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Improving growth and yield of cowpea 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

The world population is increasing at an alarming rate and is expected to reach about six billion by the end of 2050, but food productivity is decreasing due to the effect of various abiotic stresses and climatic change; minimizing these losses is a major area of concern for all nations to cope with 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), producing about 40 % of the global food supply (Somerville & Briscoe 2001). Permanent or temporary water-deficit stress limits the growth and distribution of natural and artificial vegetation and the performance of 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 irrigation water which is already not sufficient to meet all the expected demands.

The responses of plants to drought vary greatly depending on species and stress severity (Mullet & Whitsitt 1996). Higher plants respond to water deficit in several ways: stomatal closure, leaf rolling, osmotic adjustments, reductions and consequently decreases in cellular expansion, and alterations of various essential physiological and biochemical processes that can affect growth, productivity and yield quality (Costa *et al.* 2008, Lobato *et al.* 2008, Hefny 2011). For example, Carvalho *et al.* (2004) found that lupine cultivars tended to accumulate crude protein and carbon compounds in the seeds at the end of the water stress period (15 days after anthesis), but 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 summers usually occur, and drought events can have a large impact on both productivity and crop quality. Bittelli *et al.* (2001) reported that occasional or episodic drought events can be counteracted through the use of anti-transpirants, compounds applied to foliage to limit the water loss. They include both film-forming and stomata-closing compounds, able to increase leaf resistance to water vapor

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loss, thus improving plant water use in assimilating carbon, and, in turn, producing biomass or yield (Tambussi & Bort 2007). Another approach to reducing water loss due to transpiration is by increasing the reflection of sunlight from leaves, through reflectant types of anti-transpirants, thus limiting water loss from evaporative leaf cooling (Gaballah & Moursy 2004).

Among anti-transpirant compounds, chitosan has previously proved to be effective in pepper (Bittelli *et al.* 2001). Chitosan is a natural, low toxic and inexpensive compound that is biodegradable and environmentally friendly with various applications in agriculture; it is obtained by the deacetylation of chitin. In agriculture, chitosan has been used in seed, leaf, fruit and vegetable coatings, as fertilizer and in controlled agrochemical release, to increase plant productivity (New *et al.* 2004), to protect plants against microorganisms (Farouk *et al.* 2008) and 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 chitosan 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 chitosan resulted in higher vegetative growth and improvement in fruit quality of cucumber (Farouk *et al.* 2008). For other cultivated plants, Bittelli *et al.* (2001) reported that foliar application of chitosan 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 chitosan 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 total sugars showed similar trends. Recently, Sheikha & AL-Malki (2011) indicate that application of different concentrations of chitosan enhanced bean shoot and root length, fresh and dry weights of shoots, root and leaf area as well as the level of chlorophyll in leaves. The mechanisms of chitosan in counteracting the harmful effect of water stress are not well understood and there are a few reports in the literature. Transcriptional activation, induced by both chitosan and jasmonic acid, of genes encoding phenylalanine ammonia lyase and protease inhibitors, suggests that chitosan may influence pathways involving jasmonic acid (Doares *et al.* 1995). Jasmonates exhibit activities similar to the plant hormone abscisic acid, which plays a key role in the regulation of water use by plants. Increased levels of abscisic acid result in closure of stomata and reduced transpiration (Leung & Giraudat 1998). These authors demonstrated that chitosan 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 chitosan on stomatal aperture suggest the possibility that it might be a valuable anti-transpirant with useful agricultural applications.

Cowpea (*Vigna unguiculata* (L) Walp) is one of the ancient grain legumes valued for its nutritional value, especially its high protein content (25%), flavour 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). Cowpea forms an integral part of sustainable agriculture and land use (Ogbonnaya *et al.* 2003). The total cultivated area of this crop in Egypt was estimated at about 9155 feddan (3845 ha) for dry seed production in 2008, with a mean production of 980 kg/feddan (412 kg ha^{-1}). The estimates for fresh pods was 10064 kg/feddan (4227 kg ha^{-1}) with a mean production of 5.19 ton/feddan (data from the Department of Agricultural Statistics, Ministry of Agriculture, Giza, Egypt).

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

Materials & Methods

Two pot experiments were conducted in the experimental farm and laboratory of the Agricultural Botany Department, Faculty of Agriculture, Mansoura University, Egypt, during the two successive seasons of 2007 and 2008. Cowpea (*Vigna ungiculata* (L.) Walp. Cv Cream 7) seed 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) obtained from the General Organization for Agriculture Equalization Fund, Ministry of Agriculture. Egypt.

Sowing took place on 15 and 10 April in both seasons respectively. 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-day intervals until the seedlings reached the fourth leaf stage. The seedlings were then 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 start of flowering. After that the pots were divided into three groups for water-stress treatments, with each group divided into four subgroups for chitosan foliar application. The soil moisture for all pots was kept at 80% field capacity until 15 days after sowing. After that, the water stress treatments were initiated.

Pots were subjected to one of the three water-stress treatments: a well-watered control, 70% of field capacity; and two water-stress treatments, 'moderate' at 50% and 'severe' at 30% of field capacity. In the stressed treatments, moisture levels were allowed to fall from the initial 70% to 50% and 30% of field capacity, respectively. All pots were weighed every two days. Losses in pot weight represent transpiration and evaporation. Cumulative water losses were added to each pot to compensate for transpiration and evaporation. Accumulated water loss was calculated as the differences in pot weight between successive weightings. 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 until dripping, using a small pressure pump after adding tween 20 as a wetting agent at a concentration of 0.5%.

Three uniform plants were uprooted from each pot at the full blooming stage (80 days from sowing) to measure morphological and physiological characteristics as well as leaflet anatomy. For morphological measures, the plants were cleaned and plant height, number of leaves, number of branches and leaf area (using a leaf-area meter) were determined. Fresh and dry weights were estimated by drying each plant at 70°C to a constant weight. Chemical composition measures included total chlorophyll, carbohydrates and minerals. Total chlorophyll was extracted for 24 h at room temperature in methanol after adding traces of sodium carbonate, and determined spectrophotometrically (Spekol 11, Uk) according to Lichtenthaler & Wellburn (1983). Total carbohydrate content was estimated using the anthrone method as described by Sadasivam & Manickam (1996). To measure mineral constituents, a dry shoot (0.2 g) was digested using 5 ml of a mixture of sulfuric and perchloric acid ($HClO_3/H_2SO_4$ 1:1 v:v) until the sample became clear and cool; this was made up to 50 ml using deionized water. Total nitrogen was determined by the micro-Kjeldahl method. Potassium was determined by flame-photometry (Kalra 1998). Phosphorous was estimated using ammonium molybdate and ascorbic acid (Cooper 1977).

For leaflet anatomy, small pieces from the midrib region of the 3rd upper leaflet (second season) were fixed in formalin aceto alcohol for 48 h, then dehydrated in an n-butanole series and embedded in paraffin wax (52-54 ^{o}C melting points). 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 under a microscope to determine any anatomical changes in leaf structure.

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

All data were analyzed statistically using one-way ANOVA, followed by Duncan's Multiple Range Test using COSTAT software. The values presented are all mean ± SD for three samples in each group. P values <0.05 were considered as significant.

Results

Generally, severe reductions in plant growth, manifested by smaller, chlorotic, wilted, and rolled leaves were recorded due to water stress (Table 1). All plant growth characters (plant height, shoot and leaf number per plant, and shoot fresh and dry weight) significantly decreased due to water stress in both growing seasons. The largest reduction was observed under severe water stress (30% of field capacity).

Table 1: Effect of water stress, chitosan and their interaction on growth parameters during two growing seasons (1st and 2nd). Water stress values are % of field capacity; chitosan treatments are in mg l^{-1}

Treatments		Plant ht (cm)		Branch number		Leaf number		Shoot dry wt (g)		Shoot fresh wt (g)	
Water stress	Chitosan	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
70	water	38.1±0.3^{d}	37.5±2.1^{d}	4.0±0.0^{c}	4.0±0.0^{c}	30.0±1.1^{e}	29.0±0de	7.2±0.1^{d}	7.1±0.5^{c}	37.2±0.4^{def}	37.5±0.5^{de}
	125	41.1±1.5^{b}	41.1±1.2^{b}	5.0±0.0^{b}	4.7±1.2^{b}	34.3±1.2^{c}	34.3±1.2^{b}	7.8±0.1^{c}	7.7±0.1^{b}	40.3±0.8^{c}	40.0±1.8^{c}
	250	43.8±2.0^{a}	43.3±1.9^{a}	6.0±0.0^{a}	5.7±1.2^{a}	37.7±1.2^{a}	37.3±1.2^{a}	8.7±0.7^{a}	8.5±1.1^{a}	56.9±4.4^{a}	47.0±4.3^{a}
	500	36.0±2.4^{e}	35.5±2.5^{e}	4.0±0.0^{c}	4.0±0.0^{c}	28.3±1.2^{f}	27.7±1.2^{e}	6.9±0.2^{e}	6.7±0.1^{d}	37.0±1.0^{def}	36.7±1.2^{def}
50	water	30.3±2.7^{g}	29.8±1.1^{g}	3.0±0.0^{d}	3.0±0.0^{d}	25.3±1.2^{h}	24.7±1.2^{fg}	6.6±0.2^{f}	6.1±0.02^{e}	35.8±1.6^{fg}	35.6±1.0^{ef}
	125	39.0±1.6^{cd}	39.0±0.5^{c}	4.0±0.0^{c}	4.0±0.0^{c}	30.7±1.2^{e}	30.0±0.0^{cd}	7.3±0.1^{d}	7.3±0.1^{c}	38.1±1.6^{de}	37.9±0.8^{cde}
	250	42.5±2.6^{a}	42.0±1.1^{b}	5.0±0.0^{b}	5.0±0.0^{b}	35.7±1.2^{b}	35.7±1.2^{b}	8.2±0.3^{b}	7.8±0.1^{b}	44.1±2.1^{b}	43.3±3.2^{b}
	500	32.4±3.2^{f}	31.8±0.9^{f}	3.7±1.2^{c}	3.0±0.0^{d}	26.7±1.2^{g}	26.0±2.0^{f}	6.7±0.05^{f}	6.7±0.02^{d}	36.6±0.7^{ef}	36.6±1.0^{def}
30	water	26.3±1.6^{i}	25.3±2.2^{i}	2.0±0.0^{e}	2.0±0.0^{e}	21.0±2.0^{j}	21.3±4.6^{h}	5.2±0.1^{i}	5.0±0.02^{g}	29.4±2.5^{i}	28.3±5.3^{h}
	125	28.9±0.2^{gh}	28.1±0.2^{h}	3.0±0.0^{d}	3.0±0.0^{d}	24.3±1.2^{h}	23.3±1.2^{g}	6.0±0.1^{g}	5.8±0.2^{f}	34.4±1.0^{g}	34.4±2.6^{f}
	250	39.8±0.3^{bc}	39.6±1.5^{c}	4.0±0.0^{c}	4.0±0.0^{c}	32.0±0.0^{d}	31.3±1.2^{c}	7.4±0.1^{d}	7.4±0.1^{c}	38.5±0.7^{d}	38.2±0.5^{cd}
	500	27.5±1.3^{hi}	27.5±0.4^{h}	2.7±1.2^{d}	2.3±1.2^{e}	22.3±1.2^{i}	21.7±1.2^{h}	5.4±0.1^{h}	5.3±0.1^{g}	32.4±3.2^{h}	31.5±2.7^{g}

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

Foliar application of chitosan, and especially at 250 mg l^{-1}, improved all plant growth measures compared to untreated control plants. Regarding the interaction effects, application of chitosan at 250 mg l^{-1} significantly increased all growth parameter of cowpea with an increasing effect with greater stress. However, while application at 125 mg l^{-1} also counteracted the harmful effect of water stress on plant growth, application at 500 mg l^{-1} significantly decreased all the parameters.

The total content of chlorophylls and carbohydrates (Table 2) significantly decreased under water stress in both growing seasons as compared with the control plants. Foliar application of chitosan, especially at 250 mg l^{-1}, significantly increased these parameters compared with untreated plants under stress. As for its interactions with water stress, the effect of chitosan in counteracting the water stress decreased with increasing water stress.

Table 2: Effect of water stress and chitosan and their interactions on chlorophyll (mg/g fresh wt) and total carbohydrates (mg/g dry wt) contents during the two growing seasons (1st and 2nd). Water stress values are % of field capacity; chitosan treatments are in mg l^{-1}

Treatments		Chlorophyll A		Chlorophyll B		Total chlorophyll		Total carbohydrates	
Water stress	Chitosan	1st	2nd	1st	2nd	1st	2nd	1st	2nd
70	water	0.43±0.03^{bc}	0.51±0.22^{bc}	0.30±0.01^{a}	0.19±0.06^{a}	0.73±0.03^{de}	0.70±0.16^{de}	30.2±0.6^{e}	32.0±1.6^{cd}
	125	0.54±0.19^{b}	0.52±0.22^{bc}	0.29±0.22^{a}	0.29±0.20^{a}	0.81±0.02^{bc}	0.81±0.02^{bc}	35.0±1.8^{bc}	36.1±0.5^{ab}
	250	0.80±0.22^{a}	0.72±0.19^{a}	0.24±0.08^{a}	0.28±0.09^{a}	1.04±0.30^{a}	1.00±0.20^{a}	37.3±0.9^{a}	38.6±1.7^{a}
	500	0.46±0.25^{bc}	0.39±0.07^{bcd}	0.24±0.26^{a}	0.28±0.05^{a}	0.70±0.01^{def}	0.68±0.02^{de}	27.2±4.6^{f}	30.7±1.4^{cd}
50	water	0.38±0.18^{bc}	0.45±0.26^{bc}	0.25±0.15^{a}	0.24±0.21^{a}	0.64±0.03^{fg}	0.60±0.01^{fg}	25.5±0.4^{f}	30.8±1.2^{cd}
	125	0.48±0.35^{bc}	0.58±0.34^{ab}	0.25±0.34^{a}	0.15±0.32^{a}	0.73±0.01^{de}	0.73±0.02^{d}	31.8±2.3^{de}	33.5±1.0^{bc}
	250	0.61±0.24^{ab}	0.72±0.07^{a}	0.26±0.30^{a}	0.15±0.05^{a}	0.88±0.07^{b}	0.87±0.07^{b}	36.3±0.9^{ab}	37.5±1.1^{a}
	500	0.53±0.27^{b}	0.40±0.32^{bcd}	0.14±0.28^{a}	0.23±0.31^{a}	0.67±0.03^{efg}	0.63±0.05^{ef}	27.3±0.9^{f}	28.9±1.3^{de}
30	water	0.28±0.19^{c}	0.20±0.06^{d}	0.12±0.14^{a}	0.14±0.03^{a}	0.39±0.06^{i}	0.34±0.07^{h}	18.9±4.1^{h}	19.7±2.7^{g}
	125	0.40±0.30^{bc}	0.38±0.22^{bcd}	0.20±0.31^{a}	0.20±0.21^{a}	0.60±0.01^{gh}	0.58±0.02^{fg}	23.5±2.2^{g}	25.8±0.7^{ef}
	250	0.55±0.27^{b}	0.51±0.23^{bc}	0.22±0.24^{a}	0.24±0.26^{a}	0.77±0.04^{cd}	0.75±0.04^{cd}	33.3±0.5^{cd}	35.5±0.4^{ab}
	500	0.43±0.27^{bc}	0.32±0.25^{cd}	0.11±0.28^{a}	0.20±0.31^{a}	0.54±0.05^{h}	0.52±0.06^{g}	21.8±1.8^{g}	24.3±1.6^{f}

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

Water stress is generally recognized as injurious to plants by disturbing the electrolyte balance, resulting in deficiency of some nutrients. Water stress decreased significantly the percentages of nitrogen, phosphorus and potassium in both growing seasons (Table 3). The largest reduction occurred under severe water stress. Pronounced and highly significant increases in all these nutrients occurred after exogenous application of chitosan, especially at 250 mg l^{-1}. As with other measures, application at 500 mg l^{-1} reduced this effect. The impact of chitson was greater in stressed than unstressed plants.

Table 3: Effect of water stress and chitosan and their interactions on nitrogen,phosphorous and potassium percentage during the two growing seasons (1st and 2nd). Water stress values are % of field capacity; chitosan treatments are in mg l^{-1}

Treatments		Nitrogen %		Phosphorous %		Potassium%	
Water stress	Chitosan	1st	2nd	1st	2nd	1st	2nd
70	water	3.71 ± 0.14^{f}	3.43 ± 0.14^{e}	0.50 ± 0.01^{cd}	0.51 ± 0.01^{e}	1.25 ± 0.07^{e}	1.04 ± 0.08^{e}
	125	4.29 ± 0.08^{c}	4.17 ± 0.08^{b}	0.54 ± 0.03^{bc}	0.61 ± 0.04^{c}	1.56 ± 0.03^{b}	1.44 ± 0.10^{b}
	250	4.76 ± 0.14^{a}	4.48 ± 0.14^{a}	0.72 ± 0.16^{a}	0.76 ± 0.03^{a}	1.68 ± 0.02^{a}	1.59 ± 0.05^{a}
	500	3.38 ± 0.16^{g}	3.22 ± 0.14^{f}	0.48 ± 0.02^{de}	0.49 ± 0.02^{ef}	1.10 ± 0.11^{f}	0.91 ± 0.05^{f}
50	water	3.12 ± 0.08^{h}	3.01 ± 0.14^{gh}	0.43 ± 0.02^{efg}	0.45 ± 0.02^{g}	0.85 ± 0.07^{h}	0.78 ± 0.03^{h}
	125	3.99 ± 0.14^{e}	3.64 ± 0.24^{d}	0.51 ± 0.01^{cd}	0.52 ± 0.02^{e}	1.37 ± 0.10^{d}	1.19 ± 0.09^{d}
	250	4.50 ± 0.21^{b}	4.29 ± 0.08^{b}	0.59 ± 0.04^{b}	0.70 ± 0.05^{b}	1.64 ± 0.05^{a}	1.54 ± 0.05^{a}
	500	3.19 ± 0.08^{h}	3.10 ± 0.08^{fg}	0.46 ± 0.03^{def}	0.47 ± 0.01^{fg}	0.97 ± 0.07^{g}	0.84 ± 0.05^{g}
30	water	2.68 ± 0.08^{k}	2.49 ± 0.21^{i}	0.37 ± 0.05^{g}	0.38 ± 0.03^{i}	0.70 ± 0.08^{j}	0.63 ± 0.06^{j}
	125	3.01 ± 0.14^{i}	2.87 ± 0.14^{h}	0.40 ± 0.02^{fg}	0.42 ± 0.02^{h}	0.81 ± 0.05^{hi}	0.73 ± 0.03^{hi}
	250	4.17 ± 0.08^{d}	3.92 ± 0.28^{c}	0.50 ± 0.13^{cd}	0.55 ± 0.04^{d}	1.49 ± 0.10^{c}	1.30 ± 0.07^{c}
	500	2.84 ± 0.21^{j}	2.63 ± 0.22^{i}	0.39 ± 0.01^{g}	0.40 ± 0.01^{hi}	0.75 ± 0.02^{ij}	0.70 ± 0.02^{i}

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

Cross-sections of the 3rd terminal leaflet of cowpea showed that there were significant changes in leaf anatomical characteristics due to water stress (Table 4, Fig. 1). In particular, water stress resulted in a significant decrease of the thickness of almost all anatomical characters, i.e. the thickness of the leaflet, mesophyll tissue as well as main vascular bundle dimensions, xylem and phloem tissue thickness.

Table 4: Effect of water stress and chitosan and their interaction on leaflet anatomical characters of cowpea plants in the second season. Water stress values are % of field capacity; chitosan treatments are in mg l^{-1}

Treatments							Main vascular bundle dimension (µ)			
Water stress	Chitosan	Leaflet thickness (µ)	Palisade parenchyma tissue thickness (µ)	Spongy parenchyma tissue thickness (µ)	Storage tissue thickness (µ)	Thickness of midrib region (µ)	length	width	Xylem tissue thickness (µ)	Phloem tissue thickness (µ)
70	water	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	water	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	water	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

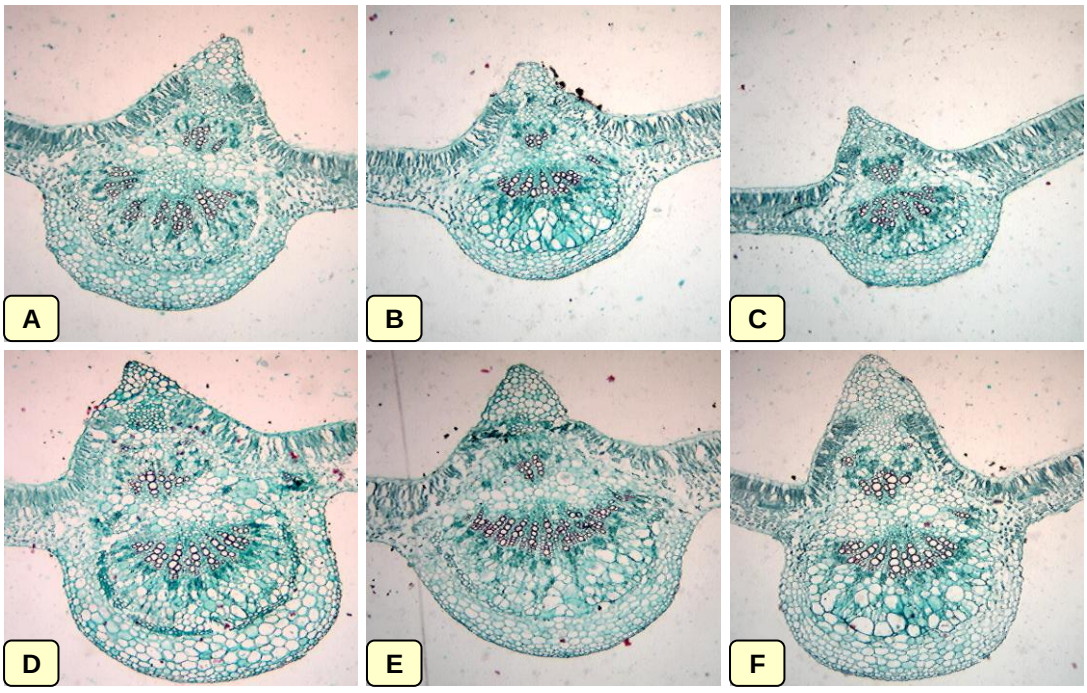

Figure 1: Effect of water stress and chitosan and their interaction on leaflet anatomical characters of cowpea plants in the second season. A, 70% of field capacity; B, 50% of field capacity; C, 30% of field capacity; D, 70% of field capacity + 250 mg/l chitosan; E, 50% of field capacity + 250 mg/l chitosan; E, 30% of field capacity + 250 mg/l chitosan. (all 40x).

Table 5: Effect of water stress and chitosan and their interactions on yield and its quality during the two growing seasons (1st and 2nd). Water stress values are % of field capacity; chitosan treatments are in mg l^{-1}.

Treatments		Pod yield per plant		Nitrogen %		Phosphorous %		Potassium %		Protein %		Carbohydrates %	
Water stress	Chitosan	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
70	water	16.5±0.7^{bcd}	16.5±0.5^{d}	3.87±0.70^{bcde}	3.87±0.70^{cde}	0.53±0.02^{de}	0.51±0.02^{de}	1.26±0.03^{cde}	1.16±0.21^{def}	24.2±4.4^{bcde}	24.2±4.4^{cde}	38.8±0.3^{e}	38.3±0.2^{d}
	125	17.7±0.4^{bc}	17.4±0.4^{bc}	4.13±1.26^{abc}	4.10±0.08^{bc}	0.61±0.07^{c}	0.62±0.07^{c}	1.36±0.14^{bc}	1.34±0.20^{bc}	25.8±7.9^{abc}	25.7±0.5^{bc}	41.1±0.8^{c}	41.6±0.5^{b}
	250	22.6±5.4^{a}	19.2±1.8^{a}	4.50±0.21^{a}	4.45±0.21^{a}	0.87±0.22^{a}	0.84±0.12^{a}	1.55±0.08^{a}	1.51±0.07^{a}	28.1±1.3^{a}	27.8±1.3^{a}	44.9±2.6^{a}	43.8±0.7^{a}
	500	16.6±0.6^{cde}	16.6±0.5^{cd}	3.75±0.49^{bcdef}	3.75±0.08^{def}	0.51±0.01^{def}	0.51±0.01^{de}	1.19±0.28^{def}	1.10±0.02^{efg}	23.5±3.1^{bcdef}	23.5±0.5^{def}	37.9±0.8^{f}	37.3±0.8^{e}
50	water	15.3±0.3^{ef}	15.7±0.6^{e}	3.52±0.08^{defg}	3.47±0.08^{fgh}	0.47±0.002^{efgh}	0.47±0.01^{ef}	1.09±0.18^{fgh}	1.01±0.15^{ghi}	22.0±0.5^{defg}	21.7±0.51^{fgh}	35.3±0.4^{h}	34.8±1.2^{g}
	125	16.5±0.5^{cde}	16.5±0.6^{d}	3.92±0.84^{bcde}	3.92±0.84^{cd}	0.56±0.03^{cd}	0.54±0.01^{d}	1.31±0.03^{cd}	1.22±0.02^{cde}	24.4±5.3^{bcde}	24.5±5.2^{cd}	39.7±0.7^{d}	38.8±0.5^{d}
	250	18.2±0.6^{b}	17.8±1.0^{b}	4.27±0.14^{ab}	4.24±0.08^{ab}	0.72±0.13^{b}	0.69±0.06^{b}	1.45±0.15^{ab}	1.39±0.13^{ab}	26.7±0.9^{ab}	26.5±1.5^{ab}	42.7±0.2^{b}	42.2±0.3^{b}
	500	15.3±0.5^{ef}	15.5±0.8^{e}	3.59±0.16^{cdef}	3.57±0.24^{efg}	0.50±0.01^{defg}	0.50±0.01^{e}	1.14±0.23^{efg}	1.04±0.10^{fgh}	22.4±1.0^{cdef}	22.3±1.8^{efg}	36.5±0.5^{g}	35.9±0.7^{f}
30	water	13.2±0.9^{g}	12.9±0.2^{g}	2.98±0.29^{g}	2.98±0.29^{i}	0.42±0.03^{h}	0.41±0.02^{g}	0.92±0.15^{i}	0.84±0.20^{j}	18.7±1.8^{g}	18.7±0.50^{i}	30.0±0.5^{k}	28.7±0.5^{j}
	125	15.6±0.3^{def}	15.4±1.7^{e}	3.38±1.12^{efg}	3.38±0.08^{gh}	0.45±0.00^{fgh}	0.44±0.03^{fg}	1.04±0.10^{ghi}	0.94±0.25^{hij}	21.1±7.0^{efg}	21.1±0.50^{gh}	33.2±0.5^{i}	32.5±1.2^{h}
	250	17.3±0.2^{bc}	17.1±0.1^{bcd}	4.03±0.08^{abcd}	4.03±0.08^{bcd}	0.60±0.06^{c}	0.59±0.06^{c}	1.32±0.15^{bcd}	1.27±0.06^{bcd}	25.2±0.5^{abcd}	25.2±0.51^{bcd}	40.7±0.3^{c}	40.5±0.3^{c}
	500	14.3±0.8^{fg}	14.3±1.4^{f}	3.26±0.08^{fg}	3.24±0.08^{hi}	0.43±0.002^{gh}	0.43±0.01^{g}	0.97±0.10^{hi}	0.89±0.20^{ij}	20.4±0.5^{fg}	20.3±0.51^{hi}	31.5±0.7^{j}	30.9±0.8^{i}

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

The data also indicated that cowpea leaflets have a well developed layer of water storage tissue, consisting of a layer 1-3 cells thick under the main vascular bundle in the midrib region. Foliar application of chitosan (especially at 250 mg l^{-1}) increased the thickness of the leaf blade by increasing the thickness of the mesophyll tissue and (through the midrib region) the midrib vascular bundle. It also increased the thickness of the water storage tissue, and increased the area of xylem and phloem by stimulating pro-cambial activity in the midrib bundle during differentiation. Concerning the interaction between water stress and chitosan, the impact of chitosan appeared to be greatest at moderate water stress levels.

Although water deficit affects all stages of growth and development, grain yield is much more depressed than vegetative growth. Pod yield per plant and seed quality significantly decreased with increasing water deficit (Table 5), but foliar application of chitosan, especially at 250 mg l^{-1}, reversed this and gave increases in yield and improved seed quality. The interactions treatment indicated that application of 250 mg/l CHI under moderate and severe water deficit significantly increased the yield and its quality. Meanwhile the low and high concentration from CHI under moderate and severe water stress counteract the harmful effect of water deficit in this respect.

Discussion

The inhibiting effects of water stress on plant growth have previously been reported for soybean (Abdalla 2011), bambara groundnuts (Vurayai *et al.* 2011) and white lupins (Hefny 2011). It is well known that water stress conditions cause a multitude of molecular, biochemical and physiological changes, thereby affecting plant growth and development (Boutraa 2010). A decline in plant growth in response to water stress might be due either to decreases in cell elongation resulting from the inhibiting effect of water shortage on growth-promoting hormones which, in turn, lead to decreases in cell turgor, volume and eventually growth (Banon *et al.* 2006), and/or to the blocking up of xylem and phloem vessels, hindering translocation. Water-stress conditions cause a marked suppression in plant photosynthetic efficiency, mainly due to the closing of stomata, which limits CO_2 diffusion into the leaf, the inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), a non-stomatal factor (Lawlor & Cornic 2002), and the impairment of ATP synthesis (Tezara *et al.* 1999). The depressive effect of water stress on growth parameters may also be attributed to a drop in leaf relative water content which reduces leaf turgor (unpublished data) and the assimilation of water and nitrogen compounds (Reddy *et al.* 2003), affecting the rate of cell division and enlargement. A reduction in the 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 relatively severely reduced plant tissues, cell size, number of cells per unit or intercellular spaces (El-Beltagy *et al.* 1984). Drought stress also reduced the uptake of essential elements and photosynthetic capacity (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 increases in abscisic acid levels in roots, which is transported from roots to shoot where it acts in the apical region of the plant as an antagonist of the auxine and cytokinin, responsible for growth and cell division, respectively (Abdalla 2011) as well as inhibiting DNA synthesis.

Foliar spraying of chitosan in most cases resulted in a significant increase in cowpea growth parameters under normal or stressed conditions, more pronounced at the intermediate concentration of 250 mg l^{-1}. This result is similar in rice, where Chibu & Shibayama (1999) showed that both 0.1 and 0.5% chitosan increased the dry weights of land rice c.v. Misatohatamochi, sweet pepper (Ghoname *et al.* 2010) and cucumber and radish plants

(Farouk *et al.* 2008, 2011). The stimulating effect of chitosan on plant growth may be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals by increasing antioxidants and enzyme activities (Guan *et al.* 2009). Khan *et al.* (2002) added that foliar application of chitosan increased the net photosynthetic rates of soybean and maize, correlated with increases in stomatal conductance and transpiration rate, without any effects on intercellular CO_2 concentration. This pattern of increases in net photosynthetic rate and stomatal conductance in the absence of any increase in intercellular CO_2 concentration indicates that the increase in net photosynthetic rate is due to enhanced uptake of CO_2 within the leaf that results in improved stomatal conductance, rather than due to having more open stomata. If an increase in stomatal aperture had been the primary cause, an increase in the leaf intercellular CO_2 concentration would have been expected (Morison 1998). In addition, the positive effect of chitosan on plant growth may be due to its effect on increasing phosphorous content, as found in our investigation. Phosphorous is an essential nutrient playing an important role in the biosynthesis and translocation of carbohydrates, and necessary for stimulating cell division and forming DNA and RNA (Nijjar 1985).

Regarding the concentration of chlorophylls and carbohydrates, the present investigation showed that water stress decreased chlorophylls and total carbohydrates concentration. The decrease in chlorophyll content under drought is thus a commonly observed phenomenon (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 to destruction of chiral macro-aggregates of the light-harvesting chlorophyll 'a' or 'b' pigment protein complexes which protect the photosynthetic apparatus, or to oxidative damage of chloroplast lipids, pigments and proteins (Lai *et al.* 2007). An additional possibility is 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) or the activity of ribulose diphosphate carboxylase leading to decreases in all sugar fractions (Stibrova *et al.* 1986). The effects of chitosan, especially at 250 mg l^{-1}, in increasing chlorophylls and total carbohydrate contents were confirmed in cucumber (Farouk *et al.* 2008) and radish (Farouk *et al.* 2011). Chitosan may alleviate the water stress effect on photosynthetic pigments by enhancing endogenous levels of cytokinins, which stimulate chlorophyll synthesis. Chibu & Shiayama (2001) referred these positive effects to the greater availability of amino compounds released from chitosan. Our results indicate significant increases in both nitrogen and potassium content in plant shoots (Table 3), which may be play an important role in increasing the number of chloroplasts per cell, cell size and number per unit area, as well as increased 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 coupled with impaired active transport and membrane permeability (Levitt 1980) resulting in reduced root-absorbing power. Nitrogen is a constituent of many plant cell components such as amino and nucleic acids, and hence its deficiency rapidly inhibits plant growth. The decrease in N content due to water stress has been reported in various crops including wheat (Singh & Usha 2003) and in soybean (Tanguilig *et al.* 1987). Phosphorus 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. Phosphorus uptake decreases with decreasing soil moisture in various crops such as pepper (Turner 1985) and wheat (Ashraf *et al.* 1998). The role of chitosan in increasing ionic content may be due to its effects on stabilizing cellular membranes through increasing

antioxidants substances, saving cell membranes from oxidative stress and hence improving plant cell permeability (unpublished data). This observation is supported by the results of Guan *et al.* (2009), where the application of chitosan significantly decreased lipid peroxidation by stimulating antioxidant enzymes, leading to decreased membrane permeability and improved function. Other reports have confirmed these results (Farouk *et al.* 2008, 2011; Ghoname *et al.* 2010).

The reduction in cowpea yield due to water stress has been reported before (Costa *et al.* 2008, Vurayai *et al.* 2011, Hefny 2011). In legume plants such as cowpea, seed yield is determined by 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 hypotheses about the influence of water stress on yield. One of them suggests that water stress decreases the number of branches and leaves per plant (Table 1) as well as leaf area (shown in unpublished data), resulting in a reduction in the supply of carbon assimilate and photosynthetic rate as well as reduced 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 that also would reduce the yield due to water stress is by increasing the rate of flower and pod abortion (Liu *et al.* 2003). A decreased rate of carbohydrate flux from leaves to reproductive structures has been reported to control pod set in well-watered plants (Kokubun *et al.* 2001, Setter *et al.* 2001). Recent evidence supports this hypothesis. In maize, low water potential disrupted carbohydrate metabolism in ovaries by reducing the activity of acid invertase, the key enzyme catalyzing the breakdown of incoming sucrose during ovary and early seed development (Anderson *et al.* 2002). Song *et al.* (1998) showed that water stress induced swollen pollen and filament development, decreased filament fertility and resulted in reductions in grain number and weight per ear.

The increase in cowpea yield due to chitosan application may be due to its effects in stimulating physiological processes, improving vegetative growth, followed by active translocation of photoassimilates from source to sink tissues, increasing leaf-blade thickness as well as the dimensions of the vascular bundles, as indicated in our results. The increases in plant biomass may be due to improving photosynthetic machinery (Khan *et al.* 2002). Ghoname *et al.* (2010) also observed that foliar application of chitosan on sweet pepper increased significantly the number of fruits per plant and the mean weight of fruit, as well as quality characteristics such as total acidity, total soluble solid and ascorbic acid content in the fruit. The role of chitosan in 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 in reducing transpiration to save water. Chitosan-treated stressed plants had a thicker entire-leaf lamina, upper epidermis and palisade mesophyll than untreated stressed plants. A thicker upper epidermis (including upper cuticle) and a thicker palisade parenchyma in these treatments may enhance survival and growth under water stress conditions by improving water relations and providing higher protection for the inner tissues (Bacelar *et al.* 2006), increasing yield.

It can be concluded that cowpea plants treated with chitosan induced the ability to grow under water stress conditions, perhaps because they can produce various metabolites which cause the closure of stomata, resulting in a reduction in transpiration.

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الملخص العربى

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

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[1] قسم النبات الزراعي، كلية الزراعة، جامعة المنصورة، [2] قسم النبات، المركز القومي للبحوث،الدقي

تعرض نبات اللوبيا للإجهاد المائي يسبب ضعف النمو وإنخفاض نسبة العناصر المغذية بالإضافة إلي محتوي النبات من الكلوروفيل والكربوهيدرات الكلية مؤدية إلي حدوث إنخفاض حاد بالمحصول مع قلة جودتة. علي الجانب الآخر فإن رش النباتات بالكيتوزان وخاصة بتركيز 250 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 250 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 250 ملليجرام/لتر كيتوزان.