

## INTERACTIVE EFFECTS OF WATERING REGIMES AND EXOGENOUSLY APPLIED OSMOPROTECTANTS ON EARLINESS INDICES AND LEAF AREA INDEX IN COTTON (*GOSSYPIUM HIRSUTUM L.*) CROP

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### Abstract

Drought is one of the major factors limiting crop production in an arid environment. The exogenous application of osmoprotectants has been found effective in reducing the adverse effects of drought stress on plant growth. A field experiment was conducted to quantify the interactive effects of water stress and exogenously applied salicylic acid, glycinebetaine and proline on cotton (*Gossypium hirsutum L.*) (cv MHN-886). The treatments included [(a) two watering regimes (well-watered 2689 m<sup>3</sup> water; drought stressed 2078 m<sup>3</sup> water) and (b) three osmoprotectants (untreated check; spray of 0.1% Tween-80 solution; salicylic acid 100 mg L<sup>-1</sup>, proline 100 mg L<sup>-1</sup>, glycinebetaine 100 mg L<sup>-1</sup>) and arranged in a split-plot design with four replications. The water stress was imposed at day 45 after sowing i.e. at the flowering stage. The chemicals were sprayed after two weeks of imposition of water stress conditions at peak flowering stage. The results showed that water stress caused an appreciable reduction in growth and yield attributes. However, more than 75% of the total seed cotton yield was gathered at first pick under drought stressed condition compared to well-watered crop. The foliar spray of salicylic acid proved its potential to a far greater extent compared to proline and glycinebetaine. The spray of glycinebetaine was comparatively more effective in improving earliness indices than proline in cotton crop. The research study reveals that salicylic acid and glycinebetaine may be foliarly applied to sustain cotton production under drought stressed ecologies.

### Introduction

Among arable crops of Pakistan, cotton (*Gossypium hirsutum L.*) is an economic engine for sustaining country's economy. The export of raw and finished products of cotton contributes >60% of the foreign exchange earnings and provides livelihood to 45% of the population. It is being cultivated on an area of 3.2 million hectares, with production of 12.9 million bales (1 bale equals to 170 kg lint) (Anon., 2013). Pakistan has experienced considerable fluctuation in cotton production over the last three decades due to increased infestation of insect pests, diseases and more particularly the non-availability of irrigation water at sowing time and critical stages of growth. The downhill trend in the availability of irrigation water is 103.5 million acre feet (MAF) during 2000-01 to 89.6 MAF during 2012-13, showing a reduction by 13.4 percent over the historical supply (Anon., 2013).

The environmental factors produce far greater extent on adaptation of crop plants than any other external cues. Of these, irrigation water and temperature stresses are the determining factors, which prospect for the growth and development of plants (Morgan, 1984). The plants strive to make osmotic adjustments in cells through increase in inorganic ions or organic solutes in response to decreased water availability (Chaves *et al.*, 2009). Under water stress, plants accumulate greater quantity of compatible organic solutes, which shield them from stress through stabilizing of membranes, tertiary structures of enzymes and proteins (Ashraf & Foolad, 2007; Kanwal *et al.*, 2013). A number of plant hormones offer their potential services to mitigate the adverse effects of water stress on plant growth and development (Shakirova *et al.*, 2003; Abbas *et al.*, 2013). Plant growth regulating substances such as auxins, salicylic acid, glycinebetaine and proline

influence the plant growth by regulating a number of physiological and biochemical processes (Ashraf *et al.*, 2008; Qureshi *et al.*, 2013). The spray of these signal molecules induces defense response in crops against reactive oxygen species (ROS) produced due to biotic and abiotic stresses (Arfan *et al.*, 2007).

Glycinebetaine (GB) and proline, compatible osmolytes, are being used as foliar spray to improve water stress and sustain the productivity of arable crops (Agboma *et al.*, 1997a; Ali *et al.*, 2007). Various researchers reported a positive response to crops in alleviating water stress in maize and sorghum (Agboma *et al.*, 1997a; 1997b) and cotton (Gorham & Jokinen, 1999; Makhdum *et al.*, 2006). Farooq *et al.*, (2009) found that foliar application of glycinebetaine at the rate of 100 mg L<sup>-1</sup> at 5-leaf stage improved drought tolerance in rice crop both under well-watered and stressed conditions. Apart from enhancement in growth and development of various crop plants (El-Tayab, 2005), the foliar applied salicylic acid (SA) improved grain yield of mungbean (Singh & Kaur, 1980), sunflower (Noreen, 2010), leaf area duration in sugarcane (Zhou *et al.*, 1999), photosynthetic rate (Waseem *et al.*, 2006) and leaf area index in cotton (Makhdum *et al.*, 2006). A strong evidence exists that external application of SA enhances tolerance in plants exposed to abiotic stresses including osmotic stress, drought and salinity through maintaining redox potential and photosynthetic activity (Ashraf, 2010).

The vagaries of water stress could be mitigated by cultivation of drought tolerant cotton varieties bred through classical and/or genetic engineering techniques. Development of drought tolerant varieties is a long process and needs a heavy investment in both capital and human resources. The exogenous application of osmoprotectants / antioxidants has been considered as a shotgun approach to withstand the ill-effects of drought

stress. Therefore, a field study was undertaken to quantify the interactive effects of watering regimes and exogenous application of osmoprotectants viz., salicylic acid, proline and glycinebetaine on cotton crop under an arid environment.

## Materials and Methods

A field study was undertaken at the Experimental Field Station of Bahauddin Zakariya University, Multan to quantify the interactive effects of watering regimes and exogenous application of applied osmoprotectants under an arid environment on earliness indices and leaf area index of cotton crop during the crop season 2011-12. The treatments included (a) two watering regimes: well-watered  $2689\text{ m}^3$  water; drought stressed  $2078\text{ m}^3$  water; and three osmoprotectants: untreated check, 0.1% Tween-80 solution, salicylic acid  $100\text{ mg L}^{-1}$ ; proline  $100\text{ mg L}^{-1}$  and glycinebetaine  $100\text{ mg L}^{-1}$ . The treatments were arranged in a split-plot design (main plot: watering regimes and sub-plot: osmoprotectants) with four replications. The solution of osmoprotectants was prepared in 0.1% Tween-80 solution (Polyoxyethylenesorbitan monolaurate, Sigma Chemicals, UK). Salicylic acid (2-hydroxybenzoic acid) was dissolved in  $100\text{ }\mu\text{l}$  diamethyl sulfoxide. A commercial cotton variety, MNH-886 was used as a test crop. Crop was subjected to drought stress (at 60% field capacity) at day 45 after sowing, i.e., at flowering stage. The chemicals were sprayed after two weeks of imposition of water stress treatments i.e. at peak flowering stage. The measured quantity of irrigation water was applied using "Cut-Throat-Flume" to different treatments. The crop was sprayed with knapsack sprayer using two-nozzles per row, at speed of  $4.0\text{ km ha}^{-1}$  and delivering  $250\text{ L ha}^{-1}$  solution at  $275\text{ kPa}$  pressure.

The planting density at 45,000 plants  $\text{ha}^{-1}$  was maintained by spacing plants at 75 cm between rows and 30 cm from plant to plant. The basal dose of fertilizers i.e., nitrogen, phosphorus, and potassium was applied at the rate of 150 kg N, 50 kg  $\text{P}_2\text{O}_5$  and 50 kg  $\text{K}_2\text{O}$   $\text{ha}^{-1}$ , respectively. The whole quantity of phosphorus and potassium and one-third nitrogen was applied at the time

of sowing and remaining quantity of nitrogen in two equal splits i.e., at flowering and peak flowering stages. The standard production practices were followed during growing season. The insect pests were controlled at economic threshold level (ETL) to reduce rank growth and avoiding fruit shedding. Data were collected on some parameters of earliness and leaf area index following one week after spray of osmoprotectants. Data analyses were done using MSTAT software (MSTAT Development Team, 1989). In case, ANOVA permitted the LSDs were applied with 0.05 and 0.01 levels of significance.

## Results

Data for leaf area duration differed significantly due to water stress condition and spray of various osmoprotectants. However, there was a non-significant interaction between water stress condition and exogenous application of osmoprotectants. Averaged across various osmoprotectants, the endurance of leaves was reduced by 6.59 days under water stress condition compared to that under well-watered condition. Averaged across water stress condition, leaves endured for 74.5 days in crop sprayed with salicylic acid at the rate of 100 mg L<sup>-1</sup> compared to 54.5 days that under untreated check. However, water stress and osmoprotectants had a little effect on endurance of leaves. The values of leaf duration ranged from 52.4 to 78.5 days under different treatments. Comparatively, the spray of salicylic acid took the lead in endurance of leaves followed by glycinebetaine and proline. The spray of water alone also leaped the duration of leaves by 2 days more compared to untreated check (Table 1). Data for leaf area ratio was significantly affected by exogenous application of osmoprotectants, whereas it was non-significantly affected by water stress environment and interaction of both factors. Averaged across water stress treatments, the crop treated with salicylic acid attained higher leaf area ratio compared to that treated with other chemicals and untreated check. The maximum leaf area ratio was recorded under salicylic acid treated crop followed by glycinebetaine and proline (Table 1).

**Table 1. Interactive effects of watering regimes and exogenously applied Osmoprotectants on some attributes of leaf duration.**

Watering regimes	Osmoprotectants					
	Untreated check	Spray of 0.1% Tween 80	Salicylic acid	Proline	Glycine betaine	Mean
<b>a. Leaf area duration (days)</b>						
Well-watered	56.5	58.5	78.5	72.9	74.8	68.2
Drought stressed	52.4	54.5	70.4	64.3	66.8	61.7
Mean	54.5	56.5	74.5	68.6	70.8	
LSD (p<0.05) Irrigation water (I) 2.76**, Osmoprotectants (O) = 2.28**, Interaction (I X O) = 3.23 <sup>NS</sup>						
<b>b. Leaf area ratio</b>						
Well-watered	0.03	0.03	0.05	0.03	0.04	0.04
Drought stressed	0.02	0.02	0.06	0.03	0.04	0.03
Mean	0.03	0.03	0.06	0.03	0.04	

Data for occurrence of event of first boll split differed significantly due to water stress environment and spray of various osmoprotectants, however, there were little differences due to interactive effects of both factors. Averaged across chemicals, the phenomenon of splitting of first boll occurred 4 days earlier under drought stress compared to well-watered crop. Averaged across water stress conditions, the spray of salicylic acid hastened the crop to get matured by 10 days earlier than untreated check. The event of first boll splitting occurred at 88, 91, 90, and 98 days by spraying of salicylic acid, proline and glycinebetaine, and untreated check, respectively (Table 2). Data for growing degree days (GDD) required for first boll splitting event, differed significantly due to watering regimes and various osmoprotectants. The interactive effects were little impacted by GDD. Averaged across osmoprotectants, the crop required 28 more GDD under well-watered compared to drought-stressed environment. Averaged across water stress conditions, crop treated with salicylic acid required 1685 GDD compared to 1727 GDD by spraying proline to the cotton crop (Table 2).

Data for production rate index (PRI) differed significantly due to watering regimes and various osmoprotectants, however, no significant effect of the

interaction of these factors was observed. The PRI was higher by 12.5% under well-watered compared to drought stressed conditions. Averaged across water stress conditions, the crop treated with salicylic acid achieved higher PRI by 46.2% over other chemicals (Table 2). Data for fruit production efficiency (FPE) were significantly influenced by water stress conditions and various osmoprotectants, while the interactive values were little affected. The crop, fully irrigated, maintained higher FPE compared to drought stress condition. Averaged across water stress conditions, the crop sprayed with salicylic acid maintained FPE by 89 days compared to 85, 83 and 81 days by spraying of proline, glycinebetaine and untreated check, respectively (Table 2). Data for percentage of seed cotton yield gathered during first picking differed significantly due to watering regimes and spray of osmoprotectants. Averaged across chemicals, the drought stressed crop produced 6% higher seedcotton yield compared to that in well-watered crop. Averaged across water stress conditions, the treated with salicylic acid contributed 85% share compared to 73, 70 and 62% of the total under proline, glycinebetaine and untreated check, respectively (Table 2).

**Table 2. Interactive effects of watering regimes and exogenously applied Osmoprotectants on cotton earliness indices.**

Data for leaf area index (LAI) differed significantly due to water stress environment and spray of various osmoprotectants. There was a non-significant interaction between water stress and osmoprotectants at various physiological growth stages. Averaged across chemicals, the crop attained lower leaf area index under water stress compared to that under well-watered conditions. Averaged across water stress conditions, the crop foliated with salicylic acid attained higher LAI followed by glycinebetaine, proline and un-treated check, respectively at different stages of growth. During the earlier part of the season, growth of leaf area progressed slowly, requiring about 60 days (first flower) to reach an LAI of 1.62. Thereafter, LAI progressed from 1.62 to 3.37 at day 90 (peak flowering) after sowing. After attaining maximums LAI, it declined gradually to a minimum of 0.88 (Table 3, Figs. 1 and 2).

Correlation coefficient between LAI and total dry weights and parameters of seed cotton yield showed a positive relationship (Table 4). The relationship between LAI and total fruit production was highly dependent on the concurrent vegetative growth. A high degree of

correlation ( $r = 0.92^{**}$ ) was measured between LAI and total dry weights under water stress environments. The regression analysis also indicated a highly significant relationship ( $r = 0.95^{**}$ ) between total fruit weight under drought-stressed conditions, and  $r = 0.77^*$  under well-watered conditions. The data show that increase in LAI due to foliar spray of osmoprotectants resulted in higher production of fruit per unit land area and thereby mitigating the adverse effects of drought conditions. There were positive correlation between leaf area duration and seed cotton yield under well-watered condition ( $r = 0.61^{**}$ ) and drought stressed condition ( $r = 0.80^{**}$ ), signifying the substantial influence of water stress on the productivity of cotton crop (Fig. 3). The higher seed cotton yield was harvested with concurrent lengthening in leaf area duration from well-watered crop. Contrarily, the lower productivity was achieved with lessening in the days of persistence of leaves. There were positive correlations between leaf area index and various parameters of seed cotton yield with the exception of total dry weights and total fruit weight under well-watered and drought stressed conditions (Fig. 4).

**Table 3. Interactive effects of watering regimes and exogenously applied osmoprotectants on leaf area index at different physiological stages.**

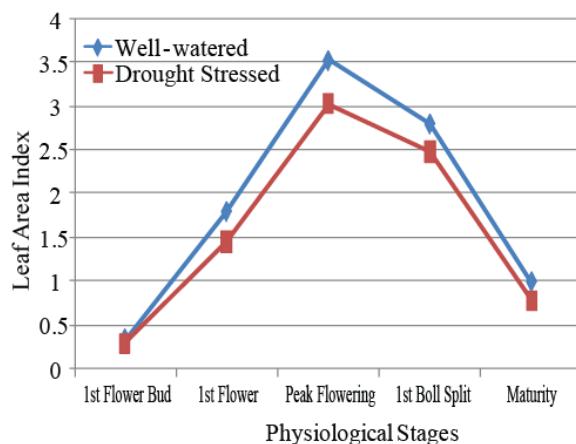


Fig. 1. Leaf Area Index as affected by watering regimes at different physiological stages of growth.

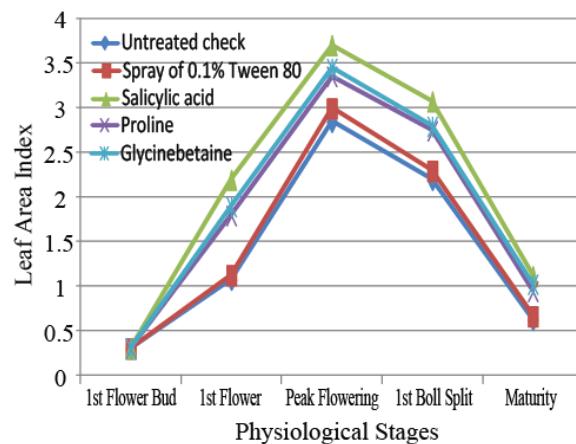


Fig. 2. Leaf Area Index as affected by exogenous application of osmoprotectants at various physiological stages of growth.

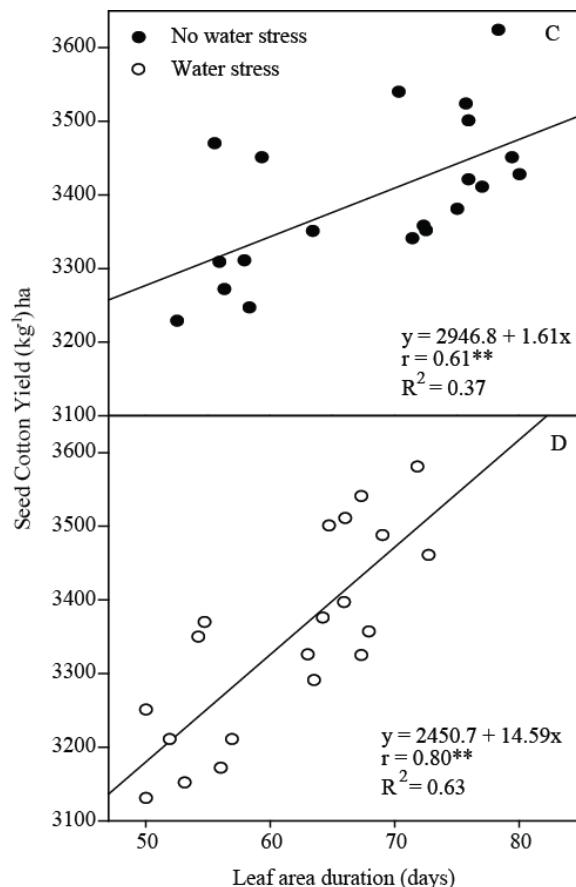


Fig. 3. Relationships between leaf area duration and seed cotton yield at well-watered (A) and drought stressed (B).

## Discussion

The results of the study show that cotton earliness indices were impacted significantly due to exogenously applied osmoprotectants under various watering regimes. Water stress caused a marked reduction in leaf area duration, fruit production efficiency, leaf area ratio and production rate index. The occurrence of event of first

boll split and percentage of seed cotton gathered at first pick were improved. The adverse effects of water stress were mitigated to a greater extent by foliar spray of salicylic acid, proline and glycinebetaine.

Russelle *et al.*, (1984) used growing degree-days (GDD) rather than calendar days, as the divisor in the growth functions, relative growth rate, net assimilation rate and to differentiate various physiological stages and thus it gave better estimates for determining various physiological stages in response to environmental conditions. The significant improvement in LAI occurred due to enhanced translocation of photo-assimilates from roots to shoots and regulation of enzymatic activities by application of salicylic acid (Noreen & Ashraf, 2008). These results agree with those of Fariduddin *et al.*, (2003) that enhancement in growth and development resulted due to increased stimulation in physiological and biochemical processes. They also found maximum increase in growth of *Brassica juncea* plant by spraying of salicylic acid at the rate of 50-100 mg L<sup>-1</sup>. The comparison of means also indicate that the spray of salicylic acid, proline and glycinebetaine triggered the leaf area index (LAI) and other earliness indices. Maximum LAI was recorded by spraying of salicylic acid compared to other chemicals under well-watered crop and drought stressed ecologies. Sakhabutdinova *et al.*, (2003) reported that application of SA diminished the alteration of phytohormones levels in wheat by preventing a decrease under indole acetic acid (IAA) and maintaining of both abscisic acid (ABA) and proline accumulations under varying water stress conditions. The production rate index and fruit production efficiency increased by the application of 3 kg ha<sup>-1</sup> glycinebetaine, resulting in higher attainment of LAI under both well-watered and drought stressed conditions. The results indicate that foliarly applied glycinebetaine possesses anti-transpirant properties and has the potential to improve drought tolerance by reducing the amount of water use for irrigation, without any significant decrease in various quantities of earliness indices (Agboma *et al.*, 1997a). The results also agree with those of other researchers (Makhdum *et al.*, 2006; Ashraf and Foolad, 2007; Noreen, 2010) that adverse effects of drought stress could be mitigated by foliar spray of osmoprotectants.

Scarcity of water due to drought and/or soil salinity influences various morphological and physiological processes and ultimately deformities at the cellular and organelle levels (Abdelkader *et al.*, 2007; Athar *et al.*, 2009). Moreover, the growth and development of a crop is greatly affected by drought stress and is dependent upon developmental stage at which it occurs (Chaves *et al.*, 2003). Jensen & Mogensen (1984) reported that drought stress reduced crop yield regardless of the growth stage at which it occurred. The water stress affects a number of biochemical and molecular processes, which results in stomatal closure, decrease in rate of transpiration, pigment content, photosynthesis and thereby partial or full inhibition in growth and development (Lawlor & Cornic, 2002); reduction in leaf size and water-use-efficiency, inhibition of enzymatic activities (Ashraf *et al.*, 1995); ionic imbalance and disturbances in solute accumulation (Khan *et al.*, 1999) and/or combination of all these factors.

The exogenous application of glycinebetaine results in enhancing the levels of endogenous glycinebetaine (GB) in non-accumulating plants such as tomato (Makela *et al.*, 1998), rape (Sulpice *et al.*, 2002) and also GB-accumulator in cotton (Gorham *et al.*, 2000), grown under stressful environment. The similar results have been reported that exogenous application of salicylic acid, proline and glycinebetaine have been found effective in alleviating drought stress and sustaining growth and development through enhancing the antioxidant activities and by scavenging ROS production. Makela *et al.*, (1996) also reported that [<sup>14</sup>C] glycinebetaine was translocated to roots within two hours of its foliar application on turnip rape (*Brassica rapa* L. ssp. *Oleifera*), soybean [*Glycine max* (L.) Merr.], pea (*Pisum sativum* L.), tomato (*Lycopersicum esculentum* Mill.) and spring wheat (*Triticum aestivum* L.). They added that glycinebetaine is quite inert in plant cell being mainly present in phloem-mobile. Its penetration by plant parts was accelerated by its combination with various surfactants. Moreover, the uptake and translocation rate of foliar applied glycinebetaine was greatly affected by environmental factors. Heikal & Shaddad (1982) reported positive effects of proline in counteracting the injury exerted through its accumulation in the whole plant organs.

Wang *et al.*, (2010) suggested that GB induces increase in osmotic adjustments for drought tolerance by

improving antioxidative defense system including antioxidative enzymes in wheat crop. Shahbaz *et al.*, (2011) also found that foliar-applied GB at the rate of 50 mM mitigated the adverse effects of drought stress by enhancing plant biomass and leaf area per plant in various genotypes of wheat crop compared to water stressed conditions. Hussain *et al.*, (2008) also found that exogenous GB and SA application significantly improved various parameters of vegetative and reproductive growth under water stress in sunflower. The results agree with those of Ali *et al.*, (2011) that plant stress at 50 and 75 percent of field capacity caused reduction in leaf area, plant height, and biomass, however, these ill-effects were reversed by spraying of synthetic cytokinins, benzyl amino purine (BAP) and leaf extract of *Moringa oleifera* at the rate of 50 mg L<sup>-1</sup> and 25 ml plant<sup>-1</sup>, respectively in maize crop. Ali *et al.*, (2011) also reported that spray of SA at the rate of 1.5 mM caused accumulation of osmolytes in chickpea (*Cicer arietinum* L.) crop. Resultantly, it intervened in greater uptake of water from the environment, reducing the immediate effect of water shortage within the plant and thereby stabilizing protein tertiary structures and cells. The results of the present study are in agreement with those of Umebese *et al.*, (2009), that the spray of SA enhanced the proline synthesis in tomato and amaranth plants under water-deficit conditions. Therefore, it is evident that spray decreased oxidative stress and increased proline and ascorbic acid contents in order to enhancing antioxidant activity levels in the cotton crop. Ali *et al.*, (2007) reported that the imposition of water stress equivalent to 60% field capacity reduced growth and photosynthetic capacity of two maize cultivars. However, exogenous application of proline (30 mM) counteracted the adverse effects of water stress. In a later study, Misra & Saxena (2009) also reported that application of SA (0.5 mM) ameliorated the stress generated by sodium chloride (100 mM) through alleviating proline metabolizing system in lentil (*Lens esculentum*). The external application of SA resulted in accumulation of higher levels of free proline in plant system. Agarwal *et al.*, (2005) reported that application of SA (1.0 mM) caused beneficial effects in terms of increased antioxidant enzyme activity and decreased oxidative stress, and thereby improved leaf area, total biomass under water stress (-0.08 MPa) over control plants of the wheat crop.

**Table 4. Relationships between watering regimes and leaf area index on components of seedcotton yield.**

Dependent variables	Independent variables	Regression equation	Correlation of coefficient (r)	Coefficient of determination (R <sup>2</sup> )
Number of intact fruit m <sup>-2</sup>	Well-watered	116.54x + 196.48	0.71**	0.50
	Drought-stressed	70.95x + 334.29	0.68**	0.46
Number of bolls plant <sup>-1</sup>	Well-watered	6.12x + 14.16	0.72**	0.52
	Drought-stressed	6.02x + 15.71	0.62**	0.38
Boll weight (g)	Well-watered	0.42x + 1.78	0.58**	0.34
	Drought-stressed	67x + 0.92	0.76**	0.58
Total dry weight (g m <sup>-2</sup> )	Well-watered	154.43x + 150.43	0.55**	0.30
	Drought-stressed	261.57x - 149.89	0.92**	0.84
Total Fruit weight (g m <sup>-2</sup> )	Well-watered	221.6x - 326.17	0.77**	0.59
	Drought-stressed	295.32x - 482.01	0.95**	0.91

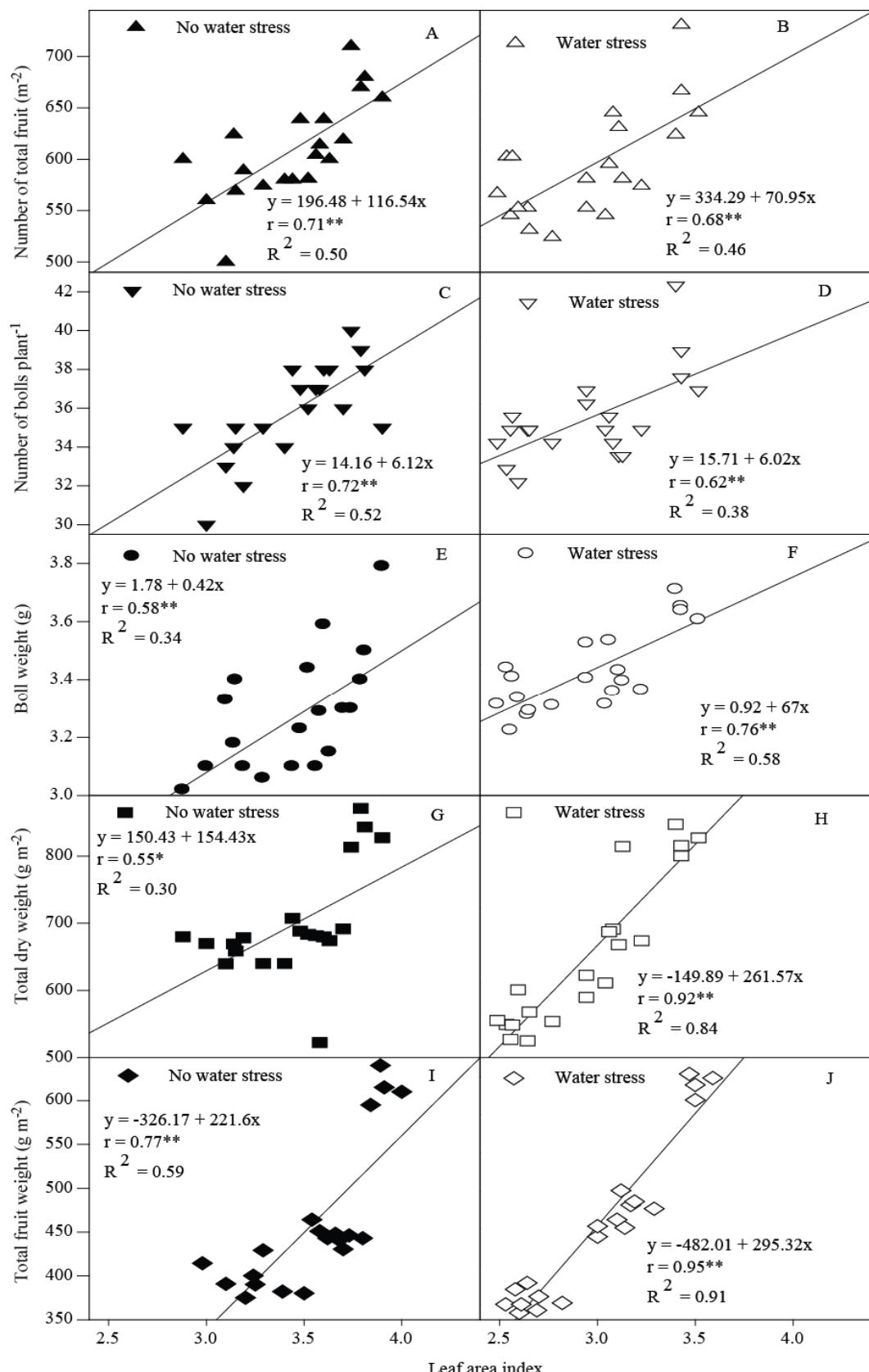


Fig. 4. Relationships between number of total fruit (A and B), number of bolls  $\text{plant}^{-1}$  (C and D), boll weight (E and F), total dry weight (G and H) and total fruit weight (I and J) with leaf area index at well-watered and drought stressed.

## Conclusion

The results of the present study reveal that various osmoprotectants could be effectively employed to reduce the adverse effects of drought stress environment on cotton crop. The sustainability of cotton crop could be attained by spraying of salicylic acid and proline @ 100 mg L<sup>-1</sup> under reduced availability of irrigation water in an arid environment.

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