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ORIGINAL ARTICLE

Glycine betaine and salicylic acid induced modification in productivity of two different cultivars of wheat grown under water stress

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A pot experiment was conducted to evaluate the beneficial effect of foliar application of glycine betaine (10mM), grain presoaking in salicylic acid (0.05 M) and their interaction on drought tolerance of two wheat (*Triticum aestivum L.*) cultivars (sensitive, Sakha 94 and resistant, Sakha 93). Water stress decreased wheat yield components (spike length, number of spikelets / main spike, 100 kernel weight, grain number / spike, grain yield / spike, grain yield / plant, straw yield / plant, crop yield / plant, harvest, mobilization and crop indices) and the biochemical aspects of grains(grain biomass, carbohydrates, total protein, total phosphorus, ions content and amino acids) in both wheat cultivars. The applied chemicals appeared to alleviate the negative effects of water stress on wheat productivity (particularly the sensitive one) and the biochemical aspects of yielded grains. The effect was more pronounced with GB+SA treatment. This improvement would result from the repairing effect of the provided chemicals on growth and metabolism of wheat plants grown under water deficit condition. In response to the applied water stress and the used chemicals, the grain yield of the sensitive and resistant wheat cultivars was strongly correlated with all the estimated yield components (shoot length, spike length, plant height, main spike weight, number of spikelets per main spike, 100 kernel weight, grain number per spike, grain weight per plant, straw weight per plant, crop yield per plant, harvest, mobilization and crop indices).

Key words: (Triticum aestivum L.)/drought/ glycine betaine/ salicylic acid/ yield/ amino acids

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Key words: (Triticum aestivum L.)/drought/ glycine betaine/ salicylic acid/ yield/ amino acids

List of abbreviations: Glycine betaine = GB, Salicylic acid = SA, Water stress = WS

Food productivity is decreasing due to detrimental effects of various biotic and abiotic stresses; therefore minimizing these losses is a major area of concern to ensure food security under changing climate. Environmental abiotic stresses, such as drought, extreme temperature, cold, heavy metals, or high salinity, severely impair plant growth and productivity worldwide (Anjum *et al*., 2011). Drought, being the most important environmental stress, severely impairs plant growth and development, limits plant production and the performance of crop plants, more than any other environmental factor (Shao *et al*., 2008; 2009).

Plant experiences drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high. Available water resources for successful crop production have been decreasing in recent years. Furthermore, in view of various climatic change models scientists suggested that in many regions of world, crop losses due to increasing water shortage will further aggravate its impacts (Anjum *et al*., 2011).

Deficit irrigation provides a means of reducing water consumption while minimizing adverse effects on yield (Zhang *et al*., 2004). Furthermore, they found that severe soil water deficit (SWD) decreased grain yield of winter wheat, while slight SWD in the growth stage from spring green up to grain-filling did not reduce grain yield or water use efficiency. Liang *et al*. (2002) demonstrated that a drying-rewatering alteration has a significant compensatory effect that can reduce transpiration and keep wheat growing.

Glycine betaine (GB) is an amino acid derivative which is naturally synthesized in several plant species. However, many important crop species, like potato or tomato are unable to accumulate glycinebetaine. Synthesis of glycinebetaine is promoted by salt and drought stress as it functions as a compatible solute regulating the intracellular osmotic balance (Abou El -Yazied, 2011). In addition, the positive effects of foliar spray of GB on yield and yield component in plants grown under water limited environment been reported in different crops such as rice (Rahman *et al*., 2002), sunflower (Iqbal *et al*., 2008) and common bean (Abou El- Yazied, 2011). There are however, some contrasting reports indicating no effect of supplied GB on yield of cotton (Meek *et al*., 2003).

Salicylic acid (SA) has been reported to cause a multitude of effects on the morphology and physiology of plants (Maghsoudia and Arvinb, 2010) and to induce a protective mechanism enhancing resistance to biotic and abiotic stresses (Zahra *et al*., 2010; Szepesi *et al*., 2011). Zaki, and Radwan (2011) observed greater wheat grain yield and higher quality under salinity conditions when treated with SA. Furthermore, Exogenous application of glycine betaine (GB) and salicylic acid (SA) has been found very effective in reducing the adverse effects of drought stress on sunflower plants (Hussain *et al*., 2010).

The present experiment was designed to evaluate the possible role of GB, SA or their interaction in modifying drought stress imposed by drought on yield attributes and grain biochemical aspects of two droughty wheat cultivars.

MATERIALS AND METHODS

Two wheat cultivars (*Triticum aestivum* L.) Sakha 94 (sensitive var.) and Sakha 93 (resistant var.), were used in this study. The variety Sakha 93 is known to be more drought resistant than Sakha 94.

A homogenous lot of wheat grains (i.e. either sensitive or resistant var.) were separately surface sterilized by soaking in 0.01 % $HgCl_2$ for 3 minutes, followed by thoroughly rinsing in sterile water. The sterilized grains from each variety were divided into two sets (≈ 500 g per set for each var.). Grains of the 1st and 2nd sets were separately soaked in distilled water or salicylic acid (0.05 M), respectively. In 20 November 2005, grains of each set were planted in plastic pots (fifteen grains per pot; 25cm width X 30cm height) filled with 6 kg mixture of soil (clay and sand = 2:1, v/v). The pots were kept in a greenhouse, and the plants were subjected to natural day/ night conditions (minimum /maximum air temperature and relative humidity were; 29.2/33.2 ϵC and 63/68 %, respectively). Irrigation to field capacity was carried out when soil water content had fallen to 60% of its initial value. Twenty days after planting, the plants were thinned to five uniform seedlings per pot.

On the day 65 (at the beginning of heading) after planting the pots of the 1st set was allocated to four groups (20 pots per each group) as follow: control (cont.), water stress (WS), glycine betaine control (GB.), glycine betaine + water stress (GB + WS). The 2nd set group was allocated as follow: salicylic acid control (SA), salicylic acid + water stress (SA+WS), control glycine betaine + salicylic acid (GB + SA) and glycine betaine + salicylic acid + water stress (GB+SA+WS). For glycine betaine (10 mM) treatment, the plants were sprayed by glycine betaine 48 hrs before starting the stress period and weekly during the stress period.

Water deficit was imposed by withholding water at the reproductive stage for 30 days within two periods: on the day 65 from planting (heading stage) and the day 80 from planting (anthesis stage). Each drought pot received 500 ml water at the end of 1st stress period. At the end of stress periods, re-watering to the field capacity was carried out. The un-drought (control) plants were irrigated to the field capacity during the stress period, and all plants were left to grow until grain maturation under normal irrigation with tap water.

At the bud stage, 20 days from planting, (i.e., tillering stage) and before heading (i.e., at ear emergence) the plants received 35 kg N ha^{-1} as urea and 35 kg P ha^{-1} as potassium dihydrogen phosphate as fertilizers.

Monitoring the water status of the soil

Soil water content (SWC) at the end of the stress period was estimated by the destructive method as recommended by Ritchie *et al*. (1990).

Determination of carbohydrates

Total soluble sugars and sucrose were extracted and determined using modifications of the procedures of Riazi et al. (1985). Glucose contents were estimated using the 0-toluidine procedure of Feteris (1965) as modified by Riazi *et al*. (1985). Polysaccharides were determined by the method of Thayennanavan and Sadasivam (1984).

Determination of protein

The protein content of fresh plant materials was determined colorimetrically as described by Lowry *et al*. (1951).

Determination of amino acids

All free amino acids were extracted with ethanol and then hydrolyzed with 6 N HCl for 24 hrs according to the Sempio and Raggi (1966). The extracted amino acids were then measured using a LKB alpha high performance amino acid analyzer (LKB Biochrom. LKD England). Retention time and area were determined using Hewlett Packard 3390 recording integrator. Concentration of each amino acid GM/16 GM nitrogen was calculated by special designed program.

Determination of some mineral ions

The extracts of the experimental plants were analyzed for the cations: Na^{+}, Ca^{+2} and Mg^{+2} measured by flam emission spectrophotometery (Williams and Twine 1960) whereas anions Cl^{-} chlorides were determined by the $AgNO_3$ titration method as described by Hansen and Munns (1988).

Determination of phosphorus

The procedure adopted for extraction of the different phosphorus compounds were essentially those described by Barker and Mapson (1964) and determined by method described by Humphries (1956).

Statistical analysis

The main effect of factors (watering regime, used chemicals, growth stages and wheat cultivar) and interaction (watering regime, both used chemicals, growth stages and wheat type) were evaluated by general linear model (two way ANOVA) using SPSS program. Tests for significant differences between means at P =0.05 were given by LSD test. The correlation coefficient between the economic yield and all evaluated criteria was also evaluated.

RESULTS

Changes in yield and yield components

The results in table 1 indicated that water stress decreased significantly (P<0.05) all yield components (shoot length, spike length, number of spikelets / main spike, 100 kernel weight, grain number / spike, grain yield / spike, grain yield / plant, straw yield / plant, crop yield / plant, harvest, mobilization and crop indices) of the two wheat cultivars. With regard to the wheat cultivar, the sensitive one was more affected by water stress than the resistant. Foliar application of GB, SA or their interaction appeared to alleviate the stress imposed by drought on all yield components of the two wheat cultivars.

Treatment with GB+SA induced additional increases (P<0.05) in shoot length, spike length, plant height and grain yield per plant of stressed wheat plants for the two cultivars. On the other hand, it caused additional increases in grain number per main spike, harvest index and mobilization index of the sensitive plants and number of spikelets per main spike of the resistant ones.

Changes in grain biomass

The data represented in table 2 indicated that, the resistant wheat cultivar had higher grain fresh and dry masses than the sensitive ones. Water stress markedly decreased (P<0.05) the grain fresh and dry masses of the two wheat cultivars. On the other hand, GB, SA or their interaction appeared to improve the grain fresh and dry masses of the two wheat cultivars. Treatments with GB+SA caused additional increases (P<0.05) in the grain fresh and dry masses of the two wheat cultivars.

CHANGES IN BIOCHEMICAL ASPECTS OF YIELDED GRAINS

Changes in carbohydrates content

Changes in soluble sugars

As compared to control values, the results indicated that, the sensitive plants accumulated more soluble sugars (glucose, sucrose and total soluble sugars) than the resistant ones (Table 1). On the other hand, water stress caused noticeable increases (P<0.05) in soluble sugars (glucose, sucrose and total soluble sugars) in the developed grains of both two wheat cultivars. Moreover, the applied chemicals induced additional increases (P< 0.05) in soluble sugars (glucose, sucrose and total soluble sugars) in the developed grains of both two wheat cultivars. This effect was more pronounced with GB+SA treatments.

Changes in polysaccharides and total carbohydrates

In relation to wheat cultivar, the developed grains of resistant plants had higher polysaccharides and total carbohydrates content than those of the sensitive ones (Table 2). Water stress led to marked decrease (P<0.05) in polysaccharides and total carbohydrates content in the developed grains of the two wheat cultivars as compared to control values.

In general, application of glycine betaine, salicylic acid or their interaction induced marked increases (P<0.05) in polysaccharides and total carbohydrates content in the developed grains of the two wheat cultivars under stressed and controlled conditions. The magnitude of increases was more pronounced with GB+SA treatment.

Table 1. Effect of glycine betaine, salicylic acid and their interaction on yield and yield components of drought wheat cultivars.

Wheat variety	Parameters / Treatments	Shoot length (cm)	Spike length (cm)	Plant height (cm)	Main spike weight (g)	Number of spikelets / main spike	100-kernel weight (g)	Grain No./ main spike	Grain yield/ plant	Straw yield/ plant	Crop yield/ plant (g)	Harvest index	Mobilization index	Crop index
Sensitive	Cont	67.49	15.17	82.66	3.07	17.01	4.59	53.17	3.63	4.24	7.87	0.85	1.88	4.11
	WS	60.34	14.38	74.72	2.73	15.67	3.80	47.17	1.99	3.08	5.07	0.62	1.66	2.71
	GB	68.85	15.52	84.37	3.17	17.33	4.65	54.33	3.92	4.22	8.14	0.96	1.92	4.19
	GB+WS	65.76	15.15	80.91	2.93	16.67	4.32	51.17	3.64	3.89	7.53	0.93	1.89	3.96
	SA	69.45	15.33	84.78	3.22	17.33	4.67	54.83	3.96	4.39	8.35	0.95	1.96	4.22
	SA+WS	66.76	15.02	81.78	3.03	16.33	4.47	53.50	3.74	3.92	7.66	0.95	1.86	4.02
	GB+SA	70.46	15.58	86.05	3.32	17.67	4.92	57.17	4.19	4.42	8.62	0.99	1.96	4.38
	GB+SA+WS	69.93	15.27	85.20	3.08	16.67	4.52	55.34	3.85	4.08	7.93	0.96	1.90	4.07
	LSD 0.05	1.25	0.10	1.52	0.09	0.32	0.08	1.29	0.05	0.14	0.20	0.05	0.04	0.11
Resistant	Cont.	62.43	16.00	78.43	3.48	17.33	4.72	58.67	4.06	4.35	8.42	0.98	1.94	4.20
	WS	57.87	14.83	72.70	3.03	16.33	4.40	53.33	3.41	3.54	6.94	0.90	1.87	3.66
	GB	63.65	16.25	79.90	3.58	17.67	4.81	59.83	4.24	4.83	9.07	1.06	1.94	4.23
	GB+ WS	60.87	15.83	76.70	3.35	17.00	4.65	57.50	4.01	4.14	8.15	0.97	1.92	4.14
	SA	63.97	16.67	80.64	3.60	17.67	4.84	60.33	4.15	4.95	9.09	1.08	1.95	4.31
	SA+ WS	61.76	16.25	78.01	3.39	17.33	4.73	58.17	4.01	4.33	8.34	1.01	1.93	4.22
	GB + SA	66.97	16.85	83.82	3.60	18.00	5.23	61.83	4.52	5.22	9.75	1.12	2.07	4.39
	GB +SA+WS	64.13	16.33	80.46	3.39	17.67	4.78	58.33	4.18	4.39	8.49	1.00	1.95	4.24
	LSD 0.05	1.14	0.32	1.29	0.10	0.09	0.11	1.50	0.09	0.40	0.41	0.05	0.04	0.133

Changes in phosphorus content

As compared to control values, water stress caused noticeable increase (P< 0.05) in the phosphorus content (inorganic, organic and total phosphorus) in the developed grains of the two wheat cultivars. On the other hand, the applied chemicals (GB, SA or their interaction) caused significant and additional increases (P<0.05) in the phosphorus content in the developed grains of the two wheat cultivars (Table 2).

Table 2. Effect of glycine betaine, salicylic acid and their interaction on some biochemical aspects of yielded grains of drought wheat cultivars.

Wheat Variety	Treatment \ Parameters	Grain biomass (mg grain^{-1})		Total protein (mg g^{-1} d wt)	Carbohydrates content (mg g^{-1} d wt)					Phosphorus content (mg g^{-1} d wt)			Ionic content (mmol g^{-1} d wt)			
		Grain fresh mass	Grain dry mass		Glucose	Sucrose	Total soluble sugar	Polysac-charides	Total carbohy-drates	Inorganic phosph.	Organic phosph.	Total phosph.	Ca^{+2}	Mg^{+2}	Cl^-	Na^{+1}
Sensitive	Cont	53.2	47.00	98.9	2.77	13.81	18.61	724.7	741.1	0.05	0.45	0.50	1.54	11.2	1.25	1.80
	WS	44.2	40.30	75.6	3.48	18.24	25.76	530.2	552.0	0.07	0.48	0.55	1.79	9.1	0.50	2.10
	GB	55.0	50.50	120.5	2.94	14.40	20.17	733.4	750.1	0.06	0.50	0.56	1.88	11.3	1.50	1.90
	GB+WS	53.8	47.30	100.5	3.95	20.93	27.12	676.7	700.6	0.10	0.61	0.71	1.85	10.4	1.00	2.20
	SA	55.9	50.10	117.1	3.02	14.77	20.93	752.5	769.9	0.07	0.51	0.59	1.72	11.9	1.75	1.95
	SA+WS	50.7	47.80	101.6	3.91	22.13	29.81	686.2	711.6	0.11	0.66	0.77	1.89	10.2	1.25	2.30
	GB+SA	59.2	54.40	128.7	3.15	14.99	21.37	759.6	777.7	0.08	0.59	0.67	1.75	12.1	1.85	1.98
	GB+SA+WS	56.5	52.70	105.3	4.49	22.53	31.93	702.1	729.5	0.12	0.68	0.80	1.96	10.5	1.25	2.40
	LSD 0.05	3.20	2.25	2.73	0.35	3.20	1.70	7.60	9.60	0.01	0.02	0.01	0.35	1.20	0.35	0.17
Resistant	Cont.	53.5	47.7	102.3	2.40	11.31	16.40	750.3	768.9	0.07	0.45	0.52	1.53	11.3	1.30	1.90
	WS	45.6	41.6	94.0	2.94	15.52	21.83	645.1	670.9	0.11	0.47	0.58	1.78	10.0	0.75	2.10
	GB	56.0	51.7	128.4	2.58	11.76	16.70	769.2	789.3	0.12	0.47	0.59	1.69	11.4	1.75	1.98
	GB+ WS	53.9	50.3	108.9	3.17	17.49	23.88	716.5	743.7	0.15	0.58	0.74	1.95	10.5	0.95	2.25
	SA	58.3	53.1	125.9	2.77	12.48	17.43	763.5	784.4	0.13	0.49	0.63	1.68	11.7	1.90	1.97
	SA+ WS	56.5	51.3	106.3	3.27	18.13	25.44	704.5	734.3	0.17	0.62	0.79	1.90	10.7	1.00	2.24
	GB + SA	64.6	60.9	135.5	2.86	12.76	18.15	783.6	805.0	0.17	0.53	0.69	1.76	12.3	1.95	1.99
	GB +SA+WS	61.7	58.3	114.0	3.30	19.86	27.40	641.4	673.3	0.27	0.57	0.85	2.14	11.0	1.00	2.26
	LSD 0.05	3.00	3.00	3.64	0.22	2.80	1.50	11.81	13.02	0.01	0.02	0.02	0.16	0.91	0.36	0.31

Changes in ionic content

It appeared from Table 2 that, water stress significantly increased (P< 0.05) calcium and sodium content but decreased the magnesium and chloride content in the developed grains of the two wheat cultivars. In general, application of GB, SA or interaction seemed to induce additional increase (P< 0.05) in ionic content of the developed grains.

Changes in total protein content

The data in Table 2 showed that the resistant plants had more protein content than the sensitive ones. In relation to control values, withholding water induced a massive decrease (P<0.05) in the protein content of the two wheat cultivars. In the majority of cases, treatments with GB, SA or their interaction caused marked and additional increases (P< 0.05) in the protein content in grains of both stressed and non-stressed plants.

Table 3. Effect of glycine betaine, salicylic acid and their interaction on amino acids content (mg/100g f wt) in the developed grains of sensitive wheat cultivar.

Treatments / Parameters	Cont	WS	GB	GB+WS	SA	SA+WS	GB+ SA	GB +SA+WS	LSD 0.05
Glutamic	8.25	8.95	8.62	10.17	8.43	9.54	8.87	10.66	0.32
Aspartic	6.12	7.71	6.94	8.51	6.47	8.24	6.66	8.75	1.32
Leucine	5.11	5.64	5.46	6.70	5.31	6.32	5.44	6.57	0.35
Tyrosine	3.26	5.15	4.88	6.44	4.23	5.83	4.65	6.21	1.23
Alanine	2.64	3.32	3.17	5.11	3.01	4.26	3.72	6.25	1.12
Isoleucine	2.55	3.15	2.95	4.01	2.53	3.42	2.81	3.73	0.63
Threonine	2.12	3.13	3.65	4.21	2.35	3.86	3.41	4.15	0.75
Serine	2.02	2.77	2.56	3.72	2.11	3.05	2.48	3.25	1.06
Proline	1.85	2.69	2.13	3.19	1.96	2.54	2.25	3.38	0.68
Arginine	1.36	2.06	1.78	2.65	1.53	2.14	1.96	2.72	0.58
Valine	1.12	2.03	2.15	2.41	1.65	1.87	1.77	2.23	0.45
Glycine	0.79	1.27	1.11	1.45	1.05	1.34	1.25	1.88	0.47
Histidine	0.72	0.97	0.83	1.05	0.76	1.04	0.86	1.12	0.17
Methionine	0.65	0.73	0.69	0.86	0.67	0.77	0.71	0.89	0.06
Pheynl alanine	0.63	0.89	0.76	1.16	0.68	0.92	0.72	1.11	0.19
Cysteine	0.53	0.62	0.57	0.78	0.55	0.66	0.55	0.73	0.04
Iysine	0.47	0.68	0.52	0.76	0.49	0.72	0.65	0.95	0.13
Total FAA	38.56	49.58	46.92	60.48	42.07	54.23	46.84	61.79	9.19
Ammonia	1.26	1.58	1.44	1.76	1.35	1.64	1.51	1.82	0.22

Changes in amino acids content

Seventeen amino acids were detected in harvested grains of the two wheat cultivars. These amino acids, together with ammonia, are presented in Tables 3 & 4. It is clear, that glutamic acid, aspartic acid, leucine, tyrosine, alanine, isoleucine, serine, threonine and proline occurred in higher amounts in the grains of control and treated plants. Furthermore, Glutamic acid appeared to be the dominating amino acid in yielded grains of control and treated plants of both wheat cultivars. As compared to control values, water stress caused remarkable increases ($P < 0.05$) in all detected amino acids. In addition, application of GB, SA or their interaction induced additional increases ($P<0.05$) in the detected amino acids content in harvested grains of the two wheat cultivars. In the majority of cases, this effect was more pronounced with plants treated with GB alone than those treated with SA or GB+SA of both wheat cultivars.

Table 4. Effect of glycine betaine, salicylic acid and their interaction on amino acids content (mg/100g f wt) in the developed grains of resistant wheat cultivar.

Treatments / Parameters	Cont	WS	GB	GB+ WS	SA	SA+WS	GB+SA	GB + SA+WS	LSD 0.05
Glutamic	7.16	8.46	7.54	9.16	7.38	8.75	7.78	9.48	0.84
Aspartic	5.32	6.54	5.95	7.38	5.44	6.85	6.03	8.14	1.05
Leucine	4.31	5.43	5.02	6.51	4.67	6.13	4.89	6.37	0.83
Tyrosine	2.43	3.55	2.73	4.99	2.56	3.95	2.7	4.54	1.32
Alanine	2.13	2.78	2.66	3.46	2.35	2.98	3.45	4.28	0.35
Isoleucine	2.04	2.67	2.84	3.59	2.33	2.92	2.63	3.24	0.74
Threonine	1.67	2.07	2.32	3.49	1.85	2.85	2.12	3.27	1.24
Proline	1.58	2.24	1.75	3.03	1.64	2.73	1.89	3.45	0.58
Serine	1.45	2.03	1.63	2.72	1.53	2.21	1.61	2.53	0.42
Valine	0.86	1.47	1.19	1.86	0.93	1.62	1.11	1.68	0.46
Glycine	0.66	0.98	0.87	1.23	0.72	1.11	0.95	1.39	0.21
Histidine	0.68	0.85	0.77	0.96	0.71	0.89	0.82	1.03	0.14
Arginine	0.62	0.89	0.72	1.39	0.68	1.14	0.78	1.57	1.05
Iysine	0.52	0.76	0.63	0.89	0.59	0.84	0.69	0.98	0.18
Pheynl alanine	0.47	0.68	0.54	0.87	0.49	0.75	0.52	0.84	0.12
Methionine	0.45	0.49	0.47	0.53	0.46	0.51	0.47	0.55	0.03
Cysteine	0.37	0.46	0.42	0.57	0.38	0.48	0.41	0.56	0.06
Total FAA	32.36	41.9	37.64	52.07	34.35	46.23	38.45	53.36	9.57
Ammonia	0.92	1.25	1.08	1.55	0.97	1.46	1.11	1.68	0.17

In response to the applied water stress and the used chemicals, the grain yield was strongly correlated with all the estimated yield criteria (shoot length, spike length, plant height, main spike weight, number of spikelets per main spike, 100 kernel weight, grain number per spike, grain weight per plant, straw weight per plant, crop yield per plant, harvest, mobilization and crop indices) (r = 0.85-0.99) and (r = 0.87-0.97) for the sensitive and resistant wheat cultivars respectively (Table 5).

Table 5. Correlation coefficients (r) between grain yield and the yield components of sensitive and resistant cultivars.

Sensitive Cultivar		**Resistant Cultivars**	
Variables	r	Variables	r
Crop index	0.99	Crop index	0.95
Crop yield	0.99	Crop yield	0.97
Grain No. /main spike	0.93	Grain No. /main spike	0.97
Harvest index	0.98	Harvest index	0.90
100 kernel weight	0.94	100 kernel weight	0.93
Mobilization index	0.98	Mobilization index	0.87
No.of spikeletes/main spike	0.85	No. of spikeletes/ main spike	0.96
Plant height	0.96	Plant height	0.97
Shoot length	0.95	Shoot length	0.96
Spike length	0.95	Spike length	0.94
Main spike weight	0.89	Main spike weight	0.91
Straw weight	0.95	Straw weight	0.93

DISCUSSION

Yield is a result of the integration of metabolic reactions in plants; consequently any factor that influences this metabolic activity at any period of plant growth can affect the yield (Ibrahim and Aldesuquy, 2003). In this investigation, Yield and yield attributes (shoot length, spike length, plant height, main spike weight, number of spikelets per main spike, 100 kernel weight, grain number per spike, grain weight per plant, straw weight per plant, crop yield per plant, harvest, mobilization and crop indices) are reduced due to water stress in both wheat cultivars. The reduction in yield of stressed wheat plants can be attributed to the decrease in photosynthetic pigments, carbohydrates accumulation (polysaccharides) and nitrogenous compounds (total nitrogen and protein). The decrease in yield and yield components in different crops under similar conditions has also been reported by many workers (Arfan *et al*., 2007; Sankar *et al*., 2008). These workers clearly indicated that drought tolerant genotypes showed less reduction in yield plants in respect of susceptible ones. Therefore, maintenance of better yield of the wheat cultivar, Sakha 93 than that of Sakha 94 under water deficit.

Drought stress during the early stage of reproductive growth tends to reduce yield by reducing seed number. During seed development stress reduces yield by reducing seed size. Prolonged moisture stress during reproductive growth can severely reduce yield because of reduced seed number and seed size (Dombos *et al*., 1989). Furthermore, water stress was showed to reduce the head diameter, 100-achene weight and yield per plant in sun flower (Shao *et al*., 2008). These authors also observed significant but negative correlation of head diameter with fresh root and shoot weight under water stress. A positive and significant relation was recorded between dry shoot weight and achene yield per plant. The yield components, like grain yield, grain number, grain size, and floret number, are decreased under pre-anthesis drought stress treatment in sunflower (Shao *et al*., 2008).

Zhang et al. (1998) have reported that in field-grown wheat soil drying during the grain filling period could enhance early senescence. They found that while the grain filling period was shortened by 10 days (from 41 to 31 days) in un-watered (during this period) plots, a faster rate of grain-filling and enhanced mobilization of stored carbohydrate minimized the effect on yield. They showed that the early senescence induced by water deficit does not necessarily reduce grain yield, even when plants are grown under normal nitrogen conditions. The gain from accelerated grain-filling rate and improved translocation outweighed the possible loss of photosynthesis as a result of shortened grain filling period when subjected to water stress during grain filling.

Water stress reduced harvest, mobilization and crop indices in the two wheat cultivars. This was in agreement with Jaleel *et al*. (2008) who reported that, water stress decreased harvest index, and biomass yield in two varieties of *Catharanthus roseus* (L.). However, in crops, the detrimental effects of water deficits on the harvest index (HI) also minimize the impact of the water limitation on crop productivity and increase the efficiency of water use (Chaitanya *et al*., 2003). Therefore, increasing transpiration, transpiration efficiency and harvest index are three important avenues for the important of agricultural productivity (Sankar *et al*., 2008). Additionally, the aerial environment plays a role in determining the ratio of carbon gain to water use, because the vapor pressure deficits between the leaf and the air determine the transpiration rate (Zhu *et al*., 2002).

The results clearly indicated that application of GB (10 mM) was significant in alleviating the adverse effects of water deficit on yield and yield components of both wheat cultivars. These results are in conformity with those obtained by Ibrahim and Aldesuquy (2003) with sorghum plant under drought stress. This improvement in yield and yield components due to GB application would result from the beneficial effect of GB on growth and metabolism and its role as osmoprotectant. However, there are some contrasting reports indicating no effect of supplied GB on yield of cotton (Meek *et al*., 2003). In this respect, Iqbal *et al*. (2005) recorded that, exogenous supply of the GB (foliar spray) showed effective role in ameliorating the effects of water stress on turgor potential and yield of two sunflower lines. The effect of GB application was more pronounced when it was applied at the time of initiation of water deficit at the vegetative or reproductive growth stages.

The positive effects of foliar spray of GB on 100- kernel weight of wheat cultivars grown under water stress was in accord with the results observed by some investigators in different crops [wheat (Diaz-Zarita *et al*., 2001) and sunflower (Iqbal *et al*., 2008)].

The application of salicylic acid (0.05 M) enhanced the yield and yield components of the two wheat cultivars. In this respect, Arfan *et al*. (2007) studied the effect of exogenous application of salicylic acid (SA) through the rooting medium of two wheat cultivars differing in salinity tolerance. They found that increase in grain yield along with increase in 100-grain weight, number of grains and number of spikelets per spike with 0.25mM SA application under saline conditions suggested that improvement in salt-induced reduction in grain yield with SA application was mainly due to increase in grain size and number.

Furthermore, the beneficial effect of SA on grain yield may be due to translocation of more photo-assimilates to grains during grain filling, thereby increasing grain weight. These results are similar to those of Zhou *et al*. (1999) who reported that maize stem injected with SA produced 9% more grain weight than those with sucrose and distilled water treatments. The second possible mechanism of SA-induced yield enhancement might be an increase in the number of spikelets and number of grains, because SA has the capacity to both directly or indirectly regulate yield. These results are in a good agreement with those obtained by Khan *et al*. (2003) with maize and soybean.

Generally, the grain fresh and dry masses, polysaccharides, total carbohydrates and total protein are decreased in response to water stress in both two wheat cultivars. The results showed that, water withholding occurred during grain filling particularly the 2nd stress period (at anthesis) might cause the following events: 1- led to an increase in ABA levels in flag leaves which in turn induced stomatal closure and consequently decreased photosynthetic activity in flag leaves (the main source of photo-assimilates towards developing grains). This effect may result in a decrease in the grain biomass, 2- water stress decreased the leaf area by inducing leaf rolling particularly in susceptible cultivar and this may decrease the dry matter production that translocate towards developing grains, 3- water stress may stimulate the early senescence in wheat leaves particularly in susceptible cultivar which also affected the translocation of the photo-assimilates from leaves (particularly flag leaf) which represents the main export source towards the main import sink (developing grain). Bearing in mind the conclusion of Egeli *et al*. (1985) that the accumulation of dry matter by grains requires the production of assimilates in the leaves, their translocation to the fruit, movement into the storage organs of seed, and the synthesis of materials to be stored.

The above-mentioned results are in accord with those obtained by Sankar *et al*. (2007). In addition, Savin and Nicolas (1996) investigated the effects of drought stress on grain growth, starch and nitrogen accumulation in barley cultivars. Water deficits decreased both individual grain weight and grain yield. Nitrogen content per grain was quite high and similar for all treatments, and nitrogen percentage increased when stress was severe enough to reduce starch accumulation. This confirms that starch accumulation is more sensitive to post-anthesis stress than nitrogen accumulation.

Application of GB, SA or their interaction appeared to mitigate the deleterious effects of water stress on grain biomass of the two wheat cultivars. The repairing effect of SA may be attributed to the fact that SA reduces the rate of transpiration from leaves (Larque-Saavedra, 1979), which could possibly lead to the accumulation of excessive water, thus resulting consequently in an increase in grain fresh mass (Abo-Hamed *et al*., 1990). Furthermore, GB application may act in the same manner as SA in inducing drastic reduction in the rate of transpiration. The results obtained from diurnal changes in transpiration rate make this postulation decisive (unpublished data).

The results indicated that, soluble sugars are accumulated in response to water stress in both wheat cultivars. On the other hand, water stress induced massive decrease in polysaccharides content in yielded grains of both wheat cultivars. This may probably due to the fact that water stress stimulates the degradation of polysaccharides and at the same time increases the dark respiration during which a part of soluble sugars was consumed as a respiratory substrate. The other part of soluble sugars may explain the massive increase in total soluble sugars occurred within the developing grains as a result of water stress. From another point of view, water stress decreased the pigments concentration in wheat leaves (unpublished data) which results in inhibition of photosynthetic activity, in turn it leads to less accumulation of carbohydrates in mature leaves and consequently may decrease the rate of transport of carbohydrates from leaves to the developing grains, where there is a good relationship between source (leaves) and sink (grain) in cereal plants. Furthermore, the noticed decrease in polysaccharides of wheat grains as a result of water stress could be explained on the fact that, water stress impaired the utilization of carbohydrates during the vegetative growth and reduced the area of conductive canals (mainly phloem and xylem), so reduction in the translocation of the assimilates toward the developed grains might have occurred. In accord with these results, several physiological studies suggested that under stress conditions nonstructural carbohydrates (sucrose, hexoses, and sugar alcohols) accumulate although to varying degree in different plant species. A strong correlation between sugar accumulations and osmotic stress tolerance has been widely reported, including transgenic experiments (Taji *et al*., 2002). In addition Singh *et al*. (2008) reported that the effect of water stress (WS) at 8 and 15 days post anthesis (DPA) on the characteristics of starch and protein separated from five wheat varieties. The starch from wheat exposed to WS at 15 DPA showed lower amylose content, lipids content and pasting temperature, and higher peak viscosity, final viscosity and setback.

Phosphorus content (organic, inorganic and total) in wheat grains increased due to water stress application. Several studies have investigated the relationship between phosphorus status and photosynthetic metabolism (Rao and Terry, 1994) but few have evaluated the effects of water deficit in this relationship (Dos Santos *et al*., 2006). El-Tayeb (2005) recorded that phosphorus increased in barley plants due to salinity. In addition, application of GB, SA or their interaction caused additional accumulation in phosphorus content in both wheat cultivars yielded grains.

Water stress stimulates the accumulation of both calcium and sodium content but decreased the magnesium and chloride content in the yielded grains of the two wheat cultivars. This increase in calcium and sodium levels may result from transportation of these elements from root to shoot through the transpiration stream to the developing grains. In addition, application of GB, SA or interaction seemed to induce additional increase in ionic content (calcium, sodium, magnesium and chloride) of the developed grains.

Under water stress, protein content of the developed grains was significantly decreased in both wheat cultivars. The decrease in protein contents in yielded grains was more pronounced in the sensitive cultivar than the resistant ones under drought, this may probably be due to less transport of protein from source (flag leaf) to the sink (grain). In support, water stress induced remarkable decrease in soluble protein in flag leaf at heading and anthesis. The decrease in protein content in yielded grains as a result of drought stress was alleviated by the application of GB, SA or their interaction. In connection with these results, Mäkelä *el al*. (2000) found increased protein in tomato plants under drought or salinity by means of foliar-applied GB. In addition, similar results are obtained by El-Tayeb (2005).

Free amino acids play an important role in maintaining the osmotic balance in the tissue of plants, yeasts, bacteria and animals (Zushi *et al*., 2005). In this investigation, water stress induced a massive increase in total amino acids detected in the harvested grains of the two wheat cultivars. This may result from the enhanced production of amino acids as a result of increased proteolytic activities which may occur in response to the changes in osmotic adjustment of their cellular contents (Greenway and Munns, 1980). The accumulation of free amino acids under stress at all the growth stages indicates the possibility of their involvement in osmotic adjustment (Yadav *et al*., 2005). The amino acid content has been shown to increase under drought conditions in sorghum (Yadav *et al*., 2005), in *Abelmoschus esculentus* (Sankar *et al*., 2007).

The obtained results indicated that glutamic acid was the most abundant amino acid in the yielded grains of the control and treated plants. These results are in a good agreement with those obtained by Caputo and Barneix (1997). They found that, the amino acid composition of phloem sap is different in different species, in barley, Glu accounts for approximately 50% of the total amino acids, while Asp accounts for roughly 20% and in wheat, Glu amounted to 30% of the total amino acids, and Asp to 20%, with these proportions changing with plant age. Also, in spinach, Glu was the most abundant amino acid, accounting for 39.1%, followed by Asp (14.7%) and Glu (10.1%) (Riens *et al*., 1991), and Glu was also the dominating amino acid in the phloem of *Beta vulgaris* L. (Lohaus *et al*., 1994). For instance, an accumulation of Glu has been reported in the wheat grains under salinity (Aldesuquy, 1998) while, in *Phragmites australis*, Glu levels rose in rhizomes (Hartzendorf and Rolletschek, 2001). In strawberry fruit, salt stress led to a considerable increase in Glu, especially in the salt-sensitive cv. Elsanta (Keutgen and Pawelzik, 2008). This accumulation of Glu could have been caused by both, the activation of biosynthesis from Glu and the inactivation of Glu degradation (Yoshiba *et al*., 1997).

Water stress induced an accumulation in proline concentration in harvested grains in both wheat cultivars. Increased proline in the grain of stressed wheat plants may help to overcome any further stress conditions. Proline accumulates under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate stress (Chandrashekar and Sandhyarani, 1996). Similar results are obtained in sorghum (Yadav *et al*., 2005), and in salt-stressed *Catharanthus roseus* (Jaleel *et al*., 2007).

Treatments with GB, SA or their interaction induced remarkable increases in amino acids detected in the harvested grains of both wheat cultivars. This was in agreement with El-Tayeb (2006) with sunflower plants treated with SA under Cu- stress conditions. Also, Hussein *et al*. (2007) studied the effect of salicylic acid and salinity on growth of maize plants. They found that all amino acid concentrations are lowered by salinity except for proline and glycine. All determinate amino acid concentrations (except methionine) are increased with the application of salicylic acid (200 ppm). On the other hand, methionine was negatively responded which slightly lowered. These chemicals may reduce proline oxidase and resulted in proline accumulation which acts as an osmolyte as well as scavenger.

The applied chemicals appeared to mitigate the effect of water stress on wheat yield and the biochemical aspects of yielded grains particularly the sensitive one. The effect was more pronounced with glycine betaine + salicylic acid treatment. This improvement would result from the repairing effect of the provided chemicals on growth and metabolism of wheat plants under water deficit condition.

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