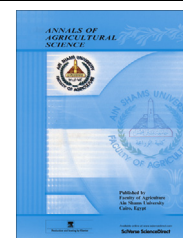




Faculty of Agriculture, Ain Shams University

Annals of Agricultural Sciencewww.elsevier.com/locate/aoas

Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract

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Received 9 February 2014; accepted 13 March 2014

Available online 28 July 2014

KEYWORDS

Wheat;
Drought;
Amino acid;
Yeast;
Water relations;
Physio-chemical;
Yield

Abstract Wheat (*Triticum aestivum* L.) is one of the most important cereal crops grown in the world. Drought is a worldwide problem, constraining global crop production seriously and recent global climate change has made this situation more serious. Two experiments (pots and field) were performed to investigate the effect of foliar spray with some biostimulants (amino acids 1.5 and 3 ml/L or yeast 3 and 6 g/L) to reduce hazards of drought stress (irrigation after the depletion of 65% and 80% of available soil water) on bread wheat (Sakha 94 cv.). The obtained results revealed that all studied characters of growth, relative water content (RWC), photosynthetic pigments, total soluble sugars (TSS), total carbohydrates (TC), total free amino acids (TAA), enzymes activities, minerals (NPK% and uptakes), yield and its attributes and grain quality were negatively affected by lower water supplies, meanwhile a significant increase was obtained in leaf water deficit (LWD), osmotic potential (OP), total phenols (TP) and proline content. The maximum decrease was recorded under high level of water stress (W2) compared to optimum level of water supply (W0). Application of amino acids and yeast extract significantly increased all measurement studied with exception of leaf water deficit, osmotic potential and proline content characters in favor of yeast application at a rate of 6 g/L compared to untreated plants in the two seasons. The interaction between the tested water stress and biostimulants was found to be significant for most characters of physiological traits and yield and its components. Also it was noticed that application of yeast (6 g/L) under normal water supply gave the best results of all studied characters. Also, it could be recommended that application of natural substances led to overcome the deleterious effect of drought and consequently resulted in improved the productivity of wheat and its grain quality.

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Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops grown in the world which plays a key role in the

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Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.

<http://dx.doi.org/10.1016/j.aoas.2014.06.018>

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economic activity. It is used as a stable food grain for urban and rural societies and as a major source of straw for animal feeding. In Egypt, the cultivated area was around 3 million feddan yearly. Increasing the cultivated area of wheat should be done in the reclaimed land due to the limited areas of the Nile Valley and the competition of the main crops. Therefore, improving both quantitative and qualitative characteristics of wheat was still the aim of many investigators. Water stress (drought) is the most important factor that affecting the productivity of wheat. Exposing plants to water stress adversely affect plant growth and productivity (Namich, 2007). Drought is a worldwide problem, constraining global crop production seriously and recent global climate change has made this situation more serious. Wheat should be irrigated when 50–55% of the available soil water is depleted in the root zone (Doorenbos and Pruitt, 1992; Mahmood and Ahmad, 2005). Water deficiency is generally considered as one of the limiting factors for crop productivity which affects physiological and biochemical processes in plants (Osborne et al., 2002). Egypt presents a typical example of the drought problem faced in some arid districts. There is a critical need to balance water availability, water requirements and water consumption. Thus, water conserving is becoming a decisive consideration for agriculture. Exploiting and increasing production in these areas are necessary to bridge the gap between production and consumption of wheat.

The application of biostimulants, i.e. amino acids and yeast extract was found to extent positive effect of plant growth which overcomes the harmful effect of some environmental stress such as drought. The importance of amino acids came from their widely use for the biosynthesis of a large variety of nonproteinic nitrogenous materials, i.e. pigments, vitamins, coenzymes, purine and pyrimidine bases. Studies have proved that amino acids can directly or indirectly influence the physiological activities in plant growth and yield (Mohamed, 2006). Yeast extract is a natural source of many growth substances (thiamine, riboflavin, niacin, pyridoxine and vitamins B1, B2, B3 and B12), cytokinins and many of the nutrient elements as well as organic compounds i.e., protein, carbohydrates, nucleic acid and lipids (Barnett et al., 1990; Nagodawithana, 1991). The positive effect of yeast extract in alleviation the deleterious effect of drought stress was observed by Hammad (2008). In this connection, Mohamed (2005) found that active dry yeast as foliar application had a beneficial effect on growth, yield and chemical constituents of plants. Therefore, the aim of this study was suggested the possibility of using some natural substances (amino acids or yeast extract) to reduce the harmful effect of water stress with studying the physiological mechanism or changes under these situation, photosynthetic pigments, water relations, chemical constituents, yield and its quality of wheat.

Materials and methods

Experimental site

Two experiments (pots and field) were carried out during the two winter seasons of 2010/2011 and 2011/2012 at the Experimental Farm, Faculty of Agriculture, Minufiya University, in Shebin El-Kom, Egypt, to investigate the performance of spring wheat cultivar Sakha 94 in relation to foliar spray with

some natural substances (amino acids “commercial name Delfan” and yeast extract) under drought conditions. Wheat cultivar was identified and obtained from Wheat Researches Institute, Agriculture Researches Center, Egypt.

Treatments

The experiments included 15 treatments which were all possible combinations of water stress and natural substances application. The plants under study were irrigation after depletion of 50% (W0), 65% (W1) and 80% (W2) of available soil water. These treatments reflecting conditions achieved as optimum level of water supply, moderate and severe water stress, respectively. The water stress treatments were applied after first irrigation until plant maturity. For the natural biostimulants, the plants were treated with delfan at 1.5 ml/L (D1), 3 ml/L (D2) and yeast extract at 3 g/L (Y1), 6 g/L (Y2) as well as untreated plants were taken as control. The treatments were sprayed three times, and the first one was 25 days after sowing and repeated each 15 days. Tween 20 was added to spraying solution at 0.5% as a surfactant. All cultural practices, other than treatment variables, were performed as recommended for the area.

Amino acids

Spanish compound known as Delfan 10% L- α -free amino acids was used as simulative compound source for amino acids mixture. Delfan is a brown liquid with pH of 5–5.5 and containing amino acids mixture as follows (mg/100 ml): Aspartic (2.3), Glycine (4.6), Threonine (1.2), Tyrosine (0.9), Glutamic (4.2), Histidine (0.3), Alanine (2.5), Cystine (0.2), Valine (1.8), Methionine (0.2), Isoleucine (1.1), Leucine (2.1), Phenylalanine (1.1), Hydroxyproline (2.7), Serine (2.8), Arginine (2.6), Proline (2.8) and Lysine (1.1). Also, contains amino nitrogen (1.4%) and organic matter (18.4%). Delfan is produced by Trade Corporation International Company Madrid and imported by TECNOGREEN Co., Egypt.

Yeast extract

The dry pure yeast powder was activated by using sources of carbon and nitrogen at a rate of 6:1 (Barnett et al., 1990) to obtain higher reactive yeast cells (1200 yeast cell/1 ml yeast extract). Then the media were frozen and thawed directly before usage.

Soil analysis

Soil samples of experimental site were randomly collected from depths of 0 to 30 cm and 30 to 60 cm using an auger before sowing to analyze for the determination of some physical and chemical properties of the soil according to Jackson (1973), Chapman and Pratt (1978) and Klute (1986) as shown in Tables 1a and 1b.

Pots experiments

Wheat grains were sowing on 15th November in the two seasons at a rate of 10 grains per pot. Pot was 25 cm inner diameter and 30 cm depth, and each holding 6 kg air-dry clay

Table 1a Physical and chemical properties of the experimental soil.

Properties	Particle size distribution (%)			Texture class	pH	E.C. ds/m	O.M. %	Available (ppm)		
	Sand	Silt	Clay					N	P	K
Seasons										
2010/2011	20.58	40.42	39.00	Clay loam	7.6	0.42	1.90	30.2	8.4	285.2
2011/2012	21.30	41.32	37.38	Clay loam	7.5	0.44	1.80	31.5	8.6	290.1

Table 1b Physical properties of the experimental soil in different depths.

Properties	Field capacity (%)	Permanent wilting point (%)	Available soil water (%)	Bulk density (g/cm ³)
Soil depth (cm)				
0–30	39.2	19.9	19.3	1.25
30–45	38.4	19.6	18.8	1.32
45–60	36.8	18.6	18.2	1.36

soil was collected from the soil surface (0–30 cm) of field experiment site. One week after sowing, the seedlings were thinned to 5 per pot. To obtain the recommended doses of fertilization in field, 0.6 g calcium super phosphate (15.5% P₂O₅)/pot was added before sowing. Ammonium nitrate (33.5% N) and potassium sulfate (48% K₂O) were also added in two equal doses at first and third irrigation at rates of 1.34 g and 0.3 g/pot, respectively. Soil moisture levels were monitored for each treatment throughout the growing seasons by tensiometer or weighing soil samples before and after placing in an electric oven. When reaching the level of drought treatment the required amount of water was added to each pot to maintain the respective moisture. The experimental design was factorial and the pots were arranged in a randomized complete blocks with four replicates.

Measurements

After 96 days from sowing, ten plants were randomly selected from each treatment and the following data were recorded.

– Growth characters

Plant height, No. of tillers/plant, No. of leaves/plant, shoots dry weight/plant, roots dry weight/plant, leaf area/plant and flag leaf area.

– Water relations

Relative water content (RWC%), leaf water deficit (LWD%), osmotic pressure of cell sap using the method of Kreeb (1990). To indicate the extent of membrane damage in leaf tissues (membrane integrity) subjected to drought condition, measurements on leakage of solutes were determined following the method of Leopold et al. (1981).

– Physio-chemical constituents

- Enzymes activities (peroxidase and phenoloxidase) were measured using the methods described by Fehrman and Dimond (1967) and Broesh (1954), respectively.
- Photosynthetic pigments were determined according to Wettstein (1957).

- Total soluble sugars and total carbohydrates, total phenols and total free amino acids in dry shoots were measured according to the methods described by Dubois et al. (1956), Snell and Snell (1953) and Rosen (1957), respectively.
- Free proline in fresh leaves was measured using the method described by Bates et al. (1973).
- Mineral concentrations were measured in dried shoots. Nitrogen was determined using the micro Kjeldahl method as described by AACC (2000), phosphorus was determined by spectrophotometer method as described by Snell and Snell (1954), potassium was estimated using flamephotometer method described by Chapman and Pratt (1978), then both their concentrations (%) and uptake (mg/plant) were calculated.

Field experiments

The experimental field was ploughed, harrowed and leveled. Sowing date was 15th November in the two seasons. Grains at a rate of 60 kg/fed were hand drilled in rows 15 cm apart in plots 3.5 m length and 3 m width with total area 10.5 m², grains covered by thin layer of soil after drilled. Calcium super phosphate (15.5% P₂O₅) was applied during soil preparation at the rate of 15.5 kg P₂O₅/fed. Nitrogen fertilization was applied at a rate of 75 kg N/fed in the form of ammonium nitrate (33.5% N) in two equal doses at first irrigation and second irrigation. Potassium fertilization was applied at a rate of 50 kg/fed in the form of potassium sulfate 48% K₂O with nitrogen fertilization. To monitor soil moisture status, tensiometer was located at various depths in the soil profile near the effective fibrous root zone to check subsoil moisture. Soil water content was measured with the tensiometer every 2 days until the targeted soil water depletion was attained to determine when irrigation is required again. The experimental design was randomized complete blocks and the treatments were arranged in a form of split plot with three replications. The water stress levels were arranged at random in the main plots, whereas the biostimulants were assigned at random in the sub-plots. There was a 2.0 m empty buffer area between all main plots and also irrigation canal in order to eliminate any effect from plot to another and from the canal to plots.

Measurements

– Yield and its attributes

At harvest, thirty spikes were taken randomly from the fifth inner row in each plot to estimate the following characters: No. of grains/spike, grains weight/spike (g), 1000 grains weight (g). Number of spikes/m², grain yield/fed, straw yield/fed and biological yield/fed were estimated from square meter from the inner rows per each plot.

– Grain quality

Protein percentage in the dry grains was calculated by multiplying N% by the factor of 5.70 which determined by using the micro Kjeldahl method as described by [AACC \(2000\)](#).

Total carbohydrate percentage in the dry grains was estimated using the method described by [Dubois et al. \(1956\)](#).

Total fibers in dry grains were determined using the method described by [Sadasivam and Manickam \(1992\)](#).

Statistical analysis

All measurements data during the two seasons in this study were analyzed according to the methods described by [Snedecor and Cochran \(1980\)](#). The differences among the means of different treatments were tested using the Least Significant Differences (LSD) at probability 5%. Statistical analysis was done using the CoStat package program, version 6.311 (cohort software, USA).

Results and discussion

Growth characters

The effect of water stress and natural substances (amino acids or yeast extract) individually or in interaction on growth characters is recorded in [Table 2](#). It is clear that all growth characters represented as plant height, number of tillers/plant, number of leaves/plant, shoots dry weight/plant, roots dry weight/plant, leaf area/plant and flag Leaf area were negatively affected by lower water supply treatments as compared with normal water supply treatment (control plants) in both seasons. Water stress could restrict internode elongation and leaf expansion through inhibiting cell expansion ([Namich, 2007](#)). Also, drought causes losses in tissue water content which reduce turgor pressure in cell, thereby inhibiting enlargement and division of cell causing of reduce of plant growth and dry mass accumulation ([Delfine et al., 2002](#)). These results are in agreement with those obtained by [Mujtaba et al. \(2007\)](#), [Maria et al. \(2008\)](#) and [Mahamed et al. \(2011\)](#) in wheat.

It is obvious from the same table that foliar spraying of simulative biostimulants (amino acids or yeast extract) significantly increased all growth characters of wheat plant compared to untreated plants in both seasons. Yeast extract was suggested to participate in a beneficial role during vegetative and reproductive growths through improving flower formation and their set in some plants due to its high auxin and cytokinins content and enhancement carbohydrates accumulation ([Barnett et al., 1990](#)) and/or the fact that this substance

enhanced cell division and nutritional status resulting in increasing number of leaves and dry weight of leaves, stems and roots. Application of delfan was improved growth which may be due to their role in raising cell division and enlargement and forming more tissues and organs. The beneficial effect of amino acids or yeast on crop growth characters was also reported by [Mohamed \(2006\)](#) and [Zaki et al. \(2007\)](#). The differences in the interaction recorded significant values for all growth characters in both seasons. The average values indicate that the biostimulant treatments significantly increased the most growth measurements of wheat plants grown under drought conditions. In this respect, application of yeast extract at higher level (Y2) under normal water supply (W0) gave the maximum increases as compared with other treatments. Moreover, spraying wheat plants grown under any water stress conditions with amino acids or yeast resulted in a significant increase in all growth characters if compared with its control. The positive effect of yeast extract may be due to the fact that it is a natural source of cytokinins, vitamins and most essential elements ([Nagodawithana, 1991](#)), which improved wheat vegetative growth and overcome the inhibitory effect of moisture stress. The obtained results are in agreement with those achieved by [El-Garhy \(2002\)](#) on faba bean, [Al-Thabet \(2006\)](#) on wheat and [Hammad \(2008\)](#) on pea.

Water relations

In both seasons, results in [Table 3](#) indicate that water stress levels significantly affected on relative water content (RWC), leaf water deficit (LWD), osmotic pressure (OP) and membrane integrity (MI), there was a gradual reduction in RWC, and meanwhile there was a gradual increase in OP and MI in leaves of wheat plants as compared with control plants. The maximum reduction in RWC reached about 15.15 but LWD, OP and MI were increased under the severe water stress (depletion 80% of available water) compared to control (depletion 50% of available water). RWC has been reported as an important indicator of water stress in leaves which is directly related to soil water content. This indicated greater resistance to water flow at the soil-root interface or decreased hydraulic conductivity of soil at low soil moisture. In addition, [Ranney et al. \(1991\)](#) proved that with osmotic adjustment mechanism, there is lowering osmotic potential of the cells and hence participates in maintaining of full turgor of tissue under water stress conduction. Osmotic adjustment is an active accumulation of solutes within the plant in response to decrease in soil water potential, thus reducing the harmful effects of water deficit. Under stressed conditions cell membranes are subject to changes often associated with the increase in the cell permeability ([Blokhina et al., 2003](#); [Iqbal, 2009](#)). These results are in line with those obtained by [Mujtaba et al. \(2007\)](#), [Maria et al. \(2008\)](#), [Zhang et al. \(2009\)](#) and [Waraich and Ahmad \(2010\)](#) on wheat and [Hammad \(2008\)](#) on pea.

Concerning the effect of spraying wheat plants with amino acids or yeast extract, data in [Table 3](#) showed clearly significant increments in RWC compared to untreated plants. Presented result showed that application of these natural extracts enhancement OP and MI compared to control plants which had the highest OP and solute leakage from leaf in both seasons. In general yeast extract especially at the highest rate

Table 2 Growth characters of wheat plants as affected by water stress, foliar application of delfan or yeast and their interaction during 2010/2011 (S1) and 2011/2012 (S2) seasons.

Water stress	Biostimulants	Plant height (cm)		No. of leaves/plant		No. of tillers/plant		Shoots dry weight/plant (g)		Roots dry weight/plant (g)		Leaf area/plant (cm ²)		Flag Leaf area (cm ²)	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
W0		70.20	62.10	16.53	13.60	3.13	2.93	3.17	3.14	0.70	0.56	206.12	182.20	36.59	24.30
W1		55.67	53.67	13.33	10.80	2.73	2.67	2.62	2.52	0.63	0.52	170.54	161.43	28.91	21.05
W2		48.20	45.03	10.93	9.27	1.53	1.67	1.83	1.71	0.50	0.40	141.74	120.98	23.94	17.18
	Control	51.78	50.67	12.00	9.56	1.89	1.89	1.85	1.93	0.47	0.37	145.13	127.08	23.81	17.38
	D1	55.22	51.72	13.11	10.56	2.11	2.22	2.21	2.13	0.58	0.44	157.50	140.37	27.46	18.81
	D2	58.78	53.44	14.11	11.44	2.44	2.44	2.50	2.45	0.67	0.49	169.87	152.03	30.95	20.11
	Y1	60.11	54.67	14.11	12.11	2.67	2.56	2.71	2.62	0.63	0.54	187.87	172.13	31.88	21.90
	Y2	64.22	57.50	14.67	12.44	3.22	3.00	3.43	3.15	0.72	0.62	204.17	182.73	34.96	25.92
W0	Control	65.00	57.50	14.00	11.33	2.67	2.33	2.51	2.46	0.59	0.48	166.50	148.30	29.80	20.06
	D1	69.00	59.50	16.33	12.67	2.67	2.67	2.62	2.80	0.63	0.52	183.50	170.10	33.63	21.70
	D2	70.33	62.67	17.00	13.67	3.00	3.00	3.02	3.30	0.75	0.55	201.10	180.00	41.81	24.00
	Y1	71.67	64.33	17.33	15.00	3.33	3.00	3.55	3.44	0.72	0.60	231.00	197.20	36.50	25.39
	Y2	75.00	66.50	18.00	15.33	4.00	3.67	4.16	3.70	0.82	0.66	248.50	215.40	41.21	30.33
W1	Control	50.66	51.50	12.33	9.33	2.00	2.00	1.92	2.00	0.45	0.39	143.30	126.77	23.11	17.30
	D1	52.33	52.33	13.00	10.00	2.33	2.33	2.25	2.10	0.64	0.44	156.50	135.10	26.51	18.40
	D2	57.00	53.00	14.00	11.00	2.67	2.67	2.63	2.30	0.70	0.50	163.90	155.10	28.73	19.11
	Y1	55.33	55.00	13.33	11.67	3.00	3.00	2.44	2.54	0.66	0.57	183.50	190.10	30.31	22.00
	Y2	63.00	56.50	14.00	12.00	3.67	3.33	3.84	3.65	0.72	0.68	205.50	200.10	35.88	28.44
W2	Control	39.67	43.00	9.67	8.00	1.00	1.33	1.11	1.33	0.36	0.25	125.60	106.20	18.51	14.77
	D1	44.33	43.33	10.00	9.00	1.33	1.67	1.76	1.50	0.47	0.37	132.50	115.90	22.24	16.33
	D2	53.00	44.67	11.33	9.67	1.67	1.67	1.85	1.74	0.55	0.41	144.60	121.00	25.11	17.21
	Y1	49.33	44.67	11.67	9.67	1.67	1.67	2.13	1.88	0.50	0.45	147.50	129.10	26.03	18.58
	Y2	54.67	49.50	12.00	10.00	2.00	2.00	2.30	2.11	0.61	0.53	158.50	132.70	27.80	19.00
LSD:	A	1.07	0.77	0.87	0.82	0.56	0.47	0.16	0.14	0.01	0.01	3.39	4.16	0.10	0.59
	B	1.37	0.99	1.12	1.06	0.72	0.63	0.20	0.18	0.02	0.03	4.37	5.37	0.13	0.77
	AB	2.39	1.72	1.90	1.77	1.04	0.77	0.44	0.31	0.03	0.04	7.45	9.30	0.22	1.24

W0, W1 and W2: Irrigation after depletion of 50%, 65% and 80% of available soil water, respectively. D: Delfan Y: Yeast.

Table 3 Water relations, osmotic pressure, membrane integrity and enzymes activities as affected by water stress, foliar application of delfan or yeast and their interaction during 2010/2011 (S1) and 2011/2012 (S2) seasons.

Water stress	Biostimulants	Relative water content (%)		Leaf water deficit (%)		Osmotic pressure C.S. (bar)		Membrane integrity (%)		Peroxidase activity (O.D./g F.W.)		Phenoloxidase activity (O.D./g F.W.)	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
W0		77.63	77.36	22.37	22.64	7.55	6.85	63.75	62.88	1.91	1.83	1.21	1.22
W1		78.13	75.72	21.87	24.28	9.61	8.83	66.96	67.99	1.87	1.86	1.17	1.23
W2		65.64	65.88	34.36	34.12	10.84	10.27	71.54	72.44	1.37	1.43	0.69	0.78
	Control	69.10	70.00	30.90	30.00	9.90	9.68	75.54	73.47	1.44	1.42	0.72	0.88
	D1	72.47	71.33	27.53	28.67	9.56	9.00	67.71	69.41	1.62	1.59	0.94	1.00
	D2	74.24	72.80	25.76	27.20	9.35	8.27	67.18	67.65	1.72	1.73	1.08	1.09
	Y1	75.60	74.60	24.40	25.40	9.10	8.19	64.48	64.91	1.84	1.83	1.17	1.18
	Y2	77.60	76.20	22.40	23.80	8.74	8.11	62.17	63.42	1.97	1.96	1.22	1.25
W0	Control	75.25	76.11	24.75	23.89	8.30	8.07	69.01	66.11	1.71	1.60	0.96	1.07
	D1	76.30	76.70	23.70	23.30	7.79	7.11	65.03	64.40	1.80	1.75	1.15	1.16
	D2	77.20	77.30	22.80	22.70	7.55	6.66	63.50	62.20	1.90	1.88	1.27	1.25
	Y1	79.00	77.10	21.00	22.90	7.20	6.30	62.80	61.20	2.05	1.90	1.33	1.30
	Y2	80.40	79.60	19.60	20.40	6.91	6.11	58.40	60.50	2.10	2.00	1.36	1.34
W1	Control	70.33	71.41	29.67	28.59	10.10	9.55	78.50	75.60	1.51	1.44	0.75	0.90
	D1	76.80	72.00	23.20	28.00	9.80	9.00	63.81	68.11	1.80	1.77	1.05	1.11
	D2	79.00	75.00	21.00	25.00	9.70	8.82	65.75	67.53	1.92	1.90	1.30	1.25
	Y1	81.10	79.20	18.90	20.80	9.45	8.56	64.22	65.41	2.00	2.05	1.35	1.40
	Y2	83.40	81.00	16.60	19.00	9.00	8.21	62.51	63.33	2.15	2.16	1.41	1.51
W2	Control	61.71	62.47	38.29	37.53	11.30	11.41	79.10	78.70	1.11	1.22	0.44	0.68
	D1	64.30	65.30	35.70	34.70	11.10	10.88	74.30	75.71	1.27	1.26	0.63	0.71
	D2	66.51	66.11	33.49	33.89	10.81	9.33	72.30	73.22	1.36	1.40	0.68	0.77
	Y1	66.70	67.50	33.30	32.50	10.66	9.71	66.41	68.11	1.48	1.55	0.82	0.83
	Y2	69.00	68.00	31.00	32.00	10.31	10.00	65.60	66.44	1.65	1.72	0.88	0.91
LSD:	A	0.59	1.33	0.59	1.33	0.20	0.15	0.81	1.16	0.09	0.04	0.04	0.03
	B	0.76	1.73	0.76	1.73	0.26	0.19	1.05	1.50	0.12	0.05	0.05	0.04
	AB	1.30	2.92	1.30	2.92	0.44	0.34	1.82	2.71	NS	0.09	0.09	0.08

W0, W1 and W2: Irrigation after depletion of 50%, 65% and 80% of available soil water, respectively. D: Delfan Y: Yeast.

(6 g/L) gave the best results. It is well known that, when transpiration exceeds water absorption, cell turgor falls as relative water content and cell volume decreased (Lawlor and Cornic, 2002) and low turgor and RWC slow plant growth and decrease of stomatal conductance. These results are in line with those achieved by El-Garhy (2002) on faba bean and Hammad (2008) on pea.

It is obvious from the same table that foliar application of biostimulants (delfan and yeast) related to all water relations studies under moisture stress conditions compared with untreated plants in both seasons. Natural substances have a better effect of drought stress on RWC and alleviated the depressive effect of drought by improving OP and MI status. In this regard the application of yeast (Y2) gave the best results under the different drought stress conditions. This confirmed those reported by Hammad (2008) who reported that foliar spraying of pea plant grown under drought conditions with yeast extract improved water status in leaves.

Physio-chemical constituents

(a) Enzymes activities

Data recorded in Table 3 indicate that peroxidase and phenoloxidase activities in wheat leaves were negatively affected under drought conditions. Enzyme activities were decreased in response to water stress and maximum decrease was noted in severe stress.

The obtained results are in agreement with those achieved by Iqbal (2009) who found that water stress treatment decreased peroxidase activity in wheat leaves. Maria et al. (2008) revealed that there was a great decrease in the activity of phenoloxidase in the leaves of wheat plants was reported with increasing the water deficit.

Results recorded in the same table demonstrated that the application of natural substances caused a significant enhancement in peroxidase and phenoloxidase activities in wheat leaves compared to untreated plants. In this connection, El-Nabarawy (2001) mentioned the importance and role of amino acids in synthesizing processes enzymes that are very important for growth and protein synthesis. However, Abbas (2013) mentioned that the incremental effect of yeast extract might attribute to the influence on photosynthetic pigments, phytohormones and enzyme activity that in turn increased vegetative growth of faba bean plant.

Concerning the interactive effect of water deficit and biostimulants, data in Table 3 showed a significant difference in peroxidase and phenoloxidase activities. The highest mean values were recorded in plants treated with yeast (Y2) under moderate water stress (W1) as compared with their controls in both seasons. However, the lowest enzyme activities were obtained under severe stress in the absence of amino acids or yeast extract.

(b) Photosynthetic pigments

Statistical analysis shows significant decrease in chlorophyll a + b and carotenoids by increasing water stress conditions compared with control as shown in Table 4. The decrease in the photochemical activities of chloroplast caused by water stress can be correlated with the decrease in the accumulation of chlorophyll. A decrease in net photosynthetic rate under water stress is also related to disturbances in biochemical

processes of a non-stomatal nature, caused by oxidation of chloroplast lipids and changes in the structure of pigments and proteins (Marcinska et al., 2013). In addition, Waraich and Ahmad (2010) mentioned that net CO₂ assimilation rate decreased with increase in water deficit developed due to limited irrigation of wheat. Decrease in stomatal conductance as a result of water deficit could be the main reason of reduced CO₂ assimilation rate. These results were generally in accordance with those obtained by Maria et al. (2008) on wheat.

Concerning the effect of spraying amino acids (D) or yeast (Y), data in Table 4 showed clearly significant increment in total chlorophyll (chl. a + b) and carotenoids of wheat leaves compared to untreated plants. Best results were obtained by the application of Y2 which gave the maximum increment followed by Y1 then D2 extract. These findings were true during the two growing seasons. Such results are connected with those reported by Alaei (2011) for amino acids on wheat leaves and Mohamed (2005) on common bean for yeast. In this connection, El-Nabarawy (2001) mentioned the importance role of amino acids in synthesizing processes of chlorophyll. Moreover, Wanas (2002) reported that yeast enhanced the formation of chlorophyll and delayed its degradation and senescence of bean plants.

As for the interaction between moisture stress and biostimulants, it can be noticed that the application of amino acids or yeast extract alleviated the negative effect of drought stress and significantly enhanced photosynthetic pigments. In this connection, El-Garhy (2002) showed that application of yeast significantly increased chlorophyll and carotenoids concentration in faba bean plants grown under least water requirements. Moreover, application of amino acids improved chlorophyll under applying drought stress as obtained by Alaei (2011) on some wheat genotypes.

(c) Total soluble sugars, total carbohydrates, total free amino acids and total phenols concentrations

In both seasons, results in Table 4 indicate that water stress levels significantly affected on total soluble sugars (TSS), total carbohydrates (TC), total free amino acids (TAA) and total phenols (TP). The lowest values of TSS, TC and TAA as well as the highest values of TP were obtained by W2 treatment. The reduction in TSS and TAA could be ascribed to water induced loss of solutes (mainly K⁺) from guard cells, which resulted in a selective reduction in guard cells turgor leading to stomatal closure. Zhang et al. (2009) mentioned that the soluble carbohydrate concentration in well-watered wheat plants was higher than those of stressed plants. Furthermore, Hammad and El-Gamal (2004) found that total phenols in pepper leaves were significantly enhanced under water conditions.

The obtained results in the same table indicated that the usage of natural substances caused a significant increase in TSS, TC, TP and TAA compared to untreated plants. The high contents of TSS, TC and TAA considered as a direct result for high rates of photosynthesis with great efficiency, which was preceded with large photosynthetic area (Table 2) and high content of photosynthetic pigments (Table 4). Generally, it was observed that the application of yeast was more effective. Yeo et al. (2000) found that yeast contains trehalose-6-phosphate synthase which is a key enzyme for trehalose biosynthesis. Trehalose affects sugar metabolism as well as osmoprotection against several environmental stresses. These

Table 4 Physio-chemical parameters in wheat plants as affected by water stress, foliar application of delfan or yeast and their interaction during 2010/2011 (S1) and 2011/2012 (S2) seasons.

Water stress	Biostimulants	Chl. a + b (mg/g D.W.)		Carotenoids (mg/g D.W.)		Total soluble sugars (mg/g D.W.)		Total carbohydrates (mg/g D.W.)		Total phenols (mg caticol/100 g D.W.)		Total free amino acids (mg/g D.W.)		Proline content (μ g/g D.W.)	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
W0		4.99	5.41	1.50	1.69	29.03	29.10	251.48	244.83	18.44	18.88	15.21	14.97	1417.95	1363.58
W1		4.68	5.06	1.27	1.43	26.06	25.93	220.56	201.57	18.58	18.93	12.41	11.53	1502.56	1465.66
W2		3.76	3.75	1.16	1.25	17.74	16.45	185.88	160.18	20.24	21.53	10.54	9.67	1661.54	1579.34
	Control	3.79	4.20	1.15	1.31	19.57	19.00	201.67	174.60	16.72	17.42	10.85	10.07	1643.22	1545.63
	D1	4.42	4.68	1.23	1.38	23.28	21.64	213.86	188.83	18.09	18.92	11.63	11.23	1588.20	1500.31
	D2	4.59	4.77	1.33	1.45	24.69	24.10	219.58	193.93	18.34	19.77	12.37	12.22	1509.83	1480.77
	Y1	4.76	4.96	1.36	1.53	25.81	26.47	227.23	204.17	20.34	20.75	13.67	13.04	1496.72	1433.99
	Y2	4.80	5.11	1.46	1.62	28.05	27.93	234.20	216.10	21.95	22.06	15.08	13.72	1398.77	1386.93
W0	Control	4.52	5.03	1.31	1.57	25.21	23.10	225.10	200.09	14.20	15.12	12.55	11.80	1523.87	1432.10
	D1	4.78	5.29	1.46	1.65	27.13	25.20	244.80	220.07	17.02	17.11	13.90	13.03	1475.80	1400.03
	D2	5.01	5.40	1.58	1.72	28.41	29.40	250.20	224.60	17.63	18.41	14.77	15.12	1400.10	1385.10
	Y1	5.23	5.60	1.5	1.70	30.30	32.40	262.10	233.80	21.55	20.82	16.80	17.00	1399.57	1330.47
	Y2	5.41	5.74	1.65	1.82	34.12	35.40	275.20	245.60	21.80	22.93	18.04	17.91	1290.40	1270.20
W1	Control	3.75	4.11	1.11	1.20	19.10	20.10	203.10	175.60	16.75	17.12	10.65	10.00	1632.30	1559.40
	D1	4.81	5.10	1.13	1.28	26.21	24.01	218.17	191.43	17.70	18.50	11.15	11.45	1581.10	1500.20
	D2	5.00	5.20	1.25	1.33	27.32	26.00	222.83	198.10	17.61	19.00	12.04	11.88	1506.10	1467.20
	Y1	5.05	5.41	1.40	1.65	28.05	29.40	228.20	215.30	18.82	19.91	13.00	12.05	1475.80	1411.50
	Y2	4.78	5.50	1.46	1.70	29.61	30.15	230.50	227.40	22.00	20.13	15.21	12.25	1317.50	1390.00
W2	Control	3.11	3.45	1.04	1.16	14.40	13.80	176.80	148.10	19.20	20.00	9.34	8.40	1773.50	1645.40
	D1	3.68	3.65	1.11	1.22	16.50	15.71	178.60	155.00	19.55	21.14	9.85	9.22	1707.70	1600.70
	D2	3.77	3.70	1.17	1.30	18.33	16.90	185.70	159.10	19.77	21.90	10.30	9.67	1623.30	1590.00
	Y1	4.00	3.87	1.20	1.25	19.07	17.62	191.40	163.40	20.65	21.51	11.22	10.06	1614.80	1560.00
	Y2	4.22	4.10	1.26	1.34	20.41	18.23	196.90	175.30	22.05	23.12	12.00	11.00	1588.40	1500.60
LSD:	A	0.09	0.06	0.03	0.03	0.21	0.12	6.95	1.74	0.43	0.42	0.17	0.36	16.34	14.81
	B	0.11	0.07	0.04	0.06	0.26	0.24	8.98	2.24	0.56	0.54	0.23	0.47	20.47	19.12
	AB	0.19	0.13	0.06	0.11	0.48	0.42	NS	3.80	1.01	0.98	0.48	0.82	36.24	31.50

W0, W1 and W2: Irrigation after depletion of 50%, 65% and 80% of available soil water, respectively. D: Delfan Y: Yeast.

Table 5 Mineral concentrations in wheat plants as affected by water stress, foliar application of delfan or yeast and their interaction during 2010/2011 (S1) and 2011/2012 (S2) seasons.

Water stress	Biostimulants	N%		N uptake (mg/plant)		P%		P uptake (mg/plant)		K%		K uptake (mg/plant)	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
W0		3.43	3.19	110.74	101.65	0.40	0.37	12.78	11.84	2.64	2.65	84.82	83.67
W1		3.38	3.14	89.16	80.07	0.36	0.31	9.64	7.92	2.61	2.36	69.71	60.40
W2		2.68	2.43	49.59	42.02	0.30	0.24	5.55	4.22	2.24	2.13	41.39	36.71
	Control	2.84	2.55	54.03	50.51	0.31	0.25	6.03	5.11	2.28	2.16	43.17	42.70
	D1	3.07	2.77	68.97	60.74	0.34	0.29	7.71	6.40	2.41	2.33	53.90	51.37
	D2	3.23	3.00	82.57	75.37	0.36	0.31	9.14	7.94	2.47	2.38	62.40	58.59
	Y1	3.30	3.10	90.60	83.44	0.37	0.33	10.12	8.99	2.58	2.45	70.67	65.64
	Y2	3.38	3.17	119.64	102.86	0.39	0.35	13.61	11.53	2.75	2.58	96.39	83.01
W0	Control	3.21	2.85	80.48	70.22	0.37	0.31	9.21	7.65	2.44	2.40	61.49	59.08
	D1	3.40	3.10	89.00	86.73	0.39	0.36	10.20	10.05	2.51	2.60	65.71	72.76
	D2	3.45	3.25	104.39	107.49	0.40	0.38	12.09	12.56	2.55	2.67	76.72	87.82
	Y1	3.50	3.33	124.28	115.03	0.41	0.40	14.49	13.73	2.74	2.71	97.18	93.20
	Y2	3.74	3.48	155.56	128.79	0.43	0.41	17.88	15.17	2.96	2.85	123.03	105.48
W1	Control	2.80	2.61	53.86	52.03	0.31	0.25	5.96	5.02	2.35	2.18	45.20	43.63
	D1	3.15	2.90	70.91	60.90	0.35	0.28	7.86	5.88	2.55	2.23	57.96	46.83
	D2	3.55	3.30	93.38	75.83	0.37	0.3	9.74	6.91	2.62	2.31	69.01	53.17
	Y1	3.65	3.41	89.04	87.42	0.38	0.33	9.27	8.34	2.66	2.45	64.83	62.34
	Y2	3.61	3.40	138.59	124.17	0.40	0.37	15.37	13.50	2.91	2.63	111.56	96.01
W2	Control	2.50	2.20	27.76	29.28	0.26	0.20	2.90	2.66	2.05	1.91	22.81	25.39
	D1	2.66	2.32	46.99	34.60	0.29	0.22	5.05	3.28	2.18	2.30	38.04	34.51
	D2	2.70	2.46	49.93	42.78	0.30	0.25	5.58	4.34	2.25	2.00	41.48	34.76
	Y1	2.75	2.55	58.48	47.85	0.31	0.26	6.62	4.92	2.35	2.20	50.01	41.36
	Y2	2.81	2.63	64.78	55.60	0.33	0.28	7.57	5.90	2.38	2.25	54.59	47.54
LSD:	A	0.08	0.10	5.48	6.66	0.01	0.01	0.74	0.68	0.13	0.17	4.94	5.88
	B	0.11	0.13	7.08	8.60	0.02	0.02	0.95	0.89	0.17	0.22	6.37	7.59
	AB	0.18	0.22	12.27	14.87	0.04	0.03	1.64	1.53	NS	NS	11.02	13.14

W0, W1 and W2: Irrigation after depletion of 50%, 65% and 80% of available soil water, respectively. D: Delfan Y: Yeast.

results are in line with that obtained by [Barnett et al. \(1990\)](#) and [Mady \(2009\)](#).

Concerning the interaction, it is clear that yeast application at the high level under normal water supply was more the most effective treatment in increasing TSS, TC and TAA which produced the highest values of the traits as compared with the other treatments. However, the untreated plants with either amino acids or yeast had the lowest values of these traits under severe stress conditions.

(d) Proline content

It is obvious from [Table 4](#) that there is a remarkable increase in leaf proline content under drought condition when compared with control plants. Proline not only acts as an osmolyte but also contributes in stabilizing subcellular structures (e.g. membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions ([Iqbal, 2009](#)). Furthermore, [Pedersen et al. \(1996\)](#) reported that there was a positive correlation between proline concentration and membrane integrity of tobacco leaves and proline believed to stabilize membrane phospholipids which helps the plants to overcome periods of drought stress. These findings were supported by those obtained by [Mujtaba et al. \(2007\)](#) and [Maria et al. \(2008\)](#) in wheat.

Concerning effects of amino acids or yeast extracts treatments, results indicated that these treatments caused a

significant reduction in proline content compared to untreated plants in both seasons. The highest depression was recorded with the application of yeast extract at the rate of 6 g/L. Generally, the application of natural substances (D or Y) with drought significantly decreased proline concentration compared to untreated plants (water stress alone). The role of amino acids in abiotic stress tolerance was reported by [Singh \(1999\)](#) who mentioned that this class of molecules includes certain amino acids (notably proline), quaternary ammonium compounds. These compounds are thought to play a pivotal role in plant cytoplasmic osmotic adjustment in response to osmotic stress. These results confirm those reported by [El-Garhy \(2002\)](#) on faba bean and [Alaei et al. \(2012\)](#) on wheat plants.

(e) Mineral concentration

Data recorded in [Table 5](#) indicate that water stress treatments affected on both NPK percentages and uptakes in stress tissues negatively compared to untreated plants. The maximum reductions were recorded under W2 treatment (high stress) which reached about 22.11%, 29.73% and 15.98% for N%, P% and K% in the first season respectively. The same trend was recorded in the second season. The reduction in P concentration may be due to the dieback of the absorbing roots during the exposure of plants to drought conditions ([Larson, 1975](#)). In addition, [Baque et al. \(2006\)](#) observed that

Table 6 Yield and its attributes of wheat plants as affected by water stress, foliar application of delfan or yeast and their interaction during 2010/2011 (S1) and 2011/2012 (S2) seasons.

Water stress	Biostimulants	No. of spikes/m ²		No. of grains/spike		1000-grain weight (g)		Grains weight/spike (g)		Grain yield (ton/fed)		Straw yield (ton/fed)		Biomass yield (ton/fed)	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
W0		319.27	325.93	62.27	60.40	42.26	44.49	2.57	2.52	2.93	3.11	3.62	3.53	6.55	6.64
W1		283.93	277.53	57.20	58.27	39.63	40.45	2.39	2.30	2.44	2.72	3.22	3.15	5.66	5.87
W2		247.53	251.13	52.40	49.93	35.67	36.15	1.79	1.82	1.74	1.80	2.47	2.44	4.21	4.24
	Control	261.22	259.67	47.78	46.89	33.36	35.63	2.06	2.00	2.02	2.18	2.41	2.39	4.43	4.57
	D1	275.67	273.89	55.89	54.22	37.84	38.56	2.15	2.09	2.28	2.52	2.92	2.76	5.20	5.28
	D2	282.67	285.56	58.33	58.11	40.47	40.68	2.22	2.23	2.44	2.62	3.19	3.15	5.63	5.77
	Y1	293.56	296.33	60.33	59.33	41.12	42.29	2.33	2.33	2.49	2.65	3.45	3.40	5.94	6.05
	Y2	304.78	308.89	64.11	62.44	43.15	44.64	2.50	2.41	2.62	2.75	3.55	3.50	6.17	6.25
W0	Control	301.67	303.33	56.67	54.67	37.44	40.70	2.37	2.35	2.54	2.68	3.07	3.05	5.61	5.73
	D1	313.00	318.33	59.33	56.67	40.80	43.12	2.41	2.45	2.74	2.99	3.48	3.25	6.22	6.24
	D2	319.33	330.67	62.00	62.00	43.68	44.80	2.47	2.52	2.98	3.19	3.57	3.45	6.55	6.64
	Y1	328.00	333.67	63.00	61.00	43.08	45.20	2.72	2.60	3.09	3.28	3.93	3.88	7.02	7.16
	Y2	334.33	343.67	70.33	67.67	46.32	48.62	2.87	2.68	3.32	3.43	4.04	4.01	7.36	7.44
W1	Control	261.00	254.67	43.00	44.00	32.82	34.68	2.25	2.02	2.14	2.36	2.25	2.17	4.39	4.53
	D1	274.33	265.00	58.67	59.00	37.20	38.36	2.29	2.05	2.39	2.75	2.87	2.77	5.26	5.52
	D2	281.67	272.67	59.00	61.00	42.14	41.73	2.40	2.36	2.51	2.80	3.40	3.38	5.91	6.18
	Y1	292.33	289.00	61.00	63.33	42.74	43.23	2.44	2.53	2.52	2.84	3.71	3.69	6.23	6.53
	Y2	310.33	306.33	64.33	64.00	43.26	44.24	2.59	2.55	2.64	2.85	3.87	3.77	6.51	6.62
W2	Control	221.00	221.00	43.67	42.00	29.82	31.52	1.57	1.65	1.39	1.52	1.90	1.94	3.29	3.46
	D1	239.67	238.33	49.67	47.00	35.52	34.20	1.75	1.79	1.72	1.83	2.40	2.28	4.12	4.11
	D2	247.00	253.33	54.00	51.33	35.60	35.52	1.79	1.81	1.83	1.86	2.61	2.63	4.44	4.49
	Y1	260.33	266.33	57.00	53.67	37.53	38.44	1.82	1.85	1.87	1.84	2.71	2.64	4.58	4.48
	Y2	269.67	276.67	57.67	55.67	39.86	41.07	2.04	2.00	1.89	1.97	2.73	2.74	4.62	4.71
LSD:	A	10.43	10.36	3.25	2.79	1.95	1.22	0.09	0.15	0.19	0.07	0.21	0.27	0.16	0.29
	B	8.45	7.79	2.11	2.29	1.80	1.50	0.10	0.10	0.11	0.06	0.17	0.21	0.17	0.23
	AB	14.36	13.25	3.59	3.90	3.06	2.56	0.17	0.17	0.20	0.11	0.29	0.37	0.29	0.39

W0, W1 and W2: Irrigation after depletion of 50%, 65% and 80% of available soil water, respectively. D: Delfan Y: Yeast Fed = 4200 m².

Table 7 Grain quality of wheat plants as affected by water stress, foliar application of delfan or yeast and their interaction during 2010/2011 (S1) and 2011/2012 (S2) seasons.

Water stress	Biostimulants	Protein (%)		Carbohydrate (%)		Fibers (%)	
		S1	S2	S1	S2	S1	S2
W0		10.95	11.87	66.52	67.89	1.79	1.74
W1		11.26	11.36	66.84	67.73	1.80	1.77
W2		8.24	9.43	56.21	55.37	1.94	1.92
	Control	8.65	9.86	58.21	58.72	1.96	1.92
	D1	9.20	10.35	58.80	60.44	1.87	1.76
	D2	9.99	10.72	61.60	61.43	1.84	1.81
	Y1	11.24	11.45	67.30	67.02	1.81	1.84
	Y2	11.69	12.06	70.03	70.70	1.76	1.74
W0	Control	9.64	10.84	63.60	68.65	1.85	1.80
	D1	10.20	11.11	61.79	65.37	1.83	1.80
	D2	11.05	11.63	63.64	63.06	1.81	1.70
	Y1	11.70	12.42	70.43	69.77	1.79	1.75
	Y2	12.16	13.35	73.13	72.62	1.71	1.67
W1	Control	9.00	10.31	60.50	63.02	1.97	1.97
	D1	9.50	10.78	62.96	64.19	1.80	1.78
	D2	10.64	10.98	65.89	65.42	1.78	1.82
	Y1	13.40	12.11	70.93	71.19	1.75	1.64
	Y2	13.78	12.62	73.93	74.83	1.70	1.66
W2	Control	7.30	8.44	50.55	44.49	2.06	2.00
	D1	7.90	9.15	51.66	51.77	1.97	1.93
	D2	8.28	9.55	55.26	55.80	1.92	1.91
	Y1	8.61	9.82	60.54	60.12	1.89	1.90
	Y2	9.12	10.21	63.04	64.65	1.86	1.88
LSD:	A	0.42	0.64	2.25	1.53	0.12	0.09
	B	0.94	0.67	2.00	2.45	0.13	0.11
	AB	1.64	1.16	3.48	4.26	NS	NS

W0, W1 and W2: Irrigation after depletion of 50%, 65% and 80% of available soil water, respectively. D: Delfan Y: Yeast.

mild and severe water stress significantly reduced the uptake of NPK in wheat plants compared to that of normal conditions. These results are in accordance with those obtained by [Maria et al. \(2008\)](#) in wheat plants.

The same table clearly indicated that the application of natural substances significantly increased NPK% and uptakes compared to control. Using yeast extract showed the greatest significant increase. Amino acids and yeast extract may be increased absorption of different elements by roots and also their translocation and accumulation in leaves. Similar results were observed by [Hammad \(2008\)](#) and [Mady \(2009\)](#).

Obtained results in the same table demonstrated that the application of natural extracts (D or Y) alleviated the negative effect of drought stress and significantly enhanced N, P and K concentrations especially under normal conditions with yeast extract. The second season followed the same trend. Similar results were obtained by [El-Garhy \(2002\)](#) on faba bean. The application of yeast under drought stress condition caused a significant increase in N%, P% and K% in pea leaves which could be attributed to its minerals, carbohydrates and hormonal contents ([Hammad, 2008](#)).

Yield and its attributes

Data in [Table 6](#) reveal that significant differences were registered in yield and its attributes (number of spikes/m², number of grains/spike, 1000-grain weight and grains weight/spike)

among the various drought stress treatments in both seasons. It is apparent that yield components were reduced due to affected by drought treatments compared to normal water supply in the first and the second seasons. The greatest reduction was observed in the severe water stress treatment (W2). The low grain weight could have occurred due to the direct effect of water stress on carbohydrate accumulation. [Iqbal \(2009\)](#) indicated that water stress affected of wheat plants might lie not only in the variations in physiological processes such as accumulation of osmolytes, antioxidant capacity, stomatal conductance but also in changes in the phytohormonal balance. Yield and its components were increased when the frequency of irrigation increased. The reduction in photosynthesis under drought stress which is the most important anabolic process in plants, resulting in reducing the efficiency of all other biological processes in plant which led to reduction of growth and yield. Delaying irrigation until soil water reached 65% or 80% depletion had the effect of decreasing grain, straw and biological yields. This could have occurred due to loss of yield components. Increasing soil moisture depletion levels decreased the grain yield as an average of the two seasons by about 14.63% and 41.37% for W1 and W2, respectively compared with W0. The results are in line with those reported by [Iqbal \(2009\)](#), [Waraich and Ahmad, \(2010\)](#), [Mahamed et al. \(2011\)](#) and [Mohammadi et al. \(2013\)](#).

Foliar spraying wheat plants with biostimulants significantly increased yield and its components in both seasons.

The different natural substances used, were more efficient in raising the photosynthetic surfaces of plants that reflected positively in raising their yield components. The highest mean values are recorded in plants treated with Y2 (6 g/L). As an average of the two seasons, it can be noticed that the plants were treated with yeast (Y2), (Y1) and delfan (D2) caused an increasing in grain yield/fed amounted to 27.93%, 22.42% and 20.49% more than untreated plants, respectively. The positive effect of yeast extract on yield and its components may be attributed the fact that it contains cytokinins, vitamin B12 and minerals, which might play a role of in orientation and translocation of metabolites from leaves into reproductive organs. Moreover, it may play a role in synthesis of protein and nucleic acids and minimize their degradation, which might lead to the improvement of yield plants. These results partially agreed with the findings of Mohamed (2006), Zaki et al. (2007) and Dromantiene et al. (2009).

The interaction between natural biostimulants (D or Y) and water stress on yield attributes had significant effect on yield and yield components. The data in Table 6 showed that application of yeast extract under W0 gave the best results in both seasons for yield components. It can be noticed that the application of natural substances inhibitory effect of water stress and improved yield and its components of wheat plants. The natural substances showed significantly good performance than other treatments in normal irrigation and drought stress conditions. It seems that natural substances supply would increase grain set or inhibit its further reduction due to water stress conditions. Several studies have convincingly demonstrated that the application of natural substances provides tolerance to various stress conditions (Al-Thabet, 2006 and Alaei, 2011).

Grain quality

The results presented in Table 7 show significant depression in total protein and carbohydrates meanwhile a gradual increase in total fibers in wheat grains as a result of plants treated with severe drought conditions (W2). Irrigation until soil water reached 50% or 65% depletion had the effect of increasing grain protein and carbohydrates content. This could have occurred due to more NPK uptake from the soil compared to irrigation until soil water reached 80% depletion. These results are in line with those obtained by Dromantiene et al. (2009) and Bakry et al. (2012).

It is obvious from the same table that foliar spraying of natural biostimulants significantly increased total carbohydrates and total protein meanwhile decreased total fiber in wheat grains compared to control plants. In general, yeast extract was superior to amino acids on grain quality in both seasons. Improving quality by application of yeast extract and amino acids were recorded by Dromantiene et al. (2009) and Abbas (2013).

Concerning the interaction effect of water deficit and natural substances, it can be noticed that the application of amino acids or yeast extracts alleviated the negative effect of drought stress and significant enhanced total carbohydrates and protein in grains. The best interaction results were recorded in plants treated with optimum water supply or moderate water stress combined with foliar application of yeast extract (6 g/L) in both seasons.

Conclusion

Sufficient water supply is fundamental for optimal growth and productivity. The decrease in the plant yield might be affected by changes in the physiological processes under water stress. Results showed that soil moisture stress causes low grain yield by inducing low growth, number of spikes, grain weight and number of grains/spike. Thus, wheat plants appear to be suffering yield losses due to deficiency of irrigation water. Therefore, wheat grower must be careful about water stress especially on critical stages which can cause tremendous yield losses. It could be recommended that application of natural biostimulants especially yeast extract at the rate of 6 g/L led to overcome the deleterious effect of drought and consequently resulted in improved the productivity of wheat and its grain quality.

References

- AACC, 2000. American Association of Cereal Chemists. Approved Methods of the AACC, 10th ed. St. Paul. Minnesota, USA.
- Abbas, S.M., 2013. The influence of biostimulants on the growth and on the biochemical composition of Vicia faba cv. Giza 3 beans. Romanian Biotechnol. Lett. 18 (2), 8061–8068.
- Alaei, Y., 2011. The effect of amino acids on leaf chlorophyll content in bread wheat genotypes under drought stress conditions. Middle-East J. Sci. Res. 10 (1), 99–101.
- Alaei, Y., Khanghah, A.M., Jafari, M., Khaneghah, A., 2012. Evaluation on leaf proline amount in three bread wheat cultivars in presence of two fertilizers containing amino acids in drought stress. World Appl. Sci. J. 18 (9), 1190–1192.
- Al-Thabet, S.S., 2006. Promotive effect of 5-amino Levulinic acid on growth and yield of wheat grown under dry conditions. J. Agron. 5 (1), 45–49.
- Bakry, A.B., Abdelraouf, R.E., Ahmed, M.A., El Karamany, M.F., 2012. Effect of drought stress and ascorbic acid foliar application on productivity and irrigation water use efficiency of wheat under newly reclaimed sandy soil. J. Appl. Sci. Res. 8 (8), 4552–4558.
- Baque, M.A., Karim, M.A., Hamid, A., Tetsushi, H., 2006. Effect of fertilizer potassium on growth, yield and nutrient uptake of wheat (*Triticum aestivum* L.) under water stress conditions. South. Pacific Stud. 27 (1), 25–35.
- Barnett, J.A., Payne, R.W., Yarrow, D., 1990. Yeast Characteristics and Identification, second ed. Press, Cambridge Univ., London, UK, 1012 p.
- Bates, L.S., Waldren, K.P., Teare, L.D., 1973. Rapid determination of free proline for water stress studies. Plant Soil 39, 205–207.
- Blokhina, O., Virolainen, E., Fagerstedt, K.V., 2003. Antioxidants, oxidative damage and oxygen deprivation stress. Ann. Bot. 91, 179–194.
- Broesh, S., 1954. Colorimetric assay of phenoloxidase. Bull. Soc. Chem. Biol. 36, 711–713.
- Chapman, H.D., Pratt, P.F., 1978. Methods of Analysis for Soils, Plants and Water. Division of Agriculture Sciences, University of California, Davis, pp. 162–165.
- Delfine, S., Tognettir, R., Loreto, F., Alvino, A., 2002. Physiological and growth responses to water stress in field grown bell pepper (*Capsicum annuum*, L.). J. Hort. Sci. Biotechnol. 77 (6), 697–704.
- Doorenbos, J., Pruitt, W.O., 1992. Calculation of crop water requirements. In: Doorenbos, J., Pruitt, W.O. (Eds.), Guideline for Predicting Crop Water Requirements: FAO Irrigation and Drainage. Paper No. 24. FAO, Rome, pp. 1–65.
- Dromantiene, R., Pranckietiene, I., Sidlauskas, G., Pranckietis, V., 2009. The effect of fertilisers containing amino acids on winter

- wheat grain yield and technological properties. *J. Zemdirbyste (Agriculture)* 96 (4), 97–109.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Robers, P.A., Smith, F., 1956. Colorimetric method for determination of sugar and related substances. *Anal. Chem.* 28 (3), 350–356.
- El-Garhy, A.M., 2002. Physiological Studies on Tolerance of Some Varieties of Faba Bean Plants Under Least Water Requirements. Ph. D. Thesis, Agric., Botany Dept., Faculty of Agric., Minufiya Univ., Shebin El-Kom, Egypt, pp. 44–120.
- El-Nabarawy, M.A., 2001. Mitigation of dark induced senescence. I – By some amino acids. *Ann. Agric. Sci. Moshtohor* 39 (1), 225–232.
- Fehrman, H., Dimond, A.E., 1967. Peroxidase activity and phytophthora resistance in different organs of the potato. *Plant. Pathol.* 57, 69–72.
- Hammad, S.A.R., 2008. Physiological and anatomical studies on drought tolerance of pea plants by application of some natural extracts. *Ann. Agric. Sci., Ain Shams Univ., Cairo* 53 (2), 285–305.
- Hammad, S.A.R., El-Gamal, S.M., 2004. Response of pepper plants grown under water stress condition to biofertilizers (Halex 2) and mineral nitrogen. *Minufiya J. Agric. Res.* 29 (1), 1–27.
- Iqbal, S., 2009. Physiology of Wheat (*Triticum aestivum* L.) Accessions and the Role of Phytohormones Under Water Stress. Ph.D. Thesis, Fac. of Biological Sci., Quaid-i-azam Univ., Islamabad, pp. 83–154.
- Jackson, M.L., 1973. Soil Chemical Analysis. Prentice Hall of India Ltd, New Delhi, India, 498 p.
- Klute, A., 1986. Methods of Soil Analysis, second ed. American Society of Agronomy, Madison, Wisconsin, 183 p.
- Kreeb, K.H., 1990. Methoden Zur Pflanzenökologie und Bioindikation. Gustav Fischer, Jena, Germany, 327 p.
- Larson, K.L., 1975. Drought injury and resistance of crop plants. In: Gupta (Ed.), U.S. Physiological Aspects of Dry Land Farming. Oxford and RHS Pub. Co., New Delhi, India, pp. 147–165.
- Lawlor, D.W., Cornic, G., 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficit in higher plants. *Plant Cell Environ.* 25, 275–294.
- Leopold, A.G., Musgrave, M.E., Williams, K.M., 1981. Solute leakage, resulting from leaf desiccation. *Plant Physiol.* 68, 1222–1225.
- Mady, M.A., 2009. Effect of foliar application with yeast extract and zinc on fruit setting and yield of faba bean (*Vicia faba* L.). *J. Biol. Chem. Environ. Sci.* 4 (2), 109–127.
- Mahamed, M.B., Sarobol, E., Hordofa, T., Kaewrueng, S., Verawudh, J., 2011. Effects of soil moisture depletion at different growth stages on yield and water use efficiency of bread wheat grown in semi arid conditions in Ethiopia. *Kasetsart J. (Nat. Sci.)* 45, 201–208.
- Mahmood, N., Ahmad, R.N., 2005. Determination of water requirements and response of wheat to irrigation at different soil moisture depletion levels. *Int. J. Agric. Biol.* 7 (5), 812–813.
- Marcinska, I., Czaczyo-Mysza, I., Skrzypek, E., Filek, M., Grzesiak, S., Grzesiak, M.T., Janowiak, F., Hura, T., Dziurka, M., Dziurka, K., Nowakowska, A., Quarrie, S.A., 2013. Impact of osmotic stress on physiological and biochemical characteristics in drought-susceptible and drought-resistant wheat genotypes. *Acta Physiol. Plant* 35, 451–461.
- Maria, A.M., Gendy, A.A., Selim, A.H., Abd El-All, A.M., 2008. Response of wheat plants grown under water stress in relation to Jasmonic acid. *Minufiya J. Agric. Res.* 33 (6), 1355–1375.
- Mohamed, A.M., 2006. Effect of Some Bio-chemical Fertilization Regimes on Yield of Maize. M.Sc. Thesis, Fac. of Agric., Zagazig Univ., Egypt, pp. 70–177.
- Mohamed, S.E., 2005. Photochemical studies on common bean (*Phaseolus vulgaris*, L.) plants as affected by foliar fertilizer and active dry yeast under sandy soil conditions. *Egypt. J. Appl. Sci.* 20 (5b), 539–559.
- Mohammadi, M.M., Maleki, A., Siaddat, S.A., Beigzade, M., 2013. The effect of zinc and potassium on the quality yield of wheat under drought stress conditions. *Int. J. Agric. Crop Sci.* 6 (16), 1164–1170.
- Mujtaba, S.M., Ali, M., Ashraf, M.Y., Khanzada, B., Farhan, S.M., Shirazi, M.U., Khan, M.A., Shereen, A., Mumtaz, S., 2007. Physiological responses of wheat (*Triticum aestivum* L.) genotypes under water stress conditions at seedling stage. *Pak. J. Bot.* 39 (7), 2575–2579.
- Nagodawithana, W.T., 1991. Yeast Technology. Universal foods corporation Milwaukee, Wisconsin. Van Nostrand Reinhold, New York, 273 p.
- Namich, Alia.A.M., 2007. Response of cotton cultivar Giza 80 to application of glycine betaine under drought conditions. *Minufiya J. Agric. Res.* 32 (6), 1637–1651.
- Osborne, S.L., Schepers, V., Francis, D.D., Schlemmer, M.R., 2002. Use of spectral radiance to estimate in-season biomass and grain yield in nitrogen and water-stressed crop. *Crop Sci.* 42, 165–171.
- Pedersen, A.L., Feldner, H.C., Rosendahl, L., 1996. Effect of proline on nitrogenase activity in symbiosomes from root nodules of soybean (*Glycine max* L.) subjected to drought stress. *J. Exp. Bot.* 47 (303), 1533–1539.
- Ranney, T.G., Bassuk, N.L., Whilow, T.H., 1991. Osmotic adjustment and solute constituents in leaves and roots of water-stressed cherry prunus tress. *J. Am. Soc. Hort. Sci.* 116 (4), 648–688.
- Rosen, H., 1957. A modified field ninhydrin colourimetric analysis for acid nitrogen. *Arch. Biochem. Biophys.* 67, 10–15.
- Sadasivam, S., Manickam, A., 1992. Biochemical Methods for Agricultural Sciences. Wiley Eastern Limited, New Delhi, India, 246 p.
- Singh, B.K., 1999. Plant Amino Acids: Biochemistry and Biotechnology. Marcel Dekker, INC., New York, USA, pp. 319–356.
- Snedecor, G.W., Cochran, W.G., 1980. Statistical Methods, seventh ed. The Iowa State Univ. Press, Ames. Iowa, USA, pp. 1–507.
- Snell, F.D., Snell, C.T., 1953. Colorimetric Method of Analysis, Including Some Turbidimetric and Nephelometric Methods, third ed. D. Van Nostrand Company Inc., New York USA, pp. 225–233.
- Snell, R., Snell, G., 1954. Colorimetric Method of Analysis. D. Van Nostrand Company, New York, USA, pp. 3–29.
- Wanas, A.L., 2002. Response of faba bean (*Vicia faba*, L) plants to seed soaking application with natural yeast and carrot extracts. *Annals Agric. Sci., Moshtohor* 40 (1), 83–102.
- Waraich, E.A., Ahmad, R., 2010. Physiological responses to water stress and nitrogen management in wheat (*Triticum aestivum* L.): evaluation of gas exchange, water relations and water use efficiency. Fourteenth International Water Technology Conference (IWTC 14), Cairo, Egypt, pp. 46–51.
- Wettstein, D.V., 1957. Chlorophyll Totale und der Submikroskopische Form- wechsel der Plastiden. *Exp. Cell. Res.* 12, 427–433.
- Yeo, E., HawkBin, K., SangEun, H., JoonTak, L., JinChang, V., MyungOk, B., Yeo, E.T., Kwon, H.B., Han, S.E., Lee, J.T., Ryu, J.C., Byun, M.O., 2000. Genetic engineering of drought resistant potato plants by introduction of the trehalose-6-phosphate synthase (TPSI) gene from *Saccharomyces cerevisiae*. *Mol. Cells* 10 (3), 263–268.
- Zaki, Nabila.M., Hassanein, M.S., Gamal El-Din, K.M., 2007. Growth and yield of some wheat cultivars irrigated with saline water in newly cultivated land as affected by biofertilization. *J. Appl. Sci. Res.* 3 (10), 1121–1126.
- Zhang, J., Dell, B., Conocono, E., Waters, I., Setter, T., Appels, R., 2009. Water deficits in wheat: fructan exohydrolase (1-FEH) mRNA expression and relationship to soluble carbohydrate concentrations in two varieties. *New Phytol.* 181, 843–850.