tomato plants with significant enhancement in the determined vegetative growth parameters.

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