

## **Studies on the Effects of Salinity and Hydrogel Polymer Treatments on the Growth, Yield Production and Solute Accumulation in Cotton and Maize**

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**Key words.** Cotton, (*Gossypium hirsutum*, L.), Maize, (*Zea mays*, L.), Salt tolerance, Salinity, Hydrogel polymer, Growth, Photosynthetic activity, Chloroplast pigments, Carbohydrates, Free amino acid, Free proline, Protein, Macronutrient and micronutrient elements.

**Abstract.** The effect of incorporating hydrogel polymer into sand on the development of selected crop plants grown under saline conditions has been investigated. The seeds of cotton (*Gossypium hirsutum*, L.) and maize (*Zea mays*, L.) were germinated in sand/swollen hydrogel polymer mixture (80:n 20, v:v) with added Hoagland nutrient solution, then transplanted into a range of sand/swollen hydrogel polymer combinations (100:0, 95:5, 90:10, 85:15 and 80:20, v:v) in plastic growbags. Saline solutions containing NaCl, CaCl<sub>2</sub>, and MgCl<sub>2</sub> (4 000, 8 000, 16 000 & 32 000 ppm) were applied to the growbags (to field capacity) twice per week, alternating with a comparable watering regime. Harvesting was carried out at the fruiting stage. Polymer incorporating reduced the effect of salinity on both species (20% > 15% > 10% > 0%). Above 4 000 ppm salinity treatments tended to reduce shoot height, leaf area, dry weight (D.W.), yield production, chloroplast pigments (chlorophyll a, chlorophyll b and carotenoids) and photosynthetic activity while levels of carbohydrate (monosaccharides, sucrose and polysaccharides) and nitrogenous components (total free amino acids, free proline and protein) tended to increase under saline conditions particularly in the presence of hydrogel polymer. In the absence of salt the proline content was 30-42% of the total free amino acids pool; in the presence of salt up to 32 000 ppm and up to 20% hydrogel polymer the figure was 85-95%. Inorganic elements (sodium, calcium, magnesium, phosphorus and chloride) tended to decline in the presence of saline and hydrogel polymer treatments, but potassium and nitrogen tended to decline in the presence of saline and hydrogel polymer incorporated into sand treatments. Hydrogel polymer appears to be highly effective for use as a soil conditioner in saline conditions resulting in improved soil characteristics, crop tolerance and growth in a sand substrate. It is intended to confirm the results of these studies by field trials.

### Introduction

Salt tolerance in higher plants may be achieved through the preferential accumulation of compatible osmotic solutes in the cytoplasm [1] in order to maintain equilibrium of the water potentials of vacuole and cytoplasm [2, 3]. Chloride may cause a sharp reduction in chlorophyll content and photosynthetic rate [4]. Protective plant responses to salinity include increases in the concentration of sugars [5] and amino acids, including proline [e.g. 6]; it is known that proline has an important role in the osmotic functions of higher plants under saline conditions [2, 7 pp. 201-241].

Water stress was repeatedly recorded to induce a pronounced alternation in the metabolism of nitrogenous compounds [8-10]. Ahmed *et al.*, [11] reported that water stress resulted in some disturbances in nitrogen metabolism. Protein synthesis is one of the biochemical processes that are affected by water stress [12, 13]. Accumulation of amino acids under drought stress has been reviewed by [14, pp. 609-635; 15, pp. 205-259; 7]. This accumulation may be important in plant adaptation to dry environment [16, 2].

Salinity levels also affect calcium and magnesium levels in the plant [17]. Increased salinity tended to increase  $Mg^{2+}$  concentration in bean plants [18] and the uptake of  $Na^+$  [18]; accumulation may be localized in the plant [19] and this may apply also to chloride [20].

The potential use of hydrogel polymers as soil conditioners or substrates for plant growth depends on a number of factors including their capacity to swell in water or water vapor, release of the contained moisture from the hydrogel to the plant roots, the partitioning, binding and release of ions nutrients. Hydrogels which are commercially available and advocated for use as soil conditioners include crosslinked acrylic copolymers such as polyacrylamide or polyacrylic acid and insolubilised starch. The hydrogel polymer used in this study is based on poly (ethylene oxide), a material widely used in industry and pharmacy in the forms of poly (ethylene glycol) and non ionic surface active agents. Poly (ethylene glycols) of high molecular weight are water soluble but can be converted into water insoluble and swellable hydrogels via the reaction of their hydroxylic end groups with diisocyanates with or without the addition of other polyols as crosslinking agents. The crosslinking can be by urethane, urea, allophanate or biurel groups. The formation of a polyurethane hydrogel is shown below:

Water soluble polymer	Crosslinker	Chain extending agent
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$$HO (CH_2CH_2-O)_n - H + HO-CH_2CH_2CH_2CH (OH) CH_2OH + Diisocyanate$$

Crosslinked Poly (ethylene oxide) Co-polyurethane hydrogel

These products absorb and hold water and are of interest as potential aids to arid land plant production [21].

El Sayed, *et al.*, [22] observed that at high salinity conditions (32 000 ppm), plants of tomato, lettuce and cucumber were reduced in growth but appeared to be tolerant at all levels of hydrogel polymer incorporation with sand (sand/swollen hydrogel polymer, 100:0, 75:25, 50:50, 25:75 and 0:100, v:v).

In the present study the effect of salinity on the growth accumulation of cotton and maize under sandy soil conditions in the presence and absence of hydrogel polymer (0 - 20%) as been investigated.

## Materials and Methods

### Hydrogel

At ambient temperature the water-holding capacity of the material used in this study was 9.7 g/g of dry hydrogel. The initially dry granules had a particle size of 0.5 - 2.0 mm and when fully swollen were rubbery grains through which liquid water could still freely drain. Typical preparations of poly (ethylene oxide)-copolyurethane hydrogels have been reported previously [21].

### Salinity treatments

In separate glasshouse experiments carried out in late spring under controlled light and temperature conditions (14 hr light, 25°C day/ 15°C night), the seeds of cotton (*Gosypium hirsutum*, L.) and maize (*Zea mays*, L.) were germinated in a sand:swollen polymer mixture 925:75, v:v) with added Hoagland's nutrient solution. At the cotyledon + first true leaf stage, three plants were transplanted into polythene growbag containing a rang of sand:swollen hydrogel polymer combinations (100:0, 95:5, 90:10, 85:15 & 80:20, v:v). Saline solutions containing NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> as molar solutions were applied to produce final concentrations of 4 000, 8 000, 16 000 & 32 000 ppm (Molar equivalents  $1.2 \times 10^{-1}$ ,  $2.4 \times 10^{-1}$ ,  $4.8 \times 10^{-1}$ , and  $9.6 \times 10^{-1}$ , respectively, Hoaglands solution being used as control. The growbags were watered to field capacity twice per week, alternating the appropriate solution with a comparable watering regime. Treatments were harvested at the vegetative, flowering and fruiting stages, the results for the latter only are presented.

### Growth, yield production and sampling of plants for analysis

At harvest recordings were made of shoot height, leaf area and shoot dry weight (D.W.) and succulence. The economic yield was assessed as weight of grain and straw per growbag and the harvest index per plant calculated.

### Chloroplast pigment analysis

An 85% aqueous acetone extract of a known F.W. of leaf was assayed spectrophotometrically (LKB NOVASPEC) at 664, 645, 420 nm [23]. The following equations were used to determine the concentration of the pigment fractions as  $\gamma$ /ml.

$$\text{Chlorophyll a} = 10.3 E_{664} - 0.918 E_{645}$$

$$\text{Chlorophyll b} = 19.7 E_{645} - 3.870 E_{664}$$

$$\text{Carotenoids} = 4.3 E_{452} - (0.0264 \text{ Chl. a} + 0.426 \text{ Chl. b})$$

The pigment fractions were calculated as  $\mu\text{g Chl.}/\text{mg D.W.}$

### Photosynthetic activity

Chloroplasts were prepared by the method of [24, 25]. Fresh leaves were shredded, ground for one min in a blender, using a buffered solution of 0.4 M sucrose, 20 mM HEPES-KOH (pH 7.8), 3 mM  $\text{MgCl}_2$ , 4 mM sodium ascorbate and 0.1% bovine serum albumin (BSA) [25]. The mush was strained through cheese-cloth, filtered and the suspension centrifuged (1 min at  $8,000 \times g$ ). The pellet was resuspended in the isolation medium, centrifuged (5 min at  $300 \times g$ ) and the supernatant recentrifuged (10 min at  $1,000 \times g$ ). The sediment was resuspended in a 2 ml buffer solution at pH 7.8 and the aggregates dispersed. The levels of chlorophyll and chlorophyll b were determined by the method described by [26]. An aliquot of 0.2 ml of the chloroplast suspension was extracted with 3.8 ml of 85% cold aqueous acetone and the density of the extract measured at 652 nm. The chlorophyll content was calculated according to the following equation:

$$C = E_{652} \times 1000 / 34.5 \text{ mg Chl. / l.}$$

Where C = Chlorophyll a & b.

The photosynthetic activity of the isolated chloroplasts was measured using potassium ferricyanide ( $5 \times 10^{-4}$  M) as an electron acceptor. Reduction of ferricyanide was monitored spectrophotometrically (LKB NOVASPEC) at 420 nm at room temperature. The reduction mixture contained 0.2 ml of chloroplast suspension, (0.2 - 0.8 mg Chl.  $\text{ml}^{-1}$ ), 3.8 ml HEPES BUFFER (pH 7.8), and  $5 \times 10^{-4}$  M potassium ferricyanide. The mixture was illuminated at  $300 \text{ Wm}^{-2}$  using a slide projector provided with a heat filter with a 24 v, 250 w quartz halide bulb, 15 - 45 cm from the well. The photosynthetic activity of the isolated chloroplasts was calculated from the standard curve and expressed as  $\mu\text{mol ferricyanide mg chl}^{-1} \text{ h}^{-1}$  [27].

### Carbohydrates analysis

300 mg of oven dry plant material was extracted with 5 ml of borate buffer (28.63 g boric acid + 29.8 g KCl + 3.5 g NaOH in a liter of hot distilled water), left for 24 hr, then centrifuged and filtered. The filtrate was used to determine the direct reducing value (DRV) and total reducing value (TRV); the residue was dried at  $80^\circ\text{C}$  for determination of polysaccharide [28, 29].

The determination of DRV (including all free monosaccharide) was carried out by evaporation, 0.1 ml of extracted cleared borate buffer was reduced to dryness and then mixed with 1 ml of modified Nelson solution [29]. The mixture was maintained on a boiling water-bath for 15 min, after which it was cooled rapidly using running tap water. Thereafter 1 ml of arsenomolybdate [30] was added, the mixture was diluted to a definite volume, and its intensity measured at 700 nm, using a colorimeter (LKB NOVASPEC Spectrophotometer). For the determination of total reducing value (TRV), 0.2 ml of cleared extract was mixed with deionized water up to 5 ml then 0.2 ml of the diluted extract was mixed with 0.1 ml of 1% invertase enzyme solution and the mixture maintained at 37°C for 0.5. Thereafter, the reducing value was determined as described before for DRV. The difference between the value obtained from this step and that of the DRV is an estimate of sucrose, in terms of glucose. 10 mg of the remaining residue was mixed with 0.2 ml of 1% taka diastase enzyme and 0.1 ml acetate enzyme and ml acetate buffer (6 ml acetic acid 0.2 N + 4 ml sodium acetate buffer 0.2 N) made up to 3 ml left overnight at 28°C and then centrifuged [28]. The reducing value of 1 ml of the filter was estimated as above.

### **Nitrogenous components**

#### **Total free amino acids and free proline**

The total free amino acids were determined by the method described by [31], and the free proline content estimated using the acide ninhydrin method described by [32].

#### **Protein content**

Dry sample collected during the growth study were analyzed for protein content according to [33], after precipitating the protein with 15% TCA at 4°C.

#### **Ion determination**

Cation contents of the milled samples were determined by the "Wet-ashing procedure" [34] and the mixed acid digests analyzed photometrically for sodium, potassium and calcium using a Corning-400 flame photometer [35,36, p.565]. The magnesium content was determined using an atomic absorption spectrophotometer. Analyses of the remaining elements were carried out as follows: phosphorus by the molybdenum-blue method [36], nitrogen using an automatic Microkjeld method [36], and chlorides by the AgNO<sub>3</sub> titration method [37].

## **Results and Discussion**

Analysis of variance was carried out on the data presented in Tables 1-5 and the results are summarized in Appendix Table 1.

### **Growth and yield production**

In both species, with the exception of 4,000 ppm salinity, treatments tended to decrease shoot height ( $p < 0.001$ ), leaf area ( $p < 0.01$ ) and dry weight (D.W.) ( $p <$

0.05) (Table 1 & Appendix Table 1). Ability to tolerate salinity increased at all levels of hydrogel polymer. Tolerance levels were 32,000 ppm in cotton with or without hydrogel polymer and < 16,000 ppm in maize. Plant growth (shoot height, leaf area & D.W.) was inhibited increasingly with increase in salinity concentration (< 32,000 ppm), mortality being recorded at 32,000 ppm at the vegetative stage in maize; cotton was tolerant to all levels of salinity. Addition of hydrogel polymer (5 - 20%) to sand resulted in the survival of plants at all salinity concentrations including 32,000 ppm.

These results confirm earlier findings that, generally D.W. decreased with increase in salinity concentration relating to the effect of water deficits [38 & 22]. Das and Mehrotra [39] found that 8,000 ppm salinity was critical, mortality being recorded at 6 weeks in oat, 7 weeks in wheat and 8 weeks in barley. At 10,000 ppm the growth of crops almost completely ceases after the addition of a second treatment of salt. Mortality began 3 weeks after treatment in wheat and oat, and after 4 weeks in barley even before the application of the last salt treatment. Among the legumes, pea and gram showed similar behavior towards salt concentrations both surviving < 2,000 ppm. At 4,000 ppm mortality occurred in pea and gram even before the last application of salt to the soil. Lentil did not show any tolerance to salt even at the lowest salinity level. Compared to the controls, reductions in growth at 2,000 ppm salt treatment were 13, 14 and 38% in pea, gram and lentil respectively. In these studies the D.W. of both species was increased at salinity levels of 4,000 ppm. El Sayed *et al.*, [22] and Das and Mehrotra [39] observed the same results in tomato, lettuce, cucumber, barley and oat while Shourbagy and Wallae [40] found that 5-10 meq dm<sup>-1</sup> of sodium increased the yield of barley.

In addition to increasing yield, the time required to produce the yield was significantly reduced ( $p < 0.01$ ) in the presence of hydrogel polymer. In both species, salinity tended to decrease the yield with the exception of 4,000 ppm in the presence of hydrogel polymer; yield increased significantly ( $p < 0.001$ ) with increase in the hydrogel polymer concentration, the maximum yield production in the presence of hydrogel polymer ranged from 1.8 - 3.3 times the level measured in the absence of hydrogel polymer (Table 1 & Appendix Table 1). El Sayed *et al.*, [22] found that sensitivity to salinity treatments was shown by tomato, lettuce and cucumber from the earliest stages of development. Leaf area and D.W. decreased with increasing salinity concentration in all three test species; inhibition at higher saline levels were reversed by hydrogel polymer incorporation with sand. Similar effects of salinity on growth have been reported by a number of workers. Lopez [41] studied the influence of four concentrations of total salts (3,500; 5,500; 8,000 & 10,000 ppm) on the development of maize and found that toxicity occurred at 5,500 ppm. Iyengar *et al.*, [42] found that diluted sea water (15,000 ppm) reduced the number of leaves, tillering and plant height of barley cultivars.

**Table 1. Effects of a hydrogel polymer and salinity stress on shoot height (cm/plant), leaf area (cm<sup>2</sup>/leaf), dry weight (g/plant) and yield production of cotton and maize plants**

S.C.(ppm)	Hydrogel polymers (%)					Hydrogel polymers (%)				
	0	5	10	15	20	0	5	10	15	20
<b>Spp.</b>	<b>Cotton</b>					<b>Maize</b>				
<b>Shoot height (cm/plant)</b>										
Control	17.5	20.3	23.9	24.7	27.8	69.3	87.1	92.5	97.1	110.3
4,000	16.5	21.8	24.9	25.7	27.9	68.1	88.3	96.3	99.3	116.8
8,000	15.3	17.8	19.7	17.3	15.3	60.2	81.2	87.1	90.2	91.4
16,000	13.4	17.8	19.7	17.3	15.3	34.1	66.7	71.3	73.2	97.1
32,000	7.1	10.9	13.8	11.9	10.8	---	34.7	40.2	44.9	51.3
<b>Leaf area (cm<sup>2</sup>/leaf)</b>										
Control	36.9	41.7	47.1	54.6	50.0	122.2	374.7	453.3	502.7	509.7
4,000	17.9	42.9	56.4	56.8	58.8	175.3	498.3	530.3	616.7	693.3
8,000	15.5	38.1	45.2	53.6	41.7	107.1	399.7	396.5	437.3	446.0
16,000	13.1	26.2	38.1	35.7	33.3	93.2	293.3	266.7	399.0	376.7
32,000	9.8	10.9	13.1	21.7	21.5	---	144.0	168.3	191.7	195.8
<b>Dry weight (g/plant)</b>										
Control	2.6	3.2	3.4	3.5	3.6	4.53	9.82	12.41	13.37	14.85
4,000	2.3	3.9	4.9	4.2	4.8	3.62	16.27	13.48	14.04	17.80
8,000	1.4	2.9	3.9	3.2	3.0	2.96	10.58	11.30	12.18	13.24
16,000	1.3	1.5	1.9	1.7	1.9	1.98	8.49	9.41	10.16	10.68
32,000	0.4	0.8	0.9	0.8	0.7	---	5.26	6.37	7.74	8.43
<b>Wt. yield/growbag</b>										
Control	1.2	1.9	2.3	2.8	2.7	19.0	56.3	59.8	63.2	62.9
4,000	1.4	2.4	2.7	3.2	3.5	22.1	62.7	69.8	74.2	73.1
8,000	1.1	2.2	2.6	3.3	3.4	18.3	64.3	70.1	70.7	69.8
16,000	0.9	2.0	2.4	3.0	3.1	10.0	42.7	55.3	60.2	59.8
32,000	0.7	1.2	1.9	2.2	2.4	---	22.1	29.1	32.4	30.1
<b>Wt. straw/growbag</b>										
Control	1.9	2.3	2.8	3.3	3.7	4.9	6.8	8.4	9.2	9.7
4,000	2.0	2.9	3.2	3.9	4.0	6.1	8.8	9.7	10.7	11.2
8,000	1.7	2.8	3.4	4.0	4.1	5.0	8.9	8.9	9.8	10.1
16,000	1.0	2.0	2.6	2.9	3.0	3.2	6.0	6.3	7.4	8.4
32,000	0.7	1.2	1.7	1.9	2.1	---	3.7	4.2	5.9	7.0
<b>Harvest index/plant</b>										
Control	14.2	17.0	17.6	19.0	23.7	23.9	53.5	67.7	78.8	89.1
2,000	15.2	19.2	19.9	21.2	27.0	24.2	69.7	72.3	79.8	92.3
4,000	13.9	20.0	20.2	20.9	28.2	20.1	73.8	79.2	91.3	97.9
8,000	10.2	16.2	17.2	18.3	21.3	13.0	80.1	90.2	82.0	93.8
32,000	7.7	10.1	10.9	11.7	12.7	---	60.2	63.7	80.3	90.2

S.C. = Salinity concentration (ppm), Spp = Species.  
 Statistical treatments; (ANOVA) See appendix table 1.

The incorporation of hydrogel polymer, overcame the inhibitory effects of salinity (20% > 15% > 10% > 5%). In the presence of hydrogel polymer the maximum yield production ranged from 1.8 - 3.3 times the level measured in the absence of hydrogel polymer. Asana and kale [43] detected a 10% decrease in grain yield of different cultivars of wheat grown under saline conditions, mainly reflecting a reduction in 1,000 grain weight. Paliwat *et al.*, [44] recorded maximum reductions of 34% in straw yield and 49% in grain yield of barley at the highest salt concentration. Reductions in grain and straw yield due to salinity have also been reported by [45].

### Physiological effects

In both species increasing salinity tended to decrease the levels of chlorophyll a-, b, carotenoids and chloroplast photosynthetic activity; generally these effects were most evident in maize > cotton (Table 2 and Appendix Table 1). In all cases, the presence of hydrogel polymer incorporation ( $p < 0.05$ ) greatly moderated the effect of salinity, 20% polymer resulting in < 100% increase in pigments at any particular salinity levels, while effects of polymer incorporation were moderate in cotton, increase of up to 300% were apparent in maize. Other workers have concluded that salinity affected the rate of photosynthesis reflecting in pigment composition [46].

Fallth *et al.*, [47] studied the effect of five irrigation water salinities (91,200 - 5, 250 ppm) on different bean cultivars under greenhouse conditions. Crop germination and emergence were not affected by any of the water salinity levels. Crop growth, green matter (G.M.) and dry matter (D.M.) components were significantly reduced with increase in water salinity. The G.M. production ranged from 81034 g per pot to 7306 g per pot and D.M. production ranged from 38.58 g per pot to 21.53 g per pot as the salinity increased.

In the present study, chlorophylls a, b and carotenoids decreased with salinity concentration in cotton and maize (Table 2 and Appendix Table 1). The results indicate that the level of chlorophyll a was always higher than that of either chlorophyll b or carotenoids. The reduction in leaf chloroplast pigments with increased salinity has been previously reported [e.g. 46; 22]. With the exception of the lowest concentrations, the photosynthetic activity of chloroplasts was markedly reduced by salinity, particularly in the absence of hydrogel polymer ( $p < 0.05$ ) (Table 2 and Appendix Table 1). This may reflect the decrease in chlorophyll content and activity and may result from disrupted chloroplast structure or reduced number and size. Helal and Mengel [48] demonstrated that salinity affected the rate of photosynthesis and they associated this with changes in pigment composition. Ahmed *et al.*, [49] found that safflower could tolerate salinity (< 0.01 M NaCl) exhibiting higher pigment content and photosynthetic activity compared to the control; in contrast maize was highly sensitive to salinity and exhibited lower pigment contents. El Sharkawi and Salama [50] found that in wheat (Mexican 'Super-X') the chlorophyll content increased with

**Table 2. Effects of a hydrogel polymer and salinity stress on pigment contents (chlorophyll a & b and carotenoids as  $\mu\text{g}/\text{mg D.W.}$ ) and photosynthetic activity (as  $\mu\text{mol. ferricyanide mg chl}^{-1} \text{h}^{-1}$ ) of cotton and maize plants**

S.C.(ppm)	Hydrogel polymers (%)					Hydrogel polymers (%)				
	0	5	10	15	20	0	5	10	15	20
<b>Spp.</b>	<b>Cotton</b>					<b>Maize</b>				
<b>Chlorophyll a</b>										
Control	17.3	20.6	22.2	24.6	24.9	13.4	16.2	17.3	18.9	19.3
4,000	17.7	21.3	25.0	25.1	25.0	14.1	17.9	18.2	19.8	20.1
8,000	14.7	19.7	20.1	21.7	22.7	12.3	18.3	19.7	20.2	21.3
16,000	9.5	13.9	13.8	15.2	16.7	10.0	13.9	13.0	17.3	19.7
32,000	4.5	8.0	9.7	10.3	12.0	---	10.1	10.7	12.1	13.9
<b>Chlorophyll b</b>										
Control	6.2	6.9	8.2	8.7	10.6	7.0	8.9	9.0	9.7	10.9
4,000	6.5	7.1	8.9	8.9	10.9	8.1	9.2	9.7	10.3	11.4
8,000	6.7	6.5	7.4	9.2	11.2	7.9	9.4	10.2	10.9	11.9
16,000	5.3	5.9	6.1	7.3	9.0	4.1	7.3	8.2	9.2	10.4
32,000	2.6	5.5	5.7	5.9	6.3	---	5.4	6.0	6.7	7.2
<b>Carotenoids</b>										
Control	3.9	4.3	5.0	5.1	6.0	4.1	4.9	5.0	5.3	5.9
4,000	4.2	4.7	5.9	6.2	6.9	4.7	5.0	5.9	6.1	6.2
8,000	4.7	5.2	6.7	6.8	6.7	5.0	5.3	6.2	6.7	7.1
16,000	3.8	4.1	6.0	5.8	5.9	3.1	4.0	5.2	6.0	6.3
32,000	1.9	3.1	4.0	3.9	4.0	---	2.9	3.8	4.0	5.0
<b>Phototsynthetic activity</b>										
Control	62.8	86.7	85.8	98.2	85.2	17.5	49.1	48.1	32.8	59.2
4,000	69.1	80.0	79.2	88.2	76.7	21.3	40.5	55.4	60.0	71.1
8,000	60.1	76.0	76.9	83.8	75.3	18.9	36.6	48.4	46.6	62.2
16,000	53.0	78.0	72.1	78.7	70.9	16.3	34.2	43.2	39.0	52.3
32,000	39.8	53.9	63.2	68.2	60.1	---	31.0	39.2	20.1	49.4

S.C. = Salinity concentration (ppm), Spp = Species.

Statistical treatments; (ANOVA) See appendix table 1.

salinity treatments, while the reverse was true in the Egyptian cultivar (Giza 155). Hekal [51] also found that the pigment content of safflower plants increased with salinity of 1,000 ppm but in sunflower plants it was reduced at salinities of 3,000 and 6,000 ppm.

In the present study, inorganic solute increased with salinity concentration ( $p < 0.01$ ) up to 16,000 ppm (potassium and nitrogen) or 32,000 ppm (sodium, calcium, magnesium, phosphorus and chloride); these levels declined with a hydrogel polymer increased ( $p < 0.05$ ) (Table 3 and Appendix Table 1) at each salinity level. The evidence presented here shows that the reduction in levels of sodium, potassium calcium, magnesium, nitrogen, phosphorus and chloride resulting from salinity treatments was reduced in the presence of hydrogel polymer; similar results were presented by [52]. Sodium tended to accumulate in response to salinity, particularly in the absence of a hydrogel polymer, confirming previous findings [e.g. 53, 22]. Generally, the potassium content decreased in response to salinity, particularly in the presence of a hydrogel polymer possibly reflecting competitive reduction in uptake due to salinity or reduced availability of  $K^+$ . Similar observations have been reported by [39, 53, 54]. The calcium content increased with increase in salinity concentration particularly in the absence of hydrogel polymer, possibly reflecting a salt induced increase in magnesium uptake, [55, 56]. At all levels of salinity, the phosphorus tended to increase while the nitrogen decreased especially in the absence of hydrogel polymer, whereas, actually nitrogen increased at 8,000 ppm then decreased with further increase in salinity. Similar reductions have been reported by [57] Glycine javinica; [58] (Clover); [59] (sugarcane) and [60] (various species). These results are contrary to the findings of some investigators, however, who reported a decrease in the absorption of phosphorus under saline conditions [e.g. 61]. At all levels of salinity, the chloride content increased progressively with salinity concentration particularly in the absence of hydrogel polymer. The accumulation of chloride in both plant species was higher than sodium and this has been reported also in bean (*Phaseolus vulgaris*, L.) [20]; Rhodes grass (*Hloris gayana*, kunth) and peanut (*Arachis hypogea*, L.) [53, 54]; Lessani and Marschner [62] found higher levels of chloride than sodium in salt stressed crops except in tolerant sugarbeet.

Salinity treatments increased the levels of monosaccharide, sucrose and polysaccharide up to salinity levels of 8,000 - 16,000 ppm, thereafter declining (Table 4 and Appendix Table 1); incorporation of hydrogel polymer increased carbohydrate levels at salinity levels up to 100%. Salinity enhanced content may protect the isolated chloroplast pigment against injury during desiccation [63 & 22].

Total free maino acids, free proline and protein levels increased with increasing salinity concentration ( $p > 0.01$ ) particularly in the presence of hydrogel polymer ( $p > 0.05$ ) (Table 5 and Appendix Table 1). Protein content increased up to salinity levels of 8,000 ppm ( $p > 0.05$ ), while total amino acid and proline levels increased up

**Table 3. Effects of a hydrogel polymer and salinity stress on inorganic solutes (sodium, potassium, calcium, magnesium, nitrogen, phosphorus, and chloride as meq/100g D.W.) of cotton and Maize plants**

S.C.(ppm)	Hydrogel polymers (%)					Hydrogel polymers (%)				
	0	5	10	15	20	0	5	10	15	20
<b>Spp.</b>	<b>Cotton</b>					<b>Maize</b>				
<b>Sodium</b>										
Control		59.4	56.7	47.0	40.2	39.8	63.8	60.2	58.3	50.9
48.1										
4,000	70.3	63.2	59.8	48.9	46.9	78.1	68.7	63.1	59.1	57.2
8,000	78.9	70.1	67.8	52.9	50.8	83.8	79.2	70.2	70.2	62.3
16,000	87.3	78.9	79.2	70.8	69.2	90.1	83.1	79.3	73.1	69.2
32,000	93.9	88.8	83.9	80.1	79.1	---	90.2	83.1	80.1	73.2
<b>Potassium</b>										
Control	57.9	53.2	50.7	49.2	47.1	71.3	69.8	65.2	64.1	63.2
4,000	79.8	62.8	63.7	71.8	69.3	82.5	73.9	70.1	66.3	62.9
8,000	73.8	70.3	69.8	62.3	52.8	70.2	62.4	57.8	53.9	50.1
16,000	52.7	50.2	49.1	48.9	43.2	63.9	52.1	50.2	49.1	45.3
32,000	49.1	47.3	42.1	40.3	34.7	---	49.3	43.7	41.2	38.1
<b>Calcium</b>										
Control	73.7	70.1	67.3	60.1	56.3	94.8	80.9	79.1	70.2	62.3
4,000	89.1	79.8	74.8	71.8	67.8	96.9	90.9	89.9	83.7	80.1
8,000	98.3	87.3	83.2	80.2	79.8	103.9	101.8	99.9	92.7	89.1
16,000	101.2	98.2	91.7	89.1	83.7	121.7	112.3	110.3	98.7	93.7
32,000	113.7	107.2	98.9	93.7	90.2	---	130.7	127.3	110.3	102.7
<b>Magnesium</b>										
Control	107.4	102.1	98.1	95.8	90.1	150.0	113.7	107.9	102.1	98.1
4,000	118.3	110.3	107.9	103.8	98.2	163.8	132.1	118.3	113.1	107.8
8,000	139.7	127.0	113.8	110.7	107.9	172.8	151.7	137.2	127.9	112.8
16,000	152.8	141.0	129.8	121.9	113.8	189.7	167.2	152.8	141.9	128.9
32,000	161.7	158.9	143.8	141.8	124.8	---	179.3	170.2	160.7	141.9
<b>Nitrogen</b>										
Control	97.6	81.3	70.2	58.1	57.1	102.3	97.6	90.2	81.3	79.1
4,000	101.3	98.3	90.8	89.8	83.2	113.7	107.9	96.9	90.3	89.1
8,000	119.8	120.8	103.7	92.7	91.7	124.7	113.4	104.8	97.6	83.3
16,000	102.1	112.1	117.2	90.3	89.7	101.2	105.1	93.7	81.7	78.3
32,000	101.7	91.7	98.3	81.7	72.9	---	92.8	85.9	78.2	70.7
<b>Phosphorus</b>										
Control	125.0	118.3	113.8	107.8	94.6	132.1	123.7	120.1	118.1	102.9
4,000	139.7	120.3	119.1	117.9	101.3	141.9	139.8	130.3	129.1	110.8
8,000	142.8	139.8	128.3	131.7	120.8	158.7	152.8	142.9	140.2	123.7
16,000	167.0	152.7	149.8	150.9	139.8	167.0	161.7	158.9	153.9	148.0
32,000	178.0	169.2	160.8	159.8	150.9	---	169.9	163.2	160.7	154.3
<b>Chloride</b>										
Control	288.4	251.7	230.7	217.4	214.8	323.9	314.8	287.7	272.6	210.1
4,000	316.0	276.8	259.8	226.1	220.7	340.1	327.1	298.3	290.9	227.8
8,000	377.9	299.8	286.1	249.8	240.8	359.7	340.8	310.3	307.7	249.4
16,000	399.8	326.1	319.7	266.8	259.1	372.2	349.1	329.7	317.8	288.9
32,000	416.7	389.7	370.3	299.7	281.7	---	380.3	367.4	358.1	310.7

S.C. = Salinity concentration (ppm), Spp = Species.

Statistical treatments; (ANOVA) See appendix table 1.

**Table 4. Effects of a hydrogel polymer and salinity stress on carbohydrates (monosaccharides, sucrose and polysaccharides as mg/100g D.W.) of cotton and maize plants**

S.C.(ppm)	Hydrogel polymers (%)					Hydrogel polymers (%)				
	0	5	10	15	20	0	5	10	15	20
<b>Spp.</b>	<b>Cotton</b>					<b>Maize</b>				
<b>Monosaccharides</b>										
Control	26.7	33.9	46.1	52.2	56.8	57.1	59.2	66.2	76.4	78.2
4,000	31.7	37.9	47.9	59.1	69.7	61.7	67.2	71.3	82.7	87.1
8,000	28.9	39.2	52.1	60.2	70.2	69.2	70.3	74.8	89.7	90.3
16,000	20.1	29.1	39.7	53.2	62.1	50.8	63.8	72.9	73.8	89.1
32,000	19.8	22.3	34.8	49.8	53.1	---	59.7	67.2	69.1	72.8
<b>Sucrose</b>										
Control	75.6	99.1	116.1	174.3	189.7	99.1	127.7	157.4	181.7	212.3
4,000	81.3	103.7	127.9	189.1	198.3	101.3	139.8	172.1	198.3	229.8
8,000	89.7	127.9	148.1	198.2	201.3	112.7	159.7	190.8	210.3	231.7
16,000	92.3	128.1	141.7	150.1	182.9	109.2	172.8	198.3	220.7	251.9
32,000	70.1	110.0	120.7	148.3	167.2	---	169.0	187.0	192.3	240.8
<b>Polysaccharides</b>										
Control	4.5	6.2	7.1	10.8	11.3	9.7	10.5	18.1	19.4	20.3
4,000	5.8	6.7	7.9	11.3	13.2	10.9	11.7	19.8	20.3	24.7
8,000	6.7	7.2	8.8	13.0	15.1	11.2	13.2	20.1	24.9	26.8
16,000	6.0	8.9	9.7	11.1	12.7	10.7	15.1	18.0	24.7	24.9
32,000	3.0	6.1	7.2	9.7	10.9	---	9.1	15.2	20.1	20.2

S.C. = Salinity concentration (ppm), Spp = Species.

Statistical treatments; (ANOVA) See appendix table 1.

to 32,000 ppm; hydrogel polymer again caused increases in all three constituents up to 100% irrespective of salinity treatment. In both plant species, the total free amino acid and free proline contents increased with rise in salinity concentrations, particularly in the presence of hydrogel polymer ( $p > 0.05$ ). Ismailov *et al.*, [6] showed that in *solanum iaciniatum* all amino acids including proline, increased as a result of salinization; proline has an important role in the osmotic functions of higher plants under saline conditions. Indeed it is possible that in higher plants increases in amino acids under saline conditions are exclusively due to accumulation of proline [2, 64, 65, 1]. It is possible that metabolites which are observed to accumulate under saline conditions may provide an alternative sources of solutes in the cells enabling them to osmotically to the external environments. Plants which have the highest ability to accumulate organic solutes under osmotic stress in the presence of hydrogel polymer, show the greatest resistance to loss of water under these conditions.

High concentrations of organic solutes in the cytoplasm may contribute to the osmotic balance when the concentration of electrolytes is lower in the cytoplasm than

**Table 5. Effects of a hydrogel polymer and salinity stress on nitrogenous components (total free amino acids, free proline and proteins as mg/100 g D.W.) of cotton and maize plants.**

S.C.(ppm)	Hydrogel polymers (%)					Hydrogel polymers (%)				
	0	5	10	15	20	0	5	10	15	20
<b>Spp.</b>	<b>Cotton</b>					<b>Maize</b>				
<b>Total free amino acids</b>										
Control	3.2	4.7	4.9	5.2	5.0	3.1	4.8	5.3	5.9	6.2
4,000	4.7	5.7	6.3	7.3	7.9	3.9	5.9	6.2	6.9	7.1
8,000	6.0	7.2	7.7	8.3	8.9	4.2	6.7	7.8	7.9	8.2
16,000	7.1	9.3	9.8	9.9	10.3	4.9	8.9	8.9	9.6	9.9
32,000	8.2	11.0	10.9	11.9	12.0	---	10.0	10.4	11.9	12.3
<b>Free proline</b>										
Control	1.2	1.5	1.7	1.9	2.1	0.9	1.3	1.5	1.7	2.9
4,000	2.8	4.2	5.9	6.2	6.9	2.3	3.7	4.0	4.9	5.2
8,000	4.9	6.7	6.3	7.9	8.2	3.9	4.9	6.2	5.8	7.0
16,000	6.1	8.9	8.7	9.1	9.8	4.8	6.9	7.9	7.9	8.2
32,000	7.9	10.3	9.2	10.3	11.3	---	7.3	9.2	8.9	10.3
<b>Protein</b>										
Control	10.2	17.2	18.1	19.8	20.2	10.0	14.8	15.9	17.2	19.3
4,000	13.1	18.3	19.3	20.3	21.3	11.8	16.2	17.3	18.1	20.1
8,000	12.0	19.2	20.2	21.2	22.7	11.9	17.8	18.9	19.2	21.0
16,000	9.8	16.2	17.2	19.0	18.0	9.9	12.3	14.0	14.4	18.2
32,000	7.4	10.8	16.1	18.2	16.7	---	10.1	12.4	13.1	15.3

S.C. = Salinity concentration (ppm), Spp = Species.

Statistical treatments; (ANOVA), See appendix table 1.

in the vacuole; they may protect the enzymes in the presence of high electrolytes in the cytoplasm [2]. The present results show that total free amino acid and free proline levels were increased under saline conditions especially in the presence of hydrogel polymer. Free proline which is present only in traces in the absence of salt, is increased to high levels under high saline concentration, particularly in the presence of hydrogel polymer. On average, free proline represented about 30 - 42% of total free amino acid pool in the absence of salt presence of hydrogel polymer, and about 85 - 95% in the presence of salt (32,000 ppm) and hydrogel polymer (20%). The rise in the level of free proline was a linear function of the increase both in salt and hydrogel polymer treatments, in culture solutions particularly at high salinity and hydrogel polymer levels. Stewart and Lee [2] reported a marked accumulation of free proline at high salt levels in *Armeria maritima* (Mill) wild., while [65] found that a rapid

increase in the level of free proline in plants was associated with a threshold salt concentration in the medium and also hydrogel polymer treatments. Increases in the total amino acids and proline levels in cytoplasmic solutes under high salinity concentrations have been reported for many species [2 & 66].

The results also show that the total soluble protein content generally decreased with increased salinity concentration particularly in the absence of hydrogel polymer. Other workers [e.g. 48] have suggested that there is a marked decomposition of proteins under saline conditions; this may offer an alternative explanation of chlorophyll destruction under saline conditions. Protein synthesis in halophytes and glycophytes is equally sensitive to salt excess [67] suggesting that the site of metabolism in halophytes may be protected from excessive salt through the compartmentation of NaCl in the vacuole. If this mechanism does exist in halophytes they must also possess an ability to raise the solute concentration of the cytoplasmic component through some other source, otherwise, there will be a loss of water from the cytoplasm.

The evidence presented here shows that under glasshouse conditions, incorporation of low concentrations of hydrogel polymer into sand has enabled both species of differing salt tolerance to be grown in saline solutions of up to 32,000 ppm concentration. In the absence of hydrogel polymer the inhibitory effects of salinity on growth, photosynthetic pigment, efficiency and protein content were greater. It is possible that metabolites which are observed to accumulate under saline conditions may provide the alternative sources of solutes in the cells to provide osmotic adjustment. The incorporation of different concentrations of hydrogel polymer appears to counteract salt inhibition; the most effective concentration being (20% > 15% > 10% > 5%); the mechanism involved is to be investigated. Saleh and Hussein [68] similarly observed the beneficial effect of incorporating polymeric material (aquastore) into a sandy soil on the seedling emergence of wheat cultivars in a sandy soil. The study indicated that in the presence of aquastore at salinity levels of less than 6,000 ppm, wheat had earlier emergence and higher germination percentage. Surface application of aquastore resulted in lower salt content of the soil than untreated. It is evident that the capacity of plants to resist water loss under saline conditions may be closely associated with their ability to accumulate organic solutes under these conditions. It is possible that organic and inorganic metabolites which are observed to accumulate under saline conditions may provide an additional source of solutes in the cells in order to enable osmotic adjustment to the external environments. The reduction in growth and yield under saline stress may be due to expenditure of energy on the synthesis of organic and inorganic components for osmotic adjustment rather than for growth. Plants which have the highest ability to accumulate organic solutes under osmotic stress in the presence of hydrogel polymer, show the greatest resistance to loss of water under these conditions.

To conclude, salinity treatments reduced plant growth and this effect was coincident with increased levels of organic and inorganic solutes (Appendix Table 1). In the presence of hydrogel polymers; growth and organic solute levels were relatively enhanced whereas ion uptake was reduced; it may be that in these circumstances improved growth is related to increase levels of amino acids, particularly proline. These may compensate osmotically for relative reduction in ion uptake and in turn this reduction may explain reduced toxicity.

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## دراسات على تأثير الملوحة و الهيدروجيل بوليمر على النمو وإنتاجية المحصول وتكدس الذائبات لنبات القطن والذرة

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ملخص البحث. تمت دراسة تأثير تداخل الهيدروجيل بوليمر مع الرمل على تطور نمو مختارات من بعض المحاصيل تحت الظروف الملحية.

زرعت بذور القطن والذرة في رمل/هيدروجيل بوليمر مشبع بالماء بنسبة (٢٠/٨٠ حجم/حجم) مع إضافة محلول غذائي للتغذية (هوجلند). تم شتل النباتات في تربة مخلوطة تحتوي على تركيزات من الرمل/الهيدروجيل بوليمر المشبع كما يلي: (١٠٠/صفر - ٥/٩٥ - ١٠/٩٠ - ١٥/٨٥ - ٢٠/٨٠ حجم/حجم) في أكياس بلاستيك للنمو. المحاليل الملحية تحتوي على ص كل - كا كل٢ - مغ كل٢ مولار بتركيزات (٤٠٠٠، ٨٠٠٠، ١٦٠٠٠، ٣٢,٠٠٠ جزء في المليون). وقد تم ري النباتات التي في أكياس النمو البلاستيكية حتى كفاءة سعة الحقل مرتين اسبوعياً بالتبادل باستخدام الماء للحفاظ على نظام الري.

الحصاد تم في مرحلة الإثمار وقد لوحظ أن تداخل الهيدروجيل بوليمر مع الرمل قلل من تأثير الملوحة على النباتين كما يلي: (١٥٪ < ١٠٪ < ٢٠٪ < ٥٪ < صفى المعاملات الملحية بأكثر من ٤٠٠٠ جزء في المليون اتجهت إلى تقليل ارتفاع المجموع الخضري ومساحة الورقة والوزن الجاف والإنتاجية وكمية البلاستيدات (محتوى الكلوروفيل «أ» و«ب» والكاروتينيدات) وأيضاً كفاءة البلاستيدات الخضراء بينما مستويات الكربوهيدرات (السكريات الأحادية والسكريات والسكريات العديدة) والمركبات النيتروجينية (كمية كل من الحموض الأمينية الحرة الكلية والبرولين الحر والبروتينات) اتجهت إلى الزيادة تحت الظروف الملحية وبالأخص مع وجود الهيدروجيل بوليمر.

كما وجد أيضاً أن في غياب الملح كان محتوى البرولين ٣٠ - ٤٢٪ من كمية الحموض الأمينية الكلية الحرة ولكن في وجود الملح حتى تركيز ٣٢,٠٠٠ جزء في المليون وعند تداخل الهيدروجيل بوليمر مع الرمل كوسط للنمو حتى تركيز ٢٠٪ بوليمر كان محتوى البرولين بالنسبة للحموض الأمينية الحرة هو ما بين ٨٥ -

٩٠٪. أما بالنسبة للعناصر غير العضوية (صوديوم، كالسيوم، مغنسيوم، فسفور، كلوريدات) اتجهت إلى النقصان في وجود الوسط الملحي واستخدام الهيدروجيل بوليمر وتداخله مع الرمل ولكن البوتاسيوم والنيتروجين نقص في وجود الوسط الملحي والهيدروجيل بوليمر المتداخل مع الرمل كوسط للنمو لذلك اتضح أن الهيدروجيل بوليمر كفاءته عالية جدًا في استخدامه كمحسن للتربة تحت الظروف الملحية لأنه أدى إلى تحسين خواص التربة الرملية وتحمل المحصول للنمو في التربة الرملية والخفيفة تحت الظروف الملحية والجافة.