Osmotic Adjustment and Yield of Cowpea in Response to Drought Stress and Chitosan

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ABSTRACT *Drought is the most significant ecological components limiting the growth and productivity of field crops. Chitosan is considered ameliorators of drought stress and could evolve drought tolerance in crop plants. To ascertain the function of chitosan in inducing osmotic adjustment in drought stressed cowpea leaf in terms of solutes accumulation, leaf water status, leaf area and yield components.*

Drought stress causes a significant reduction in osmotic potential and relative water content. On the other hand, osmotic adjustment were significantly increased due to inducing increasing the higher accumulation of osmolytes which directs to declining leaf area and yield characters. Application of chitosan, nullify the harmful effects of drought on leaf area and yield due to increasing osmolyte accumulation, sustaining osmotic potential and osmotic adjustment.

In conclusion, cowpea plants responded to drought stress by increasing the osmolytes accumulation, which further increased with chitosan application and assisted in maintaining the osmotic balance.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp) is one of the ancient grain legumes valued for its nutritional value, particularly high protein content (25%), flavour and short cooking time (*Ogbonnaya et al., 2003*). The crop furthermore has proficiency to sustain soil fertility through its excellent capacity to fix atmospheric nitrogen and thus does not need very fertile land for growth (Lobato et al., 2006). Although, cowpea is said to be rather drought tolerant, it has been shown that leaf water potential (Ψw), leaf turgor potential (Ψ_p) and osmotic adjustment (OA) are advised significant constituents for advancing drought tolerance in plants. On the base of cellular responses to water deficit, OA has been found to be one of the most effective physiological mechanisms underlying plant resistance to water deficit (*Zhang et al., 1999*). OA as a process of active accumulation of compatible osmolytes in plant cells exposed to water deficit, may enable stomatal and photosynthetic adjustments (*Morgan, 1984*), leaf development (*Cutler, 1980*), sustain root development and soil moisture extraction (*Chimenti et al., 1996*), protecting macromolecules and structures (i.e., membranes, proteins, lipids and chloroplasts) from environmental stresses induced damages and high dry matter accumulation and yield production for crops in stressful environments (*Lobato et al., 2008; Farouk and Ramadan Amany, 2012*).

Osmotic adjustment has been described to assist the turgor pressure and has drawn much attention during the last years (*Farouk, 2011*). Energy is required for the synthesis or transport of solutes for osmotic adjustment (*Munns, 2002*). Taking into consideration energy efficiency, it is predicted that the accumulation of ions, which is not needed in the metabolism and is of low molecular weight, is effective for the OA, and that the ions can be built up rapidly in response to osmotic stress (*Raven, 1985*). However, the excessive accumulation of ions may distract the balance of the absorption and the function of other ions in the cell. OA engages the net accumulation of solutes in response to a fall in the water potential of the cell's environment. As an outcome of this, the osmotic potential of the cells is lowered; this in turn attracts more water into the cell and tends to maintain the turgor pressure. This allows turgor dependent processes such as growth and stomatal activity to continue to progressively lower the leaf water potential (*Babu et al., 1999*).

In mature leaf, OA performances significant function for plant cell survival, facilitative higher stomatal conductance and leaf expansion (*Westgate and Boyer, 1985*) to sustain photosynthesis under stress conditions. It is accepted that during osmotic adjustment the cells tend to compartmentalize most of the absorbed ions in vacuoles at the same time that they synthesize and accumulate compatible organic solutes in the cytoplasm in order to maintain the osmotic equilibrium between these two compartments (*Hasegawa et al., 2000*). As an outcome of solute accumulation, the osmotic potential of the cell is lowered, this, in turn, attracts water into the cell and, thereby, tends to maintain its turgor. In fact, OA is an effective component of drought tolerance, which has an affirmative direct or indirect effect on plant productivity, because it contributes to the maintenance of turgor and cell volume (*Ludlow and Mu-Chow, 1990*).

Natural osmoprotectant concentrations in cytoplasmic compartments are osmotically significant because they have pivotal roles in maintaining cell turgor and the driving gradient for water uptake under stress (*Rontein et al., 2002*), allowing physiological processes, such as stomatal opening, photosynthesis and cell expansion (*Serraj and Sinclair, 2002*). In addition to their function in cell water relations, organic solutes accumulation may also help towards the maintenance of ionic homeostasis and of the C/N ratio, removal of free radicals, and stabilization of macromolecules and organelles, for example proteins, protein complexes and membranes (*Bray et al., 2000*). In plant the major compatible osmoprotectant solutes are glycinebetaine and proline (*Farouk, 2011*) are considered to function as osmoprotectants for protein (*Bohnert and Jenson, 1996*), these solutes furthermore supply a protective environment for enzymes and macromolecular structure and function. The contributory role of osmoprotectants i.e. glycinebetaine and proline to osmotic adjustment under environmental stress was verified by some investigations (*Meloni et al., 2001; Farouk, 2011*), but the significance of its osmotic adjustment is still in argument and varies as claimed by the species. Hence, improvement of crop performance by increasing osmotic potential-adjusting ability might be more significant in increasing plant growth and yield.

Osmotic adjustment may be carried out by application of some osmoprotectants, ions, plant growth substances, antioxidants and finally by chitosan, but there is little report in this respect. Many reports consider OA to be a causal mechanism favoring crop productivity under environmental stress (*Blum et al., 1999*). However, there are furthermore inconsistent accounts showing a contradictory connection between OA and kernel yield under stress condition (*Subbarao et al., 2000a*). Other reports show no relationship between OA and growth and/or kernel yield under stress condition (*Tangpremsri et al., 1995*). Thus, OA as an adaptation mechanism for drought resistance is rather debatable and needs farther analysis. Recently, application of chitosan has been described to competently mitigate the harmful consequences of drought on plants (*Farouk and Ramadan Amany, 2012*). However, there is little information about the role of chitosan on regulation of osmotic adjustment processes in plants under normal or stressed condition. Keeping in view the above reports on the role of exogenous chitosan on cowpea cultivar there is a require for better understanding of chitosan mechanism of action and the magnitude of its effects in cowpea plant to improve crop stress tolerance. Thus, the foremost goal of the present study was to ascertain the hypothesis that are the application of chitosan accelerating cowpea yield under drought is due to improvement plant water relations and osmotic adjustment processes.

MATERIALS AND METHODS

Two pot experiments were conducted in the experimental farm and laboratory of Agricultural Botany Department, Faculty of Agriculture, Mansoura University, Egypt throughout the two successive seasons of 2007 and 2008. Cowpea seed "*Vigna unguiculata* (L.) Walp. cv Cream 7" was got 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 young plants with 100% available water, at two days intervals till the young plants reached the fourth leaf stage. The young plants 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 throughout the seedling stage and the second at the starting of blossoming time. After that the pots were divided into three groups for water stress treatments, each group were divided into four subgroups for chitosan foliar application. The soil moisture for all pots was kept at 70% 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, 70% FC and two water stress treatments; moderate (50% FC) and severe (30% FC) water stress. In the stressed treatments, moisture levels were allowed to fall from the initial 70% 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 supplemented 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 assess leaf area per plant and certain physiological and yield characteristics. Leaf area per plant calculated based on area unit using disk method according to (Koller, 1972). Briefly, samples of ten disks were taken from the 3rd fresh leaf from plant tip and estimated their area. Leaf area per plant was calculated in square centimeters (cm²) using the following equation:

$$\text{Leaf area (cm)}^2 \text{ per plant} = \frac{\text{Disk area of 10 disks (cm2) x fresh weight of the leaves}}{\text{Fresh weight of 10 disks}}$$

Quantification of leaf water status was made by measuring the leaf water relations parameters; relative water content (RWC), osmotic potential (OP), and osmotic adjustment (OA) during the crop productive phase. Relative water content (RWC) was determined, briefly, leaf discs were weighted to obtain fresh weight (FW). The plant materials were floated in distilled water inside a closed petri dish and determined the turgid weight (TW), and then the plant materials were placed in a pre-heated oven at 80 °C for determination dry weight (DW). RWC (%) = {(FW-DW)/ (TW-DW)} x100.

Osmotic potential (Ψ_o) was determined using total soluble solids percentage (TSS) in leaf sap utilizing hand refractometer and the corresponding values of water potential were then obtained from tables given by Gossev (1960). However, osmotic adjustment was calculated as the difference in osmotic potential between stress and non-stress treatments (Blum, 1989).

Total free amino acids were extracted and determined according to the modified method of Dubey and Rani (1989a, b). Proline was determined by the modified ninhydrine methods of Magne and Larher (1992). Plant materials were placed into test tube containing distilled water. The tubes were kept for 30 min in a boiling water bath then cooled at room temperature. To 150 µL of the corresponding water extract, 1 ml of ninhydrine reagent was added and maintained in a boiling water bath for 20 min. the mixture was cooled and the product formed was extracted with toluene. Absorbance was measured at 520 nm on a spectrophotometer.

Total soluble sugars extracted by ethanol and then determined by phenol-sulphoric acid methods as described by Sadasivam and Manickam (1996). Glycinebetaine (GlyBet) content was estimated by the method of Grieve and Grattan (1983). Oven dried leaves were finally ground with deionized water at 100 °C for 60 min. GlyBet concentration was determined spectrophotometrically (Spekol-11) at 365 nm. Moreover, at harvest time (140 days from sowing) the cowpea yield characters were recorded.

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
Organic solutes accumulation:
Significant differences were observed among the drought treatments for total free amino acids (TAA), proline (Pro), total soluble sugars (TSC) and glycinebetaine (GlyBet) accumulation under drought stress. Data presented in Table (1) indicate that, cowpea plants under drought-stressed conditions responded to an increased ion influx in their cells by increasing the synthesis and accumulation of cowpea leaf organic solutes i.e TAA, Pro, TSC and GlyBet in comparison with the control, which further increased with applications of chitosan under normal or drought conditions and assisted in sustaining the osmotic balance and therefore assisted in enhanced drought tolerance. The

maximum concentration of organic solutes was recorded with the application of 250 mg/l chitosan combined with high water stressed levels in comparison with chitosan alone.

Table (1): Effect of water stress and chitosan and their interactions on total free amino acids, proline, glycinbetaine and soluble carbohydrates concentration (mg/g FW) during the second growing season. Water stress values are % of field capacity; chitosan treatments are in mg l⁻¹

Treatments		Total free amino acids	Proline	Glycinebetaine	Soluble carbohydrates
Water stress	Chitosan				
70	W	44.42±7.48i	12.11±1.42h	1.55±0.31h	7.15±0.45i
	125	61.89±1.21fg	16.22±0.39ef	2.37±0.48fg	10.99±1.15g
	250	62.66±1.10efg	16.84±0.09de	2.43±0.29fg	13.92±0.54f
	500	59.25±3.46gh	14.50±1.88g	2.05±0.34gh	9.66±1.18h
50	W	54.19±1.14h	14.35±1.28g	2.77±0.18ef	14.65±0.73ef
	125	68.06±0.62cde	17.33±0.23cd	3.29±0.33de	17.00±1.99cd
	250	70.06±0.68cd	18.16±1.10bc	4.01±0.49bc	18.16±0.39b
	500	65.47±0.97def	16.61±0.23def	3.06±0.07e	15.23±0.19e
30	W	60.20±10.99fg	15.76±1.16f	3.19±0.29 de	16.26±1.39d
	125	79.26±12.92b	18.40±0.59b	4.54±0.62ab	18.68±0.36ab
	250	85.00±4.70a	19.83±1.73a	4.96±1.70a	19.26±2.09a
	500	72.90±12.10c	17.56±0.11bcd	3.74±0.74cd	17.84±0.72bc

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).

Leaf water relations parameters:
Water status is highly sensitive to drought and is, therefore, dominant in determining the plant responses to stress. Progressively increasing drought stress, significantly affected all water relations parameters (Table 2). Osmotic potential, (Ψ_s), declined considerably (became more negative) progressively with increasing water stress, therefore, the values were the lowest at high drought level. Likewise, relative water content, (RWC), declined with increasing drought stress. The decline was more pronounced in high drought level. Osmotic adjustment (OA) capacity of cowpea leaf increased significantly with increasing drought stress regardless of stress levels.

Chitosan foliar spray increased (less negative values) leaf relative water content in cowpea leaf area as compared with unsprayed plants, meanwhile decreased osmotic potential in cowpea leaf. Osmotic adjustment increased significantly in cowpea leaf with application of chitosan due to maintaining turgor potential of leaf.

As considered to the interaction between chitosan and drought, the date in Table (2) verified that application of chitosan under normal or drought condition improved leaf water status due to decreasing leaf osmotic potential, and improving osmotic adjustment, and maintaining turgor potential. It is noted that application of chitosan increased significantly relative water content under control, then decreased under high drought level. On the other hand, application of chitosan, nullifies the harmful effect of drought on water content.

Table (2): Effect of water stress and chitosan and their interactions on relative water content percentage, leaf osmotic potential (-Mpa) and osmotic adjustment during the second growing season. Water stress values are % of field capacity; chitosan treatments are in mg l⁻¹

Treatments		Relative water content %	Leaf osmotic potential	Osmotic adjustment
Water stress	Chitosan			
70	W	80.24±0.63cd	0.224±0.04e	0.000±00h
	125	85.76±2.07ab	0.268±0.08e	0.044±0.046g
	250	87.91±1.67a	0.385±0.26bc	0.127±0.05ef
	500	80.35±1.09cd	0.266±0.08e	0.041±0.04g
50	W	78.55±0.88d	0.33±0.01d	0.105±0.03f
	125	81.09±1.90c	0.364±0.01cd	0.140±0.03def
	250	87.01±0.87a	0.387±0.01bc	0.163±0.03bcd
	500	79.24±0.82cd	0.354±0.01cd	0.130±0.03def
30	W	61.18±8.08f	0.380±0.01bc	0.155±0.02cde
	125	77.99±1.31de	0.417±0.01ab	0.193±0.03ab
	250	84.19±0.96b	0.443±0.01a	0.218±0.05a
	500	76.11±2.04e	0.403±0.01abc	0.178±0.02bc

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).

Leaf area and pod yield:
Restriction of leaf growth is among the earliest visible effects if many stress conditions, including drought. Drought stress effects on leaf expansion and functions are directly related to yield constraints under drought conditions. It is evident from results presented in Table (3), that increasing drought stress had a significant adverse effect on leaf area and pod yield of cowpea plant. The great reduction in these parameters was observed under high drought stress.

It was documented that application of chitosan under normal or drought conditions distinctly increase leaf area and cowpea yield as compared with control plants or untreated plants under such drought levels (Table 3). The highest values of both parameters were recorded due to application of 250 mg/l chitosan.

Table (3): Effect of water stress and chitosan and their interactions on leaf area per plant (cm²) and yield components during the two growing seasons (1st and 2nd). Water stress values are % of field capacity; chitosan treatments are in mg l⁻¹

Treatments Water stress	Chitosan	Leaf area per plant 1st season	Leaf area per plant 2nd season	Pod number per plant 1st season	Pod number per plant 2nd season	Seed number per pod 1st season	Seed number per pod 2nd season	100 seed weight (gm) 1st season	100 seed weight (gm) 2nd season	Pod length(cm) 1st season	Pod length(cm) 2nd season
70	W	57.74±0.94c	54.61±0.45bc	16.33±1.15cde	16.00±2.00cde	9.00 ±0.00 d	8.66±1.15 c	14.57±0.97 d	14.46±0.49cd	12.66±1.15abc	12.00±0.00bc
	125	65.69±3.73b	59.36±0.17b	18.00±2.00 bc	18.00±2.00 bc	10.00±0.00 c	10.00±0.00 b	15.69±0.33 c	15.54±0.58bc	14.00±0.00ab	14.00±0.00a
	250	71.16±10.06a	66.87±13.12a	21.33±2.30 a	20.66±2.30 a	11.66±1.15 a	11.00±0.00 a	18.35±0.57 a	17.67±0.45 a	14.66±1.15a	14.33±1.15a
	500	58.85±0.233c	54.62±3.19bc	15.33±1.15def	15.33±1.15de	8.00±0.00 e	8.00±0.00 d	14.57±0.57 d	14.59±0.56cd	13.00±5.29abc	12.00±2.00bc
50	W	53.67±2.90de	47.87±1.50de	14.00±2.00 fg	14.00±2.00 ef	8.00±0.00 e	7.00±0.00 e	13.57±0.58 e	13.55±0.24 d	12.00±2.00abc	11.00±0.00c
	125	59.81±2.08c	57.01±2.58b	17.00±2.00 cd	17.00±2.00bcd	9.00±0.00 d	9.00±0.00 c	14.60±0.13 d	14.65±0.65cd	12.00±2.00abc	13.00±0.00ab
	250	66.02±3.94b	60.05±1.97b	19.33±1.154b	18.66±1.15 b	10.66±1.15 b	10.00±0.00 b	16.44±0.50 b	15.90±1.80 b	13.66±1.15ab	14.00±0.00a
	500	56.45±0.66cd	51.57±1.02cd	15.00±2.00 ef	15.00±2.00 de	8.00±0.00 e	8.00±0.00 d	14.58±0.64 d	13.69±1.46 d	13.00±3.46abc	12.00±0.00bc
30	W	44.83±0.92f	38.52±5.27f	10.66±2.30 h	11.33±1.15 g	6.00±0.00 g	5.66±1.15 f	11.49±0.85 f	11.83±0.43 e	10.33±1.15c	10.66±1.15c
	125	52.80±1.26e	47.41±0.64de	13.00±2.00 g	13.00±2.00 fg	7.00±0.00 f	7.00±0.00 e	13.41±0.86 e	13.53±0.55 d	11.00±0.00bc	11.00±2.00c
	250	60.17±0.97c	56.25±0.68bc	17.66±1.15 bc	17.00±2.00bcd	10.00±0.00 c	9.66±1.154b	15.49±0.66 c	14.85±1.44 c	13.66±1.15ab	13.33±1.15ab
	500	50.76±1.17e	44.59±2.04e	12.33±1.15 g	12.33±1.15 fg	7.00±0.00 f	7.00±0.00 e	13.68±0.85 e	12.53±0.91 e	11.00±0.00bc	11.00±2.00c

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).

DISCUSSION

Drought stress is the major factor limiting plant growth and productivity (Farouk and Ramadan Amany, 2012). The nature of drought stress was of great importance in the water relations of the cowpea plant treated with chitosan compared with the untreated plant under normal or drought conditions. These different responses could be due to the fact that cowpea treated with chitosan has some tolerance-avoiding mechanisms, such as osmotic adjustment (OA) and decline in leaf osmotic potential, to sustain their water status at values similar to those of the control plant (Table 2). When OA has occurred, rigidity of the cell wall is necessary to maintain the cell/tissue integrity. Thus, these two processes allow an increase in the water potential gradient between the soil and plant and improving the water absorption under soil water deficit, so the tissues do not suffer water stress.

Osmotic adjustment is a mechanism used for maintaining turgor and reducing the deleterious effects of water stress on vegetative and reproductive tissue (Rhodes and Hanson, 1993). It is well known that osmotic adjustment involves the net accumulation of organic or inorganic solutes/osmolytes; total soluble sugars, total free amino acids, proline (Munns, 2005; Farouk, 2011) in a cell in response to water stress. Consequently, the cell osmotic potential decreases, which in turn attracts water into the cell and enables turgor to be maintained (Blum et al., 1996). Compatible solutes synthesis comes with energy cost and hence involved a potential growth penalty. In leaf cell, approximately seven moles of ATP are needed to accumulate one mole of NaCl as an osmoticum, whereas the amount of ATP required to synthesis one mole of an organic compatible solute is an order of magnitude higher i.e. 34 for mannitol, 41 for proline, 50 for Gly-Bet, and approximately 52 for sucrose (Raven, 1985). Upon exposure to drought stress, numerous plants accumulate organic compatible solutes that are non-toxic at high concentrations (Chen and Murata, 2002). It is usually accepted that the increase in cellular osmolarity which outcomes from the accumulation compatible solutes is accompanied by the influx of water into, or at least a reduced efflux from, cells, thus providing the turgor essential for cell expansion. Although accumulation of organic solutes increased in both non-stressed and stressed plants due to foliar applied chitosan (Table 1), leaf osmotic potential was not substantially altered due to accumulation of organic solutes. From these finding, it is plausible to propose that changes in organic solutes accumulation caused slight change in leaf osmotic potential which resulted in improved leaf turgor potential and therefore assist in osmoregulatory processes. In the present investigation, foliar application of chitosn considerably declined leaf osmotic potential in the stressed plants due to its role in increasing compatible organic solutes (Tables 1, 2).

It is well known from the present investigation that the organic osmolytes were enhanced in response to drought and/or chitosan treatments, where their interactions had an additive effect. It has been described that free amino acids contribute to osmotic adjustment, but experimental results are inconsistent (Ford, 1984). Free amino acids increased due to environmental stress in cowpea leaf. These outcomes were verified by Farouk (2011). The accumulation of amino acids in stressed plant could be caused by 1) protein degradation (Yadav et al., 1999) for providing amino acids required for synthesis of new proteins suited for growth or survival under the modified conditions, and 2) inhibition of protein synthesis. The results of present investigation proved that application of

Proline "Pro" concentrations, of all the organic solutes investigated, showed the highest relative increase in response to drought stress or chitosan application or their interactions (Table 1). Proline accumulation may contribute to osmotic adjustment at the cellular level (Tripathi et al., 2007); therefore, these solutes play an important role in osmoregulation. The significance of proline accumulation in osmotic adjustment is still argued and varies according to the species. However, convincing evidence is still lacking as to whether accumulation of proline can provide any biochemical adaptation for plants during drought stress. Chitosan is directly involved in the changes taking place in the plant under drought stress. Pro has multiple functions, such as osmotic pressure regulation, protection of membrane integrity, stabilization of enzymes/proteins, maintain appropriate $NADP^+$/NADPH ratios and scavenger of free radicals (Misra and Saxena, 2009), a major source of energy and nitrogen during immediate post-stress metabolism, thereby inducing stress tolerance (Jain et al., 2001). The increase in proline content under drought stress or chitosan application may be correlated with the increased

synthesis of Δ^1pyrroline carboxylate synthetase (P5CS) and P5CS mRNA levels (Hare and Cress, 1996) and Pyrroline 5 carboxylate reductase (P5CR) (Misra and Gupta, 2006), and γ-glutamyl kinase activity (Misra and Saxena, 2009) or the low activity of degrading enzyme, proline oxidase (EC 1.5.99.8), localized in the inner mitochondrial membrane (Misra and Saxena, 2009) and cytoplasmic proline dehydrogenase (EC 1.5.1.2) (Delauney and Verma, 1993) to negligible rate. But till date, proline metabolism in presence of chitosan is not known and need more studies.

Glycinebetaine (GlyBet), a quaternary ammonium compound, is considered as one of the most effective osmoprotectants is obliged to its numerous benefits in addition to its efficacy as a compatible solute. The molecular characteristics of GlyBet enable its interaction with both the hydrophobic and hydrophilic domains of macromolecules without perturbing the cellular functions (Sakamoto and Murata, 2002). It has been described that GlyBet protects the cells from stresses by sustaining an osmotic balance between the intracellular and extracellular environments and by stabilizing the quaternary structures of complex proteins like antioxidants enzymes and biomembranes and other functional units like oxygen-evolving photosystem II complex (Rhodes and Hanson, 1993). In the present study, it has been observed that GlyBet over-accumulated contributes to the maintenance of OA in antioxidants treated plants under normal or drought conditions. Some researchers have furthermore described that GlyBet induced the accumulation of osmolytes, such as soluble sugars, and free proline (Ma et al., 2004).

Among the organic solutes, soluble carbohydrates contributed the most to the leaf osmotic potential, and they furthermore appeared to be important in the leaf osmotic adjustment under drought stress conditions. The increment in soluble carbohydrates due to drought or chitosan application may in turn play an important role in increasing the osmotic pressure of the cytoplasm. This conclusion is in agreement with the results obtained by Greenway and Munns (1980) who stated that these organic molecules act as osmotica and play an important role in osmotic adjustment in non-halophytes, moreover, sugars as osmolytes enable plants to keep better water relation under drought stress conditions. The present hypothesis is that sugars act as osmotica and/or protect specific macromolecules and assist to the stabilization of membrane structure. The accumulation of sugars was the result of an enhanced efficiency in the use of carbon coupled to a reduction in cellular metabolism, which could favor the accumulation of respiratory substrate to support the osmotic adjustment required to survive in stressed media (Schnapp et al., 1990).

It is well known from the result of the present investigation that cowpea leaf area was progressively declined with the increase of drought (Table, 3). The declined rate of leaf growth after an increase in drought is prime due to decrease in water potential in the root zone which transmitted via the xylem to the leaves, causing leaf cells to loss water and reduced its elongation rates (Fricke and Peters, 2002). Over days, decrease in cell elongation and furthermore cell division lead to smaller final size. It is well known that cell expansion is dependent on water uptake, which relies on water potential gradients between the expanding cells and the water source. Soluble sugars and other organic solutes for example proline (Farouk, 2011) builds up in the leaves under environmental stress conditions and assists to osmotic adjustment. They furthermore assist to maintain cell wall synthesis. The actual relationship between turgor and leaf growth is complex. In affirmation with latest concepts about the mechanism of cell wall extensibility, cell enlargement beings with a decrease or relaxation of wall stress. As a consequence, turgor pressure and water potential are decreased, and water is drawn into the cell. The result is that the cell enlarges by uptake of water, initiated by a yielding of the wall. Synthesis and deposition of new wall materials is required throughout or after cell en-

largement to prevent wall rupture in subsequent growth. This was apparent in our results, where the thickness of the mesophyll tissue, epidermis cell and vascular bundles decreased in the stressed plants indicating a decrease in cell dimensions (Farouk and Ramadan Amany, 2012) due to inhibition of the pro-cambial activity from, primary vascular tissues as well as with a decline in the number and dimensions of mesophyll tissue. On contrast, application of chitosan increased significantly leaf area under control or drought conditions (Table 3) due to hyper-accumulation of compatible solutes (Tables 1). Such accumulation provides the turgor necessary for cell expansion resulting in increasing leaf area (Table 1). This conclusion was supported by our results which indicate that application of chitosan declined leaf water osmotic potential which resulted in increasing water uptake to cells and increasing relative water contents, resulted in increasing leaf cell elongation and finally leaf area. In addition, application of chitosan increased total soluble sugars which serve as a substrate for increasing initiation of leaf primordial and declined plastochron duration (Munns et al., 1979) which directs to increasing leaf area. This result was sustained by some investigations which verified that application of chitosan increased significantly leaf area (Farouk et al. 2008; 2012). This was apparent in our results, where the thickness of the mesophyll increased in plant treated with antioxidants under normal or salinized conditions indicating an increase in cell dimension (Farouk and Ramadan Amany, 2012).

Osmotic adjustment has received increasing interest throughout latest years. Associations between OA and pod yield under water deficit in wheat (Moustafa et al., 1996), and sorghum (Santamaria et al., 1990) have been reported. However, the utility of OA as a mechanism of drought tolerance is open to debate. Such a favorable effect of OA on yield and its components could presumably by attributed to the well-established role of OA in sustaining turgor and plant growth under water deficit as observed in various crops (Grammatikopoulos, 1999). Recently, Subbarao et al. (2000b) have recorded a significant positive relationship between OA and RWC under water deficit that lead to a significantly positive association between OA and leaf area, indicating maintenance of crop growth by OA under stress condition. That is, genotypes that adjusted osmotically, could sustain high photosynthetic rate because of more favorable leaf water status, which could, in turn, lead to higher crop growth rate and dry matter production, maintaining, finally, a higher productivity under drought stress. Thus, it could be inferred that maintenance of higher RWC at high drought level in this study (Table 2) could sustain growth and metabolic activities in plants, including, photosynthesis and other physiological processes (Subbarao et al., 2000b). Additionally, chitosan treated plants could, presumably, translocate the pre-anathesis carbohydrates reserves to developing pods more effectively than untreated plants. Finally, it could be said that OA could play a role in maintenance of turgor and better water content of leaves, which might help the plant, under drought stress, to survive and maintain growth and metabolic activities so as to result, ultimately, in improved crop productivity. Water stress reduced the number of pods and 1000-seed weight in our experiment. Negative correlations were reported between seed weight and number of pods per plant as well as number of seeds per pod (Jensen et al., 1996). Similar results reported with Sinaki et al. (2007). Among yield components, seed weight was reported to be lesser extent affected by environmental conditions. By limiting leaf area development, water deficit reduces radiation interception by plants and consequently less biomass is produced 28. Furthermore, the reduction of stomatal conductance by water deficits leads to reduced carbon assimilation and consequently low biomass production (Issarakraisila et al., 2007). Water deficit can also negatively affect the photosynthetic machinery of the plant, for instance through inactivation of enzymes (Li et al., 1994). Decreasing root dry weight under water stress and its association with yield production has been reported in canola (Richards and Thurling, 1978).

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