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IMPROVING GROWTH AND YIELD OF COWPEA PLANT BY FOLIAR APPLICATION OF CHITOSAN UNDER WATER STRESS

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ABSTRACT

Water stress impaired cowpea plant growth and decreased ion percentage and chlorophyll and carbohydrate concentration in the shoot as well as yield and its quality. Foliar-applied chitosan, in particular 250 mg/l, increased plant growth, yield and its quality as well as physiological constituents in plant shoot under stressed or nonstressed conditions as compared to untreated plants.

Anatomically, water stress decreased thickness of leaf blade at midrib region, thickness of mesophyll tissue, thickness of midrib vascular bundle. Treatment with chitosan, in particular, 250 mg/l and their interactions with stress conditions increased all the above mentioned parameters in either non-stressed or stressed plants. It is suggested that the severity of cowpea plants damaged from water stress was reduced by 250 mg/l chitosan application.
Keywords: water stress, chitosan, cowpea, anatomy, growth, yield

INTRODUCTION

World population was increased at an alarming rate and it was expected to reach about six billion by the end of year 2050. On the other hand, food productivity is decreasing due to the effect of various abiotic stresses and climatic changes; therefore minimizing these losses is a major area of concern for all nations to cope with the increasing food requirements. In the face of a global scarcity of water resources, drought has already become a primary factor in limiting crop production in the world. At present, around 18 % of the global farmland is irrigated (more than 240 million hectares) produced about 40 % of the global food supply (Somerville and Briscoe 2001). Permanent or temporary water deficit stress limits the growth and distribution of natural and artificial vegetation and the performance of the cultivated plants more than any other environmental factor (Shao *et al*. 2009). In Egypt, water availability is considered the prime constraint that determines the addition of new cultivated areas. Agricultural expansion needs a huge amount of available irrigation water which is already not sufficient to me*et al*l the expected demands.

The responses of plants to drought vary greatly depending on species and stress severity (Mullet and Whitsitt 1996). Higher plants respond to water deficit in several ways, stomatal closure, leaf rolling, and osmotic adjustments and reduction and consequently a decrease in cellular expansion and alteration of various essential physiological and biochemical processes that can affect growth and productivity and yield quality (Costa *et al*. 2008, Lobato *et al*. 2008, Hefny 2011). In this concern, Carvalho *et al*. (2004) found that, lupines cultivars tended to accumulate crude protein and

carbon compounds in the seeds at the end of the water stress period (15 days after anthesis). However, Jansen (2008) recorded insignificant effect of water stress on protein content when imposed at the same stage.

The sustainable management of water resources is a priority for agriculture also for the temperate regions, e.g. the Mediterranean basin, where dry and hot summer usually occurs, and drought events may have a large impact on both productivity and crop quality. In this context, Bittelli *et al*. (2001) reported that occasional or episodic drought events may be counteracted through the use of antitranspirants. These compounds are applied to foliage to limit the water loss. They include both film-forming and stomata closing compounds, able to increase the leaf resistance to water vapor loss thus improving plant water use to assimilate carbon, and, in turn, the production of biomass or yield (Tambussi and Bort 2007). Another approach to reduce water loss due to transpiration is by increasing the reflection of sunlight from leaves, through reflectant type of antitranspirants, thus limiting the water loss deputed to evaporative leaf cooling (Gaballah and Moursy 2004).

Among antitranspirant compounds, chitosan (CHT) has previously proved to be effective in pepper (Bittelli *et al*. 2001). CHT is a natural, low toxic and low expensive compound biodegradable, and environmentally friendly with various applications in agriculture, obtained by deacetylation of chitin. In agriculture, CHI has been used in seed, leaf, fruit and vegetable coating, as fertilizer and in controlled agrochemical release, to increase plant product (New *et al*. 2004), to protect plants against microorganisms (Farouk *et al*. 2008), to protect plants against oxidative stress (Guan *et al*. 2009) and to stimulate plant growth (Farouk *et al*. 2008, 2011). In the latter studies, a positive effect of CHI was observed on the growth of roots, shoots and leaves of various plant species. Similar results were determined within sweet pepper and radish (Ghoname *et al*. 2010, Farouk *et al*. 2011). In addition, foliar applications with CHI resulted in higher vegetative growth and improvement fruit quality of cucumber (Farouk *et al*. 2008). For other cultivated plants, Bittelli *et al*. (2001) reported that foliar application of CHI decreased transpiration in pepper plants, and reduced water use by 26-43% while maintaining biomass production and yield. Abdel-Mawgoud *et al*. (2010) on strawberry showed that CHI application improved plant height, number of leaves, fresh and dry weights of the leaves and yield components. Fruit quality in terms of average weight of individual fruits and TSS showed similar trends. Recently, Sheikha and AL-Malki (2011) indicate that application of the Chitosan's different concentrations enhancing bean shoot and root length, fresh and dry weights of shoots, roots and leaves area as well as the level of chlorophyll in leaves. The mechanisms of CHI on counteracting the harmful effect of water stress are not well understood and there are a few reports on this concern. Transcriptional activation, induced by both CHI and jasmonic acid, of genes encoding phenylalanine ammonia lyase and protease inhibitors, suggests that CHI may influence pathways involving jasmonic acid (Doares *et al*. 1995). Jasmonates exhibit some activities similar to the plant hormone abscisic acid (ABA), which plays a key role in the regulation of water use by plants. Increased levels of ABA result in closure of stomata and

reduced transpiration (Leung and Giraudat 1998). These authors demonstrated that CHI inhibited light-induced opening of stomata in tomato and *Commelina communis* via inducing H_2O_2 production in the guard cells. The reported effects of CHI on stomatal aperture suggest the possibility that CHI might be a valuable antitranspirant with useful agricultural applications.

Cowpea (*Vigna unguiculata* (L) Walp) is one of the ancient grain legumes valued for its nutritional value, especially high protein content (25%), flavor and short cooking time (Ogbonnaya *et al*. 2003). The crop also has ability to maintain soil fertility through its excellent capacity to fix atmospheric nitrogen and thus does not require very fertile land for growth (Lobato *et al*. 2006). Moreover, cowpea forms an integral part of a sustainable agriculture and land use system (Ogbonnaya *et al*. 2003). The total cultivated area of this crop in Egypt was estimated by about 9155 feddan for dry seed production in the year of 2008 with a mean production of 980 kg/feddan. Also, the estimated area for fresh pods was 10064 kg/feddan with a mean production of 5.19 ton/feddan (Dept. Agric. Statistics, ministry of Agriculture, Giza, Egypt, 2008).

The improvement in water economy may probably help water stressed plants in maintaining their physiological and biochemical processes, at least, at an acceptable base line. To the best of our knowledge there has also been no previous report regarding the effects of foliar applied CHI on cowpea plant growth and yield. Therefore, the objective of this work was to explore the possible role of CHI on improving drought tolerance in cowpea plants.

MATERIALS AND METHODS

Two pot experiments were conducted in the experimental farm and laboratory of Agricultural Botany Department, Faculty of Agriculture, Mansoura University, Egypt during the two successive seasons of 2007 and 2008. Cowpea seed "*Vigna unguiculata* (L.) Walp. cv Cream 7" was obtained from the legume Research Institute, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. The seeds were sterilized with 1.5% chlorox, washed three times with distilled water, and then coated with N-fixer okadeen (Rhizobia) that was obtained from General Organization for Agriculture Equalization Fund (GOAFE), Ministry of Agriculture. Egypt.

Sowing was took place on 15th and 10th April in both seasons respectively. The pots were arranged in a complete randomized block design with three replications. Plastic pots (50 cm inner diameter and 30 cm in length) filled with 25 kg air dried soil were used. The soil characteristics were as follows: sandy loam in texture, sand, 80%; silt, 15.5%; clay, 4.5%; pH, 7.8; EC, 0.4 dSm^{-1} and organic matter 0.45%. After sowing, irrigation was applied to supply seedlings with 100% available water, at two days intervals till the seedlings reached the fourth leaf stage. The seedlings were thinned to leave seven plants per pot. Phosphorous and potassium fertilizers were added to the soil before sowing at the rate of 5 g P_2O_5 in the form of calcium super phosphate (15.5% P_2O_5) and 2 g K_2O in the form of potassium sulphate

(48%). Ammonium nitrate (33.5%) was added at the rate of 4 g N/pot in two equal portions; the first during the seedling stage and the second at the beginning of flowering time. After that the pots were divided into three groups for water stress treatments, each group was divided into four subgroups for chitosan foliar application. The soil moisture for all pots was kept at 80% field capacity "FC" until 15 days after sowing (DAS). After that, the water stress treatments were initiated. Pots were subjected to one of the three water stress treatments; a well watered control, 80% FC and two water stress treatments; moderate at 50% FC and severe 30% FC water stress. In the stressed treatments, moisture levels were allowed to fall from the initial 80% FC to 50% FC and 30% FC, respectively. All pots were weighed every two days. The loss in pots weight represents transpiration and evaporation. Cumulative water loss was added to each pot to compensate transpiration and evaporation. Accumulated water loss was calculated as the differences in pots weights between successive weights. At 40, 50 and 60 days from sowing, the plants were sprayed with either tap water or chitosan at 125, 250 or 500 mg/l till dripping using small pressure pump after adding tween 20 as a wetting agent at concentration of 0.5%.

Data Recorded:

Three uniform plants were uprooted from each pot at the full blooming stage (80 days from sowing) to measure certain morphological and physiological characteristics as well as leaflet anatomy

Morphological characteristics:

The plants were cleaned and the following parameters were determined: plant height, number of leaves/plant and number of branches/plant. Shoot fresh and dry weight/plant were estimated by drying plant at 70°C until constant weight.

Chemical Composition of Leaves:

1. Total chlorophyll was extracted for 24 hr at room temperature in methanol after adding traces of sodium carbonate and determined spectrophotometrically (Spekol 11, Uk) according to Lichtenthaler and Wellburn (1985).
2. Total carbohydrates concentration was estimated using the anthrone method as described by Sadasivam and Manickam (1996).
3. Mineral constituents: Dry shoot (0.2g) was digested using 5 cm from the mixture of sulfuric and perchloric acid ($HClO_3/H_2SO_4$ 1:1 v:v) until a sample had become clear, cooled and made up to 50 ml using deionized water. Total nitrogen was determined by micro-kjeldahl method. Potassium was determined by flame-photometerically (Kalra 1998). Phosphorous was also estimated by using ammonium molybdate and ascorbic acid (Cooper 1977).

Leaflet structure*:* Small pieces from the midrib region of the 3rd upper leaflet (second season) were fixed in formalin aceto alcohol for 48 h, then dehydrated *via* n-butanol series and embedded in paraffin wax (52-54 ^{o}C melting point). Sections were prepared using a rotary microtome at 15-17 µm thickness and stained with safranin/light green and finally mounted in canada balsam. Selected sections were examined using light microscope to determine the anatomical changes in leaflets.

Total Yield and its quality:

At harvest time (140 days from sowing) the total yield per plant was recorded. Seed quality represented by their concentrations of nitrogen, phosphorous, potassium, protein and carbohydrates were determined in the dry seeds as previously described in shoots. Finally, the protein percentage in dry seeds was accounted by multiplying nitrogen content by 6.25.

Statistical analysis:

All data were analyzed statistically using One-way ANOVA to follow by Duncan's Multiple Range Test (DMRT) by COSTAT software. The values are mean ± SD for three samples in each group. P values <0.05 were considered as significant.

RESULTS

Growth parameters

Generally, severe reduction in plant growth, manifested by smaller, chlorotic, wilted, and rolled leaves was recorded due to water stress. From the results presented in Table (1), it could be concluded that plant growth characters i.e. plant height, branches and leaf number per plant, and shoot fresh and dry weight were significantly decreased due to water stress in both growing seasons.

Table (1): Effect of water stress and chitosan and their interactions on certain growth parameters during the two growing seasons.

Treatments		Plant height (cm)		Branches number		leaf number		shoot dry weight		Shoot fresh weight	
Water stress % FC	Chitosan (mg/l)	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
70% FC	W	38.06± 0.305d	37.53± 2.138d	4.00± 0c	4.00± 0 c	29.66± 1.154 e	29± 0de	7.25± 0.052d	7.11± 0.516 c	37.25± 0.430def	37.47± 0.473de
	125	41.06± 1.474b	41.10± 1.248b	5.00± 0 b	4.66± 1.154b	34.33± 1.154 c	34.33± 1.154b	7.77± 0.121c	7.75± 0.050b	40.31± 0.780 c	40.04± 1.847 c
	250	43.76± 1.962a	43.26± 1.900a	6.00± 0 a	5.66± 1.154a	37.66± 1.154 a	37.33± 1.154a	8.73± 0.692a	8.53± 1.063a	56.91± 4.359a	47.04± 4.300a
	500	35.96± 2.369e	35.46± 2.500e	4.00± 0c	4.00± 0 c	28.33± 1.154 f	27.66± 1.154 e	6.88± 0.190e	6.74± 0.144d	36.98± 1.040def	36.69± 1.224 def
50% FC	W	30.26± 2.663g	29.80± 1.113g	3.00± 0 d	3.00± 0 d	25.33± 1.154 h	24.66± 1.154fg	6.56± 0.190f	6.13± 0.023 e	35.80± 1.626fg	35.57± 0.995 ef
	125	39.03± 1.616cd	38.96± 0.503c	4.00± 0c	4.00± 0 c	30.66± 1.154 e	30± 0 cd	7.34± 0.090d	7.30± 0.131 c	38.10± 1.621de	37.86± 0.840 cde
	250	42.53±. 2.577a	42.00± 1.058b	5.00± 0 b	5.00± 0 b	35.66± 1.154 b	35.66± 1.154b	8.22± 0.253b	7.83± 0.115b	44.08± 2.085b	43.27± 3.161b
	500	32.36± 3.1643f	31.76± 0.901f	3.66± 1.154c	3.00± 0 d	26.66± 1.154 g	26± 2 f	6.66± 0.046 f	6.66± 0.02 d	36.57± 0.733ef	36.55± 0.979 def
30% FC	W	26.30± 1.562i	25.33± 2.247i	2.00± 0 e	2.00± 0 e	21± 2 j	21.33± 4.618h	5.16± 0.072 i	5.03± 0.02 g	29.41± 2.487 i	28.33± 5.280h
	125	28.90± 0.200gh	28.10± 0.200h	3.00± 0 d	3.00± 0 d	24.33± 1.154 h	23.33± 1.154g	5.97± 0.115g	5.77± 0.207 f	34.45± 1.012g	34.36± 2.618 f
	250	39.80± 0.346bc	39.56± 1.474c	4.00± 0c	4.00± 0 c	32± 0 d	31.33± 1.154 c	7.44± 0.057d	7.42± 0.117 c	38.52± 0.664d	38.17± 0.488 cd
	500	27.53± 1.331hi	27.50± 0.400h	2.66± 1.154 d	2.33± 1.154 e	22.33± 1.154 i	21.66± 1.154h	5.40± 0.128h	5.29± 0.133g	32.44± 3.190h	31.53± 2.708g

Values are given as mean± SD of three replicate. Means in columns by different letters are significantly different at P < 0.05 by (Duncan's Multiple Range Test).

The great reduction was observed under severe water stress (30% FC). From the same table, it is well noted that foliar application of CHI, in particular, 250 mg/l improved all plant growth through parameter comparing with untreated control plant. Regarding the interaction effects, the data presented in the same table revealed that application of CHI at 250 mg/l significantly increased all growth parameter of cowpea under stress and nonstressed conditions. The only exception was detected at 500 mg/l which significantly decreased all these parameters. Moreover, application of 125 mg/l CHI counteracted the harmful effect of water stress on plant growth due to increasing plant growth under such water stress level.

Chlorophylls and total carbohydrates concentrations:

Data presented in Table (2) indicate that the concentration of total chlorophylls and total carbohydrates were significantly decreased under water stress in both growing seasons as compared with the control plants.

Foliar application of CHI, in particular, 250 mg/l significantly increased the concentration of both chlorophylls and total carbohydrates as compared with untreated plants under such stress levels. As for its interactions with water stress, the data presented in the same table indicate that chitosan (500 mg/l) alleviated the harmful effect of moderate and severe water stress in this respect.

Table (2): Effect of water stress and chitosan and their interactions on chlorophyll (mg/g FW) and total carbohydrates (mg/g DW) concentrations during the two growing seasons.

Treatments		Chlorophyll A		Chlorophyll B		Total chlorophylls		Total carbohydrates	
Water stress % FC	Chitosan (mg/l)	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
70% FC	W	0.428± 0.033 bc	0.507± 0.217 bc	0.299± 0.008 a	0.195± 0.056 a	0.728± 0.026 de	0.702± 0.165 de	30.18± 0.588 e	31.98± 1.57 cv
	125	0.539± 0.194 b	0.516± 0.217 bc	0.291± 0.218 a	0.293± 0.203 a	0.814± 0.024 bc	0.809± 0.018 bc	35 ± 1.756 bc	36.15± 0.515 ab
	250	0.800± 0.225 a	0.724± 0.194 a	0.236± 0.077 a	0.278± 0.090 a	1.037± 0.298 a	1.002± 0.202 a	37.29± 0.889 a	38.61± 1.66 a
	500	0.462± 0.248 bc	0.394± 0.068 bcd	0.239± 0.261 a	0.284± 0.049 a	0.702± 0.013 def	0.679± 0.020 de	27.25± 4.622 f	30.70± 1.39 cd
50% FC	W	0.382± 0.180 bc	0.450± 0.263 bc	0.253± 0.146 a	0.237± 0.206 a	0.637± 0.035 fg	0.596± 0.011 fg	25.46± 0.355 f	30.77± 1.25 cd
	125	0.482± 0.351 bc	0.576± 0.338 ab	0.246± 0.344 a	0.150± 0.318 a	0.729± 0.011 de	0.727± 0.020 d	31.85± 2.276 de	33.55± 0.954 bc
	250	0.613± 0.237 ab	0.725± 0.071 a	0.264± 0.305 a	0.146± 0.046 a	0.878± 0.075 b	0.872± 0.075 b	36.26± 0.854 ab	37.52± 1.060 a
	500	0.530± 0.274 b	0.401± 0.325 bcd	0.143± 0.284 a	0.228± 0.310 a	0.674± 0.027 efg	0.629± 0.047 ef	27.28± 0.900 f	28.87± 1.34 de
30% FC	W	0.276± 0.188 c	0.202± 0.058 d	0.117± 0.138 a	0.136± 0.033 a	0.394± 0.057 i	0.338± 0.073 h	18.86± 4.12 h	19.72± 2.72 g
	125	0.398± 0.304 bc	0.379± 0.220 bcd	0.200± 0.314 a	0.201± 0.213 a	0.599± 0.011 gh	0.581± 0.019 fg	23.5± 2.16 g	25.77± 0.712 ef
	250	0.546± 0.275 b	0.513± 0.234 bc	0.223± 0.241 a	0.242± 0.264 a	0.769± 0.036 cd	0.755± 0.042 cd	33.33± 0.487 cd	35.51± 0.401 ab
	500	0.429± 0.274 bc	0.322± 0.254 cd	0.113± 0.276 a	0.201± 0.310 a	0.543± 0.047 h	0.524± 0.061 g	21.84± 1.79 g	24.31± 1.65 f

Values are given as mean± SD of three replicate. Means in columns by different letters are significantly different at P < 0.05 by (Duncan's Multiple Range Test).

Ions percentage:

Water stress is generally recognized as injurious to plants by disturbing the electrolyte balance, resulting in the deficiency of some nutrients. Data presented in Table (3) prove that water stress decreased significantly shoot nitrogen, phosphorus and potassium percentage in both growing seasons. The great reduction occurred under severe water stress. On the other hand, data presented in the same table show that pronounce and highly significant icrease in nitrogen, phosphorous and potassium percentages in the shoot due to exogenous application of CHI, in particular, 250 mg/l in both growing seasons.

Table (3): Effect of water stress and chitosan and their interactions on nitrogen,phosphorous and potassium percentage during the two growing seasons.

Treatments		Nitrogen %		Phosphorous %		Potassium%	
Water stress % FC	Chitosan (mg/l)	1st season	2nd season	1st season	2nd season	1st season	2nd season
70% FC	W	3.71± 0.140 f	3.43± 0.140 e	0.498± 0.008 cd	0.512± 0.011 e	1.25± 0.072 e	1.04± 0.080 e
	125	4.29± 0.080 c	4.17± 0.080 b	0.539± 0.032 bc	0.615± 0.045 c	1.56± 0.034 b	1.44± 0.100 b
	250	4.76± 0.140 a	4.48± 0.140 a	0.725± 0.161 a	0.762± 0.032 a	1.68± 0.023 a	1.59± 0.050 a
	500	3.38± 0.161 g	3.22± 0.140 f	0.478± 0.017 de	0.493± 0.018 ef	1.10± 0.113 f	0.913± 0.050 f
50% FC	W	3.12± 0.080 h	3.01± 0.140 gh	0.428± 0.016 efg	0.449± 0.017 g	0.85± 0.070 h	0.78± 0.034 h
	125	3.99± 0.140 e	3.64± 0.242 d	0.514± 0.011 cd	0.518± 0.024 e	1.37± 0.100 d	1.19± 0.092 d
	250	4.50± 0.213 b	4.29± 0.080 b	0.592± 0.043 b	0.705± 0.051 b	1.64± 0.050 a	1.54± 0.050 a
	500	3.19± 0.080 h	3.10± 0.080 fg	0.460± 0.030 def	0.470± 0.011 fg	0.97± 0.072 g	0.836± 0.050 g
30% FC	W	2.68± 0.080 k	2.49± 0.213 i	0.369± 0.049 g	0.378± 0.029 i	0.703± 0.080 j	0.626± 0.057 j
	125	3.01± 0.14 i	2.87± 0.140 h	0.404± 0.016 fg	0.420± 0.020 h	0.813± 0.050 hi	0.73± 0.034 hi
	250	4.17± 0.080 d	3.92± 0.280 c	0.499± 0.131 cd	0.547± 0.045 d	1.49± 0.100 c	1.30± 0.072 c
	500	2.84± 0.213 j	2.63± 0.224 i	0.393± 0.008 g	0.400± 0.010 hi	0.753± 0.023 ij	0.696± 0.023 i

Values are given as mean± SD of three replicate. Means in columns by different letters are significantly different at P < 0.05 by (Duncan's Multiple Range Test).

It has been observed that any of CHI, in particular, 250 mg/l alleviated the harmful effect of water stress especially at high level on nitrogen, phosphorous and potassium percentages.

Leaf anatomy

Cross section of 3rd terminal leaflet of cowpea showed that there were significant changes in leaf anatomical characteristics due to water stress (Table 4 and figure, 1). In particular, water stress resulted in a significant decrease of the thickness of almost all anatomical characters i.e.

components of the mesophyll, as well as of the entire lamina thickness (Table 4 and figure, 1). Data presented in Table (4) and illustrated in Figure (1) indicate that water stress decreased thickness of leaflet and mesophyll tissue as well as main vascular bundle dimensions, xylem and phloem tissue thickness.

The data also indicated that cowpea leaflet has a well developed layer of water storage tissue which consists of 1-3 cell thick laying under main vascular bundle in the midrib region. Foliar application of chitosan,in particular, 250 mg/l increased the thickness of cowpea leaf blade, due to the increase in the thickness of mesophyll tissue. In addition, the thickness of leaf blade through midrib region was also increased, due to the increase in the midrib vascular bundle. Moreover, chitosan application increased the thickness of water storage tissue thickness. chitosan resulted in increasing the area of xylem and phloem tissues, due to the stimulation of pro-cambium activity in the midrib bundle during their differentiation. Concerning the interaction between water stress and chitosan, the interactions increased the cowpea leaflet anatomical characteristics as compared with untreated plants under such stress levels.

Table (4): Effect of water stress and chitosan and their interactions on leaflet anatomical characters of cowpea plants in the second season.

Treatments		Leaflet thickness (µ)	Palisade parenchyma tissue thickness (µ)	Spongy parenchyma tissue thickness (µ)	Storage tissue thickness (µ)	Thickness of midrib region (µ)	Main vascular bundle dimensions (µ)		Xylem tissue thickness (µ)	Phloem tissue thickness (µ)
Water stress % FC	Chitosan (mg/l)						length	width		
70% FC	W	68	32	36	16	276	48	96	36	12
	125	68	36	32	24	328	60	104	44	16
	250	68	36	32	44	336	60	116	40	20
	500	56	32	24	32	284	52	112	32	20
50% FC	W	52	28	24	24	216	32	68	24	8
	125	72	40	32	44	308	52	124	36	16
	250	72	36	36	52	316	56	128	40	16
	500	56	32	24	40	316	56	92	44	12
30% FC	W	48	24	24	16	188	28	60	20	8
	125	56	36	20	28	268	36	88	20	16
	250	60	28	32	44	324	48	96	32	16
	500	52	28	24	20	212	36	68	24	12

Since leaves are the main organs of internal water removal, CHI treated plants under normal or stressed conditions undertaken leaf anatomical alterations in order to save water. In fact, cowpea leaves treated with CHI showed a thicker entire leaf lamina and palisade mesophyll than untreated plants under such stress levels. A thicker palisade parenchyma in this treatments may enhance survival and growth under water stress (WS) conditions by improving water relations and providing higher protection for the inner tissues (Bacelar *et al*. 2006).

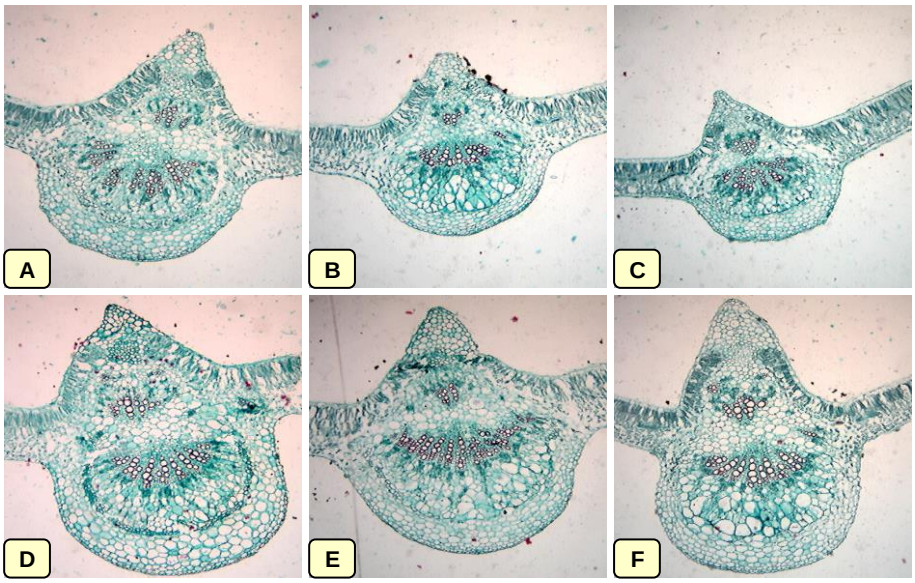

Figure (1): Effect of water stress and chitosan and their interactions on leaflet anatomical characters of cowpea plants in the second season (A, 70% of field capacity; B, 50% of field capacity; C, 30% of field capacity; D, 70% FC +250 mg/l chitosan; E, 50% FC +250 mg/l chitosan; E, 30% FC +250 mg/l chitosan).

Yield and its components:

Although water deficit affects all stages of the growth and development of crops, pod yield is much more depressed by water deficit than vegetative growth. In this concern, data presented in Table (5) show that pod yield per plant (g/plant) and seed quality (Nitrogen, phosphorous, potassium, protein and carbohydrates percentages) were significantly decreased with an increase in water deficit. On the other hand, foliar application of CHI concentrations, in particular, 250 mg/l resulted in a highest increase in cowpea yield and improved seed quality. The interaction treatments indicated that application of 250 mg/l CHI under moderate and severe water deficit significantly increased the yield and its quality. Meanwhile, the lowest and highest concentrations from CHI under moderate and severe water stress alleviated the harmful effect of water deficit in this respect.

DISCUSSION

The inhibiting effects of water stress on plant growth were previously supported by Abdalla (2011), Vurayai *et al* (2011) and Hefny (2011) using soybean, bambara groundnuts and white lupin plants respectively. It is well known that, water stress conditions, causes a multitude of changes in molecular, biochemical and physiological phenomena, thereby affecting plant growth and development (Boutraa 2010). Such decline in plant growth in response to water stress might be due to either decrease in cell elongation resulting from the inhibiting effect of water shortage on growth promoting hormones which, in turn, led to a decrease in each of cell turgor, cell volume and eventually cell growth (Banon *et al*. 2006), and/ or due to blocking up of xylem and phloem vessels thus hindering any translocation through. Moreover, water stress conditions cause a marked suppression in plant photosynthetic efficiency, mainly due to closing of stomata, which limits CO_2 diffusion into the leaf, or due to inhibition in Ribulose-1,5-bisphosphate carboxylase/oxygenase (Ribusco), a non-stomatal factor (Lawlor and Cornic 2002) and impairment of ATP synthesis (Tezara *et al*. 1999). Also, the depression effect of water stress on growth parameters may be attributed to drop in leaf relative water content in which reduce the leaf turgor (unpublished data), the assimilation of water and nitrogen compounds (Reddy *et al*. 2003), which affects the rate of cell division and enlargement. In this concern, a reduction in vegetative growth of plants under drought, in particular shoot growth, reduced cyclin-dependent kinase activity resulting in slower cell division as well as inhibition of growth and/or due to the relatively severe reduction pertaining to plant tissue, cell size, number of cell per unit or intercellular space (EL-Beltagy *et al*. 1984). Also, drought stress reduced the uptake of essential elements and photosynthetic capacity reduction (Kandil *et al*. 2001) as well as the excessive accumulation of intermediate compounds such as reactive oxygen species (Yazdanpanah *et al*. 2011) which cause oxidative damage to DNA, lipid and proteins and consequently a decrease in plant growth. Finally, water stress leads to increase in abscisic acid levels in roots, which will transport from roots to shoot and will act in the apical region of the plant with antagonist of the auxins and cytokinins, responsible for growth and cell division, respectively (Abdalla 2011) as well as inhibit DNA synthesis. On contrast, foliar spraying of chitosan in both seasons showed in most cases, a significant increase in cowpea growth parameters under normal or stressed conditions, the effect was more pronounced with chitosan at 250 mg/l. This result was supported by Chibu and Shibayama (1999) who revealed that dry weights of dry land rice c.v. Misatohatamochi grown with both 0.1 and 0.5% of CHI were increased. Similar results were reported by Ghoname *et al*. (2010) on sweet pepper and Farouk *et al*. (2008, 2011) on cucumber and radish plants. The stimulating effect of CHI on plant growth may be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting osmotic pressure in plant cells and reducing the accumulation of harmful free radicals through its effect on increasing the antioxidants compound and enzymes activities (Guan *et al*.

2009). Khan *et al*. (2002) added that foliar application of CHI increased net photosynthetic rate (P_N) of soybean and maize, this increase was correlated with increase in stomatal conductance (g_s) and transpiration rate (E), without any effects on intercellular CO_2 concentration (C_i). The increase in P_N and g_s in the absence of any increase in C_i indicates that the increase in P_N is due to enhanced uptake of CO_2 within the leaf that results in improved g_s, rather than due to more open stomata leading to increased P_N. If an increase in stomatal aperture had been the primary cause of the increase in P_N, an increase in the leaf C_i would have been expected (Morison 1998). In addition, the positive effect of CHI on plant growth may be due to its effect on increasing phosphorous content as presented in the present investigation. Phosphorous is an essential nutrient and plays an important role in the biosynthesis and translocation of carbohydrates and is necessary in stimulating cell division and the formation of DNA and RNA (Nijjar 1985)

Regarding the concentrations of chlorophylls and carbohydrates, the present investigation proved that water stress decreased chlorophylls and total carbohydrates concentration. The decrease in chlorophyll content under drought is thus a commonly observed phenenmene (Nikolaeva *et al*. 2010, Kumar *et al*. 2011). The decrease in chlorophyll under water stress might be due to reduced synthesis of the main chlorophyll pigment complexes encoded by the cab gene family (Allakhverdiev *et al*. 2003) or destruction of chiral macro-aggregates of light harvesting chlorophyll 'a' or 'b' pigment protein complexes (CHCIIs) which protect the photosynthetic apparatus or due to oxidative damage of chloroplast lipids, pigments and proteins (Lai *et al*. 2007). In addition, it may be due to impairment in the supply of magnesium and iron to the leaves. Similarly, the reduction in total sugar content induced by water stress treatments may be due to its inhibitory effect on photosynthetic activities, photosynthetic pigment concentrations (Table, 2) as well as on the activity of ribulose diphosphate carboxylase leading to decrease in all sugar fractions (Stibrova *et al*. 1986). As regard to, the effects of chitosan especially at 250 mg/l on increasing chlorophylls and total carbohydrates contents were confirmed with Farouk *et al*. (2008 on cucumber plant) and Farouk *et al*. (2011 on radish plant). The influence of CHI on alleviating the water stress effect on the photosynthetic pigments might be due to the fact that CHI enhanced the endogenous level of cytokinins, which stimulates chlorophyll synthesis. Chibu and Shiayama (2001) referred these positive effects to more availability of amino compounds released from CHI. Data in the present investigation indicate that foliar application of CHI increased significantly both nitrogen and potassium content in plant shoot (Table, 3) which may be play an important role in increasing the number of chloroplast per cell, the cell size and number per unit area as well as increased the synthesis of chlorophyll (Possingham 1980).

Water stress affects the availability of nutrients in the soil by its effects on the solubility and precipitation of salt and alters physiological processes within the plant, including nutrient uptake and translocation (Power 1990). Nutrient uptake by plants is generally decreased under water stress conditions owing to a substantial decrease in transpiration rates and impaired active transport and membrane permeability (Levitt 1980), and resulting in a

reduced root-absorbing power of crop plants. Nitrogen serves as constituents of many plant cell components like amino and nucleic acids. Therefore, N deficiency rapidly inhibits plant growth. The decrease in N content due to water stress has been reported in various crops including wheat (Singh and Usha 2003) and in soybean (Tanguilig *et al*. 1987). Phosphorous is one of the most important nutrients in the growth and development of plants. It plays a key role in cellular energy transfer, respiration, photosynthesis. Phosphorous uptake decreased with decreasing soil moisture in different crops like pepper (Turner 1985) and wheat (Ashraf *et al*. 1998). The role of CHI on increasing ion contents may be due to its effects on stabilizing cellular membrane through increasing the antioxidants substances and saving cell membrane from oxidative stress so improved plant cell permeability (unpublished data) leading to increasing ion content. This observation was supported with the results of Guan *et al*. (2009) who indicate that application of CHI significantly decreased lipid peroxidation, due to stimulation of some antioxidants enzymes leading to decreasing membrane permeability and improved its functions. More reports confirmed these results i.e. Farouk *et al*. (2008 and 2011) and Ghoname *et al*. (2010).

The reduction in cowpea yield due to water stress was confirmed with Costa *et al*. (2008), Vurayai *et al*. (2011) and Hefny (2011). In legume plants like cowpea, seed yield is determined by the production of three components; the number of pods per plant that reach maturity, the average number of seeds in each pod and dry weight of seeds. There are many hypothesis show the influence of water stress on yield. One of them, proved that water stress may be decreased number of branches and leaves per plant (Table, 1) as well as leaf area as indicate in this study (unpublished data), resulting in a reduction in the supply of carbon assimilate and photosynthetic rate as well as reduces radiation interception by plants and consequently less biomass produced as well as decreased translocation of photoassimulate towards the developing fruits (Kumar *et al*. 1994). Another possibility to reduce the yield due to water stress is increasing the rate of flower and pod abortion (Liu *et al*. 2003). A decreased rate of carbohydrates flux from leaves to reproductive structures has been reported to controlling pod set in well watered plants (Kokubun *et al*. 2001, Setter *et al*. 2001). Recent evidence supports this hypothesis. In maize, low water potential disrupt carbohydrates metabolism in ovaries by reducing the activity of acid invertase, which is the key enzymes catalyzing breakdown of incoming sucrose during ovary and early seed development (Anderson *et al*. 2002). Moreover, Song *et al*. (1998) showed that water stress, induced swollen pollen and filament development, decreased filament fertility and resulted in reeducation in grain number and weight per ear. However, the increase in cowpea yield due to CHI application may be due to its effects on stimulation of physiological processes which reflect on improving vegetative growth that followed by active translocation of the photoassimilates from source to sink in cowpea plant due to increasing leaf blade thickness as well as dimensions of vascular bundles as indicated from the present investigation. The increased in plant biomass may be due to improving photosynthetic machinery (Khan *et al*. 2002). These results were supported by Ghoname *et al*. (2010) who observed that foliar application of

CHI on sweet pepper increased significantly no. of fruits per plant and mean weight of fruit as well as some quality characteristics like total acidity, total soluble solid and ascorbic acid content in the fruit. The role of CHI on alleviating the harmful effect of water stress on yield may be due to an increase in stomatal conductance and net photosynthetic CO_2 fixation activity under water stress (Khan *et al*. 2002) and to its role as antitranspiration to save water. CHI treated plants showed a thicker entire leaf lamina, upper epidermis and palisade mesophyll than untreated plants under such stress levels. A thicker upper epidermis (including upper cuticle) and a thicker palisade parenchyma in this treatments may enhance survival and growth under water stress (WS) conditions by improving water relations and providing higher protection for the inner tissues (Bacelar *et al*. 2006) which leading to increasing plant yield.

It could be concluded that treated cowpea plants with chitosan inducedits ability to grow under water stress conditions. It may be explaind that cowpea plants with chitosan can produce some metabolites which cause closure of the stomata resulting in reduction of the transpiration.

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تحسين نمو وإنتاجية نبات اللوبيا النامي تحت ظروف الإجهاد المائي بواسطة الكيتوزان

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تعرض نبات اللوبيا للإجهاد المائي يسبب ضعف النمو وإنخفاض نسبة العناصر والمغذية بالإضافة إلي محتوي النبات من الكلوروفيل والكربوهيدرات الكلية مؤدية إلي حدوث إنخفاض حاد بالمحصول مع إنخفاض جودتة. علي الجانب الآخر فإن رش النباتات بالكيتوزان وخاصة بتركيز ٢٥٠ ملليجرام/لتر سواء تحت الظروف الطبيعية أو ظروف الإجهاد المائي يعمل علي تحسن وزيادة صفات النمو مع زيادة نسبة العناصر بالنبات وارتفاع محتوي النبات من صبغات الكلوروفيل والكربوهيدرات، الذي يستتبعة زيادة المحصول وإرتفاع صفات الجودة به.

تشريحيا، يعمل اللإجهاد المائي علي تقليل سمك كل من الوريقة بمنطقة العرق الوسطي، النسيج المتوسط وسمك الحزمة الوعائية الرئيسية. بينما إستعمال الكيتوزان خاصة ٢٥٠ ملليجرام/لتر تحت الظروف الطبيعية أو ظروف الإجهاد المائي يعمل علي تحسن جميع الصفات التشريحية للوريقة. نخلص مما سبق إلي إمكانية دفع نبات اللوبيا لتحمل نقص الماء من خلال إستخدام ٢٥٠ ملليجرام/لتر كيتوزان.

قام بتحكيم البحث

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Table (5): Effect of water stress and chitosan and their interactions on yield and its quality during the two growing seasons.

Treatments		Pod yield per plant		Nitrogen %		Phosphorous %		Potassium %		Protein %		Carbohydrates %	
Water stress % FC	Chitosan (mg/l)	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
70% FC	W	16.49± 0.746 bcd	16.53± 0.508 d	3.87± 0.704bcde	3.87± 0.704cde	0.526± 0.016de	0.515± 0.023 de	1.26± 0.034cde	1.16± 0.210def	24.20± 4.40bcde	24.20± 4.409cde	38.84± 0.298 e	38.28± 0.174d
	125	17.75± 0.360 bc	17.43± 0.450 bc	4.13± 1.26 abc	4.10± 0.080 bc	0.613± 0.072 c	0.617± 0.070 c	1.36± 0.136 bc	1.34± 0.200 bc	25.81± 7.88 abc	25.66± 0.508 bc	41.13± 0.833 c	41.65± 0.480b
	250	22.63± 5.381 a	19.19± 1.806 a	4.50± 0.213 a	4.45± 0.213 a	0.875± 0.221 a	0.839± 0.120 a	1.55± 0.072 a	1.51± 0.072 a	28.14± 1.33 a	27.85± 1.335 a	44.92± 2.601a	43.77± 0.703a
	500	16.58± 0.560cde	16.63± 0.491 cd	3.75± 0.491bcdef	3.75± 0.080def	0.511± 0.007def	0.508± 0.007 de	1.19± 0.283def	1.10± 0.023efg	23.47± 3.07bcdef	23.47± 0.508def	37.86± 0.805 f	37.34± 0.805 e
50% FC	W	15.26± 0.336 ef	15.67± 0.571 e	3.52± 0.080 defg	3.47± 0.080fgh	0.472± 0.002efgh	0.474± 0.009 ef	1.09± 0.183fgh	1.01± 0.150ghi	22.01± 0.50 defg	21.72± 0.508fgh	35.32± 0.413h	34.77± 1.207g
	125	16.٤٩± 0.488 cde	16.54± 0.570 d	3.92± 0.84 bcde	3.92± 0.84 cd	0.556± 0.030 cd	0.542± 0.009 d	1.31± 0.034 cd	1.22± 0.023cde	24.39± 5.26 bcde	24.49± 5.250 cd	39.70± 0.727d	38.82± 0.480d
	250	18.21± 0.647 b	17.85± 0.960 b	4.27± 0.14 ab	4.24± 0.080 ab	0.722± 0.127 b	0.687± 0.064 b	1.45± 0.152 ab	1.39± 0.128 ab	26.68± 0.87 ab	26.53± 1.512 ab	42.73± 0.238b	42.23± 0.282b
	500	15.27± 0.520 ef	15.54± 0.762 e	3.59± 0.161 cdef	3.57± 0.242efg	0.499± 0.014defg	0.496± 0.011 e	1.14± 0.235efg	1.04± 0.100fgh	22.45± 1.01 cdef	22.30± 1.820efg	36.53± 0.504g	35.89± 0.718 f
30% FC	W	13.21± 0.935 g	12.89± 0.243 g	2.98± 0.291 g	2.98± 0.291 i	0.422± 0.034 h	0.411± 0.021 g	0.923± 0.152 i	0.84± 0.201 j	18.66± 1.82 g	18.66± 0.496 i	30.00± 0.511k	28.67± 0.470 j
	125	15.64± 0.336 def	15.37± 1.694 e	3.38± 1.122 efg	3.38± 0.080 gh	0.447± 0.000 fgh	0.445± 0.026 fg	1.04± 0.100ghi	0.94± 0.251 hij	21.14± 7.01 efg	21.14± 0.496 gh	33.22± 0.521 i	32.46± 1.156h
	250	17.32± 0.22 bc	17.09± 0.150bcd	4.03± 0.080 abcd	4.03± 0.080bcd	0.601± 0.059 c	0.586± 0.060 c	1.32± 0.152bcd	1.27± 0.057bcd	25.22± 0.50 abcd	25.22± 0.508bcd	40.67± 0.282 c	40.51± 0.306 c
	500	14.33±	14.32±	3.26±	3.24±	0.429±	0.430±	0.97±	0.89±	20.41±	20.26±	31.52±	30.92±

		0.831 fg	1.392 f	0.080 fg	0.080 hi	0.002 gh	0.012 g	0.105 hi	0.200 ij	0.50 fg	0.508 hi	0.680 j	0.765 i

Values are given as mean± SD of three replicate. Means in columns by different letters are significantly different at P < 0.05 by (Duncan's Multiple Range Test).