Pak. J. Bot., 47(6): 2223-2230, 2015.

ASSESSMENT OF ECONOMIC BENEFITS OF FOLIARLY APPLIED OSMOPROTECTANTS IN ALLEVIATING THE ADVERSE EFFECTS OF WATER STRESS ON GROWTH AND YIELD OF COTTON (*GOSSYPIUM HIRSUTUM* L.)

SIBGHA NOREEN[2*], ZAFAR ULLAH ZAFAR[1], KAUSAR HUSSAIN[1], HABIB-UR-REHMAN ATHAR[1] AND M. ASHRAF[2]

[1]*Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan 60800, Pakistan*
[2]*Pakistan Science Foundation, Islamabad Pakistan*
[*]*Corresponding author: sibgha_noreen@yahoo.com*

Abstract

Water stress reduces crop growth and productivity by affecting various physiological and biochemical processes. Although foliar application of osmoprotectants alleviates the detrimental effects of drought stress growth and productivity of crops, its economic benefits on large scale has not been explored yet. The studies were carried out to quantify the interactive effects of some osmoprotectantsand various watering regimes on cotton crop. The treatments consisted of water stress and osmoprotectant applications [(a) two watering regimes (well watered, 2689m^3 water; drought stressed, 2078m^3), and (b) three osmoprotectants (untreated check; water spray containing 0.1 % Tween-80; salicylic acid (100 mg L^{-1}); proline (100 mg L^{-1}); glycine betaine (100 mg L^{-1})] in split plot design. The crop was subjected to drought stress at day 45 after sowing, i.e., at the flowering stage. The solutions of osmoprotectants were foliarly applied after two weeks of imposition of water stress (at the peak flowering stage). The results showed that imposition of water stress caused substantial reduction in plant growth, biological yield, fruit production, and fiber characteristics as compared to fully irrigated cotton crop. However, the application of osmoprotectants was found effective in off-setting the negative impacts of drought stress. The exogenous application of salicylic acid (100 mgL^{-1}) caused improvement by 47.9%, 36.5%, 17.4%, 4.86% and 9.9% in main stem height, biological yield, fruit production, fiber length and seedcotton yield over an untreated check, respectively. The efficiency of various osmoprotectants was in order of salicylic acid > glycinebetaine > proline in alleviating the harmful effects of drought stress. The usage of osmoprotectants was also found most cost-effective and the value for money. The cost-benefit ratio was 1:9.1, 1:3.9 and 1:1.7 by spraying of salicylic acid, proline and glycinebetaine, respectively. The research study reveals that salicylic acid may be foliarly applied to sustain growth, productivity, fiber characteristics and ultimately accruing higher profits under water stress environment.

Key words: Exogenous application; Salicylic acid; Proline; Glycinebetaine; Fiber quality; Yield components.

Introduction

The drought stress affects severely the growth, physiological processes and productivity of crop plants, in contrast to the compound effects produced by other environmental vagaries (Sinclair, 2011). It is estimated that drought stress lowered the crop yield by 50-73% in most of agricultural production zones (Langridge & Reynolds, 2015). Drought stressed plants experience oxidative stress resulting in generation of reactive oxygen species (superoxide, hydroxyl, hydrogen peroxide and alkoxy radicals) in response to drought condition (Apel & Hirt, 2004; Mittova *et al.*, 2015). These radical species could be scavenged simultaneously by stimulation of antioxidative battery system and accumulation of greater quantities of organic osmolytes in the plant system (Ashraf, 2010). The adversity of drought stress could be targeted by developing drought tolerant cultivars through conventional plant breeding techniques or through advanced genetic engineering tools (Ashraf *et al.*, 2011). However, the overexpression of traits for drought tolerance in most of crop plants is quite erratic under different ecologies (Ashraf *et al.*, 2008a; Todaka *et al.*, 2015). Alternatively, some of the compatible osmoprotectants have shown their potential usage for counteracting the stressful condition (Athar *et al.*, 2008; 2009a; 2015; Nazar *et al.*, 2015). There are growing evidences that exogenous application of osmoprotectants cause stimulation of growth and yield related physiological and biochemical processes (Ashraf *et al.*, 2008a; Noreen *et al.*, 2013; Khalid *et al.*, 2015). Thereby, the overproduction of osmolytes arrest the generation of reactive oxygen species (ROS) and resulting in sustain ability and the productivity of crops (Hu *et al.*, 2015; Uzilday *et al.*, 2015).

Among the various osmoprotectants, salicylic acid has been found to be potential endogenous growth regulator, which is vital for sustaining a number of physiological processes (Bandurska, 2013). The higher concentration of salicylic acid in the plant system, results in preventing reduction in of phytohormones and indole acetic acid with simultaneous greater accumulation of proline content (Janda & Ruelland, 2015). The foliar spray of salicylic acid has also been proved effective in enhancing the growth and productivity of many crop plants, such as okra, barley, maize, cotton, soybean, oilseed crops (Khan *et al.*, 2006; Waseem *et al.*, 2006; Arfan *et al.*, 2007; Athar *et al.*, 2009b; Nazar *et al.*, 2015). The growth and development parameters of cotton crop with respect to seedcotton yield, number of bolls per plant and fruit production were substantially improved by foliar spray of salicylic acid at the rate of 100 mg L^{-1} (Noreen *et al.*, 2013).

The glycinebetaine (quarternary ammonium compound) has been reported an effective chemical in stabilizing the structure of enzymes, lipids, photosynthetic apparatus and maintaining the osmotic adjustment under water stressful conditions (Chen & Murata, 2008;2011; Kurepin *et al.*, 2015). In most of the cases, sufficient quantity of glycinebetaine (GB) is not produced in the plant system and needs external application for greater accumulation to reduce the oxidative stress (Ashraf & Foolad, 2007). Various researchers have demonstrated that exogenous application of GB caused appreciable increase in vegetative and reproductive growth of different crops such as canola (Athar *et al.*, 2009a; 2015; Khalid *et al.*, 2015), maize, wheat (Ashraf *et al.*, 2008b) and cotton (Noreen *et al.*, 2013). Similarly, application of proline has shown its potential to induce abiotic stress tolerance in plants (Ali *et al.*, 2007; Ashraf & Foolad, 2007; Ashraf *et al.*, 2007; 2008a). However, efficiency of these osmoprotectants is quite differential in different crop species. It has been suggested that efficiency of osmoprotectants in inducing stress tolerance depends on growth stage at which it is applied, degree of water stress or prevailing soil-plant-atmosphere water continuum. Therefore, the present investigation was aimed to quantify the interactive effects of varying watering regimes and exogenously applied SA, GB and proline on growth and yield of cotton, particularly fiber quality in terms of economic benefits of exogenous application.

Materials and Methods

The field experiment was conducted at the Experimental Farm of Bahauddin Zakariya University Multan, Pakistan (30^o11N and 71^o28E, and an altitude of 123 meter above mean sea level). The temperature ranged from +30 ºC to +45ºC during the crop season. The treatments consisted of [(a) two watering regimes (well watered, 2689 m^3 water; drought stressed, 2078 m^3), and (b) three osmoprotectants (untreated check; water spray containg 0.1 % Tween-80; salicylic acid, 100 mg L^{-1}; proline, 100 mg L^{-1}; glycine betaine, 100 mg L^{-1}) and were arranged in a split plot design (main plot: watering regimes and sub-plot: osmoprotectants) with four replications. The solution of all osmoprotectants was prepared in 0.1% Tween-80 (poly ethylene sorbitol monolaurate, Sigma Aldrich, UK.) to enhance the penetration of osmoprotectants into the leaf tissues. Salicylic acid (2-hydroxybenzoic acid) was dissolved in 100 µl diamethylsulfoxide to ensure its solubility in water. A commercially grown cotton variety MNH-886 was used as a test crop. The plants were spaced at 75 cm between rows and 30 cm from plant to plant by maintaining the plant density of 45,000 plants ha^{-1}. The crop was fertilized with basal dose of fertilizers (nitrogen, phosphorous and potassium at the rate of 150 kg N, 50 kg $P_2O_5$and 50 kg K_2O ha^{-1}, respectively. The whole quantity of phosphorous, potassium and one-third nitrogen was broad cast and incorporated in the soil prior to sowing crop. The remaining quantity of nitrogen fertilizer was added in two equal splits, i-e., at flowering and peak flowering stages. The measured quantity of irrigation water was applied by using “Cut-Throat Flume” to irrigate various treatments. Crop was stressed to 60% field capacity at day 45 after sowing, i-e., at flowering stage. Thereafter, various osmoprotectants were foliarly applied after two weeks of imposition of water stress, i.e. at peak flowering stage. The solutions of osmoprotectants were sprayed with Knapsack sprayer using two- nozzles per row at the speed of 4.0 km h^{-1} and delivering 250 L ha^{-1} solution at 275 kPa pressure. The crop was protected against insect-pests by spraying the recommended dose of insecticides for controlling the insects at an economic threshold level (ETL) during the whole season. Data on plant structure, biological yield and fruit production were collected by harvesting of plants from one square meter area. The plant height was measured from cotyledonary node to the apex. The plants were dissected into leaves, stems and fruiting parts. The plant material was oven-dried at 70ºC to attain the constant weight. The biological yield was estimated according to formula (Wells & Meredith, 1984). At maturity, cotton crop was handpicked for two times and data on seedcotton yield calculated on an area bases. The various fiber characteristics were determined by employing High Volume Instrument (HVI) (Bednarz *et al.*, 2005). The economic analysis was done to quantify the cost- effectiveness of various osmoprotectants on cotton crop. Data were subjected to statistical analysis by using MSTAT software (Anon., 1989). In case, ANOVA permitted, the LSD was applied at 0.05 and 0.01 level of significance.

Results

Plant structure: Main stem height, number of nodes per plant and internodal length were significantly ($p<0.01$) reduced due to water stress and these attributes were increased by application of various osmoprotectants. However, the interactive effects of watering regimes and osmoprotectants remained statistically non-significant (Table 1). Data further revealed that the water stress caused reduction in plant height by11.06% compared to well watered crop. Of osmoprotectants, salicylic acid (SA) caused a maximum increase in plant height. The number of nodes per plant were decreased significantly ($p<0.01$) due to imposition of water stress. However, spray of osmoprotectants sustained the crop to maintain higher number of nodes compared to unsprayed crop. The foliar spray of salicylic acid (100 mg L^{-1}) caused increase in number of nodes by 4.68% higher than untreated check. Similarly, the intermodal length was increased in plants receiving osmoprotectants particularly by salicylic acid (100 mg L^{-1}) (Table 1)

Biological yield: Analysis of variance (ANOVA) of data depicted that biological yield at maturity significantly ($p<0.01$) varied by imposition of various watering regimes and spray of osmoprotectants at maturity. The interaction between watering regimes and osmoprotectants was found statistically non-significant (Table 2). The imposition of water stress at flowering stage resulted in reduction in total biological yield by 11.09% compared to well-watered crop at flowering stage. However, the foliar spray of various osmoprotectants, particularly salicylic acid (100 mg L^{-1}) caused improvement in biological yield by 36.49% over untreated check. All the treatments varied significantly ($p<0.01$) amongst each other with regard to spraying of osmoprotectants. Data further indicated that dry matter was less accumulated vegetative organ due to spraying of osmoprotectants, while greater quantity was accumulated in fruiting parts (Table 2).

Table 1. Interactive effects of watering regimes and exogenously applied osmoprotectants on plant structure of cotton crop at maturity.

Watering regime	Osmoprotectant					
	Untreated check	Water spray (0.1% Tween-80)	Salicylic acid (100 mg L^{-1})	Proline (100 mg L^{-1})	Glycine betaine (100 mg L^{-1})	Mean
	Main stem height (cm)					
Well watered	58.98	60.10	85.78	63.80	68.75	67.48
Drought stressed	51.08	52.86	76.93	60.83	62.15	60.76
Mean	54.99	56.49	81.35	62.31	65.45	
LSD ($p<0.05$): Watering regimes (W)= 6.42**; Osmoprotectants (O) = 6.76**; Interaction (WxO) = 8.15 ^{NS}						
	Number of nodes per plant					
Well watered	33	33	35	34	34	34
Drought stressed	31	32	33	33	33	33
Mean	32	32.5	33.5	33.5	33.5	
LSD ($p<0.05$): Watering regimes (W) =0.94**; Osmoprotectants (O) = 0.52**, Interaction (WxO) = 2.15 ^{NS}						
	Internodal length (cm)					
Well watered	1.79	1.81	2.44	1.998	1.98	2.00
Drought stressed	1.59	1.64	2.27	1.83	1.87	1.84
Mean	1.69	1.72	2.35	1.91	1.92	
LSD ($p<0.05$): Watering regimes (W)= 0.27**, Osmoprotectants(O) = 0.23**, Interaction (WxO) = 0.32 ^{NS}						

Table 2. Interactive effects of watering regimes and exogenously applied osmoprotectants on biological yield (gm^{-2}) of cotton crop at maturity.

Watering regime	Osmoprotectant					
	Untreated check	Water spray (0.1% Tween-80)	Salicylic acid (100 mg L^{-1})	Proline (100 mg L^{-1})	Glycine betaine (100 mg L^{-1})	Mean
	Plant organ: Leaves					
Well watered	68.20	74.25	84.85	76.83	78.53	76.44
Drought stressed	64.10	69.53	71.25	60.83	70.15	67.17
Mean	66.15	71.80	78.05	68.60	74.34	
LSD ($p<0.05$): Watering regimes (W) =0.97**; Osmoprotectants(O) = 3.38**; Interaction (WxO) = 4.79						
	Plant organs: Stems					
Well watered	198.60	186.63	138.85	160.80	163.95	169.77
Drought stressed	170.83	180.93	128.13	139.80	155.05	154.95
Mean	184.71	183.78	193.49	150.30	159.50	
LSD ($p<0.05$): Watering regimes (W)= 8.59 **; Osmoprotectants(O) = 7.48**; Interaction (WxO) = 10.57 ^{N}						
	Plant organ: Fruits					
Well watered	390.08	400.40	615.0	451.03	440.83	459.47
Drought stressed	300.15	310.18	589.93	401.23	435.65	407.43
Mean	345.1	365.29	602.46	426.13	438.24	
LSD ($p<0.05$): Watering regimes (W)= 1.88**; Osmoprotectants (O) = 14.98**; Interaction (WxO) = 21.18 ^{NS}						
	Total					
Well watered	656.88	661.23	838.00	688.20	683.30	705.64
Drought stressed	535.15	560.83	789.30	601.85	689.15	635.20
Mean	596.01	611.03	813.50	645.03	686.23	
LSD ($p<0.05$) Watering regimes (W)= 20.22**; Osmoprotectants (O) = 26.11**; Interaction (WxO) = 36.93 ^{NS}						

Fruit production: The statistical analysis of data revealed that number of total fruiting positions, number of total intact fruit and fruit shedding percentage parameters were significantly ($p<0.01$) affected due to imposition of water stress and spraying of various osmoprotectants on cotton crop. However, the interactive effects of these two factors were statistically non-significant (Table 3). The imposition of water stress caused reduction in number of total fruiting positions by 11.31% compared of well-watered crop. On the other hand, number of total fruiting positions was improved significantly by spray of various osmoprotectants. The maximum (640) number of fruiting positions m^{-2}) were produced by crop sprayed with salicylic acid (100 mg L^{-1}) , while the minimum (545 number of fruiting positions m^{-2}) under untreated check. Similarly, the number of total intact fruit m^{-3} was also reduced in crop subjected to water stress condition. The crop retained 10.86% lower number of fruit with respect to well watered crop. Furthermore, the crop sprayed with various osmoprotectants retained higher number of fruit per unit area compared to untreated check. The maximum (193 number of total intact fruit m^{-2}) was retained by crop sprayed with salicylic acid (100 mg L^{-1}), while the maximum (118 number of total intact fruit m^{-2}) under untreated check with regard to fruit shedding percentage. The crop subjected to water stress shed high proportion of fruit compared to well-watered crop. However, fruit shedding percentage decreased by foliar spray of osmoprotectants. The minimum (69.1 fruit shedding percentage) was recorded in crop sprayed with salicylic acid (100 mg L^{-1}) while, maximum (78.4 fruit shedding percentage) under unsprayed crop (Table 3).

Table 3. Interactive effects of watering regimes and exogenously applied osmoprotectants on fruit production of cotton crop at maturity.

Watering regime	Osmoprotectant					
	Untreated check	Water spray (0.1% Tween-80)	Salicylic acid (100 mg L^{-1})	Proline (100 mg L^{-1})	Glycine betaine (100 mg L^{-1})	Mean
	Number of total fruiting positions (m^{-2})					
Well watered	560	589	680	600	619	610
Drought stressed	530	520	600	540	550	548
Mean	545	555	640	570	585	
LSD (p<0.05): Watering regimes (W)= 36.71**; Osmoprotectants (O) = 25.92**; Interaction (WxO) = 36.86 ^{NS}						
	Number of total intact fruit (m^{-2})					
Well watered	125	140	200	150	150	153
Drought stressed	110	115	185	140	140	138
Mean	118	128	193	145	145	
LSD (p<0.05): Watering regimes (W)= 12.46**; Osmoprotectants (O) = 15.54**; Interaction (WxO) = 21.97 ^{N}s						
	Fruit shedding percentage					
Well watered	77.6	76.3	69.2	74.9	75.8	74.8
Drought stressed	79.2	77.8	69.1	74.7	74.5	75.1
Mean	78.4	77.0	69.1	74.8	75.2	
LSD (p<0.05): Watering regimes (W) =3.21**; Osmoprotectants (O) = 3.05**;Interaction (WxO) = 4.31 ^{NS}						

Table 4. Interactive effects of watering regimes and exogenously applied osmoprotectants on seedcotton yield and yield components of cotton crop.

Watering regime	Osmoprotectant					
	Untreated check	Water spray (0.1% Tween-80)	Salicylic acid (100 mg L^{-1})	Proline (100 mg L^{-1})	Glycine betaine (100 mg L^{-1})	Mean
	Seedcotton yield (kg ha^{-1})					
Well watered	3368	3450	3690	3610	3600	3544
Drought stressed	3210	3250	3540	3375	3396	3354
Mean	3289	3350	3615	3492	3498	
LSD (p<0.05): Watering regimes (W) =70.13**; Osmoprotectant (O) = 83.78**; Interaction (WxO) = 118.40 ^{NS}						
	Number of bolls per plant					
Well watered	33	34	38	37	37	36
Drought stressed	32	32	39	34	34	34
Mean	33	33	39	36	36	
LSD (p<0.05): Watering regimes (W) =1.81**;Osmoprotectants (O) = 1.97**; Interaction (WxO) = 2.97 ^{NS}						
	Boll weight (g)					
Well watered	3.10	3.18	3.50	3.23	3.30	3.26
Drought stressed	2.70	2.75	3.45	3.90	2..96	2.95
Mean	2.90	2.97	3.48	3.07	3.13	
LSD (p<0.05): Watering regimes (W)= 0.17**;Osmoprotectants (O) = 1.07**; Interaction (WxO) = 2.97 ^{NS}						
	Lint percentage					
Well watered	35.10	35.80	39.30	36.20	36.80	36.64
Drought stressed	34.00	33.98	38.28	35.35	35.88	35.50
Mean	34.56	34.89	38.79	35.78	36.34	
LSD (p<0.05): Watering regimes (W) =0.56**;Osmoprotectants (O) = 0.86**; Interaction (WxO) = 1.20 ^{NS}						

Yield and yield component: Analysis of variance of data indicated that yield and yield components differed significantly (p<0.01) due to watering regimes and spray of various osmoprotectants. However, interactive effects of these two factors were found statistically non-significant (Table 4). Data further revealed that imposition of water stress caused reduction in seedcotton yield by 5.66 % against the well watered crop. However, the productivity was improved appreciably by application of osmoprotectants. The maximum (3615 kg ha^{-1}) seedcotton yield was harvested from crop treated with salicylic acid (100 mg L^{-1}), while minimum (3289 kg ha^{-1}) under untreated check. The crops sprayed with salicylic acid, glycinebetaine and proline produced seedcotton of 3615, 3498 and 3492 kg ha^{-1}, respectively. The values of seedcotton ranged from 3210 to 3690 kg ha^{-1} in various treatments. The increase in seedcotton yield resulted due to significant enhancement in number of bolls per plant and boll weight by application of various osmoprotectants (Table 4).

The number of bolls per plant was also reduced by 5.88% by imposition of water stress in comparison with well-watered crop. However, crop sprayed with various osmoprotectants retained higher number of bolls per plant as compared to untreated check. The maximum (39 number of bolls) were produced by crop treated with salicylic acid (100 mg L^{-1}), and the minimum (33 number of bolls per plant) under untreated check. Analogous to these parameters, the boll weight was also reduced by 10.50% in crop subjected to water stress compared to well-watered crop. On the other hand, there was substantial enhancement in boll weight by spraying of various osmoprotectants. The maximum (3.48 g) was obtained in crop treated with salicylic acid (100 mg L^{-1}), while minimum (2.90 g) under unsprayed crop. The yield parameters regarding lint percentage was also affected significantly due to imposition of water stress and spraying of osmoprotectants. The water stress caused reduction in lint percentage by 3.21% with respect to well-watered crop. The maximum (38.79 lint percentage) was found in crop treated with salicylic acid (100 mg L^{-1}), while minimum (34.56 lint percentage) under untreated check (Table 4).

Table 5. Relationships between main stem height and biological yield and fruit production under varying watering regimes at maturity.

Independent variable	Dependent variable	Watering regimes	Regression equation	Correlation of coefficient (r)	Coefficient of determination (R^2)
Main stem height (cm)	Total dry weight (g m^{-2})	Well watered	Y= 5.80x+314.21	0.90***	0.80
		Drought stressed	Y=6.99x+209.17	0.75**	0.57
Main stem height (cm)	Fruit dry weight (g m^{-2})	Well watered	Y=7.04x+15.78	0.91**	0.84
		Drought stressed	Y=8.51x+113.9	0.85**	0.72
Main stem height (cm)	Number of total fruit (m^{-2})	Well watered	Y=3.55x+370.19	0.81**	0.66
		Drought stressed	Y=2.37x+402.96	0.70**	0.49
Main stem height (cm)	Number of intact fruit (m^{-2})	Well water	Y=2.18x+5.63	0.79**	0.62
		Drought stressed	Y=2.34x-5.70	0.85**	0.72

Table 6. Relationships between biological yield and fruit production and seedcotton seed yield under varying watering regimes at maturity.

Independent variable	Dependent variable	Watering regimes	Regression equation	Correlation of coefficient (r)	Coefficient of determination (R^2)
Total biological yield (gm^{-2})	Number of total fruit (m^2)	Well watered	Y=0.54x+229.06	0.80**	0.63
		Drought stressed	Y=0.26x+381.56	0.71**	0.51
Vegetative dry weight (gm^{-2})	Seedcotton yield (kg ha^{-1})	Well watered	Y= -2.32x+3970.2	0.40ns	0.16
		Drought stressed	Y= -3.34x+4091.6	0.54*	0.30
Reproductive dry weight (g m^{-2})	Seedcotton yield (kg/ha^{-1})	Well watered	Y=0.80x+3031.5	0.65**	0.42
		Drought stressed	Y=1.00x+2936.6	0.81**	0.65
Total biological yield (g m^{-2})	Seedcotton yield (kg ha^{-1})	Well water	Y=0.98x+2706.6	0.67**	0.45
		Drought stressed	Y=1.03x+2691.4	0.76**	0.58

The correlation co-efficient between main stem height and total dry weight, fruit dry weight, number of total fruit and number of intact fruit showed positive relationships under well waterd and drought stressed conditions. However, the relationship between main stem height and number of intact fruit was negatively correlated under drought stress environment. A high degree of correlation (r= 0.91**) was measured between main stem height and dry fruit weight under well watered crops. Similarly, regression analysis indicated a highly significant relationship (r=0.90**) due to increased main stem height and total dry weight under well-watered conditions followed by correlation of co-efficient (r= 0.85**) between main stem height and fruit dry weight under drought stress conditions (Table 5).

Analogous to existence of these positive relationships, correlation coefficient between total biological yield, vegetative dry weight, reproductive dry weight and number of total fruit, seedcotton yield were found highly positive under both well watered and drought stress conditions (Table 6). However, the relationship between vegetative dry weight and seedcotton yield remained statistically non-significant under well-watered conditions. A high degree of correlation (r= 0.81**) was measured between reproductive dry weight and seedcotton under drought stress, followed by (r=0.80**) between total biological yield and number of total fruit under well-watered conditions. The positive correlations signify that close relationships exist between total biological yield, particularly, due to greater proportion of reproductive organs towards production of higher seedcotton yield under both well watered and drought stress conditions (Table 6).

The increased production of total fruiting production and greater retention capacity under water stress conditions resulted in positive correlations (Table 7). A high degree of correlations (r = 0.80**, 0.74**, 0.72** and 0.64**) were found between seedcotton yield and total intact fruit under drought stress, total intact fruit under well watered, fruit shedding percentage under drought stress and total fruiting positions under drought stress, respectively. The crop subjected to water-stress maintained greater quantity of fruit and withstood against excessive fruit shedding which resulted in higher production of seedcotton yield (Table 7).

Fiber characteristics: The quality parameters of cotton lint were greatly influenced by application of various osmoprotectants compared to watering regimes (Table 8). There was a little improvement in quality parameters due to interactive effects of watering regimes and osmoprotectants. The spray of osmoprotectants caused substantial improvement in fiber length. Maximum (30.2 mm) fiber length was produced by crop sprayed with salicylic acid (100 mg L^{-1}), while minimum (28.8mm) under untreated check. Furthermore, the fiber fineness was improved by spraying osmoprotectants. The spray of salicylic acid (100 mg L^{-1}) increased fiber fineness by 10.52% with respect to unsprayed crop. Similarly, the fiber strength was also enhanced by spray of osmoprotectants. The maximum (99.0 thousand pounds per square inch) was recorded in lint produced by crop sprayed with salicylic acid (100 mg L^{-1}), while the minimum (94.0 thousand pounds per square inches) under untreated check. Similarly, the fiber uniformity ratio was improved by spraying of osmoprotectants. The spray of salicylic acid (100 mg L^{-1}) caused enhancement in fiber uniformity ratio by 7.5% compared to untreated check (Table 8).

Economic analysis: The economic analysis of data revealed that spray of osmoprotectants is cost-effective and value for money for pocketing higher income from cotton crop under water stress condition. The maximum value cost ratio (1:9.1) was recorded by spraying of crop with salicylic acid (100 mg L^{-1}) followed by proline (1:3.9) and glycine betaine (1:1.7) (Table 9).

Table 7. Relationships between fruit production and seedcotton yield under varying watering regimes at maturity.

Independent variable	Dependent variable	Watering regimes	Regression equation	Correlation of coefficient (r)	Coefficient of determination (R^2)
Total fruiting positions (m^{-2})	Seedcotton Yield (kg ha^{-1})	Well watered	Y=1.33x=2587.8	0.62**	0.38
		Drought stressed	Y=2.38x+2045.4	0.64**	0.42
Total Intact fruit (m^{-2})	Seedcotton yield (kg ha^{-1})	Well watered	Y=.50x+3015.0	0.74**	0.54
		Drought stressed	Y=3.62x+2848.9	0.80**	0.63
Fruit shedding (% age)	Seedcotton yield (kg ha^{-1})	Well watered	Y= -14.47x+4478.9	0.59**	0.34
		Drought stressed	Y= -22.25x+5020.1	0.72**	0.52

Table 8. Interactive effects of watering regimes and exogenously applied osmoprotectants on fibre characteristics of cotton crop.

Watering regime	Osmoprotectant					
	Untreated check	Water spray (0.1% Tween-80)	Salicylic acid (100 mg L^{-1})	Proline (100 mg L^{-1})	Glycine betaine (100 mg L^{-1})	Mean
	Fibre length (mm)					
Well watered	29.0	29.4	30.4	29.6	29.8	29.6
Drought stressed	28.5	28.7	30.0	29.1	29.2	29.1
Mean	28.8	29.1	30.2	29.4	29.5	
	Fibre fineness (µg $inch^{-1}$)					
Well watered	4.0	4.0	3.7	4.1	3.8	3.9
Drought stressed	4.3	4.4	3.9	4.3	4.0	4.2
Mean	4.2	4.2	3.8	4.4	3.7	
	Fibre strength (thousand pounds per square inch)					
Well watered	93.4	94.0	98.2	97.6	97.6	96.2
Drought stressed	94.5	95.0	99.8	98.0	98.0	97.1
Mean	94.0	94.5	99.0	97.8	97.8	
	Fibre uniformity ratio					
Well watered	44.3	45.8	48.6	47.3	47.9	46.8
Drought stressed	43.6	43.9	46.0	44.0	44.0	44.3
Mean	44.0	44.9	47.3	45.7	46.0	
	Reflectance degree of fibre (Rd%)					
Well watered	71.6	72.8	76.3	74.5	74.9	74.0
Drought stressed	72.1	72.3	78.9	75.2	75.8	74.9
Mean	71.9	72.6	77.6	74.9	75.4	

Table 9. Economic analysis of exogenously applied osmoprotectants in improving cotton yield under water stress.

Watering regime	Osmoprotectant					
	Untreated check	Water spray (0.1% Tween-80)	Salicylic acid (100 mg L^{-1})	Proline (100 mg L^{-1})	Glycine betaine (100 mg L^{-1})	Mean
	Increase in seedcotton over control (kg ha^{-1})					
Well watered	--	82	322	242	232	220
Drought stressed	--	40	330	165	186	180
Mean	--	61	326	203	209	
	Cost of osmoprotectants (Rs ha^{-1})					
Well watered	--	500	2,500	3,700	8,500	3350
Drought stressed	--	500	2,500	3,700	8,500	3350
Mean	--	500	2,500	3,700	8,500	
	Value of increased seedcotton yield (Rs ha^{-1})					
Well watered	--	5,740	22,540	16,940	16,240	15,365
Drought stressed	--	2,800	23,100	11,550	13,020	12,617
Mean	--	2,470	22,840	14,245	14,630	
	Net profit (Rs ha^{-1})					
Well watered	--	5,240	20,040	13,240	7,740	12,015
Drought stressed	--	2,300	20,600	7,850	4,520	9,203
Mean	--	3,770	20,320	10,545	6,130	

Discussion

The prolonged or intermittent water supply during the growth of most of crop plants exerts potential risk for profitable production of cotton crop across different ecologies. The results of the present study indicated that plant structure was greatly affected by water stress. However, the foliar spray of osmoprotectants stimulated crop plants to withstand against water stress. The growth is a progressive function of cell division, differentiation and progression with the ontogeny of plant species. The foliar spray of osmoprotectants resulted in increase in accumulation of these compounds in the plant system and promoted growth related physiological and developmental activities. For example, the impairment occurred by oxidative stress due to water stressful condition was scavenged by overproduction and greater accumulation of osmolytes thereby resulting in increased growth. The similar results have been reported in wheat (Athar *et al.*, 2008; 2009b), canola (Athar *et al.*, 2015) and mazie (Ali *et al.*, 2007). The protective and growth promotive effect of salicylic acid, glycine betaine and proline was also reported by other researchers (Bandurska, 2013; Kurepin *et al.*, 2015; Wutipraditkul *et al.*, 2015). The spray of osmoprotectants caused significant increase in enhancement in total number of fruiting positions and their retention per unit land area, however, these parameters witnessed reduction due to water stress environment. On the other hand, productivity of fruiting positions and retention capacity was significantly elevated by spraying of osmoprotectants. The reason being that accumulation of greater quantities of osmolytes simulated the cotton crop to withstand the stressful conditions through rapid translocation of assimilates from vegetative to reproductive organs (Arfan *et al.*, 2007). The greater retention of fruits by crop treated with osmoprotectants maintained lower fruit shedding percentage compared to untreated check. Thus, productivity of crop could be enhanced by foliar spray of salicylic acid, glycinebetaine and proline compounds.

The seedcotton yield and components of yield were significantly ($p<0.01$) affected by watering regimes and various osmoprotectants. The reduction in seedcotton yield due to water stress occurred by 5.6% compared to well-watered crop. However, the application of salicylic acid at the rate of 100 mg L^{-1}caused improvement in yield by 9.9% over untreated check. Similarly, the number of bolls per plant, boll weight and lint percentage parameters were also severely affected by water stress. However, values of these parameters were increased by spraying of osmoprotectants. The efficiency of osmoprotectants in sustaining the parameters of yield was in order of salicylic acid>glycinebetaine>proline. Efficacy of different osmoprotectants depends on type of plant species and growth conditions as has described earlier (Kurepin *et al.*, 2015; Nazar *et al.*, 2015; Wutipraditkul *et al.*, 2015).

The existence of positive correlations between total production and retention of fruit and seedcotton yield signify that sustainability of cotton crops could be achieved by enhancing the components of yield. The lint quality of cotton was affected to a little extent by imposition of water stress. The lint quality parameters such as fiber length, fiber fineness and fiber strength were improved by application of various osmoprotectants. The spray of salicylic acid at the rate of 100 mg L^{-1}caused improvement in fiber length, fiber fineness and fiber strength by 3.78%, 9.52%, 4.76%, respectively over untreated check. The improvement in fiber quality resulted due to higher translocation of photosynthates from source (vegetative organs) to sink (reproductive organs) due to greater accumulation of osmolytes and soluble sugars by spraying various osmoprotectants (Raza *et al.*, 2006; Raza *et al.*, 2007). The overproduction and greater accumulation of osmolytes stimulated the cotton crop to withstand drought conditions.

The economic analysis indicated that foliar application of osmoprotectants is the potential, cost effective and value for money option to grow cotton crop successfully under limited water supply as suggested by other researchers (Ashraf *et al.*, 2011; Chen & Murata, 2011; Bandurska, 2013). The results of this study have shown that exogenous application of salicylic acid at the rate of 100 mg L^{-1} could sustain production of cotton crop under water stress conditions. The foliar spray of salicylic acid is more cost-effective and value for money, because, cotton farmers are already fairly accustomed to spray cotton crop to control insect-pests during the season. Farmers could pocket substantial income by foliar spray of salicylic acid, glycinebetaine and proline on cotton crop.

Acknowledgements

The authors are thankful to the Higher Education Commission (HEC) of Pakistan for its financial support to undertake this research study under the project No. PM-IPFP/HRD/HEC/2011/2255.

References

Ali, Q., M. Ashraf and H.-u.-R. Athar. 2007. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. *Pak. J. Bot.*, 39: 1133-1144.

Anonymous. 1989. MSTAT User's Guide, a micro computer program for the design management and analysis of agronomic research experiments. Michigan State University, USA.

Apel, K. and H. Hirt. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.*, 55: 373-399.

Arfan, M., H.R. Athar and M. Ashraf. 2007. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *J. Plant Physiol.*, 164: 685-694.

Ashraf, M. 2010. Inducing drought tolerance in plants: Recent advances. *Biotechnol. Adv.*, 28: 169-183.

Ashraf, M., N.A. Akram, F. Al-Qurainy and M.R. Foolad. 2011. Drought tolerance: roles of organic osmolytes, growth regulators, and mineral nutrients. In: *Adv. Agron.* (Ed.): Donald, L.S. Academic Press, pp. 249-296.

Ashraf, M., H.R. Athar, P.J.C. Harris and T.R. Kwon. 2008a. Some prospective strategies for improving crop salt tolerance. *Adv. Agron.*, 97: 45-110.

Ashraf, M. and M.R. Foolad. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.*, 59: 206-216.

Ashraf, M., K. Nawaz, H.-u.-R. Athar and S.H. Raza. 2008b. Growth enhancement in two potential cereal crops, maize and wheat, by exogenous application of gllycnnnebetainne. In: *Biosaline Agriculture and High Salinity Tolerance.* (Eds.): Abdelly, C., M. Öztürk, M. Ashraf and C. Grignon. Birkhäuser Basel, Basel, Switzerland, pp. 21-35.

Ashraf, M., S. Nawazish and H.-u.-R. Athar. 2007. Are chlorophyll fluorescence and photosynthetic capacity potential physiological determinants of drought tolerance in maize (*Zea mays* L.). *Pak. J. Bot.*, 39: 1123-1131.

Athar, H.-u.-R., M. Ashraf, A. Wahid and A. Jamil. 2009a. Inducing salt tolerance in canola (*Brassica napus* L.) by exogenous application of glycinebetaine and proline: Response at the initial growth stages. *Pak. J. Bot.*, 41: 1311-1319.

Athar, H.-u.-R., A. Khan and M. Ashraf. 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environ. Exp. Bot.*, 63: 224-231.

Athar, H.-u.-R., A. Khan and M. Ashraf. 2009b. Inducing salt tolerance in wheat by exogenously applied ascorbic acid through different modes. *J. Plant Nutr.*, 32: 1799-1817.

Athar, H.-u.-R., Z.U. Zafar and M. Ashraf. 2015. Glycinebetaine improved photosynthesis in canola under salt stress: Evaluation of chlorophyll fluorescence parameters as potential indicators. *J. Agron. Crop Sci.*, 201: 428-442.

Bandurska, H. 2013. Salicylic acid: an update on biosynthesis and action in plant response to water deficit and performance under drought. In: *Salicylic Acid: Plant growth and development.* (Eds.): Hayat, S., A. Ahmad and M.N. Alyemeni. Springer Netherlands, The Netherlands, pp. 1-14.

Bednarz, C.W., W.D. Shurley, W.S. Anthony and R.L. Nichols. 2005. Yield, quality, and profitability of cotton produced at varying plant densities. *Agron. J.*, 97: 235-240.

Chen, T.H. and N. Murata. 2008. Glycinebetaine: an effective protectant against abiotic stress in plants. *Trends Plant Sci.*, 13: 499-505.

Chen, T.H. and N. Murata. 2011. Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant, Cell Environ.*, 34: 1-20.

Hu, Y., B. Wang, T. Hu, H. Chen, H. Li, W. Zhang, Y. Zhong and H. Hu. 2015. Combined action of an antioxidant defence system and osmolytes on drought tolerance and post-drought recovery of Phoebe zhennan S. Lee saplings. *Acta Physiol Plant*, 37: 1-13.

Janda, M. and E. Ruelland. 2015. Magical mystery tour: Salicylic acid signalling. *Environ. Exp. Bot.*, 114: 117-128.

Khalid, A., H.-u.-R. Athar, Z.U. Zafar, A. Akram, K. Hussain, H. Manzoor, F. Al-Qurainy and M. Ashraf. 2015. Photosynthetic capacity of canola (*Brassica napus* L.) plants as affected by glycinebetaine under salt stress. *J. Appl. Bot. Food Qual.*, 88: 78-86.

Khan, A., M.S.A. Ahmad, H.U.R. Athar and M. Ashraf. 2006. Interactive effect of foliarly applied ascorbic acid and salt stress on wheat (*Triticum aestivum* L.) at the seedling stage. *Pak. J. Bot.*, 38: 1407-1414.

Kurepin, L., A. Ivanov, M. Zaman, R. Pharis, S. Allakhverdiev, V. Hurry and N.A. Hüner. 2015. Stress-related hormones and glycinebetaine interplay in protection of photosynthesis under abiotic stress conditions. *Photosynthesis Res.*, 126: 221-235.

Langridge, P. and M.P. Reynolds. 2015. Genomic tools to assist breeding for drought tolerance. *Curr. Opin. Biotechnol.*, 32: 130-135.

Mittova, V., M. Volokita and M. Guy. 2015. Antioxidative systems and stress tolerance: insight from wild and cultivated tomato species. In: *Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants.* (Eds.): Gupta, K.J. and A.U. Igamberdiev. Springer International Publishing, Switzerland, pp. 89-131.

Nazar, R., S. Umar, N.A. Khan and O. Sareer. 2015. Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. *S. Afr. J. Bot.*, 98: 84-94.

Noreen, S., H.-u.-R. Athar and M. Ashraf. 2013. Interactive effects of watering regimes and exogenously applied osmoprotectants on earliness indices and leaf area index in cotton (*Gossypium hirsutum* L.) crop. *Pak. J. Bot.*, 45: 1873-1881.

Raza, S.H., H.R. Athar, M. Ashraf and A. Hameed. 2007. Glycinebetaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. *Environ. Exp. Bot.*, 60: 368-376.

Raza, S.H., H.U.R. Athar and M. Ashraf. 2006. Influence of exogenously applied glycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. *Pak. J. Bot.*, 38: 341-351.

Sinclair, T.R. 2011. Challenges in breeding for yield increase for drought. *Trends Plant Sci.*, 16: 289-293.

Todaka, D., K. Shinozaki and K. Yamaguchi-Shinozaki. 2015. Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants. *Frontiers in Plant Science*, 6.

Uzilday, B., R. Ozgur, A.H. Sekmen, E. Yildiztugay and I. Turkan. 2015. Changes in the alternative electron sinks and antioxidant defence in chloroplasts of the extreme halophyte Eutrema parvulum (*Thellungiella parvula*) under salinity. *Ann. Bot.*, 115: 449-463.

Waseem, M., H.U.R. Athar and M. Ashraf. 2006. Effect of salicylic acid applied through rooting medium on drought tolerance of wheat. *Pak. J. Bot.*, 38: 1127-1136.

Wells, R. and W.R. Meredith. 1984. Comparative growth of obsolete and modern cotton cultivars. II. Reproductive dry matter partitioning. *Crop Sci.*, 24: 863-868.

Wutipraditkul, N., P. Wongwean and T. Buaboocha. 2015. Alleviation of salt-induced oxidative stress in rice seedlings by proline and/or glycinebetaine. *Biol. Plant.*, 59: 547-553.

(Received for publication 24 November 2014)