

## Physiological Response of Two wheat Cultivars to $\alpha$ -tocopherol

Iman M. Talaat, M.S. Abd El-Wahed, M.E. El-Awadi,  
M.A.T. El-Dabaa and M.A. Bekheta

Botany Department, National Research Centre, 33 El-Bohoothst, (former El-Tahrirst.), Dokki,  
P.O. Code 12622, Cairo, Egypt.

**Abstract:** Two field experiments were carried out in The Experimental Farm of National Research Centre, Nubaria, El-Behaira governorate, during two successive seasons, to study the effect of foliar application of  $\alpha$ -tocopherol on improving the growth and grain yield of two wheat cultivars (Gemiza 7 and Gemiza 10) in new reclaimed area. Gemiza 10 cultivar significantly surpassed Gemiza 7 cultivar in all studied growth and yield criteria. Application of  $\alpha$ -tocopherol, especially at 1000 mg/l, resulted in significant increases in plant height, number of tillers, number of active leaves and number of spikes / plant. Tillers-sheats dry weight, blades dry weight, spikes dry weight and flag leaf blade area of wheat plants. Blades area, leaf area index, grain yield, straw yield and biological yield followed the same trend. Treatment of wheat plants with  $\alpha$ -tocopherol significantly affected number of spikes/plant, spikes dry weight, main spike length, number of spiklets/main spike, number of grains/plant, grain yield/plant and straw yield. Total carbohydrates, total soluble sugars (TSS) % and total polysaccharides %, proline and free amino acids were also increased as a result of  $\alpha$ -tocopherol treatment.

**Key Words:**  $\alpha$ -tocopherol; wheat cultivars; free amino acids, proline.

### Introduction

Wheat (*Triticum aestivum* L.) is considered one of the most widely grown crops of high nutritive value in the world as well as in Egypt. The wheat grains contain large amounts of proteins, carbohydrates in addition to some minerals and vitamins. In Egypt, wheat has a special importance because the local production is not sufficient to meet the annual demands. In Egypt, wheat sown at normal sowing date (1-15) November may be exposed to high temperature stress during grain filling (at March or April) due to the hot wind of El-Khamaseen for one or more days which in turn reduces growth, yield and quality of grains mainly by shortening the reproductive and ripening growth phases<sup>1,2</sup>. Therefore, increasing the local production of wheat is the target to cover the local consumption. This could be achieved by introducing more productive varieties, improving the culture practices such as sowing the wheat in the newly reclaimed area or application of some growth promoters during different growth stages.

$\alpha$ -Tocopherol (Toc) is a lipophilic membrane located compound present in chloroplasts. Toc is believed to protect chloroplast membranes from photo-oxidation and to help provide an optimal environment for the photosynthetic machinery<sup>3</sup>. The most prominent function of Toc is protection of polyunsaturated fatty acids from lipid peroxidation<sup>4</sup>.

Tocopherols are a group of compounds synthesized only by photosynthetic organisms. The best characterized and probably most important function of tocopherols is to act as recyclable chain reaction

terminators of polyunsaturated fatty acid free radicals generated by lipid oxidation. From a biosynthetic perspective, tocopherols are members of a large, multifunctional family of lipid soluble compounds called prenylquinones that also include tocotrienols, plastoquinones, and phyloquinones (vitamin K1)<sup>7</sup>. Orabi and Abdelhamid<sup>8</sup>, reported that foliar application of  $\alpha$ -tocopherol to faba bean plants at concentrations of 50 or 100 mg L<sup>-1</sup> recorded significant increments in growth parameters compared with control.

In plants, tocopherols are believed to protect chloroplast membranes from photooxidation and help to provide an optimal environment for the photosynthetic machinery<sup>7</sup>. Many of the proposed tocopherol functions in animals and plants are related to their antioxidant properties, the most prominent of which is protection of polyunsaturated fatty acids from lipid peroxidation by quenching and scavenging various reactive oxygen species (ROS) including singlet oxygen, superoxide radicals, and alkyl peroxy radicals<sup>7</sup>. Tocopherol levels and composition vary in different tissues and fluctuate during development and in response to abiotic stresses. Significant increases in leaf  $\alpha$ -tocopherol levels are observed during aging and senescing of plants<sup>8</sup>, possibly to protect cellular components from increased oxidative stress<sup>9</sup>. Enhanced tocopherol accumulation also occurs in response to a variety of abiotic stresses including high light, drought, salt, and cold that may provide an additional line of protection from oxidative damage<sup>7,10</sup>.

## Material and Methods

Two field experiments were carried out at the experimental station of Agricultural Production and Research Station, National Research Centre, El Nubaria Province, El Behaira Governorate, Egypt during two successive winter seasons (2011/2012) and (2012/2013). Two Wheat cultivars (Gemiza 7 and Gemiza 10) were obtained from Egyptian Ministry of Agriculture. Soil was ploughed twice, ridged and divided into plots during seed preparation. Area of each plot was 10.5 m<sup>2</sup> (15 rows; 3.5 meter long and 20 cm apart between rows). The normal agronomic practices of growing wheat were carried out from sowing till harvest as recommended by wheat research Dept., A.R.C. Wheat grains were drilled at seed rate of 60 kg/fed., during the first week of December for two successive growing seasons. The plants were sprayed on both surfaces of the leaves with different concentrations of  $\alpha$ -tocopherol at 0, 400, 600, 800, 1000 and 1200 mg/l once at 40 days old plant.  $\alpha$ -tocopherol was supplied from Sigma-chemical Co. The experimental design in this study was a randomized complete block design with four replicates. Samples of ten guarded plants were taken at random from each plot of the four replications for determination of vegetative growth parameters at 100 days after sowing (milky ripe stage; A) and 120 days after sowing (dough ripening stage; B). The following characteristics were determined: Plant height (cm), number of tillers, active leaves and spikes/plant and blades area (cm<sup>2</sup>/plant), dry weight of tillers + sheaths, blades and spikes (g/plant) as well as flag leaf blade area (cm<sup>2</sup>/plant) and leaf area index at the two stages of the plant growth. Flag leaf blade area and blade area/plant were determined according to Bremner and Taha<sup>11</sup>, meanwhile, leaf area index (LAI) was determined according to Watson<sup>12</sup>.

At harvest time, wheat plants were collected from each plot to determine grain yield and yield attributes. The following yield criteria were determined: number of spikes/plant, dry weight of spikes /plant, length of main spike, number of spikelets / main spike, number of grains/plant, grain yield and straw yield (g) /plant, as well as grain yield , straw yield and biological yield (tons / feddan). Biological yield = grain yield + straw yield.

## Chemical analyses

The chemical constituents; carbohydrate constituents (total carbohydrates, total soluble sugars, and polysaccharides) and protein content were determined in dried leaf tissues of wheat plant at 100 days old plant (Stage A). At harvest, the collected grains were cleaned and dried then ground to a fine powder for determination of carbohydrate constituents (total carbohydrates, total soluble sugars, and polysaccharides), protein content, proline and free amino acids. Total carbohydrates were determined using the colorimetric method described by Dubois *et al.*<sup>13</sup>. Total soluble sugars were determined using the method described by Smith *et al.*<sup>14</sup>. Polysaccharides were calculated by the difference between total carbohydrates and total soluble sugars. Protein was determined according to the official and modified methods of analysis<sup>15</sup>. Proline was estimated according to Bates *et al.* (1973)<sup>16</sup>. Total free amino acids were determined according to Muting and Kaiser (1963)<sup>17</sup>.

**Statistical analysis**

All data were subjected to statistical analysis according to procedure outlined by Gomez and Gomez<sup>18</sup>. Treatments means were compared by L.S.D. at 5% level test. Combined analysis was made for the two growing seasons as results followed similar trend.

**Results and Discussion**

Data presented in Table (1) indicate that, the growth of wheat plants cultivars Gemiza 10 and Gemiza 7 significantly differed in plant height, number of tillers per plant, number of leaves per plant, number of spikes per plant and blades area. Moreover, it was observed that, Gemiza 10 cultivar significantly surpassed Gemiza 7 cultivar in all studied growth and yield criteria (Tables 1-3). Data presented in Table (1) indicate that, the differences between wheat cultivars may be attributed to differences in genetical structures, as well as, the range of cultivar response<sup>17</sup>. In addition, differences in yield and yield components and biological yield could be due to the cultivar differences in carbon equivalent for vegetative components, production value of vegetative matter, net assimilation rate and crop growth rate<sup>20</sup>.

Generally, the obtained results of cultivars differences in growth parameters were in full agreement with those obtained by Abo El-Kheir *et al.*<sup>21</sup>, Ahmed and Badr<sup>22</sup> and Baz *et al.*<sup>23</sup>.

Data also indicate that, foliar spray of wheat plants with  $\alpha$ -tocopherol significantly increased tillers-sheaths dry weight at both stages, especially in plants treated with 1000 mg/l. These results hold true for blades dry weight, spikes dry weight and flag leaf blade area. Leaf area index (LAI) followed the same trend (Table 2).

**Table (1): Effect of  $\alpha$ -tocopherol treatments on plant height, number of tillers, and number of active leaves, number of spikes / plant, Blades area and Leaf area index of wheat plants**

Cultivars	$\alpha$ -tocopherol (mg/plant)	Plant hight (cm)		No. of tillers/ plant		No. of active leaves / plant		No. of spikes / plant		Blades area (cm <sup>2</sup> /plant)	
		A	B	A	B	A	B	A	B	A	B
Gemiza 7		93.28	117.91	4.79	4.08	28.52	24.68	3.29	3.94	385.36	329.09
Gemiza 10		99.11	121.70	5.17	4.74	29.82	26.03	3.96	4.61	419.18	377.95
	LSD (5%)	1.07	2.19	0.08	0.15	1.02	0.69	0.21	0.18	10.85	15.16
	Control	84.02	107.29	3.79	3.25	26.65	23.15	2.63	3.25	331.40	266.00
	400 mg/l	92.31	111.35	4.25	3.98	27.91	24.43	2.92	3.67	355.39	323.05
	600 mg/l	93.82	118.41	4.71	4.25	29.00	24.94	3.65	4.15	382.88	345.14
	800 mg/l	96.01	122.15	5.07	4.59	29.67	25.70	3.75	4.49	420.88	367.61
	1000 mg/l	108.30	131.95	6.17	5.50	31.19	27.20	4.44	5.44	470.3	424.88
	1200 mg/l	102.70	127.68	5.89	4.92	30.60	26.70	4.35	4.75	452.79	394.46
	LSD (5%)	1.34	1.22	0.07	0.09	0.24	0.15	0.08	0.34	11.55	13.42
Gemiza 7	Control	80.64	105.90	3.71	3.00	26.00	22.50	2.25	3.00	317.63	243.86
	400 mg/l	91.40	108.70	4.00	3.67	27.14	23.85	2.67	3.33	342.83	300.9
	600 mg/l	92.00	117.12	4.53	4.00	28.30	24.00	3.30	3.80	367.5	326.4
	800 mg/l	92.75	102.70	4.88	4.17	29.00	25.00	3.50	4.00	397.87	335.48
	1000 mg/l	102.90	129.40	6.00	5.00	30.67	26.70	4.00	5.00	450.11	399.75
	1200 mg/l	100.00	125.66	5.60	4.66	30.00	26.00	4.00	4.50	436.28	368.16
Gemiza 10	Control	87.40	108.67	3.86	3.50	27.60	23.80	3.00	3.50	345.18	288.14
	400 mg/l	93.22	114.00	4.50	4.28	28.67	25.00	3.17	4.00	367.95	345.19
	600 mg/l	95.64	119.70	4.08	4.50	29.70	25.88	4.00	4.50	398.25	363.88
	800 mg/l	99.27	123.60	5.25	5.00	30.33	26.39	4.00	4.80	443.89	399.75
	1000 mg/l	113.70	134.50	6.33	6.00	31.70	27.70	4.87	5.88	490.50	450.00
	1200 mg/l	105.40	129.70	6.17	5.17	31.20	27.40	4.70	5.00	469.31	420.75
	LSD (5%)	2.21	2.01	0.12	0.15	0.40	0.25	0.13	0.56	19.06	22.14

A: Milky ripe stage (100 day after sowing) B: Dough ripening stage (120 day after sowing)

**Table (2): Effect of  $\alpha$ -tocopherol treatments on tillers-sheats dry weight, blades dry weight, spikes dry weight and flag leaf blade area of wheat plants.**

Cultivars	$\alpha$ -tocopherol Conc. mg/plant	Tillers-sheats dry wt(g/plant)		Blades dry wt. (g/plant)		Spikes dry wt. (g/plant)		Flag leaf blade area (cm <sup>2</sup> )		Leaf area index (LAI)	
		A	B	A	B	A	B	A	B	A	B
Gemiza 7		8.24	7.62	5.39	4.56	6.65	9.15	19.96	23.47	2.57	2.20
Gemiza 10		8.7	8.24	5.84	4.92	7.28	11.62	21.7	25.88	2.80	2.52
	LSD (5%)	0.19	0.13	0.25	0.16	0.28	0.19	1.02	0.81	0.90	0.06
	Control	7.99	6.84	4.55	4.02	5.1	8.62	17.42	21.95	2.21	1.77
	400 mg/l	7.88	7.25	4.91	4.22	6.06	9.53	19.14	22.79	2.37	2.15
	600 mg/l	8.15	7.71	5.47	4.58	6.97	10.24	20.35	24.12	2.55	2.3
	800 mg/l	8.5	7.98	5.81	4.91	7.33	10.75	21.28	25.45	2.81	2.45
	1000 mg/l	9.87	9.25	6.58	5.39	8.6	11.78	23.69	27.3	3.14	2.84
	1200 mg/l	9.14	8.57	6.38	5.32	7.74	11.38	23.11	26.46	3.02	2.63
	LSD (5%)	0.3	0.28	0.12	0.03	0.49	0.21	0.25	0.1	0.05	0.04
Gemiza 7	Control	7.2	6.55	4.33	3.9	4.73	7.87	16.53	20.86	2.11	1.63
	400 mg/l	7.83	7.00	4.65	4.09	5.59	8.13	18.47	21.34	2.29	2.00
	600 mg/l	7.94	7.45	5.32	4.47	6.7	9.1	19.54	22.53	2.45	2.18
	800 mg/l	8.18	7.69	5.6	4.68	7.17	9.51	20.09	24.29	2.66	2.24
	1000 mg/l	9.46	8.85	6.29	5.16	8.18	10.36	22.75	26.6	3.00	2.66
	1200 mg/l	8.83	8.16	6.17	5.08	7.52	9.92	22.39	25.2	2.91	2.45
Gemiza 10	Control	7.38	7.12	4.75	4.15	5.47	9.38	18.32	23.3	2.3	1.92
	400 mg/l	7.93	7.49	5.18	4.35	6.53	10.92	19.81	24.23	2.45	2.30
	600 mg/l	8.35	7.96	5.62	4.68	7.23	11.38	21.16	25.71	2.66	2.42
	800 mg/l	8.82	8.27	6	5.12	7.49	11.99	22.47	26.6	2.92	2.66
	1000 mg/l	10.27	9.69	6.87	5.63	9.02	13.21	24.63	28	3.27	3.00
	1200 mg/l	9.44	8.97	6.6	5.56	7.96	12.83	23.81	27.72	3.13	2.81
	LSD (5%)	0.5	0.46	0.2	0.05	0.81	0.35	0.41	0.18	0.08	0.07

A: Milky ripe stage (100 day after sowing) B: Dough ripening stage (120 day after sowing)

In this concern, Zhang *et al.*<sup>24</sup> showed positive correlation between  $\alpha$ -tocopherol and shoot or root growth in the two grass species of tall fescue and creeping bentgrass. El-Bassiouny *et al.*<sup>35</sup> also reported that foliar spray with  $\alpha$ -tocopherol on faba bean plants induced increments in growth parameters, yield components, chlorophyll a, chlorophyll b and carotenoids contents.

These results are in agreement with El-Bassiouny *et al.*<sup>25</sup> who reported that foliar spray with  $\alpha$ -tocopherol on faba bean plants induced increments in growth parameters and yield components.  $\alpha$ -tocopherol helps to maintain the integrity of the photosynthetic membranes under oxidative stress<sup>7</sup>.

Data shown in Table (3) indicate that wheat plants cultivar Gemiza 10 and Gemiza 7 significantly differed in number of spikes / plant, spikes dry weight / plant, main spike length and number of spikelets / main spike.

Data also indicate that foliar treatment of wheat plants cultivar Gemiza 10 with 1000 mg/l  $\alpha$ -tocopherol resulted in the best values of number of spikes / plant, spikes dry weight, main spike length as well as number of spikelets / main spike. Number of grains / plant, grain yield (g / plant) and straw yield (g / plant) followed the same trend (Table 3).

Data presented in Table (3) indicate that foliar spray of wheat plants with  $\alpha$ -tocopherol significantly increased Grain yield (Ton/Fed), especially in plants treated with 1000 mg/l. Straw yield, and biological yield followed the same trend.

The cultivars differences in yield and its components in our study are in good agreement with the results obtained by Ahmed *et al.*<sup>26</sup> and Hassanein *et al.*<sup>27</sup>.

**Table (3): Effect of  $\alpha$ -tocopherol treatments on number of spikes/plant, spikes dry weight, main spike length, number of Spiklets/main spike, number of grains/plant, grain yield/plant and straw yield.**

Cultivars	$\alpha$ -tocopherol Conc. mg/plant	No. of spikes/plant	Spikes dry wt g/plant	Main spike length (cm)	No. of spiklets /main spike	No. of grains / plant	Grain yield g/plant	Straw yield g/plant	Grain yield (Ton/ Fed)	Straw yield (Ton/ Fed)	Biologic al yield (Ton/ Fed)
Gemiza 7		4.95	33.94	15.00	38.52	379.60	25.35	41.27			
Gemiza 10		5.17	36.85	15.81	40.23	400.63	28.01	43.18	2.42	3.38	5.80
	<i>LSD (5%)</i>	<i>0.14</i>	<i>0.27</i>	<i>0.23</i>	<i>0.91</i>	<i>14.65</i>	<i>0.54</i>	<i>0.68</i>	<i>2.48</i>	<i>3.53</i>	<i>6.01</i>
	Control	3.59	30.34	13.64	37.55	335.10	22.73	38.89	0.03	0.05	0.13
	400 mg/l	4.42	32.64	14.27	38.50	372.15	24.38	41.00	2.26	3.23	5.49
	600 mg/l	4.94	34.58	15.09	39.00	386.90	26.52	42.14	2.34	3.29	5.63
	800 mg/l	5.17	36.39	15.96	39.90	400.90	28.40	43.47	2.42	3.46	5.88
	1000 mg/l	6.38	40.03	17.28	41.40	432.50	30.45	44.80	2.51	3.54	6.05
	1200 mg/l	6.00	38.38	16.20	39.90	413.15	27.56	43.05	2.63	3.64	6.27
	<i>LSD (5%)</i>	<i>0.22</i>	<i>0.28</i>	<i>0.32</i>	<i>0.91</i>	<i>9.02</i>	<i>0.63</i>	<i>0.93</i>	<i>2.57</i>	<i>3.58</i>	<i>6.15</i>
Gemiza 7	Control	3.50	28.24	13.51	36.70	329.70	21.71	38.5	0.04	0.03	0.1
	400 mg/l	4.33	30.60	14.11	37.50	361.00	24.11	40.19	2.25	3.16	5.41
	600 mg/l	4.88	33.00	14.87	38.20	374.80	25.60	41.27	2.31	3.25	5.56
	800 mg/l	5.00	35.48	15.40	39.00	385.80	26.20	42.18	2.41	3.37	5.78
	1000 mg/l	6.25	38.76	16.50	40.50	425.00	28.00	43.59	2.48	3.46	5.94
	1200 mg/l	5.75	37.54	15.59	39.20	401.30	26.49	41.86	2.57	3.54	6.11
Gemiza 10	Control	3.67	32.43	13.77	38.40	340.50	23.75	39.28	2.52	3.51	6.03
	400 mg/l	4.50	34.68	14.42	39.50	383.30	24.65	41.80	2.27	3.3	5.57
	600 mg/l	5.00	36.15	15.30	39.80	399.00	27.44	43.00	2.36	3.33	5.69
	800 mg/l	5.33	37.29	16.51	40.80	416.00	30.70	44.76	2.43	3.54	5.97
	1000 mg/l	6.50	41.30	18.06	42.30	440.00	32.89	46.00	2.54	3.61	6.15
	1200 mg/l	6.25	39.22	16.80	40.60	425.00	28.62	44.24	2.68	3.74	6.42
	<i>LSD (5%)</i>	<i>0.36</i>	<i>0.46</i>	<i>0.53</i>	<i>1.50</i>	<i>14.88</i>	<i>1.04</i>	<i>1.53</i>	<i>2.61</i>	<i>3.65</i>	<i>6.26</i>

**Table (4): Effect of  $\alpha$ -tocopherol treatments on Total carbohydrates, Total soluble sugars and total polysaccharides %, total protein %, proline, and free amino acids contents in wheat leaves and grains**

Cultivars	$\alpha$ -tocopherol	Leaves				Grains					
	Conc. mg/plant	Total Carbo- hydrates %	Total soluble sugars (TSS) %	Polysaccharides %	Total protein %	Total Carbo- hydrates %	Total soluble sugars (TSS) %	Polysacchari- des %	Total protein %	Proline (mg/100 g dry wt)	Free amino acids (mg/100 g dry wt)
Gemiza 7		20.05	3.35	16.7	12.31	81.12	61.33	19.79	16.3	36.89	215.28
Gemiza 10		18.19	2.23	15.96	12.94	82.15	61.36	20.79	16.59	38.82	272.47
	<i>LSD</i> (5%)	0.53	0.02	0.09	0.40	0.70	0.44	0.26	0.29	0.55	7.89
	control	14.00	2.96	17.00	11.00	80.77	61.08	19.69	14.42	25.65	147.71
	400 mg/l	18.99	2.77	16.60	11.40	81.32	61.30	20.02	15.56	32.55	201.14
	600 mg/l	19.42	2.87	11.13	12.6	81.79	61.51	20.28	16.76	38.51	235.05
	800 mg/l	20.195	2.81	16.76	12.83	82.10	61.4	20.7	17.10	43.63	307.59
	1000 mg/l	21.42	2.7	17.89	14.08	82.37	61.22	21.15	17.56	44.84	386.97
	1200 mg/l	20.71	2.64	18.60	14.02	81.49	61.54	19.95	17.21	40.90	184.78
	<i>LSD</i> (5%)	0.53	0.20	0.09	0.06	1.10	0.80	0.30	0.23	1.50	15.69
Gemiza 7	control	17.43	3.09	13.86	11.29	80.40	61.13	19.27	14.71	25.69	147.33
	400 mg/l	19.53	3.15	16.07	11.46	80.92	61.30	19.62	15.28	29.94	176.75
	600 mg/l	20.30	3.33	16.79	13.40	81.02	61.27	19.75	16.99	37.47	210.73
	800 mg/l	20.61	3.51	17.46	13.40	81.55	61.48	20.07	17.21	42.12	254.42
	1000 mg/l	21.39	3.57	18.06	14.08	81.83	61.59	20.6	17.90	45.51	336.41
	1200 mg/l	21.04	3.46	17.95	14.08	81.00	61.23	19.41	17.33	40.60	166.02
Gemiza 10	control	10.56	2.03	8.38	10.66	81.13	61.02	20.11	14.08	25.61	148.09
	400 mg/l	18.44	2.06	16.07	11.29	81.72	61.30	20.42	15.85	35.15	225.53
	600 mg/l	18.54	2.18	16.41	11.74	82.55	61.32	20.81	16.47	39.55	259.36
	800 mg/l	19.78	2.19	17.72	12.26	82.64	61.48	21.33	16.99	45.14	360.76
	1000 mg/l	21.45	2.47	19.26	14.08	82.91	61.74	21.59	17.16	46.26	437.52
	1200 mg/l	20.38	2.46	17.92	13.97	81.97	61.32	20.49	17.10	41.19	203.53
	<i>LSD</i> (5%)	0.85	0.030	0.14	0.06	1.80	1.30	0.50	0.17	1.08	11.30

Data presented in Table (4) indicate that total carbohydrates %, total soluble sugars (%) and polysaccharides % in wheat grains and leaves were significantly increased as a result of  $\alpha$ -tocopherol treatments, especially in plants treated with 1000 mg/l.

These results are in good harmony with those reported by El-Tohamy and El-Greadly<sup>28</sup>, Sadak and Dawood<sup>29</sup> who reported that  $\alpha$ -tocopherol increased total carbohydrates in snap bean and flax, respectively. Maeda and Della<sup>30</sup> also indicated that  $\alpha$ -tocopherol plays crucial role in maintaining the photo-assimilated substances and affects the sugar transport from the source (leaves) to sink (seeds) because of its effect on the plant metabolism processes.

It is also worth to mention that total carbohydrates, total soluble sugars (TSS) and total polysaccharides in grains were higher in cv Gemiza 10 than cv Gemiza 7. In the same time, these constituents were higher in the leaves of cv Gemiza 7 than cv Gemiza 10. In this concern, the differences between wheat cultivars may be attributed to differences in genetical structure, as well as, the range of cultivar response<sup>19</sup>.

Data also indicate that all concentrations of  $\alpha$ -tocopherol under study significantly increased protein % in leaves and grains. The highest protein content was obtained in plants treated with 1000 followed by 1200 mg/l  $\alpha$ -tocopherol. Total proline content (mg/100 g dry weight) and free amino acids content (mg/100 g dry weight) followed the same trend. These results are similar to those reported by El-Tohamy and El-Greadly<sup>28</sup> and El Rokiek *et al.*<sup>31</sup> on different plant species. Azzi<sup>32</sup> stated that tocopherol has an important function in regulating signal transduction and gene expression and has been found out that changes in amino acid, total soluble-N, protein-N and total-N indicate that tocopherol promotes the biosynthesis of amino acid and protein.

Proline accumulation, accepted as an indicator of environmental stress, is also considered to have important protective roles. Heavy metal stress leads to proline accumulation<sup>33</sup>. Proline accumulation in plant tissues has been suggested to result from (a) a decrease in proline degradation, (b) an increase in proline biosynthesis, (c) a decrease in protein synthesis or proline utilization, and (d) hydrolysis of proteins<sup>34</sup>.

Free radical generation is one of the initial responses of plants to stress. Generation of free radicals and reactive oxygen species is stimulated in the presence of metals<sup>35</sup>, and this can seriously disrupt normal metabolism through oxidative damage to cellular components. To mitigate and repair the damage initiated by active oxygen, plants have developed a complex antioxidant system. These antioxidants play an important role in the cellular defense strategy against oxidative stress, inducing resistance to metals by protecting labile macromolecules<sup>36</sup>. It is widely accepted that detoxification of metal ions within plant tissues usually depends on chelation by appropriate ligands. Antioxidants like cysteine, proline, ascorbic acid and nonprotein thiol (sulfhydryl) play an important role in detoxification of toxic metal ions<sup>37</sup>. Ascorbic acid was found to act as a chain-breaking scavenger for peroxy radicals and also as a synergist with vitamin E (tocopherol), since vitamin C can donate a hydrogen atom to the vitamin E-derived phenolate radical, thus regenerating its activity. There is a need to increase our understanding of this enigmatic molecule, since it could be involved in a wide range of important functions from antioxidant defense and photosynthesis to growth regulation<sup>38</sup>.  $\alpha$ -tocopherol is the major form found in green parts of plants. Tocopherols protect lipids and other membrane components by physically quenching and reacting chemically with singlet oxygen. Scavenging of singlet oxygen by  $\alpha$ -tocopherol in chloroplasts results in the formation of, among other products,  $\alpha$ -tocopherol quinone, a known contributor to cyclic electron transport in thylakoid membranes, thereby providing photoprotection for chloroplasts. Furthermore,  $\alpha$ -tocopherol may affect intracellular signaling in plant cells. The effects of this compound in intracellular signaling may be either direct, through interaction with key components of the signaling cascade, or indirect, through prevention of lipid peroxidation or the scavenging of singlet oxygen. In the latter case,  $\alpha$ -tocopherol may regulate the intracellular concentrations of reactive oxygen species and plant hormones, such as jasmonic acid, which controls both the growth and development of plants, and also the plant response to stress<sup>7</sup>. More recently it has been shown that the extent of  $\alpha$ -tocopherol quinone accumulation in Mediterranean plants exposed to drought stress depends on the severity of the stress, the stress sensitivity of the species, and the presence of alternative mechanisms of antioxidant protection (e.g., diterpenes, flavonoids)<sup>9</sup>. It has also been shown that  $\alpha$ -tocopherol quinone correlates with dynamic photoinhibition of photosynthesis, supporting the contention that this compound may be involved in cyclic electron transport around photosystem II<sup>39,40</sup>.

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