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Comparative impact of different iso-osmotic solutions on osmotic adjustment in *Gossypium barbadense*

Abeed A.H.A.* **and Dawood M.F.A.**
Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut, 71516, Egypt
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*to whom all correspondence should be addressed: e-mail: dramany2015@aun.edu.eg
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Abstract

To discriminate the specific response of ion toxicity versus osmotic stress on altering leaf solute contents, contributing of organic and/or inorganic components in osmotic adjustment and its reflection on plant performances under ionic and osmotic stresses, two cotton (*Gossypium barbadense* L.) cultivars, Giza 90 and Giza 83, were subjected to iso-osmotic concentration (–0.57 and –1.05 MPa) created by; NaCl, KCl and polyethylene glycol-6000. The three used osmotica altered seedling length, chlorophyll, leaf dry weight, relative water content, organic and inorganic solutes and proline. Contribution of organic solutes to osmotic adjustment tittered among the two cultivars, it was higher in PEG > KCl > NaCl in Giza 83, suggesting that the character of osmotic adjustment via salt attuned to high yield with moderate ion toxicity is effectively achieved by KCl than NaCl. At high-stress intensities, regardless to cultivar, the salt stress-induced nutritional imbalance, leaf chlorosis than osmotic stress that could be attributed to specific ion toxicity, not to osmotic stress of salt. In salt sensible cultivar only NaCl, among different osmotica, reduced leaf K^+ content implying that avoidance of Na-induced K^+ deficiency in leaf might stimulate salt tolerance in cotton. In our study, the capacity of plants to regulate their metabolic and physiological functions had superiority in water stress tolerance rather than osmotic adjustment.

Keywords: Osmotic adjustment, organic acids, osmotic stress, salt stress, proline, sugars, ion toxicity, solutes content, relative water content.

1. Introduction

Salinity is a complex environmental restriction that impact plants development via two main routes: an osmotic route through the decrease in the osmotic potential of the soil solution (osmotic stress) hindering water uptake, and an ionic route where ions (mainly Na^+ and Cl^-) accumulates to become toxic at high concentrations (ion-toxicity) leading to imbalance of essential elements, such as potassium and calcium (antagonism). A rapid response due to salinity-induced osmotic stress is a reduction in leaf growth by inhibiting cell expansion and cell division followed by chlorosis, necrosis and senescence of mature leaf as a result of ionic toxicity (Carillo *et al.*, 2011). However, the magnitude of the deteriorations to plants by salt stress relies on the existing saline solution concentration and stage of growth (Annunziata *et al.*, 2017). For instance, seedling stage is considered the most susceptible stage to salinity medium (Carillo *et al.*, 2008). Moreover, it is still not clear whether the salt stress mediated ionic or osmotic effects have similar impacts on physiological properties associated with plant growth and if their relative significance is the same for different cultivars of the same species. Thus, it is necessary to differentiate between damages caused by the osmotic and ionic damages of salt stress (Hossain *et al.*, 2017).

Ionic effects may be recognized from osmotic ones by comparison the influences of saline solutions and isotonic solutions of an inert osmotic medium such as polyethylene glycol (PEG) that cannot penetrate into the cell wall (Luan *et al.*, 2014). Ionic stress might be imitated by NaCl or KCl, causing specific ionic phyto-toxicities; but high salt concentration of both salts surrounding the plants may be prohibited water uptake causing osmotic stress (Kumar *et al.*, 2017). Whilst non-ionic and non-penetrating, inert osmoticum, like polyethylene glycol (PEG) propagated non-ionic osmotic and water stresses by decreasing the water potential of the plants nutrient solution (Ahmad *et al.*, 2007). Moreover, the growth in saline soils may be also affected by the total amount of solutes dissolved (or the osmotic pressure applied) as well as by the kind of the used salts. Thus, The use of different iso-osmotic concentrations of salinizing agents as KCl or NaCl and osmotic agent like PEG could present valuable information in this respect that the growth depression in PEG-growing medium could be ascribed to osmotic effects, and any change in growth of by any salinizing agent relative to PEG-provided plants, could be ascribed to ionic effects of NaCl or KCl.

After the rise of salinity in the growing medium, plants instantly suffered from osmotic stress followed by ionic stress simultaneously with abrupt ions accumulation (Deinlein *et al.*, 2014). In a response, cells satisfactorily adjust osmotic potential, converse the water flow as well as ion homeostasis to lessen stress damage and to

maintain active growth (Woodrow *et al.*, 2016) either via synthesis of organic compounds or by the uptake of ions from the growing medium. Thus, osmotic adjustment allows plant cells to withstand salt stress and water shortages via maintaining sufficient turgor for growth (Carvajal *et al.*, 1999). Since it's a prerequisite for osmotic adjustment to regulate the intracellular levels of certain carbon and nitrogenous compounds, many of which are supposed to be compartmented mostly in the cytoplasm, whereas inorganic ions (mainly Na^+, K^+ and Cl^-) are impounded in the vacuoles or distributed between the vacuole and cytoplasm (Voetberg and Sharp, 1991). Organic solutes in the cytoplasm contribute to an intracellular osmotic balance once inorganic ion concentrations are high in the vacuole, and may have a role in cytosolic enzymes protection when ion concentrations increase (Khan *et al.*, 2016). Therefore, the nature of the salts involved is crucial for studying the response of plants to salinity.

Cotton is considered a dual-purpose crop, broadly used for fiber and oil production all over the world. Although cotton is considered a salt tolerant crop (Mahajan and Tuteja, 2005), its growth and yield are retarded markedly under high salinity stress, especially during germination, emergence and seedling stages (Hussain *et al.*, 2012). Thus, identification of physiological attributes that confer salt tolerance during seedling stage could serve as valuable constituents to differentiate the deteriorations attained by osmotic and ionic routes of salt stress. In order to unravel the modalities of ionic and osmotic stress impact on leaves growth and physiological responses, the osmotic adjustment contribution of the inorganic (Na^+, K^+, Ca^{+2}, Mg^{+2}, Cl^- and NO_3^-) as well as organic solutes (amino acids, organic acids and soluble sugars) was conducted in relation to iso-osmotic solutes of different saline and osmotic agents on two cotton cultivars varying in their salt resistance. Due to increasing the drought and saline problems in Egyptian soils and worldwide. The main object of our study is to compare the growth of two cotton cultivars grown in iso-osomtic solutions of NaCl or/and KCl as well as PEG. This study gives highlight about the different mechanisms of tolerant and sensitive cultivars which likely to be of great interest to the plant physiology and agricultural studies.

2. Materials and methods

2.1. Plant material and treatments

Two cotton (*Gossypium barbadense* L.) cultivars, Giza 90 and Giza 83, were obtained from Horticulture department, Faculty of agriculture, Assiut University.

The experiment was conducted during summer season during the year of 2017 at Botany and Microbiology Department, Faculty of science, Assiut University, Egypt. Seeds were surface washed by 0.05% (w/v) sodium hypochloride, rinsed thoroughly by tap water for 3 minutes. The sterilized seeds of the two cultivars were sown in peetmoss-filled trays in the rate of one seed for each hole. A seedling was considered emerged when the hypocotyl hook was visible above the substrate (peetmoss) surface. 168 uniform seedlings (84 for each cultivar) on the seventh day after emergence were prepared for next step: Each three uniform seedlings were transferred into 56 Aluminum foil covered glass containers (28 for each cultivar), to keep roots in dark conditions with capacity of 250 ml, were filled with Hoagland's nutrient solution (Hoagland and Arnon, 1950) of 2 dSm^{-1}. Transferred seedlings were allowed to grow in half strength Hoagland's nutrient solution for 3 days to obtain the uniform stand of seedling, nutrient solution was continuously aerated with aeration pumps along the experiment period, containers were placed in greenhouse to simulate natural conditions (an air temperature ranged from 30 to 44 °C with a mean temperature of 35 °C, a relative humidity of 55–57% and a 12 h photoperiod).

To create the two levels of iso-osmotic solutions, 100 and 200 mM of NaCl or KCl as well as 18 and 24% PEG-6000 were added to the nutrient solution. To check whether the three stressing agents has no significant difference in their isosmotic concentration, the final osmotic potential of the nutritive solutions was tested by using Beckman differential thermometer (calibrated to 0.01 °C) as described by caryoscopic method by Walter (1931a). Final osmotic potential of the nutritive solutions was –0.57 ± 0.028 MPa for 100 mM NaCl or KCl and 18 % PEG-6000 as well as –1.05 ± 0.019 MPa for 200 mM NaCl or KCl and 24% PEG-6000. To determine the response of cotton seedlings upon exposure to these osmotica, ten-day-old seedlings were transferred to these osmotica and the control plant were grown in Hoagland's nutrient solution only were the nutrient solutions of each treatment were renewed every two days. Four replicates of three seedlings were used in each treatment (cultivar x osmoticum x osmotic potential) seedlings were harvested 5 days after treatment. Leaf fresh weight, leaf dry weight (after drying at 65°C for 3 days) and the total height (hypocotyl + epicotyl) were determined and the following measurements have been done.

2.2. Chlorophyll content

Chlorophyll (chl) content was determined by suspending 0.25 g leaf sample in 10 ml ethyl alcohol (95%) in water bath at 60–70 °C absorbance was measured with a Unico UV-2100 spectrophotometer at wavelengths 663 and 644. Lichtenthaler (1987) equations were used for total chlorophyll determinations as mg/g DW.

2.3. Electrolyte leakage

Electrolyte leakage was assessed according to Dionisio-Sese and Tobita (1998). Leaf sample (0.1 g) was divided into smaller pieces and then put in closed test tubes containing 15 ml distilled deionized water. Test tubes were heated at 40 °C for 10 min. After cooling, initial electric conductivity (EC1) was measured using conductimeter, YSI Model 35 Yellow Springs, OH, USA. Test tubes were then boiled at 121 °C for 20 min using an autoclave. After cooling at room temperature, the final electric conductivity was recorded as EC2. Electrolyte leakage was calculated by the following formula: Electrolyte leakage (%) = (EC1/EC2) × 100.

2.4. Leaf water relations

2.4.1. Relative water content (RWC)

RWC was estimated from fresh, turgid and dry weight of leaf discs, as described by (Silveira *et al.*, 2009).

2.4.2. Total osmotic potential

Leaf sap was obtained by grounding fresh leaves in ice cold mortar centrifuged at 10,000 g for 15 min and the collected plant extract was divided into two part one used to determine mineral analysis and the other to measure the osmotic potential (Ψs) by the Cryoscopic method of walter (1931a) using a Backman differential thermometer (calibrated to 0 ± 01 °C). Correction of Walter and Thren (1934) and Walter (1936) was used to calculate the real freezing point depression for the super-cooling. The osmotic potentials (-bar) were then obtained from tables compiled by Walter (1931b).

2.5. Solute contribution to leaf osmotic potential

The Calculated osmotic potential for each solute was evaluated by the van't Hoff equation.

2.6. Analysis of inorganic and organic solutes

Inorganic (Na^+, K^+, Ca^{+2}, Mg^{+2}, Cl^-, NO_3^-) and organic (soluble sugars, free amino acids and organic acids) solute contents were analyzed in leaf samples. Sodium and potassium were determined by the flame emission technique (Carl-Zeiss DR LANGE M7D flame phtometer) (Williams and Twine, 1960). Calcium and magnesium were determined with an atomic absorption/flame emission spectrophotometer (Shimadzu- model AA-630-02). Cl^- was determined by titration with $AgNO_3$ of the aqueous extract as described by Jackson (1958). NO_3^- content was analyzed spectrophotometrically (Unico UV-2100 spectrophotometer) using sulfanilic acid and α-naphthylamine method (Lambert and Dubois, 1971). Soluble sugars were quantified with the anthrone reagent according to (Yemm and Willis, 1954). Free amino acids were determined by a ninhydrin method (Moore and Stein, 1984). Organic acids as total acidity of the plants were determined by titration of extracts against standard alkaline solution (NaOH 0.001 N) by using phenolphthalein as an indicator and the pH values of these extracts were measured by using a pH-meter. Proline content was quantified spectrophotometrically by the tagged method of Bates *et al.* (1973).

2.7. Statistical analysis

The data were subjected to one-way ANOVA using SPSS 10.0 software program. Means were calculated for four replicate values. Means were compared by the Duncan's multiple range tests and statistical significance was determined at 5% level.

2.8. Experimental results

Osmotic stress induced by 18% PEG-6000 significantly reduced RWC by 14 and 10% for cv. Giza 83 and cv. Giza 90, respectively and the decrement was intensified as much as 33 and 20 % at 24 % PEG-6000 for the same cultivars (Table 1). Conversely, saline agents (NaCl and KCl) did not affect RWC of cv. Giza 90 whatever the ionic agent used, whilst RWC was only reduced, in average, 3 and 12% at 100 and 200 mM, respectively for cv. Giza 83. However, saline agents exerted no effect on osmotic potential of cv. Giza 83, whenever both agents lessened significantly Ψs but much more so for NaCl- than KCl-medium. Osmotic agent, PEG-6000, increased the Ψs by the same extent for the two tested cultivars as well as for both concentrations. These results implied that cv. Giza 90 leaves were able to sustain adequate hydration status even under high saline treatment which were strongly attributed to the ionic toxicity of saline medium rather than osmotic one that will be tackled in discussion.

The studied inorganic solutes in terms of ion content (sum of Na^+, K^+, Ca^{+2}, Mg^{+2}, Cl^-, NO_3^-) of leaves was higher for saline agents and lower under osmotic agent for the two cultivars compared to stress free- medium (Figure 1). As registered in Table 1, the contribution of inorganic solutes to Ψs predominately was higher than that of organic solutes. Both saline agents tended to reinforce the contribution of inorganic solutes at expense of organic ones for the two cultivars. But the PEG-osmoticum lessened the contribution of inorganic solutes to be lowered than the organic solutes for cv. Giza 83 relative to control plants while the contribution of inorganic solutes to Ψs was buffered in favor of organic ones to be 55.6%: 44.4% for cv. Giza 90 at 24% PEG, without any effect of 18% PEG on the sharing contribution percentage of both solutes. The data represented in Table 1 denoted that under control conditions, both K^+ and NO_3^- not only represented more than 50% of Ψs for the two tested cultivars (Table 1), but also were the most contributors to Ψs under both KCl- and PEG-treatments. Interestingly, the studied ions shared equally in their contribution to Ψs at 100 mM NaCl medium for cv. Giza 83 and to some extent cv. Giza 90 (some depletions of NO_3^- contribution percentage).

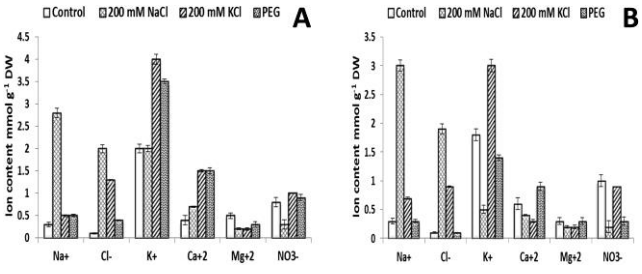

Figure 1. Leaves ion content (total inorganic solutes) in mmol g^{-1} DW (sum of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^-) of two cotton cultivars; Giza 90 (A) and Giza 83 (B), control and plants treated with the highest stress intensity (-1.05 MPa) induced by iso-osmotic concentration of 200 mM NaCl, 200 mM KCl and 24% PEG. Each value is the mean of four replicates per treatment

Table 1. Relative water content (RWC %), leaf sap osmotic potential Ψs (-MPa), and percent contribution of the estimated minerals and solutes to Ψs in leaves of cotton cultivars, Giza 83 & Giza 90. Means having no common letter within columns are significantly different at P ≤0.05

			RWC %	Ψs (- MPa)	% contribution of solutes to Ψs		% contribution of individual solutes to Ψs							
							Inorganic solutes					Organic solutes		
					Inorganic	Organic	Na^+	K^+	Cl^-	NO_3^-	SS	OA	FAA	Pro
Giza 83	Control		90a±1.5	0.661a±0.02	72.20	27.80	0.80	30.40	4.40	36.60	6.40	18.30	2.70	0.40
	-0.57 MPa	NaCl 100 mM	87b±1.2	0.721a±0.03	79.60	20.40	20.00	18.80	19.00	21.80	7.50	5.20	6.90	0.80
		KCl 100 mM	88b±2.2	0.766a±0.05	81.00	19.00	5.10	38.70	4.80	32.40	6.70	4.10	8.00	0.20
		PEG 18%	77c±2.5	0.482b±0.06	41.00	59.00	4.80	29.80	3.40	3.00	34.00	17.00	5.50	2.50
	-1.05 MPa	NaCl 100 mM	80d±2.3	0.675a±0.04	75.80	24.20	30.00	13.90	20.00	11.90	10.10	9.30	4.50	0.30
		KCl 100 mM	79d±3.3	0.699a±0.005	85.00	15.00	11.10	30.70	12.80	30.40	9.70	2.20	3.00	0.10
		PEG 24%	60e±3	0.332c±0.006	45.80	54.20	4.80	20.60	3.40	17.00	35.70	14.00	2.80	1.70
Giza 90	Control	88a±2.6	0.842a±0.03	71.30	28.70	8.50	20.00	10.00	32.80	9.00	16.40	3.00	0.30	
	-0.57 MPa	NaCl 100 mM	90a±2	1.633b±0.01	75.80	24.20	23.30	19.60	22.50	10.40	11.60	9.00	3.00	0.60
		KCl 100 mM	87a±1.1	0.912ab±0.03	81.30	18.70	6.50	34.80	3.90	36.10	9.20	6.00	3.00	0.50
		PEG 18%	79b±2.3	0.531c±0.004	71.60	28.40	9.00	25.90	16.10	20.60	13.20	9.90	3.90	1.40
	-1.05 MPa	NaCl 100 mM	89a±1.5	2.224d±0.08	84.60	15.40	29.00	18.40	26.20	11.00	9.90	3.10	1.70	0.70
		KCl 100 mM	88a±2.6	1.866d±0.07	82.30	17.70	8.50	28.00	20.20	25.60	9.50	6.00	2.10	0.10
		PEG 24%	70b±2	0.494c±0.003	55.60	44.40	3.00	26.40	5.20	21.00	23.40	15.20	4.20	1.60

The sum of K^+ and NO_3^- percent contribution to Ψs of the studied cultivars were 67%, 71.1%, 61.1%, 32.8% and 37.6% in cv. Giza 83 as well as 52.8%, 70.9%, 53.6%, 46.5% and 47.4% in cv. Giza 90 for control, 100, 200 mM KCl, 18% and 24% PEG, respectively. However, Na^+ and Cl^- were the dominant species contributed to Ψs for 200 mM NaCl medium where their corresponding percent contribution to Ψs were 55% and 50% for cv. Giza 90 as well as cv. Giza 83, respectively. These data also revealed that the contribution of Na^+ and Cl^- to Ψs were higher in cv. Giza 83 (the tolerant cultivar) than in cv. Giza 90 under 100 and 200 mM NaCl. The contribution of Na^+ to Ψs increased from 0.8% at control to 20 and 30% at 100 and 200 mM NaCl in cv. Giza 83, but from 8.5% at control to 23.3 and 29% at 100 and 200 mM NaCl in cv. Giza 90.

NaCl treatments imposed nutritional imbalance via reduction of K^+ and NO_3^- levels in the sensitive cultivar. Conversely, a slight reduction in leaves K^+ and NO_3^- contents accompanied with accretion of Na^+ under the highest level of NaCl in the tolerant cultivar Giza 90. It is interesting to note that however the contribution of inorganic solutes to Ψs in cv. Giza 83 under osmotic stress was 35–38% lower than under both saline treatments, cv. Giza 90 exhibited high inorganic solutes contribution mainly K^+ and NO_3^- at the expense of the contribution of organic solutes (Table 1).

As discriminated in Figure 1, Mg^{+2} generally reduced irrespective of the stress imposed whilst Ca^{+2} content raised except for cv. Giza 83 plants grown under both salinizing agents which exhibited reduction compared to non-stressed plants. It is worth mentioning that the contribution of both bivalent cations to Ψs was too small can be neglected so the dominant inorganic solutes contributed to Ψs were Cl^-,NO_3^-, Na^+ and K^+.

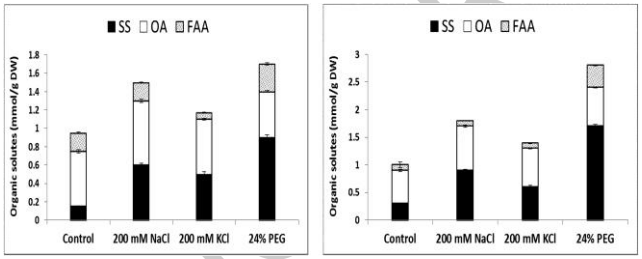

Figure 2. Leaves organic solutes (sum of soluble sugars, organic acids, free amino acids) in mmol g^{-1} DW of two cotton cultivars; Giza 90 (A) and Giza 83 (B), control and plants treated with the highest stress intensity (-1.05 MPa) induced by iso-osmotic concentration of 200 mM NaCl, 200 mM KCl and 24% PEG. Each value is the mean of four replicates per treatment. From left to right Giza 83 and Giza 90

The total organic solutes (sum of SS, OA, proline and FAA) in leaves and their contribution to Ψs declined under saline treatments compared to control plants, regardless of the tested cultivar. Under PEG-induced osmotic stress, their contribution to Ψs was slightly increased compared to inorganic solutes in cv. Giza 83. However, in cv. Giza 90 the contributions of inorganic and organic solutes were comparable to control plants under low PEG-osmotic stress. As compared to unstressed plants (control), variable increments of carbohydrate content in cotton leaves of both cultivars treated with saline (NaCl and KCl) and osmotic (PEG) treatments (Figure 2), which was more obvious for PEG treated plats compared to saline agents where soluble sugars increased, in average, 5 times higher than control in cv. Giza 83 at both stress intensities (18% and 24%), whilst 1.5- and 2.5 times for cv. Giza 90 at 18 and 24% PEG, respectively.

OA represented the major contributor of organic solutes to osmotic potential for control plants. Total organic acid contents decreased in both cultivars under both saline agents more than PEG. Both OA and SS represented nearly 47% of Ψs in cv. Giza 83 and only 22–38% in cv. Giza 90 under PEG-osmotic stress. The contribution of free amino acids to osmotic potential (Table 1) increased for cv. Giza 83 notably at iso- osmotic solutions corresponding to -0.57 MPa. In cv. Giza 90, the contribution of free amino acids to osmotic potential rose only under both levels of PEG, reduced at 200 mM NaCl or KCl as well as did not influence at 100 mM NaCl or KCl. As illustrated in Table 1, proline was the minor contributor of organic solutes to osmotic potential for control plants and this contribution induced by the used treatments except for 200 mM NaCl and KCl of cv. Giza 83. High levels of proline were found in PEG-treated plants compared with salt-treated plants. For saline agents, proline contribution to Ψs was higher under NaCl than KCl (Table 1).

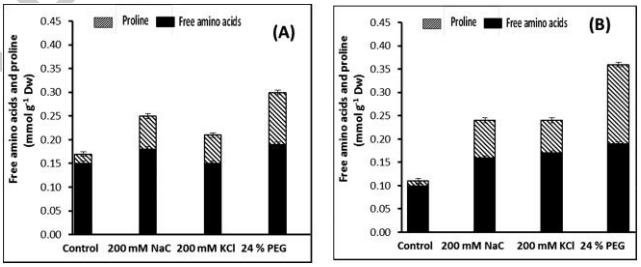

Figure 3. Proline and other free amino acids in mmol g^{-1} DW of two cotton cultivars; Giza 90 (A) and Giza 83 (B), control and plants treated with the highest stress intensity (-1.05 MPa) induced by iso-osmotic concentration of 200 mM NaCl, 200 mM KCl and 24% PEG. Each value is the mean of four replicates per treatment. From left to right Giza 83 and Giza 90

As illustrated in Figure 4, plant growth assessed in terms of chlorophyll content, leaves dry weight and plant height was significantly ($p < 0.05$) decreased by the different tested iso-osmotic solutions where 200 mM NaCl reduced chlorophyll by 50% as compared to control in cv. Giza 83 and comparable decrease nearly 27% of control was recorded for high levels of KCl- and PEG-medim. The reduction in total chlorophyll content reflected on leaves dry weight and plant height which in turn declined by 50 and 28%, respectively at 200 mM NaCl as well as 40 and 20%, in average, for 200 mM KCl- and 24 % PEG-treatment, respectively. Adversely, in cv. Giza 90 the decrement in chlorophyll was relatively moderate by about 11 and 20% under 200 mM NaCl- and KCl-treatment, respectively, which consequently induced

analogous reduction percent by about 16 and 10% in leaves dry weight as well as 8 and 4 % for seedling height relative to control. Distinguishably, under PEG-osmoticum, the reduction in chlorophyll content was only 10%, that unequally comparable to the change of leaf growth (i.e. leaves dry weight which severely reduced by more than 40% in relation to control). On the other hand, seedling height in Giza 90 was not adversely affected at the same extent of leaves dry weight where the total seedling height was lessened only by 17.3% of control at 24% PEG. Such episode may indicate different capacities of morphological and physiological acclimatization among cultivars that will be discussed.

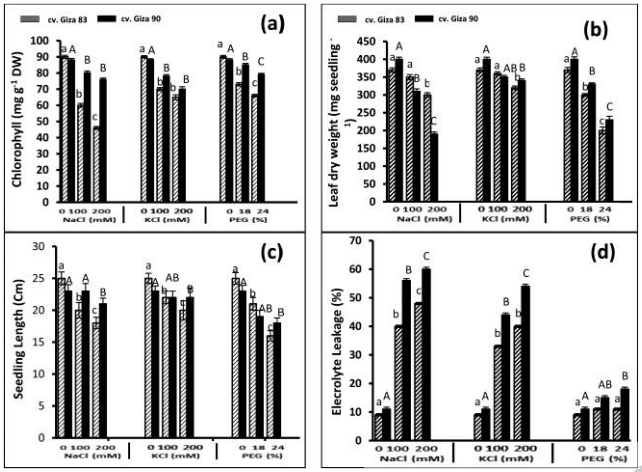

Figure 4. Effect of iso-osmotic concentrations of NaCl, KCl and PEG-6000 on different parameters Chlorophyll (a), leaf dry weight (b), seedling length (c) and electrolyte leakage (d) in two cotton cultivars. Means (±SE) of four replicates for each treatment. Values of different small letters are significantly different at P ≤0.05

The leaf electrolyte leakage, a membrane integrity indicator, generally cv. Giza 83 seedlings displayed higher electrolyte leakage than cv. Giza 90, regardless of iso-osmotic agent or concentration. The values of electrolyte leakage elevated significantly with increasing the osmoticum concentration. However, 200 mM NaCl and KCl (saline) treated seedlings exhibited 60 and 54% increment of electrolyte leakage, respectively at the maximum stress level compared to control. While there were no remarkable changes in electrolyte leakage between control and PEG-treated seedlings particularly in cv. Giza 90.

3. Discussion

Plant growth retardation due to saline environment was soundly ascribed to raising osmotic pressure of growing medium, discrepancy with mineral supplies of plants as well as decline water absorption by plants in terms of osmotic or water-deficit effect of salinity especially at high saline content in irrigation solution (Machado and Serralheiro, 2017). In atrial to mimic osmotic and ionic components of salinity stress, different iso-osmotic solutions were experienced on leaves growth and of two cotton cultivars. The increased leaves osmotic potential of PEG-stressed plants and its decrement or stability for cv. Giza 90 and cv. Giza 83, respectively under saline agents (NaCl and KCl) relative to control at iso-osmotic concentrations, revealed that PEG mimic osmotic component of salinity stress, whilst NaCl- and KCl-damaging impacts on cotton cultivars resulted from their toxic ion effect. The data of seedling length and leaves growth revealed that both cultivars were moderately osmotic stress tolerance where the osmotic agent, PEG, was frequently inhibitorier than saline agents at iso-osmotic concentrations. Apparent tolerant behaviors were obvious for cv. Giza 90 compared to cv. Giza 83 especially for saline agents and comparable response was noted for osmotic agent. Thus PEG osmotically affect the plant, thereby dehydration of leaves (lower relative water content) was the result. But NaCl and KCl treatments maintain adequate water absorptivity; thereby leaf relative water content was more or less around the control plants which reflecting that osmotic adjustment was efficiently maintained for the saline agents rather than osmotic one. Osmotic adjustment which included transfer, accumulation, and compartmentation of inorganic ions and compatible solutes, infer resistance against stresses via preserving adequate turgidity and metabolism for active growth (Carillo *et al.*, 2011; Wu *et al.*, 2015). The contribution to osmotic adjustment in stressed plants involved either inorganic ions or organic metabolites. In non- stressed plants, the contribution of inorganic ions to osmotic adjustment in leaves of both cotton cultivars constituted (71.3–72.2%) as compared with that of organic components (27.8–28.7%) where K^+,NO_3^- and organic acids which are the dominant contributors to osmotic potential. The marked sensitivity of plants grown at PEG showed higher restrictions in leaf growth compared to NaCl or KCl at the same osmotic potentials could be due to the great shift of osmotic adjustment towards organic solutes for the former and intensified the contribution of inorganic ions to osmotic adjustment for the latter. In this regard, the formation of organic osmolytes is energetically cost that 50–70 moles ATP consumed for 1 mole production (Shabala, 2013), hence the tremendous organic compounds production for osmotic adjustment could expenditure many of energy and carbon compounds, which are indispensable for growth or other metabolic processes (Sonnewald, 2001). At the level of 24% PEG reduced the contribution of inorganic solutes by 15.7% and 31.2% for cv. Giza 90 and cv. Giza 83, respectively which may be due to strong diminution of NO_3^- and K^+ contributions accompanied with increased Na^+ contribution from 0.8% to 4.8% for the former and NO_3^- only for the latter.

On the other hand, saline agents increased inorganic solutes contribution to osmotic adjustment more than 10% compared to control for both cultivars at the level of 200 mM. In this regard, NaCl and KCl shifted the highest percentages of osmotic adjustment contribution towards Na^+/Cl^- and K^+/Cl^-, respectively, affirming ion toxicity rather than water stress effect of these ionic components on these plants. The escalation in soluble solutes

decelerated water uptake and nutrients ensuing osmotic effects and ionic toxicity (Jiang *et al.*, 2014). Although the studied plants did not suffer from water stress under NaCl and KCl, but nutrients deficiency was apparently recorded throughout the preset work. Both stress agents lowered growth with various degrees which points to differences in their cytotoxicity. The theory of the osmotic effect of the salt could be valuable if the toxic effect of its ions can be avoided. KCl as an osmoticum can provide that dual advantages as the toxicity effect of its ionic component (K^+ and Cl^-) is relatively less than NaCl (Amjad *et al.*, 2015) that can be confirmed by the growth criteria that have been studied in our experiment. K^+ displayed relatively less reduction in growth criteria involving total chlorophyll content rather than iso-osmotic NaCl.

Although the leaves of cv. Giza 90 had the same Na^+ content of cv. Giza 83, cv. Giza 90 suffered less growth reduction and moderate membrane injury than cv. Giza 83. The toxic effect of ionic component of NaCl was more declared in cv. Giza 83, and it may be attributed to Na^+ rather than Cl^-. This may contribute to genotypic differences in salt tolerance if the detrimental impact of high leaves Na^+ content is a function of concentration as explained by the values of electrolyte leakage where in cv. Giza 83 under iso-osmotic NaCl, electrolyte leakage was the highest among other osmotica (KCl & PEG). The increase in stress intensity increased the damage in membrane stability. One of the most effectiveness mechanisms to cope with salinity stress is Na^+ and/or Cl^- tissue tolerance (Munns and Tester, 2008). Tissue tolerance is the capability of cells and tissues to accumulate high concentrations of Na^+ and Cl^- without or with relatively low injurious effects on tissue performance (Flowers *et al.*, 2015). These results accrue evidence of higher tissue tolerance in cv. Giza 90 than cv. Giza 83 which can be realized probably in cotton either through regulating Na^+ accumulation in mesophyll cells or having lower Na^+ in cytoplasm by compartmentalizing more Na into vacuoles (Tester and Daveport, 2003). Tissue tolerance to Na^+ should be studied in detail for a wide range among cotton cultivars. The maintenance of K^+ in high concentrations in leaves or higher cytosolic K^+/Na^+ ratios contributes to salt tolerance (Kronzucker and Britto, 2011). This trait may be regarded as a determinable factor in the differences in salt tolerance between these two cultivars (growth relatively higher in salinized cv. Giza 90 seedlings which maintain higher leaf K^+ content than cv. Giza 83 seedlings, (Table 1 and Figure 4). Cellular and intracellular Na^+ and K^+ partitioning should be scouted to highlight tissue tolerance mechanism in these and other cultivars of cotton.

The scenario of ionic imbalance by suboptimal media appeared by dramatic reduction of bivalent cation Mg^{+2}, but not for osmotic agent, PEG. As Mg^{+2} is the central atom the porphyrin ring of chlorophyll, so its reduction by NaCl and KCl interpreted chlorophyll degradation at both medium. Severe effect of NaCl on chlorophyll compared to KCl for the sensitive cultivar could be ascribed to K^+ diminishing. From one hand, impaired potassium/sodium ratio, due to increased sodium uptake, affects the bio-energetic processes of photosynthesis under salt stress, and these effects are further aggravated under potassium deficiency (Farooq *et al.*, 2015).

But drought induced by the osmoticum PEG, reduces photosynthesis, for a number of reasons: (i) hydro-active stomatal closure and low stomatal and low mesophyll conductance reduce the CO_2 supply to the leaves and/or impairments in carbon assimilation metabolism; (ii) water deficiency damages the cytoplasm ultrastructure and enzyme activity; (iii) dehydrated cuticles, cell walls, and plasma membranes are less permeable for CO_2 (Amirjani and Mahdiyeh, 2013). Dehydration results in cell shrinkage, and consequently a decline in cellular volume which makes cellular contents more viscous. Therefore, an increase in the probability of protein-protein interaction leads to their aggregation and denaturation. Increased concentration of solutes, leading to increased viscosity of the cytoplasm, may become toxic and maybe deleterious to the functioning of enzymes, including those of the photosynthetic machinery (Hoekstra *et al.*, 2001).

Sodium accretion also disturbs calcium nutrition (Kaya *et al.*, 2010; Shahzad *et al.*, 2012). Some calcium is required to uphold cell membrane integrity for proper functioning (Hu *et al.*, 2007). Thus, Ca^{+2} induction by PEG controlled ions leakage to a large extent to be slightly increased compared to control, but its reduction by saline components, especially NaCl, folded electrolyte leakage immensely reached 6- and 5-folds at 200 mM NaCl and KCl, respectively for cv. Giza 83. The damaging impacts of studied stressors on membrane stability of cv. Giza 90 was vastly lower than that of cv. Giza 83, due to the ability of this cultivar to raise Ca^{+2} content under these conditions compared to control. Another advantage from elevated Ca^{+2} content under saline agents for cv. Giza 90 was stabilization of K^+ even at high saline levels as Ca^{2+} affect K^+/Na^+ selectivity by maintaining the Na^+ influx via nonselective ion channels (Melgar *et al.*, 2006) and vice versa was reported for cv. Giza 83.

Besides K^+, Mg^{+2} and Ca^{+2}, NO_3^- content and its contribution to osmotic adjustment observed to be depleted by NaCl salinity, but not KCl or PEG. Similar hindering of nitrogen uptake and translocation owing to saline medium was obtained by (Gadalla *et al.*, 2007; Turan *et al.*, 2010).

The accumulation of metabolites that act as compatible solutes is one of the common responses of plants to changes in the external osmotic potential (Munns and Tester, 2008) such as proline (Shamshiri and Fattahi, 2014), soluble sugars (Chelli-Chaabouni *et al.*, 2010), amino acids (Dijksterhuis and De Vries, 2006), organic acids and so on (Zhou and Yu, 2009).

The carbohydrate content and its involvement in osmotic adjustment were more conspicuous for osmotic stressor compared to saline stressor and for sensitive cultivar compared to tolerant one. The participation of carbohydrates to osmotic adjustment in PEG-stressed plants was at expense of the other metabolites and even

inorganic ions. In this case, the massive accumulation of organic solutes for osmotic adjustment will cost lots of energy and carbon substances, which are available and necessary for plant growth or other metabolic activities (Sonnewald *et al.*, 2001), as the case of sensitive cultivar Giza 83. Whilst, cv. Giza 90 experienced a high inorganic solutes consequently high contribution to osmotic adjustment under PEG treatment so suggested reduction in growth was more relatively mitigated than in cv. Giza 83 which depend on organic solutes by more than 50%. It is worth mentioning that the studied cultivars exhibited some morphological acclimation attributable to the genetic differences. This was more obvious in cv. Giza 90 where the reduction in leaves dry weight (more than 40 % of control) was not in accordance with the relative slight reduction in the sum length of hypocotyl and epicotyl (only 17.3% of control). This may be in favor of root growth that may be prolonged at the expense of shoot growth in a trail to raise the root efficiency in water uptake compensating water shortage in outer media imposed by PEG, as explained by the data of shoot/root ratios (data not shown) the lowest value of shoot/root ratio was belonged to PEG treatment in case of Giza 90. Sinclair and Muchow (2001) who have assessed traits for yield increases under water-limited conditions, the trait that consistently increased crop yields was an increase in rooting depth. This can be attributed to the possibility role of osmotic adjustment in root tips so that root growth can be sustained at the onset of soil drying and roots penetrate deeper into the soil and into new water reserves. This has been confirmed by Serraj and Sinclair (2002) who stated that if genetic limitations of the crop or soil conditions limit rooting into deeper wet soil, the yielding capability of the crop can be severely limited.

On the other hand, in salt-growing plants, the elevated sugar content in mesophyll cells of leaves may be indispensable feedback inhibition of photosynthesis. These high sugar concentrations may be developed from deterioration of normal sugar usage in the growing tissues (Munns *et al.*, 1982). This outcome may be another reliable interpretation of chlorophyll decline under salt stress. The role of TSS as compatible solutes may be masked by their different additional functions in plants, as direct products of photosynthesis, components of primary metabolism and signaling and regulatory molecules, making sometimes difficult to assess their specific contribution to stress tolerance (Lokhande *et al.*, 2012; Gil *et al.*, 2013). Although organic acids exacerbated by the applied stressors, their contribution to osmotic adjustment exhibited stressor-specific responses. PEG intensified the participation of organic acids to osmotic adjustment at expense inorganic ions especially at high doses, whereas the saline agents reduced this contribution which may be implicated in recompensing for ionic imbalance.

Amino acids and proline also enhanced by saline and osmotic agents, which exhibited dose, stressor- and cultivar-specific responses in relation to their contribution to osmotic adjustment. Generally, PEG-induced participation of amino acids and proline in osmotic adjustment compared to control especially for the level of 18%. The role of amino acids in osmotic adjustment of plants grown under water stress conditions is well documented (Braam *et al.*, 1997; Dijksterhuis and de Vries, 2006), ensuing in production of greater concentration of amino acids useful in osmoregulation (Nayyar and Walia, 2003).

In cv. Giza 90, the contribution of amino acids to osmotic adjustment was maintained constant at the level of 100 mM and reduced for 200 mM of both saline agents, whilst accretion of the contribution of proline to osmotic adjustment was displayed at 100 and 200 mM NaCl or KCl compared to control which indicating that proline only rather than other amino acids contributed to osmotic adjustment for these saline agents and for this cultivar. In cv. Giza 83, NaCl as well as KCl triggered the contribution of amino acids rather than proline in osmotic adjustment compared to control which reflecting that proline was not involved in the osmotic adjustment, alternatively other amino acids participated in this labor. Such increase in proline may be invested in other activities rather than osmotic adjustment, it can also contribute to scavenging reactive oxygen species (ROS), stabilizing subcellular structures, modulating cell redox homeostasis, supplying energy, and functioning as a signaling molecule to interact with other metabolic pathways under stress conditions (Szabados and Savouré, 2010; Sharma *et al.*, 2011; Wang *et al.*, 2015). Another common reaction to abiotic stress is the synthesis and accumulation of specific osmolytes in the stressed plants, although it is often difficult to establish whether the stress dependent increase in the concentration of a particular osmolyte has a functional role in the mechanisms of tolerance of a given species.

4. Conclusion

Accordingly, salt- and PEG-treatments were applied in cv. Giza 83 behaved as a sensitive cultivar since PEG and salt stresses reduced its growth equally while lower reduction in cv. Giza 90 was recorded being a good indicator of the degree of salt tolerance between the two cultivars (cv. Giza 90 and Giza 83). In cv. Giza 83 under osmotic stress, the increasing organic solute content did not lead to an osmotic adjustment level preventing plant biomass reduction and the internal water deficit. Actually, osmotic potential increased inversely with salt conditions; this suggested the importance of decreasing inorganic solute content and/or the shift in metabolites to osmotic adjustment. Tolerance of cv. Giza 90 to NaCl imposition induced osmotic stress seemed to be associated with more accumulation and storage of ions in the vacuole. Cellular and intracellular Na^+ and K^+ partitioning should be scouted to highlight tissue tolerance mechanism in these and other cultivars of cotton.

On the other hand, PEG induce osmotic stress tolerance seemed to be associated with greater proline accumulation and a slight increase in cations (Na^+, K^+, and Ca^{+2}) in the leaves tissues, serving energy to sink in growth process by depending on inorganic solutes as low

energetic cost osmoticum instead of the highly cost one (organic) revealing different capacities for osmotic adjustment.

Osmotic adjustment seems to be a substantial response to salt and osmotic stresses; sugar accumulation and growth inhibition of PEG-treated sensitive cultivar are linked to reducing organic acid contents, whose contribution to osmotic potential, decreased by about 47% relative to control, Thus, the low organic acid contents of the PEG-treated cv. Giza 83 would suggest an impaired respiration and a decreasing energy supply, while the costs of adaptation are high.

Abbreviations: cv(s), cultivars; PEG, polyethylene glycol; Ψs, osmotic potential; Chl, chlorophyll; DW, dry weight; RWC, relative water content; Pro, proline; SS, soluble sugars; FAA, free amino acids; OA, organic acids.

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