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ORIGINAL ARTICLE

Role of glycine betaine and salicylic acid in improving growth vigour and physiological aspects of droughted wheat cultivars

Heshmat. S. Aldesuquy[1], Samy. A. Abo- Hamed[1], Mohmed. A. Abbas[1] and Abeer. H. Elhakem [2]

[1] *Department of Botany, Faculty of Science, Mansoura University, Egypt.*

[2] *Department of Biology, Faculty of Science & Humanitarian Studies, Salaman Bin Abdelaziz University, KSA.*

E mail: HS-Aldesuquy@hotmail.com

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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 two droughted wheat (*Triticum aestivum L.*) cultivars (sensitive, Sakha 94 and resistant, Sakha 93). In general, water stress caused noticeable increases in root length, number of adventitious roots, soluble sugars and nitrogen but a massive reduction in fresh and dry masses of root, growth vigor of shoot, leaf area, pigments content, polysaccharides, protein-N and total nitrogen in both wheat cultivars. Vice versa, exogenous application of GB, SA or their interaction could counteract the adverse effects of drought by improvement of growth vigor of root and shoot, leaf area, retention of pigments content, increasing the concentration of organic solutes (soluble sugars and soluble nitrogen) as osmoprotectants, keeping out the polysaccharides concentration and/or stabilization of essential proteins in both wheat cultivars. Finally, we can conclude that GB, SA or their interaction could improve the drought tolerance of both two wheat cultivars particularly the sensitive ones.

Key words: Triticum aestivum L / Drought / Glycine betaine / Salicylic acid / Flag leaf.

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Key words: Triticum aestivum L / Drought / Glycine betaine / Salicylic acid / Flag leaf.

Yield in wheat is a function of many factors among which, shoot growth and morphological characteristics of root and shoot as well as pigments content of leaves are the most important. Water stress is a very important limiting factor at the initial phase of plant growth and establishment (Shao *et al*., 2008). There was a significant reduction in shoot height in *Populus cathayana* under deficit stress (Nautiyal *et al*., 2002). In soybean, the stem length decreased under water-deficit stress, but this decrease was not significant when compared to well-watered control plants (Shao *et al*., 2008). The plant height reduced up to 25% in water-stressed Citrus seedlings (Panneerselvam *et al*., 2007). Stem length was significantly affected under water stress in potato (Ravindra *et al*., 1991).

Root characteristics, especially root length, root length density, and the number of thick roots, are important for a plant to have comparatively well-established aboveground parts by exploiting the available water, as in rice (Manivannan *et al*., 2007a). Drought avoidance due to a profound root system that enhances the ability of a plant to capture water is a fundamental adaptation mechanism to drought (Liu *et al*., 2005).

Drought stress decreases mean plant biomass, whereas it increases both the relative variation in plant biomass and the concentration of mass within a small fraction of the population (*Shao et al*., 2008). Drought stress decreased the plant biomass in *Cyamopsis tetragonoloba* (Wang *et al*., 2005) and spring wheat (Shao *et al*., 2008). The reduction in total biomass was reported in groundnut cultivars under water stress due to the reduction in the pod mass rather than in the vegetative mass (Tschaplinski *et al*., 1998). Morphological parameters like fresh and dry weights have a profound effect in water-limited conditions (Shao *et al*., 2008). There was a one-third reduction in fresh and dry weights of the *Ziziphus rotundifolia* plant under drought conditions (Tsialtas *et al*., 2001).

Drought stress decreases photosynthetic rate and disrupts carbohydrate metabolism in leaves of stressed maize both may lead to a reduced amount of assimilate available for export to the sink organs, and thereby increasing the rate of reproductive abortion. (Kim *et al*., 2000). Furthermore, they reported that, loss of kernel set is correlated with the extent of loss in photosynthesis and the photosynthate influx into kernels.

Many studies indicate that GB might play an important role in enhancing plant tolerance to some abiotic stresses such as salt, drought, and extreme temperatures (Quan *et al*., 2004). On the other hand, salicylic acid (SA) has been reported to cause a multitude of effects on the morphology and physiology of plants (Levent *et al*., 2007) and to induce a protective mechanism enhancing resistance to biotic and abiotic stresses (Lopez-Delgado *et al*., 1998). There is also evidence that SA can alter the antioxidant capacity in plants (Rao *et al*., 1997). Many studies support the SA-induced increases in the resistance of wheat and maize to salinity (Sakhabutdinova *et al*., 2003; Shakirova *et al*., 2003; Güneş *et al*., 2005) and osmotic stress (Bhupinder and Usha, 2003) and of rice on heavy metal stress (Mishra and Choudhuri, 1999).

This study was planned to investigate the effect of foliar application of glycine betaine, grain presoaking in salicylic acid and their interaction on growth criteria (growth vigor of root and shoot and leaf area as well as fresh and dry mass of flag leaf), pigments content and some metabolites (carbohydrates and nitrogen) of droughted wheat (*Triticum aestivum L.*) cultivars.

MATERIALS AND METHODS

Plant material and growth condition

Two wheat cultivars (*Triticumn 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. These two varieties are common in Egypt.

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 1st set and 2nd set were separately soaked in distilled water or salicylic acid (0.05 M) respectively. In 20 November 2009, grains of each set were planted in plastic pots (fifteen grains per pot; 25cm width X 30cm height) filled with 6 kg mixed 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 єC 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, thinning to five uniform seedlings per pot took place.

On the day 65 after planting (at the beginning of heading) the pots of the 1st set was allocated to four groups (20 pots per each group) as follows: control (cont.), water stress (WS), glycine betaine control (GB.), glycine betaine + water stress (GB + WS). The 2nd set group was allocated to four groups as follows: 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 droughted pot received 500 ml water at the end of 1st stress period. At the end of stress periods, rewatering to the field capacity was carried out. The undroughted (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, 21 days from planting (tillering stage), and before heading (at ear emergence) the plants received 35 kg N ha^{-1} urea and 35 kg P ha^{-1} 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 photosynthetic pigments

The protocol of measurements of the plant photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) determined at the two stages of plant development is based on methods of Arnon (1949) for chlorophylls and Horvath *et al.* (1972) for carotenoids.

Determination of Carbohydrates:

Total soluble sugars and sucrose were extracted and determined according to the procedures of Riazi *et al.* (1985) and modified by Ibrahim (1999) and Handel (1968). 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 nitrogenous constituents

The method used in this investigation was essentially that adopted by Yemm and Willis (1956).

Ammonia - N was estimated spectrophotometrically by the method adopted by Delory (1949) using Nessler's reagent as modified by Naguib (1964)

The methods used for Determination of amide-N and Amino-N were that recommended by Naguib (1964) and Muting and Kaiser (1963) respectively.

The total soluble nitrogen and total nitrogen were determined by the conventional semimicro-modification of Kjeldahl method (Pine, 1955) and Chibnal *et al.* (1943) respectively.

Statistical Analysis

The main effect of factors (watering regime, used chemicals, growth stages and wheat type) 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 AND DISCUSSION

Changes in growth vigor of root

The pattern of results in table 1 showed that, root length, root mass (fresh and dry), number of adventitious roots and root/shoot ratio were increased in control and droughted plants from heading to anthesis in both wheat cultivars. In relation to wheat cultivar, the sensitive one had higher root criteria values than the resistant ones. As compared to control values, water stress led to marked decrease ($P< 0.05$) in fresh and dry masses of root but increased ($P< 0.05$) the root length, number of adventitious roots and root plasticity of both wheat cultivars at heading and anthesis stages. The growth vigor of root of droughted plants was in general, stimulated by GB, SA or GB+SA treatments in both wheat cultivars at heading and anthesis. The magnitude of increase was more pronounced with SA than GB treatment.

Changes in growth vigor of shoot

The data presented in tables 2a & 2b showed that, there is a tendency among control and droughted plants to a progressive increase in the growth vigor of shoot from heading to anthesis. In relation to control values, water stress caused noticeable decrease ($P< 0.05$) in the shoot characteristics (shoot length, plant height, shoot diameter, fresh and dry masses, number of tillers, leaf area and degree of succulence) of both wheat cultivars at heading and anthesis. This effect was more obvious with the sensitive cultivar. On the other hand, application of glycine betaine, salicylic acid or their interaction induced marked increases ($P< 0.05$) in the all shoot growth characteristics of both wheat cultivars at heading and anthesis.

Changes in pigments content

In relation to wheat cultivar, the flag leaves of the control resistant plants had higher pigments (chl a, chl b, chl a+b, chl a/b, carotenoids and total pigments) content than the sensitive one at heading and anthesis (Table 3). Water stress resulted in a massive decrease ($P< 0.05$) in the pigments content of the two wheat cultivars at heading and anthesis. The magnitude of decrease was pronounced with the sensitive ones. Foliar application with GB, presoaking in SA or their interaction enhanced the accumulation of pigments in flag leaves of both wheat cultivars at heading and anthesis.

Changes in carbohydrates content

Changes in the soluble sugars

Data presented in table 4 showed that, glucose, sucrose and total soluble sugars in flag leaves increased in control and droughted plants from heading to anthesis stages of both wheat cultivars. With regard to the wheat cultivar, the sensitive plants accumulated more soluble sugars than the resistant ones at heading and anthesis satages.

As compared to control values, water stress caused noticeable increase ($P< 0.05$) in the soluble sugars of the two wheat cultivars at heading and anthesis stages. In the majority of cases, treatments with GB, SA or their interaction caused additional increases ($P< 0.05$) in the soluble sugars of the two wheat cultivars at heading and anthesis stages.

Changes in the polysaccharides and total carbohydrates

It can be seen that from table 4, the control plants of resistant cultivar had higher values of polysaccharides and total carbohydrates at heading and anthesis stages. On the other hand, water stress led to marked decreases ($P< 0.05$) in the polysaccharides and total carbohydrates in flag leaves of the two wheat cultivars at heading and anthesis. Application of GB, SA or their interaction induced massive increases (P< 0.05) in the polysaccharides and total carbohydrates at heading and anthesis stages.

Table 1. Effect of glycine betaine, salicylic acid and their interaction on growth vigor of root of droughted wheat cultivars at heading .and anthesis stages

varietyWheat	**Parameters**	**Growth vigor of root**										
		(Root length (cm		**Root Plasticity**	**(Root fresh mass (g**		**(Root dry mass (g**		**No. of adventitious roots**		**Root / shoot Ratio**	
	Treatments	**Heading**	**Anthesis**		**Heading**	**Anthesis**	**Heading**	**Anthesis**	**Heading**	**Anthesis**	**Heading**	**Anthesis**
Sensitive	**Cont**	20.90	24.11	1.15	1.56	2.54	1.23	2.10	15.40	18.60	0.24	0.37
	WS	24.90	29.70	1.19	1.19	2.04	0.98	1.72	17.10	22.80	0.24	0.38
	GB	21.60	25.22	1.17	1.61	2.71	1.31	2.30	16.10	19.10	0.24	0.37
	GB+WS	25.35	30.32	1.16	1.43	2.47	1.18	2.11	18.20	23.80	0.27	0.45
	SA	21.95	24.75	1.13	1.72	2.83	1.42	2.38	16.80	18.90	0.25	0.38
	SA+WS	26.50	30.30	1.14	1.45	2.66	1.20	2.16	19.10	24.30	0.26	0.44
	GB+SA	22.50	24.20	1.08	1.87	3.06	1.57	2.58	16.70	19.40	0.27	0.41
	GB+SA+WS	26.20	30.85	1.14	1.49	2.59	1.20	2.18	20.90	24.40	0.24	0.37
	LSD 0.05	**2.85**	**2.65**	**0.05**	**0.15**	**0.24**	**0.17**	**0.11**	**1.63**	**1.70**	**0.01**	**0.01**
Resistant	**.Cont**	15.85	18.40	1.16	1.17	2.07	0.93	1.66	11.80	12.90	0.22	0.33
	WS	20.96	24.50	1.17	0.95	1.73	0.74	1.35	12.40	14.20	0.21	0.31
	GB	16.95	19.00	1.12	1.29	2.29	0.97	1.86	11.80	13.20	0.19	0.32
	GB+ WS	22.35	25.80	1.15	1.07	1.83	0.80	1.62	12.90	14.80	0.21	0.36
	SA	18.20	20.30	1.12	1.30	2.47	1.07	2.11	11.90	13.50	0.20	0.35
	SA+ WS	22.40	25.14	1.12	1.11	1.87	0.90	1.59	12.60	14.90	0.23	0.33
	GB + SA	19.80	20.84	1.05	1.47	2.77	1.20	2.27	11.90	13.90	0.21	0.36
	GB +SA+WS	22.84	25.86	1.13	1.13	1.91	0.94	1.61	13.10	15.10	0.24	0.33
	LSD 0.05	**1.86**	**2.58**	**0.08**	**0.17**	**0.31**	**0.11**	**0.24**	**0.92**	**0.80**	**0.01**	**0.01**

Table 2a. Effect of glycine betaine, salicylic acid and their interaction on growth vigor of shoot of droughted wheat cultivars at .heading and anthesis stages

Wheat variety	Parameters	Growth vigor of shoot									
		(Shoot length(cm		(Plant height (cm		Shoot fresh mass ((g		(Shoot dry mass (g		Shoot diameter ((mm	
	Treatments	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis
Sensitive	**Cont**	56.38	60.96	69.95	75.69	12.85	15.31	5.21	5.99	3.64	3.81
	WS	47.74	55.42	60.59	69.14	9.28	11.23	4.05	4.24	3.17	3.20
	GB	56.96	61.84	70.66	77.01	13.56	16.43	5.43	6.13	4.02	4.15
	GB+WS	53.70	58.63	67.33	73.42	12.14	14.41	4.34	4.68	3.51	3.69
	SA	58.43	62.92	72.18	78.17	13.90	17.51	5.69	6.18	3.97	4.10
	SA+WS	55.04	59.22	68.64	74.05	12.23	14.76	4.56	4.92	3.60	3.83
	GB+SA	60.51	63.58	74.51	79.00	15.01	17.21	5.82	6.26	4.04	4.24
	GB+SA+WS	55.66	59.47	69.36	74.40	12.98	15.07	4.95	5.92	3.75	4.02
	LSD 0.05	1.53	1.28	2.10	1.31	0.70	1.15	0.42	0.14	0.28	0.22
Resistant	**.Cont**	52.76	56.83	67.33	72.10	10.07	13.47	4.17	4.96	4.12	4.34
	WS	46.79	52.81	60.79	67.13	8.80	11.00	3.48	4.39	3.98	4.13
	GB	55.66	57.44	70.41	73.22	12.51	15.35	4.99	5.80	4.23	4.65
	GB+ WS	50.71	55.03	65.26	70.36	10.03	12.73	3.75	4.55	4.09	4.28
	SA	57.09	58.59	71.93	74.67	13.54	15.50	5.43	6.09	4.14	4.47
	SA+ WS	49.77	53.41	64.37	68.91	10.55	12.26	3.92	4.75	4.08	4.30
	GB + SA	59.25	61.88	74.32	78.17	14.52	16.60	5.69	6.26	4.28	4.71
	GB +SA+WS	52.37	56.52	67.06	72.21	11.09	12.88	4.00	4.89	4.13	4.30
	LSD 0.05	2.03	0.56	3.09	1.11	1.67	1.28	0.77	0.59	0.11	0.29

Table 2b. Effect of glycine betaine, salicylic acid and their interaction on growth vigor of shoot of droughted wheat cultivars at heading and anthesis stages

Wheat variety	Parameters	Growth vigor of shoot											
		Number of tillers		(Leaf area (cm2		Flag Leaf fresh (mass (g		Flag Leaf dry (mass (g		Degree of succulence		Specific leaf area	
	Treatments	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis
Sensitive	**Cont**	2.70	2.80	17.33	19.10	0.282	0.318	0.095	0.107	92.65	90.52	182.37	178.50
	WS	2.30	2.20	13.81	2.59	0.219	0.127	0.080	0.067	99.32	42.77	172.58	38.91
	GB	2.80	2.90	18.28	19.58	0.288	0.327	0.105	0.114	99.86	91.75	174.05	172.35
	GB+WS	2.60	2.80	14.52	6.96	0.274	0.187	0.096	0.102	81.57	81.53	151.25	68.53
	SA	3.00	3.20	18.33	20.27	0.299	0.336	0.108	0.115	95.97	91.69	169.72	176.44
	SA+WS	2.70	2.80	15.74	8.17	0.278	0.263	0.099	0.102	87.96	50.65	159.04	80.21
	GB+SA	3.20	3.40	19.20	20.91	0.324	0.335	0.116	0.120	92.33	97.26	165.55	174.25
	GB+SA+WS	2.90	2.90	16.25	10.15	0.298	0.258	0.102	0.107	82.91	67.34	159.32	94.57
	LSD 0.05	0.28	0.26	0.91	1.13	0.035	0.027	0.010	0.009	1.70	1.12	2.30	2.51
Resistant	**.Cont**	1.90	2.30	26.81	29.52	0.387	0.438	0.147	0.159	111.70	105.81	182.37	185.66
	WS	1.50	2.10	22.80	25.56	0.341	0.380	0.138	0.146	112.29	109.23	165.18	175.07
	GB	1.90	2.30	27.22	31.33	0.405	0.446	0.166	0.171	113.88	113.93	163.96	183.22
	GB+ WS	1.70	2.20	25.26	27.94	0.372	0.410	0.141	0.149	109.34	107.05	179.13	187.52
	SA	2.30	2.80	27.56	31.69	0.419	0.468	0.174	0.183	112.49	111.19	158.39	173.17
	SA+ WS	2.10	2.40	24.47	27.63	0.380	0.418	0.142	0.150	102.81	103.10	172.32	184.20
	GB + SA	2.30	2.80	28.27	32.02	0.448	0.499	0.180	0.198	105.49	106.38	157.06	161.72
	GB +SA+WS	1.90	2.50	25.60	28.05	0.406	0.421	0.145	0.152	98.09	104.28	176.55	184.54
	LSD 0.05	0.22	0.23	0.41	1.77	0.040	0.038	0.011	0.017	1.02	1.23	1.85	1.97

Table 3. Effect of glycine betaine, salicylic acid and their interaction on pigments content (mg g^{-1} d wt) in flag leaf of droughted wheat cultivars at the heading and anthesis stages.

Wheat variety	Parameters Treatments	Pigments content (mg g^{-1} d wt)											
		Chl a		**Chl b**		**Chl (a +b)**		**Chl (a /b)**		**Carotenoids**		**Total pigments**	
		Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis
Sensitive	**Cont**	3.08	4.54	1.37	1.49	4.45	6.03	2.25	3.04	1.16	1.48	5.61	7.50
	WS	2.54	3.24	0.97	1.17	3.51	4.41	2.63	2.77	0.93	1.18	4.44	5.59
	GB	3.16	4.70	1.72	1.85	4.89	6.55	1.83	2.55	1.27	1.69	6.16	8.24
	GB+WS	2.96	4.02	1.15	1.31	4.11	5.33	2.57	3.07	1.05	1.30	5.16	6.64
	SA	3.21	5.30	1.79	1.90	5.00	7.19	1.80	2.79	1.28	1.70	6.28	8.89
	SA+WS	3.03	4.10	1.21	1.34	4.24	5.45	2.49	3.05	1.09	1.36	5.33	6.81
	GB+SA	3.40	5.60	1.89	2.08	5.28	7.68	1.80	2.69	1.33	1.78	6.61	9.46
	GB+SA+WS	3.10	4.32	1.29	1.43	4.39	5.75	2.41	3.01	1.14	1.41	5.53	7.16
	LSD 0.05	**0.08**	**0.10**	**0.09**	**0.15**	**0.13**	**0.19**	**0.14**	**0.33**	**0.07**	**0.12**	**0.18**	**0.24**
Resistant	**Cont.**	3.32	5.36	1.60	1.90	4.92	7.26	2.08	2.82	1.21	1.95	6.13	9.21
	WS	2.80	4.11	1.26	1.51	4.07	5.62	2.22	2.73	1.02	1.31	5.09	6.92
	GB	3.56	5.52	1.70	1.94	5.27	7.46	2.09	2.84	1.26	2.15	6.52	9.62
	GB+ WS	3.15	4.38	1.52	1.65	4.67	6.04	2.08	2.65	1.10	1.71	5.78	7.74
	SA	3.64	5.70	1.86	2.04	5.50	7.73	1.96	2.80	1.32	2.22	6.83	9.95
	SA+ WS	3.26	4.65	1.54	1.66	4.81	6.31	2.11	2.80	1.16	1.76	5.97	8.07
	GB + SA	3.72	6.33	2.03	2.19	5.75	8.52	1.83	2.89	1.35	2.27	7.11	10.79
	GB +SA+WS	3.30	5.11	1.73	1.86	5.03	6.97	1.91	2.75	1.18	1.88	6.20	8.85
	LSD 0.05	**0.09**	**0.10**	**0.14**	**0.09**	**0.15**	**0.15**	**0.19**	**0.14**	**0.13**	**0.06**	**0.22**	**0.12**

Table 4. Effect of glycine betaine, salicylic acid and their interaction on carbohydrates content (mg g^{-1} d wt) of flag leaf of droughted wheat cultivars at heading and anthesis stages

Wheat variety	Parameters / Treatments	Carbohydrates content (mg g^{-1}dwt)									
		Glucose		Sucrose		Total soluble sugar		Polysaccharides		Total carbohydrates	
		Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis
Sensitive	**Cont**	5.73	6.51	32.80	36.61	42.49	50.09	133.40	155.04	175.89	205.14
	WS	6.06	7.12	39.23	44.69	50.49	59.62	93.60	113.72	144.09	173.34
	GB	5.80	6.64	33.09	37.73	43.90	50.86	158.85	170.39	202.75	221.25
	GB+WS	6.78	7.94	43.65	46.35	55.81	61.92	111.81	132.37	167.62	194.29
	SA	5.81	6.58	34.17	36.93	43.64	51.49	165.93	176.25	209.57	227.74
	SA+WS	6.40	7.26	45.33	48.85	55.16	62.63	116.77	136.73	171.94	199.35
	GB+SA	5.96	6.64	34.35	38.02	44.15	51.64	171.48	188.59	215.63	240.24
	GB+SA+WS	6.87	7.36	46.08	50.80	58.73	63.85	129.39	143.12	188.12	206.97
	LSD 0.05	**0.33**	**0.32**	**0.86**	**1.82**	**3.19**	**3.25**	**8.71**	**8.20**	**3.84**	**4.22**
Resistant	**Cont.**	5.44	5.84	28.75	33.95	39.19	45.10	155.14	174.07	194.33	219.17
	WS	5.97	6.95	34.29	38.27	45.88	52.21	133.10	140.14	178.98	192.34
	GB	5.59	5.93	29.39	34.53	39.55	46.13	168.48	180.32	208.02	226.46
	GB+ WS	6.40	7.57	38.11	41.76	49.01	56.73	145.13	154.54	194.15	211.27
	SA	5.52	5.71	29.15	33.65	38.47	45.96	171.72	192.04	210.18	238.00
	SA+ WS	6.47	6.52	37.65	42.45	52.41	55.77	146.19	159.49	198.60	215.27
	GB + SA	5.56	5.96	30.01	34.77	40.32	47.51	176.26	196.24	216.58	243.75
	GB +SA+WS	6.53	7.02	41.44	45.47	53.00	58.76	153.66	162.63	206.67	221.39
	LSD 0.05	**0.20**	**0.27**	**0.69**	**1.75**	**2.95**	**2.19**	**9.30**	**9.45**	**3.97**	**4.82**

Table 5. Effect of glycine betaine, salicylic acid and their interaction on nitrogen content (mg g^{-1} d wt) in flag leaf of droughted .wheat cultivars at heading and anthesis stages

Wheat variety	Parameters	(Nitrogen content (mg g^{-1} d wt											
		Amino-N		Ammonia-N		Amide-N		Total soluble-N		Total nitrogen		Protein	
	Treatments	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis	Heading	Anthesis
Sensitive	Cont	2.00	2.54	1.71	2.12	0.099	0.111	3.89	4.90	20.16	15.76	16.27	10.86
	WS	3.19	3.63	2.09	2.91	0.125	0.133	5.44	6.71	14.46	11.34	9.02	4.63
	GB	2.87	3.46	1.91	2.49	0.100	0.126	4.90	6.18	22.26	17.03	17.37	10.84
	GB+WS	3.67	4.00	2.43	3.20	0.131	0.142	6.23	7.41	17.70	13.22	11.48	5.81
	SA	2.67	3.07	1.86	2.31	0.111	0.131	4.70	5.70	22.04	16.75	17.34	11.04
	SA+WS	3.44	3.80	2.30	3.07	0.128	0.148	5.92	7.21	16.55	13.05	10.63	5.84
	GB+SA	3.07	3.57	1.98	2.56	0.110	0.137	5.18	6.42	24.24	18.10	19.06	11.68
	GB+SA+WS	3.94	4.24	2.66	3.21	0.138	0.156	6.80	7.74	19.98	14.39	13.18	6.66
	LSD 0.05	**0.24**	**0.30**	**0.06**	**0.09**	**0.03**	**0.004**	**0.27**	**0.35**	**1.62**	**1.87**	**0.17**	**0.12**
Resistant	.Cont	1.87	2.3	1.60	2.02	0.090	0.108	3.67	4.50	20.77	16.86	17.10	12.36
	WS	2.74	3.22	2.03	2.74	0.114	0.127	5.29	6.34	17.62	14.35	12.33	8.01
	GB	2.54	3.09	1.85	2.32	0.108	0.115	4.50	5.66	22.91	17.90	18.41	12.24
	GB+ WS	3.12	3.54	2.31	3.11	0.119	0.133	5.97	7.19	18.93	15.68	12.96	8.49
	SA	2.17	2.87	1.74	2.18	0.101	0.120	4.11	5.20	23.26	17.31	19.16	12.11
	SA+ WS	3.09	3.45	2.21	2.99	0.123	0.146	5.88	6.82	19.71	16.48	13.83	9.66
	GB + SA	2.87	3.15	1.90	2.51	0.104	0.123	4.92	6.10	27.02	24.20	22.10	18.10
	GB +SA+WS	3.19	3.62	2.48	3.08	0.131	0.151	6.40	7.21	20.44	17.93	14.04	10.71
	LSD 0.05	**0.19**	**0.17**	**0.05**	**0.08**	**0.005**	**0.004**	**0.21**	**0.30**	**2.19**	**2.11**	**0.19**	**0.16**

Changes in nitrogen content

Changes in the soluble nitrogen

It appeared from table 5 that, soluble nitrogen (amino-N, ammonia-N, amide-N and total soluble nitrogen) accumulated in control and droughted plants from heading to anthesis in flag leaves of both wheat cultivars. With regard to the wheat cultivar, the sensitive plants had higher soluble nitrogen than the resistant ones. In relation to control values, water stress caused noticeable increases ($P< 0.05$) in the soluble nitrogen of the two wheat cultivars at heading and anthesis stages.

Foliar application of GB, presoaking in SA or their interaction induced marked increases ($P< 0.05$) in the soluble nitrogen at heading and anthesis in the flag leaves of the two wheat cultivars. Plants treated with GB had higher soluble nitrogen content than those treated with SA under stress and controlled conditions.

Changes in the total nitrogen and soluble protein

Total nitrogen and soluble protein decreased in the flag leaves from heading to anthesis in control and droughted plants of both wheat cultivars (Table 5). The resistant cultivar had higher total nitrogen and soluble protein concentrations than the sensitive one. With respect to control values, water stress caused drastic decreases ($P< 0.05$) in the total nitrogen and soluble protein in the flag leaves of both wheat cultivars at heading and anthesis. In general, GB, SA or their interaction caused additional increases ($P< 0.05$) in total nitrogen and soluble protein in the flag leaves of the two wheat cultivars.

DISCUSSION

Growth is influenced by various internal and external factors besides its genetic makeup and is an important tool for assessing crop productivity in various crops. The aforementioned pattern of results revealed that, drought stimulates root growth by increasing root length, number of adventitious roots and root plasticity. On the other hand, root fresh and dry masses appeared to decrease in response to water stress. Furthermore, water stress led to a drastic decrease in shoot growth vigor (shoot length, plant height, fresh and dry masses, shoot diameter and leaf area). The inhibitory effect of water stress was more pronounced on the sensitive cultivar than on the resistant ones. The variation in response of wheat cultivar for stress (drought and salt) tolerance was known in many studies (Iqbal and Ashraf, 2005; Arfan *et al.*, 2007). In this respect Sankar *et al.* (2007), have recoded that the root length, shoot length, total leaf area, fresh weight and dry weight of bhendi were significantly reduced under drought stress treatment.

Continuation of root growth under drought stress through stimulation in root length and number of adventitious roots is an adaptive mechanism that facilitates water uptake from deeper soil layers. These results were in accordance with those obtained by (Sundaravalli *et al.*, 2005; Yin *et al.*, 2005). Furthermore, arrest of plant growth during stress conditions largely depends on the severity of the stress. Mild osmotic stress leads rapidly to growth inhibition of leaves and stems, whereas roots may continue to elongate (Spollen *et al.*, 1993). The degree of growth inhibition due to osmotic stress depends on the time scale of the response, the particular tissue and species in question, and how the stress treatment was given (rapid or gradual).

Morphological characters like fresh and dry masses have a prefund effect in water-limited conditions (Shao *et al.*, 2008). The reduction in fresh and dry masses in root, shoot and flag leaves of droughted wheat cultivars may be responsible for the suppression of plant growth and consequently affected crop productivity. Overall, there is a sharp contrast between the root and the shoot in their response to water deficit. It could be explained by different rate of osmotic adjustment of shoot and root cells (Hsiao, 2000) or various loosening ability of leaf cell walls from roots cell walls. Loosening ability of the growing cell wall could be affected by auxins and also by ABA. Under water stress, the concentration of endogenous ABA increases in both leaves and roots and more ABA is transported from root to the shoot (Davies and Zhang, 1991). Simultaneously, convincing evidence was obtained indicating that ABA maintains root growth while inhibiting shoot growth in soybean (Creelman *et al.*, 1990) and in maize (Saab *et al*., 1990) at low water potential conditions.

The root/shoot ratio increases under water stress conditions to facilitate water absorption (Nicholas, 1998). The growth rate of wheat and maize roots was found to decrease under moderate and high water-deficit stress (Noctor and Foyer, 1998). However, the development of the root system increases water uptake and maintains the right osmotic pressure through higher proline levels (Pan *et al.*, 2003). An increased growth was reported in mango under water stress (Jaleel *et al*., 2007). The root dry weight decreased under mild and severe water stress in sugar beet (Pan *et al*., 2002). A significant decrease in root length was reported in water stressed *Populus* species by (Panda and Khan, 2003).

Water stress caused noticeable decreases in the shoot length and plant height. The reduction in plant height may probably due to the decline in the cell enlargement that result from low turgor pressure and more leaf senescence under water stress (Manivannan *et al*., 2007b). In fact, water stress is a very important limiting factor at the initial phase of plant growth and establishment (Shao *et al*., 2008). There was a significant reduction in shoot height in *Populus cathayana* under deficit stress (Nautiyal *et al*., 2002). In soybean, the stem length decreased under water-deficit stress, but this decrease was not significant when compared to well-watered control plants (Shao *et al*., 2008).

Reduction in leaf area by water stress is an important cause of reduced crop yield through reduction in photosynthesis (Rucker *et al*., 1995). The growth retardation in leaf area of droughted wheat plants is mainly due to water stress decreased the turgor which may diminish both cell production and cell expansion within the leaves. These results were in a good agreement with those obtained by Hsiao (2000); Choluj *et al*. (2004).

Rolling of leaves was observed to occur mainly in the susceptible wheat cultivar. There are two possible ways in which a plant in a droughted environment may benefit from rolling its leaves. Firstly, damage by increased leaf temperature resulting from high levels of solar radiation incident on leaf surfaces could be minimized by reducing the effective leaf area presented to the sun's rays, so that less radiation is intercepted by leaf tissue (Begg, 1980). Secondly, transpiration rates could be reduced through the creation, by leaf rolling, of a microclimate with both higher humidity and boundary layer resistances near the leaf surfaces, thereby conserving scarce water resources (Oppenheimer, 1960).

The applied chemical appeared to improve the growth vigor by increasing the plant height, fresh and dry masses, and leaf area and root length of both wheat cultivars. The protective role of GB on wheat growth can be related to its role in osmotic adjustment where it acts as a non-toxic cytoplasmic osmolyte (Ibrahim and Aldesuquy, 2003). In addition, the beneficial effect of GB application on droughted wheat plants may probably be due to GB induced production additional vacuoles in root cells, which resulted in a greater accumulation of Na^+ in the root and a decrease in its transportation to the shoot (Lutts *et al*., 1999). In this respect, Demiral and Turkan (2004) studied the ability of exogenously applied glycine betaine (GB) for the alleviation of growth inhibition and senescence resulting from NaCl stress. They found that shoot fresh weight of *Pokkali* and shoot and root dry weight of IR-28 showed a decrease under salinity but an increase with exogenous GB application. In addition to its direct protective roles, either through positive effects on enzyme and membrane integrity or as an osmoprotectant, GB may also protect cells from environmental stresses indirectly via its role in signal transduction. For example, GB may have a role in Na^+/K^+ discrimination, which substantially or partially contributes to plant salt tolerance. Ion homeostasis in plants is governed by various membrane transport systems (Ashraf and Foolad, 2007).

Exogenous application of SA had a promotive effect on growth vigor of root, shoot and leaf area of both wheat cultivars under non-stress and stress conditions. These results can be related to earlier studies which observed that exogenous application of SA promotes growth and counteracts the stress induced growth inhibition due to abiotic stresses in a range of crop species (Tari *et al*., 2002; Shakirova *et al*., 2003; Singh and Usha, 2003; Khodary, 2004; El-Tayeb, 2005; Arfan *et al*., 2007). For example, salinity stress-induced growth inhibition was alleviated by exogenous SA application through the rooting medium on the growth of tomato (Tari *et al*., 2002) and *Phaseolus vulgaris* (Stanton, 2004). Similarly, foliar spray with SA also mitigated the adverse effects of salt stress on growth of maize (Khodary, 2004) or promoted the growth in soybean (Gutierrez-Coronado *et al*., 1998). Singh and Usha (2003) reported that foliar spray with SA counteracted growth inhibition in wheat caused by water stress, one of the major factors caused by salinity stress in plants. Salicylic acid-induced increase in growth of wheat under non-saline or saline conditions can be attributed to an increase in photosynthesizing tissue, i.e., leaves (Dhaliwal *et al*., 1997; Zhou *et al*., 1999), which is in agreement with our results.

Gutierrez–Coronado *et al*. (1998) observed significant effect of SA on soybean increases in shoot growth, root growth and plant height. Also, Khodary (2004) reported that SA increased the fresh and dry weight of shoot and roots of salt stressed maize plants. Furthermore, Sawada *et al*. (2008) recorded that salicylic acid (SA) accumulates in salt-stressed rice (*Oryza sativa* L.cv. Nipponbare) seedlings and they hypothesized that the accumulation of SA might potentate oxidative injury in rice seedlings since the inhibition of SA synthesis alleviated the growth inhibition under high salinity.

The prevention of water loss from leaves of droughted wheat plants as results of SA application might be the principal reason for the significant increase in shoot fresh weight. The observed increase in growth of droughted wheat plants resulting from SA (antitranspirant) may due to its effect on improvement of turgidly at a time when the growth of that particular plant part was more dependent on water status than in photosynthesis (Davenport *et al*., 1972).

Increasing the efficiency of photosynthesis has long been a goal of plant research (Nartr and Lawlor, 2005). The site of the photosynthesis in plants is directly depends upon the chlorophyll bearing surface area, irradiance and its potential to utilize CO_2 (Hirose *et al*., 1997). Photosynthesis is a key metabolic pathway in plants. In fact, maintaining good photosynthetic rate leads to maintenance of growth under water stress (Dubey, 2005). The present results indicated that water stress caused marked decreases in the pigment contents in the flag leaves of the wheat plants. There is a common observation that leaf yellowing can occur when leaves have had low water potentials for a considerable time. Chlorophyll is more sensitive to drought than carotenoids and consequently the ratio of total chlorophyll to carotenoids decreases with increasing drought severity (Barry *et al*., 1992).

In accordance with these results, Manivannan *et al*. (2007c) found that the water deficit affected the early growth, biomass allocation and pigment of five varieties of sunflower (*Helianthus annuus L.*) plants. They found that there was a significant difference in early growth, dry matter accumulation and pigment among the studied varieties. The root length, shoot length, total leaf area, fresh and dry weight, chlorophyll a, b, total chlorophyll and carotenoids were significantly reduced under water stress treatments. Moreover, Kamel *et al*. (1995) working with cotton plants (*Gossypium barbadense* cv Giza 75) reported two opposite trends regarding the relative concentration of photosynthetic pigments throughout the experimental period in plants under constant water stress. The first was a reduction in different photosynthetic pigments comparing to the normally irrigated plants, while the second was an enhancing effect. Subjecting cotton plants to drought whether during the vegetative growth or at maximum flowering, showed a decrease in the concentration of chlorophylls a, b and carotenoids. However, at budding or flowering a remarkable increase occurred compared with controls.

It is clear that, the decline in pigments (chl a, chl b and carotenoids) content in flag leaves of sensitive and resistant cultivates under drought, may accelerated the ageing process in the sensitive cultivar more than in the resistant ones. The stimulating effect of GB on photosynthetic pigments may probably be due to GB had a protective role on photosynthetic apparatus of sensitive cultivar and decrease the rapid senescence of flag leaves occurred as a result of water stress (Mдetak *el al*., 2000).

In general, SA application induced noticeable increases in pigments content (chl a, chl b and carotenoids) in flag leaves of droughted wheat cultivars (Table 4). The stimulating effect of SA may be due to the fact that SA led to increase leaves longevity on droughted plants by retaining their pigments content, therefore inhibit their senescence. In relation to these results, Chandra and Bhatt (1998) observed that an increasing or decreasing effect of SA on chlorophyll content of cowpea (*Vigna unguiculata*) depends on the genotype. In another study, treatment with SA increased pigment contents in soybean (Zhao *et al*., 1995), maize (Khodary, 2004), and wheat (Singh and Usha, 2003; Arfan *et al*., 2007)) grown under normal or stress conditions. Furthermore, Arfan *et al*. (2007) found that the improvement in growth and grain yield of wheat salt-tolerance due to SA application was associated with improved photosynthetic capacity.

Carbohydrates that represent one of the main organic constituents of the dry matter were found to be affected by water stress. Glucose, sucrose and total soluble sugars were increased in the flag leaves of two wheat cultivars at heading and anthesis. This is consistent with the widely observed of increase soluble sugars in response to water stress in both resistant and sensitive plants (Al-Hakimi *et al*., 1995; Ibrahim, 1999). Drought stress decreases photosynthetic rate and disrupts carbohydrate metabolism in leaves (Kim *et al*., 2000); both may lead to a reduced amount of assimilate available for export to the sink organs, and thereby increasing the rate of reproductive abortion.

Liu *et al*. (2004) studied the effect of drought stress on carbohydrate concentration in soybean leaves and pods during early reproductive development. They found that drought did not affect the activity of soluble invertase in leaves. In flowers and pods, sucrose concentrations were higher under drought as compared with well-watered controls.

Hexose and starch concentrations of flowers and pods were also higher under drought; thereafter they were significantly lower than those of the well-watered controls. Soluble invertase activity was decreased by drought in pods. The total amount of non-structural carbohydrate accumulated in pods during the sampling periods was substantially reduced by drought.

Drought stress also reduces photosynthesis, for a number of reasons: i) hydroactive stomatal closure reduces the CO_2 supply to the leaves, ii) water deficiency damages the cytoplasm ultrastructure and enzyme activity, iii) dehydrated cuticles, cell walls and plasma membranes are less permeable to CO_2. An analysis of the correlation between drought and the carbohydrate metabolism reveals that one characteristic symptom of water deficiency is the mobilization of the starch stored in the chloroplasts. As there is also a reduction in the translocation of carbohydrates during drought stress, this leads to a change in source-sink relationships (Liu *et al*., 2004).

Growth arrest resulted from water withholding can be considered as a possibility to preserve carbohydrates for sustained metabolism, prolonged energy supply, and for better recovery after stress relief (Bartels and Sunkar, 2005). The inhibition of shoot growth during water deficit is thought to contribute to solute accumulation and thus eventually to osmotic adjustment (Osorio *et al*., 1998). For instance, hexose accumulation accounts for a large proportion of the osmotic potential in the cell elongation zone in cells of the maize root tip (Sharp *et al*., 1990).

As sucrose is both the principal and the preferred form of photosynthate for long-distance transport to sink organs, its concentration in leaves should represent the current availability of assimilate for reproductive development (Westgate and Grant, 1989). The results also indicated that the increase in the sucrose with a concomitant increase with glucose content in flag leaves of both wheat cultivars and this is in conformity with the work of Ibrahim (1999) who observed that sorghum leaves accumulated sucrose, glucose and total soluble sugar in response to water stress. It has been suggested that drought induced sucrose accumulation in crop reproductive organs may be partially due to a low activity of acid invertase, which fails to cleave the incoming sucrose into hexose under drought conditions (Andersen *et al*., 2002). As a result of this, the ratio of hexose to sucrose, which has been suggested to play an important role in regulation ovary and seed development (Weschke *et al*., 2000), may thus be reduced under drought stress.

Results in table 5 indicated that polysaccharides and total carbohydrates contents were decreased in droughted wheat flag leaves. The sensitive cultivar suffered more reduction than the resistant ones. This is in agreement with the observed accumulation of starch in sorghum plants by Ibrahim (1999) in response to water stress. This increase would be advantageous in terms of carbohydrate reserve for growth e.g. more root (Chaves *et al*., 1995) or leaves osmoregulation (Ackerson, 1981). In pigeon pea (*Cajanus cajan*), leaf starch and sucrose concentrations decreased rapidly and becomes close to zero, while the concentrations of glucose and fructose significantly increased in response to drought stress (Keller and Ludlow, 1993). Similar results have been observed in several plant species under drought conditions (Lawlor and Cornic, 2002).

Applications of GB (osmoprotectant) induced additional increases in soluble sugars and stimulate the biosynthesis of polysaccharides and consequently total carbohydrates in flag leaves of the two wheat cultivars. This was in agreement with the finding of Ibrahim and Aldesuquy (2003); Ibrahim (2004) who found that droughted sorghum plants treated with GB accumulated more soluble sugars than the droughted plants only.

Under water stress conditions, SA-pretreated plants showed significant increases in the soluble sugars, polysaccharides and total carbohydrates content than the SA-untreated plants. Similar results were obtained by Khadary (2004) and El-Tayeb *et al*, (2006) with sunflower plants under Cu stress. In this respect, El-Tayeb, (2005) investigated that; total soluble sugars were accumulated in salt-stressed barley plants treated with 1 mM salicylic acid (SA). Furthermore he reported that, exogenous application of SA appeared to induce preadaptive response to salt stress leading to promoting protective reactions to the photosynthetic pigments and maintain the membranes integrity in barley plants, which reflected in improving the plant growth.

Water stress led to massive increases in the soluble nitrogen (amino-N, ammonia-N, amide-N and total soluble nitrogen) but decreased total nitrogen and soluble protein (Table 6). In order to increase its ability to overcome water stress, the resistant cultivar was able to keep out its nitrogen and protein content under water stress at higher levels than the sensitive ones. These results were in good conformity with that of Khalil and Mandurah (1990) who studied the effect of water stress on nitrogen metabolism of cowpea plants. They observed that water stress decreased shoot total-N and protein-N but increased the soluble-N content. This change in nitrogen content may be related to the inhibition of translocation from root to shoot, inhibition of protein synthesis or the increase of protease activity.

The increase of soluble nitrogen compounds are of importance in plant osmoregulation in response to water deficit. In this respect, Mohammadkhani and Heidari (2008) found that, the initial increase in total soluble proteins during drought stress was due to the expression of new stress proteins, but the decrease was due to a severe decrease in photosynthesis. Photosynthesis decreased in drought stress (Havaux *et al*., 1987) and materials for protein synthesis weren't provided; therefore, protein synthesis dramatically reduced or even stopped. In addition, the increase in total soluble proteins under drought stress was consistent with the findings of Riccardi *et al.* (1998) and Ti-da *et al.* (2006) in maize, and Bensen *et al.*(1988) in soybean. These authors reported that drought stress resulted in an increase of some soluble proteins and a decrease of others.

Foliar application of GB caused marked increases in the soluble nitrogen and enhancing the total nitrogen as well as soluble protein in the droughted wheat plants of the two cultivars. GB application may lead to increase free amino acids especially proline in the water stressed wheat plants and consequently increased the soluble nitrogen as well as total-N. In this respect, Ibrahim (2004) found that, in correcting the N concentration to total shoot dry weight found that, salinity had a negative effect on N content, and GB improved total-N of salinity stressed sorghum plants.

Salicylic acid treatment led to an accumulation in the soluble nitrogen, the total nitrogen as well as soluble portion in the flag leaves of droughted wheat plants of the two cultivars. These results were in consistant with (El-Tayeb *et al.* 2006). In addition, SA treatment protected nitrate reductase activity and maintained the protein and nitrogen contents of the leaves, compared to water-sufficient plants. The results signify the role of SA in regulating the drought response of plants and suggest that SA could be used as a potential growth regulator to improve plant growth, under water stress (El-Tayeb *et al.* 2006).

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