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Effects of NaCl Salinity and Hydrogel Polymer Treatments on Viability, Germination and Solute Contents in Maize *(Zea meays)* Pollen

By

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With 1 figure

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Summary

EL SAYED H. & KIRKWOOD R. C. 1992. Effects of NaCl salinity and hydrogel polymer treatments on viability, germination and solute contents in Maize *(Zea mays)* pollen. – Phyton (Horn Austria) 32 (1) 143–157, 1 figure. English with German summary.

Pollen grains from maize plants subjected to 0, 40, 80, 120 and 160 meq l^{-1} NaCl showed adverse effects on pollen grain structure, viability, germination, bursting and pollen tube growth. Incorporation of a swollen hydrogel polymer into the sand growing medium in the ratios 0, 25, 50, 75 & 100 %, (v:v) enhanced pollen grain viability, germination, tube length and bursting in proportion to the level of hydrogel polymer incorporation with sand. Pollen grains from salinized plants had more soluble carbohydrate, free amino acids (especially proline), phenols and DNA, and less starch, protein and RNA compared to the non-saline controls. Salinity also resulted in the accumulation of ions such as Na^+, K^+ and Cl^- but a reduced B^{3+}content. Hydrogel polymer incorporation with sand caused a reduction in ions but enhanced soluble carbohydrate, free amino acid especially proline, protein, DNA, RNA and phenols. The significance of these metabolic changes in relation to viability, germination and tube growth of pollen grains and the role of hydrogel polymer is discussed.

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Zusammenfassung

El Sayed H. & Kirkwood R. C. 1992. Der Einfluß von NaCl Salinität und Behandlung mit Hydrogelpolymer auf Lebensfähigkeit, Keimung und gelöste Stoffe in Pollen von Mais *(Zea mays)*. – Phyton (Horn, Austria) 32 (1) 143–157, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Pollenkörner von Maispflanzen werden 0, 40, 80, 120 und 160 meql^{-1} NaCl ausgesetzt. Sie führen zu negativen Auswirkungen auf die Struktur der Pollenkörner, die Lebensfähigkeit, Keimung und Wachstum des Pollenschlauchs. Das Einbringen eines gequollenen Hydrogelpolymers in Konzentrationen von 0, 25, 50, 75 und 100 % im Sand als Wachstumsmedium erhöht die Lebensfähigkeit der Pollenkörner, deren Keimung und Pollenschlauchwachstum je nach dem Verhältnis der Menge an Hydrogelpolymer im Sand. Pollenkörner von Salz behandelten Pflanzen haben im Vergleich zur Kontrolle mehr lösliche Kohlenhydrate, freie Aminosäuren (besonders Prolin), Phenole und DNA hingegen weniger Stärke, Proteine und RNA. Salzbehandlung führt auch zur Akkumulierung von Ionen wie Na^+, K^+ und Cl^- aber zu einer Reduzierung des B^{3+} Gehaltes. Sandkulturen, versetzt mit Hydrogelpolymer, führen zu einem reduzierten Ionengehalt aber größeren Mengen von Kohlenhydraten, freien Aminosäuren (besonders Prolin), Proteine, DNA, RNA und Phenole. Die Bedeutung dieser Stoffwechseländerungen in bezug auf die Lebensfähigkeit, Keimung und das Pollenschlauchwachstum der Pollenkörner, aber auch die Rolle des Hydrogelpolymers werden diskutiert.

Introduction

The adverse effects of salt stress on growth, dry matter production and economic yield of a number of cultivated plant species have been extensively reported (e. g. Greenway, Munns & Wolfe 1983, Mass & Hoffman 1983), but information on the effect of salt stress on development of reproductive structures of flowering plants is meagre. Kapp 1947 observed that salinity had no effect on straw yield of rice but adversely affected grain production. Failure of seed formation subsequent to pollination with compatible pollen under saline conditions may be due to several factors. These include sterility or inability of pollen to germinate on the stigmatic surface; slow growth or bursting of the pollen tube after its entry into the stylar tissue or to the failure of zygote development possibly due to non-availability of metabolites. The development and behaviour of pollen under conditions of salt stress has received little attention, though adverse effects of salinity on viability and germination of pollen grains have been reported for *Petunia hybrida* (Reddy & Goss 1971) and wheat (Adullah & al. 1978). Associated metabolic changes in pollen however, have not been studied by these workers.

In general the adverse effects of salt stress on plant metabolism are well known. These include increase in soluble sugars (Downton 1977, Hawker 1980); decrease in starch content (Dowton 1977); reduction in the rates of synthesis of proetins, RNA and DNA (Nieman & Poulson 1964, Rauser & Hanson 1966, Prisco & O'Leary 1972); accumulation of amino acids such

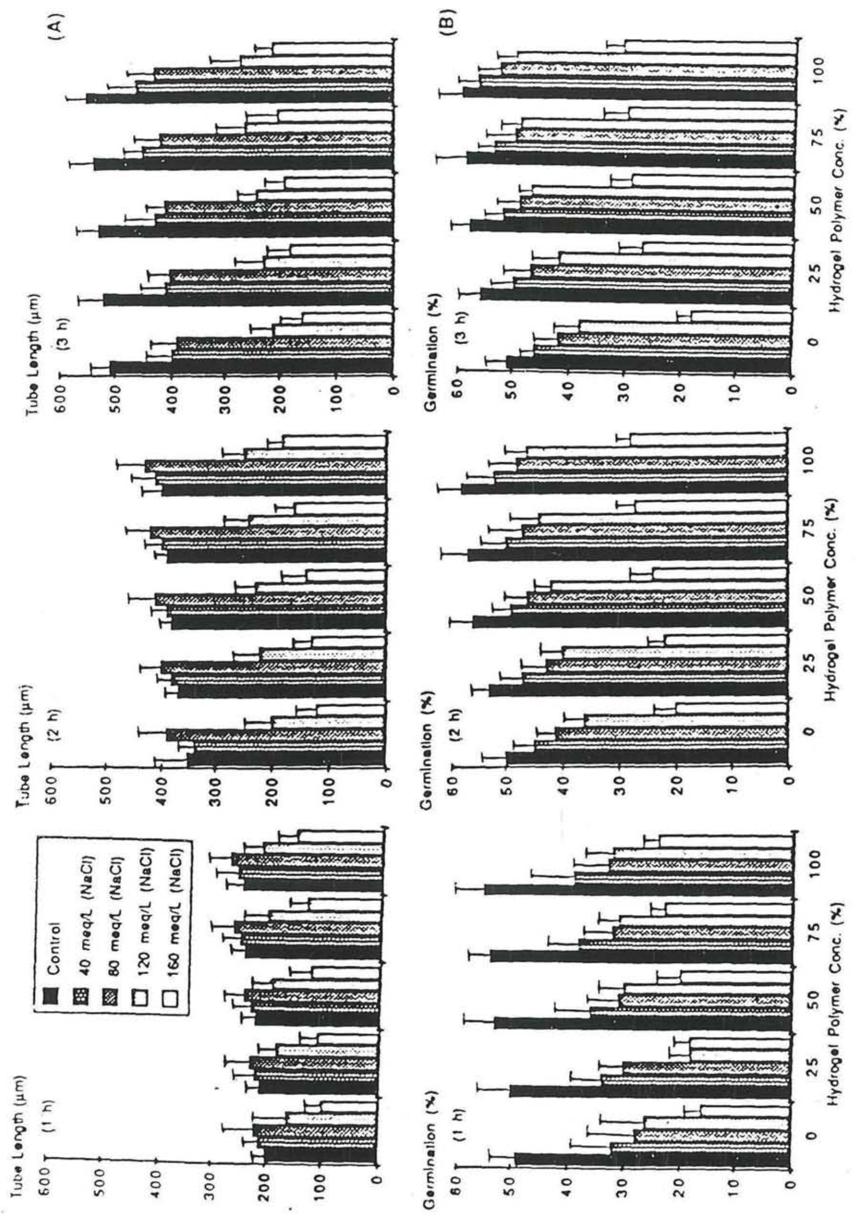

Fig. 1 A & B: Effect of NaCl salinity and hydrogel polymer treatments of PMP on the tube growth (µm) (A) and germination (%) (B) of maize pollen as Influenced by hours after Incubation.
Each value is the mean of 25 observations; Vertical bars, Standard Error means.

as proline (PALFI & JUHASZ 1970, RAKOVA & KLYSHEV 1973) and a decrease in glutamic acid content (MINIBERG & LE 1974). Furthermore salt stress may lead to an accumulation of ions including Na^+ und Cl^- and to a deficiency in certain others including K^+, in the vegetative parts of plants (TAL & al. 1978, HAWKER & SMITH 1982, IMAMUL–HUQ & LARHER 1983). It is not yet known, however as to how salinity affects the different metabolic activities of pollen which may possibly account for the reduced viability and germination. Proline generally accumulates in response to salt stress in both halophytes and non halophytes (ASPINALL & PALEG 1981). In general, the amount of proline accumulation correlated well with the degree of salinity. Drought stressed soybean leaves did not accumulate prline without prolonged wilting either in a chamber (WALDREN & TEARE 1974) or in the field (WALDREN & al. 1974). BHASKARAN & al. (1985) found no correlation between proline levels and stress tolerance in cultured sorghum cells exposed to low water potential from polyethylene glycol and they conclueded that proline increase in their system was an incidental consequence of, rather than an adaptive response to, stress. HANSON & NELSON 1978 suggested that proline accumulation is merely a symptom of injury. GREENWAY & MUNNS 1980 although not accepting the latter view because of the way in which the stress was imposed, presented evidence that the role of proline is related to survival rather than to growth maintenance.

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 vapour, release of the contained moisture from the hydrogel to the plant roots, the partitioning, binding and release of ions and 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 biuret groups The formation of a polyurethane hydrogel is shown below

Water soluble polymer		Crosslinker		Chain Extending Agent
$HO(CH_2CH_2\text{-}O)_n\text{-}H$	+	$HO\text{-}CH_2CH_2CH_2CH(OH)CH_2OH$	+	DIISOCYANATE

$$\downarrow$$

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 (GRAHAM & al. 1982).

The present study was undertaken to examine the effects of salinity (NaCl) and hydrogels based on polymers of poly(ethylene oxide) incorporated into the sand substrate of maize plants, on the metabolic activity and germination of pollen grains from these plants.

Materials and Methods

Hydrogel: The water-holding capacity of the material used in this study was at ambient temperature 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) -co-polyurethane hydrogels have been reported previously (GRAHAM & al. 1982).

Culture Conditions:

The seeds of maize *(Zea mays* L. Cv. Golden Bantam) were germinated in a sand: swollen hydrogel polymer mixture (25:75) with added Hoagland's nutrient solution (HOAGLAND & ARNON 1950). After two weeks, when the seedlings were 10 cm in height, three plantlets were transplanted into each of a series of polythene 'growbags' containing nearly 2 liter of sterilized sand and swollen hydrogel polymer at a range of combinations (0:100, 25:75, 50:50, 75:25 & 100:0). One week after transplanting the plants were subjected to 0, 40, 80, 120 & 160 meq l^{-1} salinity by the addition of appropriate amounts of NaCl to the Hoagland solution every third day until the desired level of salinity was reached. There were nine replicates per treatment. In order to maintain these levels, 100 ml per bag of nutrient solution containing the desired amounts of NaCl were added once every week. There were sixty plants per treatment.

Pollen Viability and Germination:

At anthesis, pollen grains were collected between 07.00 am and 08.00 am and used directly for experimentation. Pollen viability was tested by using 2,3,5-triphenyl tetrazolium chloride (HAUSER & MORRISON 1964). For germination studies, pollen grains were inoculated on solid media as defined by PFAHLER (1967). The effect of treatments on the rate of pollen grain germination and tube growth was determined at hourly intervals. After the incubation period, the metabolic activity was inhibited by flooding the surface of media with a killing and preserving solution (PFAHLER 1967).

Biochemical Analysis:

One hundred mg of freshly collected pollen was homogenized in 80 % ethanol (v:v) using acid washed sand as an abrasive. The mixture was refluxed on a steam bath and then centrifuged. The residue was further refluxed twice with 80 % ethanol. The supernatants were pooled together and used for estimation of total soluble carbohydrates (YEMM & WILLIS 1954); free amino acids (YEMM & COCKING 1955) and phenols (SWAIN & HILLIS 1959). Pellets were hydrolyzed with 0.2 N $HClO_4$ and the hydrolysates were used for estimation of starch (MECREDDY & al. 1958). The pellets left after $HclO_4$ hydrolysis were further hydrolyzed with 0.3 N NaOH for extraction of nucleic acids (NIEMAN & POULSON 1963); RNA and DNA contents were determined by recording absorbance at 260 and 290 nm using a U. V. Spectrophotometer (Carl Zeiss Model USV-2). Proteins were estimated by the method of LOWRY & al. (1951) by dissolving the pellet left after nucleic acid extraction with 1 N NaOH for 24 h. Proline was extracted with 3 % sulphosalicylic acid (BATES & al. 1973) and the content estimated using acid ninhydrin and toluene.

Mineral Analyses:

For estimation of Na^+, K^+ and Cl^-, 100 mg of freshly collected pollen was boiled in 5 ml of distilled water for 5 h and the final volume made upt to 10 ml with distilled water. The Na^+ and K^+ contents of these aqueous extracts were determined using a Digital Flame photometer (Systronics 121). Chloride and boron were estimated by the methods of REITEMEIR (1943) and HATCHER & WILCOX (1950) respectively.

Results

Pollen tube length increased almost linearly with time in the case of pollen collected from plants raised under non-saline conditions. Substrate salinities of 80 and 120 meq L^- marginally increased tube growth in the first hour of incubation but growth was almost negligible by the third hour (Fig. 1 A & B). Growth of pollen from plants which had been grown in the presence of hydrogel polymer with sand was greater than the corresponding treatment without polymer.

The viability and germination of pollen from pollen mother plants (PMP) grown in pure sand declined with increasing salinity level (Table 1). In the absence or presence of salinity treatments, incorporation of hydrogel polymer increased the viability and germination of pollen from mother plants receiving these treatments. For example, at 160 meq L^{-1} salinity pollen grain viability and germination were reduced by 7 % to 32.9 and 30.1, respectively, in the absence of hydrogel polymer, and by 50.7 % to 41.0 and 39.0, respectively, where it was present in the medium of the mother plants. Pollen grains obtained from plants raised under non-saline conditions attained maximum germination during the first hour of incubation, whereas, those obtained from plants grown under saline conditions at all levels of hydrogel polymer showed appreciable germination during the second hour of incubation. Bursting of the pollen grain was commonly observed especially with increasing levels of salinity and hydrogel polymer treatment of the mother plants (Table 1).

Increasing salinity of PMP substrate resulted in increased contents of Na^+, Cl^-, K^+ & B^{3+} in quiescent pollen; with the exception of B^{3+} these levels decreased with increasing hydrogel polymer incorporation with sand. The increase in Cl^- content with salinity treatments was relatively greater than that of the Na^+ and K^+ contents (Table 2).

The effect of increasing the level of salinity of PMP is shown in Table 3. Salinity treatments had an adverse effect on proteins, RNA and starch contents of pollen grains but increased the free amino acid content especially proline, total phenols and DNA contents. Hydrogel polymer treatments tended to reverse these effects.

Table 1 : Effect of different levels of NaCl salinity and hydrogel polymers on Viability, germination, bursting and tube length of maize pollen.

Polymer (%)	NaCl (meq L^{-1}) 0	40	80	120	160	Variable S	P	SXP
Viability (%)						**	*	***
0	39.9	73.4	63.8	48.9	32.9			
25	90.8	75.2	67.3	49.9	39.7			
50	93.8	78.9	69.8	53.7	42.9			
75	96.7	81.3	71.3	59.8	43.8			
100	97.9	84.9	74.8	60.4	47.2			
Germination (%)						***	***	***
0	37.1	35.9	34.1	32.2	30.1			
25	62.9	47.8	44.9	39.9	33.2			
50	67.9	49.3	46.2	42.8	35.0			
75	70.8	50.7	47.3	44.9	37.2			
100	80.8	53.3	50.3	47.8	39.0			
Bursting (%)						***	***	***
0	14.7	19.3	19.8	21.1	22.7			
25	16.7	22.3	23.7	28.1	29.8			
50	18.3	24.8	25.8	30.2	33.7			
75	20.0	26.7	27.8	32.7	39.8			
100	21.3	29.3	33.2	35.8	40.1			
Tube Length (μm)						***	***	***
0	474.8	397.3	368.8	287.7	201.8			
25	478.9	398.7	372.9	291.8	209.9			
50	482.8	409.8	378.3	297.9	218.3			
75	489.7	418.9	382.5	299.7	220.7			
100	491.6	423.7	388.6	307.8	228.6			

S = Salinity Treatments, P = Polymers, S X P = Salinity X Polymer Interaction, H.P. = Hydrogel Polymers.

Statistical treatments, where relevant, the experimental data were subjected of Two-Way analysis of variance (*ANOVA*).

Note : *F* values * = *p* < 0.05, ** = *P* < 0.01, *** = *P* < 0.001, ns = not signigicant.

Discussion

The viability and germination of pollen grains produced by maize plants grown in a saline substrate, was reduced in proportion to salinity level; the effects, However, were ameliorated by the incorporation of hydrogel polymer with sand. This confirms the observations of REDDY & GOSS 1971 in *Petunia hybrida* and ABDULLAH & al. 1978 in wheat pollen.

Analysis of the quiescent pollen for its biochemical constituents revealed that alterations in the levels of these metabolites are similar to those taking place in the vegetative parts. These include increases in soluble sugars (DOWNTON 1977); reduction in the rates of synthesis of proteins, RNA and DNA (NIEMAN & POULSON 1964, RAUSER & HANSON 1966, PRISCO & O'LEARY 1972); accumulation of amino acids such as proline (PALFI & JUHASZ 1970, RAKOVA & KLYSHEV 1973) and a decrease in glutamic acid content (MINIBERG & LE 1974). Furthermore, salt stress may lead to an

Table 2 : Effect of different levels of NaCl salinity and hydrogel polymers on the mineral contents (as mg/g D.W.) of maize pollen.

	NaCl (meq L^{-1})					Variable		
Polymer (%)	0	40	80	120	160	S	P	SXP
Na^+						***	ns	ns
0	1.63	2.56	4.60	6.03	7.09			
25	1.62	3.40	4.30	5.67	6.09			
50	1.55	3.32	4.20	5.30	5.80			
75	1.40	3.10	3.90	4.20	4.80			
100	1.33	3.00	3.70	3.90	4.20			
K^+						***	*	ns
0	4.46	6.82	9.75	14.38	17.80			
25	4.32	6.70	9.80	14.08	17.30			
50	4.17	6.43	9.20	13.81	16.10			
75	4.08	6.32	8.70	13.60	15.80			
100	3.89	6.13	8.30	13.20	14.10			
Cl^-						***	**	***
0	7.04	23.07	34.51	58.35	64.91			
25	7.00	20.09	33.50	53.39	62.83			
50	6.80	19.10	30.62	50.48	60.92			
75	6.50	18.50	28.81	48.56	59.30			
100	6.21	16.93	24.91	46.78	53.91			
B^{3+}						**	*	***
0	42.1×10^{-3}	39.3×10^{-3}	34.6×10^{-3}	29.3×10^{-3}	21.2×10^{-3}			
25	41.3×10^{-3}	38.3×10^{-3}	30.9×10^{-3}	28.3×10^{-3}	20.1×10^{-3}			
50	39.8×10^{-3}	33.8×10^{-3}	29.8×10^{-3}	26.9×10^{-3}	19.8×10^{-3}			
75	37.9×10^{-3}	30.7×10^{-3}	27.9×10^{-3}	24.8×10^{-3}	18.1×10^{-3}			
100	35.1×10^{-3}	29.0×10^{-3}	24.9×10^{-3}	23.1×10^{-3}	17.3×10^{-3}			

S = Salinity Treatments, P = Polymers, S X P = Salinity X Polymer Interaction, H.P. = Hydrogel Polymers.

Statistical treatments, where relevant, the experimental data were subjected of Two-Way analysis of variance (*ANOVA*).

Note : *F* values * = $p < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not signigicant.

accumulation of ions including Na^+ and Cl^- and to a deficiency in certain others including K^+, in the vegetative parts of plants (TAL & al. 1979, HAWKER & SMITH 1982, IMAMUL-HUQ & LARHER 1983). Thus, it appears that a decrease in pollen grain viability and germination may be related to salinity induced disturbances of metabolic processes associated with increased formation of soluble carbohydrates, free amino acid (especially proline) and phenols and reduced contents of starch and protein.

In general, the NaCl selected pollen grain tissues do maintain higher levels of K^+ and proline compared to the sensitive tissues confirming previous reports for callus of other plant species (CROUGHAN & al. 1978, DIX & PEARCE 1981, WATAD & al. 1983, PANDEY & GANAPATHY 1985). The fact that the selected cell lines demonstrated growth and accumulated proline at NaCl levels which completely inhibit growth of normal cells suggests the possibility of an osmoregulatory role for proline. Such a conclusion have been drawn by HANDA & al. (1986) and BINZEL & al. (1987) for other cell

Table 3 : Effect of different levels of NaCl salinity and hydrogel polymers on starch, total soluble carbohydrate, protein, free amino acids, proline, phenol, RNA and DNA content (mg/g D.W.) of maize pollen.

	NaCl (meq L^{-1})					Variable		
Polymer (%)	0	40	80	120	160	S	P	SXP
Starch						***	***	ns
0	78.9	37.5	22.5	15.9	10.2			
25	81.3	39.7	23.7	17.1	11.3			
50	83.7	40.2	24.9	19.8	13.7			
75	87.9	42.8	26.8	20.3	15.8			
100	89.1	44.7	28.1	21.7	16.9			
Total Soluble Carbohydrates (TSC)						ns	*	***
0	269.0	294.0	298.3	302.6	257.3			
25	273.8	296.7	301.7	309.8	262.8			
50	276.7	298.3	309.7	317.2	268.7			
75	280.1	303.7	313.5	321.8	272.8			
100	284.8	317.2	319.8	327.8	278.1			
Protein						*	ns	***
0	23.7	20.7	19.9	19.4	17.3			
25	28.8	22.8	20.3	19.8	18.7			
50	29.1	24.8	22.8	20.9	19.2			
75	32.1	27.0	24.8	23.2	21.3			
100	33.7	29.0	26.1	24.5	22.9			
Free amino acids (FAA)						***	ns	***
0	4.3	5.7	7.7	14.3	16.2			
25	4.8	6.8	9.1	15.8	17.3			
50	5.3	8.3	11.3	17.2	18.2			
75	6.1	9.2	13.7	18.1	19.1			
100	6.9	10.7	15.0	18.9	20.3			
Proline						***	**	***
0	3.1	5.1	6.5	10.1	13.7			
25	3.2	6.0	7.9	13.9	15.9			
50	3.9	6.9	9.5	15.8	16.8			
75	4.0	7.8	10.8	16.9	17.8			
100	4.8	9.9	13.7	17.8	18.9			
Total Phenols						***	**	**
0	2.7	2.9	3.5	5.0	5.9			
25	3.0	3.4	4.2	5.3	6.4			
50	3.2	3.8	4.9	5.8	7.2			
75	3.9	4.7	5.2	6.2	7.9			
100	4.1	5.2	5.9	6.9	8.3			
RNA						**	ns	***
0	15.7×10^{-2}	12.7×10^{-2}	10.2×10^{-2}	9.6×10^{-2}	8.7×10^{-2}			
25	16.9×10^{-2}	13.9×10^{-2}	10.9×10^{-2}	10.8×10^{-2}	9.8×10^{-2}			
50	18.3×10^{-2}	14.2×10^{-2}	13.1×10^{-2}	11.2×10^{-2}	10.2×10^{-2}			
75	19.7×10^{-2}	15.7×10^{-2}	14.0×10^{-2}	12.4×10^{-2}	10.9×10^{-2}			
100	20.8×10^{-2}	17.2×10^{-2}	15.9×10^{-2}	13.0×10^{-2}	12.3×10^{-2}			
DNA						*	*	***
0	2.8×10^{-3}	3.2×10^{-3}	3.8×10^{-3}	3.4×10^{-3}	2.7×10^{-3}			
25	2.9×10^{-3}	3.7×10^{-3}	4.2×10^{-3}	3.6×10^{-3}	2.9×10^{-3}			
50	3.2×10^{-3}	4.0×10^{-3}	4.8×10^{-3}	3.9×10^{-3}	3.2×10^{-3}			
75	3.7×10^{-3}	4.2×10^{-3}	5.2×10^{-3}	4.2×10^{-3}	3.5×10^{-3}			
100	3.9×10^{-3}	4.8×10^{-3}	5.9×10^{-3}	4.8×10^{-3}	3.9×10^{-3}			

S = Salinity Treatments, P = Polymers, S X P = Salinity X Polymer Interaction, H.P. = Hydrogel Polymers.

Statistical treatments, where relevant, the experimental data were subjected of Two-Way analysis of variance (*ANOVA*).

Note : *F* values * = *p* < 0.05, ** = *P* < 0.01, *** = *P* < 0.001, ns = not signigicant.

lines. It is possible however, that the accumulation of free proline in selected cells occurred as a result of injury/stress (HANSON & al. 1977, DIX & PEARCE 1981, HANSON & HITZ 1982, HASEGAWA & al. 1986).

ELHAAK & EL SAYED (1990) reported that the accumulation of proline in response to decrease in leaf solute potentials with greater values in Clark than in Williams cultivars of soybean. When the leaf solute potential approached –1.9 to–2.0 MP_a, as a result of salt stress, proline accumulation was inhibited specially in old leaves of Clark. In soybean the effective leaf solute potential for proline accumulation varied with cultivar and leaf age. It ranged from –1.12 to –2.5 MP_a and from –1.4 to –2.2 MP_a for both young and old leaves of Clark, while in Williams proline increased slowly but with increasing rate between –1.04 to –1.20 and –1.24 to –1.40 MP_a in old and young leaves respectively. Proline accumulation was low in leaves of Williams in comparison with Clark although it accumulated by 6 and 13 times its control values in old and young leaves respectively at the highest stress treatment (ELHAAK & EL SAYED 1990). In young tobacco plants old leaves showed the highest proline content (SANO & KAWASHIMA, 1982), but AMBERGER & OBENDORFER (1988) found an increase in proline in younger leaves of *Chrysanthemum*.

Despite a drastic reduction in leaf water potential, the turgor maintenance may be due to osmotic adjustment as a result of accumulation of solutes, including higher levels of proline under stress conditions (JONES 1978, JONES & TURNER 1980, TURNER & STEWART 1986). Proline accumulation in water stressed plants (BANSAL & NAGARAJAN 1986, NEWTON & al. 1986, BINZEL & al. 1987, REDDY 1987) is due to enhanced synthesis (HANDA & al. 1986), and decreased oxidation (STEWART & al. 1977). Furthermore, this proline accumulation was found to be independent of the levels of ABA which accumulates more rapidly in stressed leaves (STEWART & VOETBERG 1987).

ELHAAK & EL SAYED (1990) found that the less important role played by proline in osmoregulation could be indicated by the proline to NaCl ratios. The ratios decreased from the 10 mM to 100 mM NaCl treatments in both young and old leaves of the two cultivars except for the slight increase between 40 to 60 mM in old leaves of Clark cultivars. This indicates that the increase in NaCl did not increase proline content with equal value suggesting an inhibition in proline synthesis with NaCl osmotic stess.

It is possible that increase in salinity may cause a decrease in incorporation of the precursors of starch and proteins into these macromolecules such as have been reported for the vegetative parts of flowering plants by KAHANE & POLJAKOFF-MAYBER (1968) and HASSON-PORATH & POLJAKOFF-MAYBER (1973). A decrease in the RNA/DNA ratio under saline conditions is possibly indirect evidence of a decrease in the transcriptional activity of DNA. Salt stress induced disturbances in the metabolic processes of pollen may change the nutritional requirements for germination of pollen obtained from salinized plants.

Salinity treatments applied to the PMP subsequently reduced pollen tube growth, though this effect was reduced by all levels of hydrogel polymer incorporation with sand. A similar effect of salinity has been reported for pollen from *Petunia hybrida* (REDDY & GOSS 1971) and wheat pollen (ABDULLAH & al. 1978). SALEH & HUSSEIN (1987) observed the effect of application of polymeric material (aquastore) on the germination and emergence of wheat cultivars in a sandy soil. In the presence of aquastore at salinity levels of less than 6,000 ppm, earlier emergence and higher germination percentages were recorded for wheat compared with untreated soil. Surface application of aquastore resulted in a lower salt content of the soil than untreated controls.

Mineral analysis of quiescent pollen from plants grown under salt stress has revealed an accumulation of Na^+, K^+ and Cl^-, whereas boron content decreased. A similar trend with regard to Na^+ and Cl^- in the anthers of *Petunia hybrida* grown under saline conditions has been demonstrated by REDDY & GOSS (1973). This suggests that, like the vegetative portion, pollen is selective in the accumulation of ions. Increased accumulation of Na^+ and Cl^- together with a decrease in boron content may be an important factor responsible for poor viability and germination of pollen from plants raised under saline conditions. Thus it may be inferred that accumulation of Na^+ and Cl^- causes disturbance in the metabolic processes which are reflected in the decreased viability and germination of pollen. The decline in mineral level at all levels of hydrogel polymer incorporation may minimise the adverse effects of salinity treatments by reducing the level of ions available for uptake by the PMP.

Boron (B) ist an essential plant element but it can become toxic to some plants when soil concentrations only slightly exceed that required for optimum plant growth (CHRISTIES 1987). Symptoms of excess B often include characteristic chlorotic and necrotic patterns of leaves although some sensitive fruit crops such as stone and pome fruits may be damaged without exhibiting visible leaf-injury symptoms. Crop leaves normally contain about 40 to 100 mg of B Kg DW^{-1} but often contain more than 250 mg/kg when soil B approaches toxic levels. Boron concentrations in leaves may exceed 700 to 1000 mg kg^{-1} under extreme conditions of B toxicity (CHRISTIES 1987).

To conclude, these studies show that the response of pollen of maize plants subjected to NaCl salinity treatments involved the following effects: (1) Reduced germination, viability, bursting and pollen tube growth. (2) Increased levels of soluble carbohydrate, free amino acids (especially proline), phenol and DNA. (3) Reduced levels of starch, protein and RNA. (4) Accumulation of ions such as Na^+, K^+ and Cl^-. (5) Reduction in boron content. (6) Incorporation of hydrogel polymer with sand caused a reduction in these ions but enhanced the levels of soluble carbohydrate, nucleic acids (DNA and RNA), amino acids (especially proline), phenol, germination, viability, bursting and pollen tube growth.

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