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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5(3): 375-381, 2002

The Role of Trace Elements in Hardening of Three Wheat Cultivars to be Irrigated with Sea Water

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Abstract: The grains of the wheat cultivars Giza 155, Sakha 8 and Sakha 92 were pretreated and germinated in 0 (control), 25, 50 and 75% sea water (v/v) in petridishes for 16 days. The germination percentage and growth parameters in addition to the content nutrients of common elements were determined. Low (25%) sea water enhanced germination and growth, but higher treatments inhibited them with variant degrees in the three cultivars. Mean inhibition (MI) proved improvement of germination by trace elements pretreatment under sea water. The best effect was for $CoCl_2$ followed by $AlCl_3$ while the bad one was for $CuCl_2$ pretreatment. $CoCl_2$ has led to 100% seed germination under 50% sea water in Sakha 92. Tolerance index (TI) to sea water stress was also improved for the three cultivars by the pretreatments with $CoCl_2$ and $AlCl_3$. This improvement paralleled the increase in plant lengths, dry weight and root/shoot. The nutrient elements accumulated by low sea water but by high sea water uptake or translocation of most was retarded at the root. The order of effect for the pretreatment was $AlCl_3$ > $CoCl_2$ > $CuCl_2$ on nutrients accumulation in the three cultivars under sea water treatments.

Key words: Wheat, hardening, trace elements, Al, Co, Cu, MI, TI, germination, nutrient elements.

Introduction

Hardening is a term invented to semi-quantitatively express the increase in tolerance of water or salt stressed plants (Henckel, 1964). The difficulties involved in measuring tolerance are discussed by Shannon (1984). Meanwhile, Glenn *et al.* (1998) reported that none of the top five plants eaten by people-wheat, corn, rice, potatoes and soybeans can tolerate the increased salt. They wilt, shrivel and die within days when exposed, for example, to sea water. Hence rapid diagnostic methods of differentiating the tolerant and resistant materials are needed for hardening programs in order to use alternative sources of water and land for growing crops. Glenn *et al.* (1998) tested the feasibility of sea water agriculture and have found that it works well in the sandy soil of desert environments but with limits. Unfortunately, subsequent efforts to increase the salt tolerance of conventional crops through selective breeding and genetic engineering, in which genes for salt tolerance were added directly to the plants, have not produced good candidates for sea water irrigation trials (Glenn *et al.*, 1998). Also, the screening of an increasing number of varieties for salt tolerance through laboratory and field trials involves much time and expenditure.

The effect of trace elements on seed germination and plant activity under extreme environmental conditions has long been attracted the attention of many investigators (Smirnova, 1970 & Shkolnik and Bozhenko, 1974). Popoff (1931) was the first to study in detail the effect of pre-sowing treatment with trace elements and found it possible to increase germination and enhance the yield of crops by enriching their seeds with trace elements. Al^{3+}, Co^{2+} and Cu^{2+} have been shown to increase resistance of plants to drought both under experimental or natural conditions, in arid region (Shkolnik *et al.*, 1970; Shkolnik and Bozhenko, 1974). Furthermore, Migahid and Sadek (1994) found that presoaking of wheat grains cv. Giza 155 in $AlCl_3$ has increased their salt tolerance, while their presoaking in $CoCl_2$ and $CuCl_2$ exerted a negligible effect. Yagodin (1970) revealed that cobalt sulfate effect depends on concentration, where lower one enhanced the germination, stimulated the growth and increase the yield of legume, while higher doses (1% solution) reduced the germination, led to the development of cobalt chlorosis and sharply reduced the growth process. Effects of Al on growth remains largely speculative (Foda *et al.*, 2000) although Matsumoto *et al.* (1976) had reported a stimulating effect of Al^{3+} on the growth of tea seedlings.

Growth processes are especially sensitive to the effects of salt, so that growth rate and biomass production provide a reliable criteria for assessing the degree of salt stress and the ability of a plant to withstand it. Whereas extreme salt stress leads to dwarfism and to inhibition of root growth (Larcher, 1995), several workers (e.g. Shannon, 1984) have predicted salt tolerance characteristics on the basis of different parameters in the plants, i.e. the existence of Na ion exclusion mechanism in salt tolerant varieties of rice (Gelu SR. 26-R).

The aim of the present study was to elucidate the importance of using some trace elements (Al^{3+}, Co^{2+} and Cu^{2+}) in hardening and increasing the tolerance of wheat grains for different sea water percentage treatments.

Materials and Methods

Giza 155, Sakha 8 and Sakha 92 wheat grains were obtained from the Egyptian Ministry of Agriculture. Equal size grains from each variety were surface sterilized using 0.1% $HgCl_2$. Then the grains were soaked in bi-distilled water or in 0.25% of each $AlCl_3$, $CoCl_2$ and $CuCl_2$ solutions (weight/volume) for 5 minutes after which they were thoroughly air dried (Shkolnik *et al.*, 1970). The treated grains were germinated on filter papers wetted by 0, 25, 50 and 75% sea water concentrations in petri dishes. Wetting of filter papers in each perti dish was done by adding 6ml solution from each treatment. The entire experimental design became four soaking treatments x four sea water concentrations and each combination (from 16 petri dishes) was replicated five times, resulting in 80 experimental units for each cultivar and 240 experimental units in all. These units were arranged in a randomized complete block design in an incubator having 22± 2°C incubation temperature and 25 Wm^{-2} light intensity.

The cultures were observed daily to keep the germination filter paper continuously and uniformly moist by adding either the prepared sea water or bi-distilled water. After 2 days from sowing the grains showing radicle or plumule emergence were daily recorded till the 16th day (plants at the 3-leaf stage), then the time and percentage of germination were determined. Because there were remarkable differences in the time for radicle and plumule emergence, even some grains failed to produce plumule as a result of the used treatment, the percentages of plumule and radicle were calculated for each treatment. The maximum percentages of plumule and radicle emergence and their times were recorded. The mean inhibition for the germination of the study plants (MI) was calculated as:

Plumule inhibition = (100 - maximum percentage of plumule) + Time of this maximum

Radicle inhibition = (100 - maximum percentage of radicle) + Time of this maximum

The MI = (Plumule + Radicle) Inhibition/2

The increase in MI indicates decrease in the maximum percentage of germination and/or delay in germination (increase in time of the maximum percentage of germination).
The length, fresh and dry weights for both shoot and root were measured. Drying was performed at 60°C for constant weight. The plant biomass was determined as the dry weight per individual and the plant root/shoot ratio was calculated. Tolerance index was calculated according to Brown and Wilkins (1985) equation:

Tolerance Index (TI) = Dry weight of treated plant / Dry weight of control plant

Dry samples (0.5g) from root and shoot of the three cultivars under different treatments were suspended in 3 ml distilled water and placed in a boiling water bath for 10 minutes. The suspension was cooled, and filtered through Micro-cloth. The process was repeated 2 more times and the 3 filtrates were summed together and adjusted accurately to 5 ml with bi-distilled water. The extracts content of Na^+, Mg^{2+}, Mn^{2+}, Cu^{2+} and Fe^{3+} were assayed by flame or atomic absorption spectrophotometry (Allen, 1989).
Two ways analysis of variance was applied to evaluate the significance of variations due to sea water treatments and pretreatments with the trace elements in addition to their interaction. The used statistical methods were according to Snedecor and Corchran (1973).

Results

Germination percentage: Pretreatment of grains of Giza 155, Sakha 8 and Sakha 92 wheat cultivars with the chlorides of Al, Co and Cu and subjecting these treated grains to growth under different sea water treatments, generally, affected their germination significantly (Table 1). The recorded percentages of germination (radicle emergence) indicated 30, 15 and 15% by maximum sea water treatment for Giza 155, Sakha 8 and Sakha 92 wheat cultivars respectively . Also, growing processes of radicle and plumule were disturbed by the used treatments giving rise to failure for producing plumule in about 10% of the germinated grains which produced radicle only, especially with the maximum sea water treatment (75%). Under the control conditions, the two Sakha cultivars (8 and 92) showed only 95% germination, while Giza 155 showed 100% germination. Also, the emergence of radicle was after 2, 4 and 4 days while the emergence of plumule was delayed to 4, 6 and 10th day for Sakha 92, Giza 155 and Sakha 8 cultivars respectively. Seawater treatment did not affect the time of radicle and plumule emergence greatly in Sakha 8. In Sakha 92 there was a gradual increase in time of plumule emergence with the increase in the percentage of sea water, while the emergences of radicle in this cultivar and of plumule and radicle in Giza 155 cultivar were in the same time under all the sea water treatments. Pretreatment grains with $AlCl_3$ and $CoCl_2$ under the control increased the maximum percentage of germination, the best effect was for the pretreatment with $CoCl_2$ meanwhile, it decreased the time of maximum percentage of germination in the three wheat cultivars. On the contrary, $CuCl_2$ pretreatment increased the time of germination, especially radicle emergence, and decreased the maximum percentage of germination in sea water treated or not treated cultivars (Table 1).
The maximum percentage of germination (100% indicated from the percent of radicle emergency) under the highest sea water treatment (75% sea water) was obtained after the pretreatment of Sakha 92 with $AlCl_3$ and $CoCl_2$. The pretreatment by the two elements counteracted also, the inhibition of plumule emergency by sea water treatments. The percentage of plumule emergency increased to 95% under the highest sea water treatment. On the opposite, similar and low percentages of germination under the highest sea water treatment were for not or $CuCl_2$ pretreated grains of Giza 155 and Sakha 8.

It is also important to record here that using 25% sea water did not affect the germination percentage of Sakha 92 in comparison with the control. Pretreatment grains of the three cultivars with $AlCl_3$ and $CoCl_2$, under this low sea water treatment, improved germination where the percentage reach to 100% for Giza 155, Sakha 8 and Sakha 92, but after slightly longer time for the first cultivar. However, this low sea water treatment (25%) enhanced 100% plumule emergence for the three cultivars without the pretreatment with the used trace elements for Giza 155 or after their pretreatment for Sakha 8 and 92.

Mean inhibition (MI): As the inhibition in germination was in aptitude and time needed for germination, it is suitable to consider the overall inhibition as sum of both types and represent it as mean inhibition (MI). The MI (Table 2) showed an inhibition pattern in both plumule and radicle emergence as Sakha 8 < Sakha 92 < Giza 155 under the control. In general, MI was many times greater under different sea water treatments than under the control, but it was lower in radicle than in plumule. The pretreatment with $AlCl_3$ and $CoCl_2$ decreased the MI in the three cultivars under different sea water treatments as compared with the control values. Also, the decrease in MI of plumule and radicle emergence in Sakha 92 exceeded that in Sakha 8 and Giza 155 under different sea water treatments. Pretreatments with $CoCl_2$ still acquiring the best counteracting effect for sea water stress treatment as indicated from the recorded lowest MI. On the opposite, $CuCl_2$ pretreated grains of the three cultivars exhibited greater MI than the control.

Growth parameters: The present results demonstrated highly significant sharp reductions in the shoot and root lengths of the three cultivars of wheat by sea water treatments (Table 5 and Fig. 1). The percent reduction by the maximum sea water treatment ranged between 91 to 98 for pretreated or not treated plants of the three cultivars. The maximum reduction was recorded in Sakha 92 as compared with the other cultivars under all the used treatments. The decrease was slightly less in root length than in shoot length, but the decrease in two organs length was still over 92%. Pretreatment of grains with $AlCl_3$ under control increased shoot and root lengths in both Giza 155 and Sakha 8, while with $CoCl_2$ and $CuCl_2$ decreased both organs length, with a greater effect by the latter element and with high percentage in root of the three cultivars, especially Giza 155 (94.3%). Under 25% sea water treatment $AlCl_3$ and $CoCl_2$ pretreatment ameliorated the exhibited reductions by sea water and increased the shoot and root lengths over those pretreated with water or $CuCl_2$. In present work the pretreatment with $CuCl_2$ induced a decrease in shoot and root length of three cultivar plants under unstressed (control) and further decreases occurred under stress by sea water conditions. In exception of the slight increase in shoot length exhibited with $AlCl_3$ over that without pretreatment, in Giza 155, pretreatment with the three trace elements further inhibited the shoot and root growth of three cultivars under sea water stress by over 50 %. The results also, showed that the length of both shoot and root of Giza 155 cultivar exhibited the greatest response to pretreatment, especially with $AlCl_3$, under unstressed treatment when compared with other cultivars.
The plant dry weights of the three cultivars of wheat decreased in response to the increase in sea water concentrations when applied alone or in combination with most of grains pretreatments (Fig. 2). Pretreatment with $CuCl_2$ decreased the dry weight of the three cultivars Giza 155, Sakha 8 and Sakha 92 by 54, 35 and 39% respectively under control. The noticeable increase in the dry weight by $CuCl_2$ was only for Giza 155 and Sakha 92 under 25% sea water stress and was by 52 and 10% respectively. On the opposite, $AlCl_3$ increased the dry weight of the three cultivars under most sea water treatments in addition to control for Giza 155 and Sakha 8 cultivars. Under control (no sea water

Table 1: The time (day) and maximum percentage of germination for seeds of Giza 155, Sakha 8 and Sakha 92 wheat cultivars under the treatment by sea water and $AlCl_3$, $CoCl_2$ and $CuCl_2$ pretreatments.

Treatments			Cultivars											
			Giza 155				Sakha 8				Sakha 92			
			0%	25%	50%	75%	0%	25%	50%	75%	0%	25%	50%	75%
H_2O	Plumule	Time	6	14	14	14	10	10	10	14	4	6	8	14
		%	100	100	90	60	95	95	95	75	95	85	100	70
	Radicle	Time	4	6	6	6	4	4	6	6	2	8	8	8
		%	100	100	95	70	95	100	95	85	95	95	100	85
$AlCl_3$	Plumule	Time	4	8	14	14	4	4	14	14	4	6	14	14
		%	100	100	90	70	100	100	95	60	90	100	95	95
	Radicle	Time	2	2	2	8	4	4	8	14	4	4	8	6
		%	100	100	85	80	100	100	95	85	90	100	100	100
$CoCl_2$	Plumule	Time	6	8	8	10	6	6	14	14	4	6	10	10
		%	100	100	85	80	100	100	90	70	90	100	100	95
	Radicle	Time	2	2	8	14	4	4	6	10	10	4	4	10
		%	100	100	90	95	100	100	90	90	100	100	100	100
$CuCl_2$	Plumule	Time	6	6	14	14	14	14	14	14	6	8	14	14
		%	90	80	65	65	100	95	95	60	95	95	100	70
	Radicle	Time	6	10	8	8	6	6	8	10	4	4	8	6
		%	100	100	90	70	95	90	95	70	95	95	100	75

Table 2: The mean inhibition (MI) in plumule and radicle for Giza 155, Sakha 8 and Sakha 92 wheat cultivars under the treatment by sea water and $AlCl_3$, $CoCl_2$ and $CuCl_2$ pretreatments

Treatments		Cultivars											
		Giza 155				Sakha 8				Sakha 92			
		0%	25%	50%	75%	0%	25%	50%	75%	0%	25%	50%	75%
	Plumule	6	14	24	54	15	15	15	39	9	21	8	4
H_2O	Radicle	4	6	11	36	9	4	11	21	7	13	8	23
	Mean	5	10	18	45	12	10	13	30	8	17	8	34
$AlCl_3$	Plumule	4	8	24	44	4	4	19	54	14	6	19	19
	Radicle	2	2	17	28	4	4	13	29	14	4	8	6
	Mean	3	5	20	36	4	4	16	42	14	5	14	13
$CoCl_2$	Plumule	6	8	23	30	6	6	24	44	14	6	10	15
	Radicle	2	2	18	19	4	4	16	20	10	4	4	10
	Mean	4	5	20	25	5	5	20	32	12	5	12	13
$CuCl_2$	Plumule	16	26	46	49	14	19	19	54	11	13	14	44
	Radicle	6	10	18	38	11	16	13	40	9	9	8	31
	Mean	11	18	32	44	13	18	16	47	10	11	11	38

Table 3: Tolerance index for Giza 155, Sakha 8 and Sakha 92 wheat cultivars under the treatment by sea water stress and $AlCl_3$, $CoCl_2$ and $CuCl_2$ pretreatments

Treatments	Cultivars											
	Giza 155				Sakha 8				Sakha 92			
	0%	25%	50%	75%	0%	25%	50%	75%	0%	25%	50%	75%
H_2O	1.00	0.82	0.56	0.13	1.00	0.55	0.48	0.11	1.00	0.75	0.45	0.10
$AlCl_3$	1.15	0.88	0.64	0.16	1.04	0.89	0.42	0.14	0.78	0.82	0.29	0.03
$CoCl_2$	0.90	0.77	0.47	0.10	1.07	0.83	0.38	0.09	1.02	0.55	0.37	0.08
$CuCl_2$	0.46	0.48	0.28	0.03	0.65	0.45	0.31	0.03	0.61	0.65	0.34	0.06

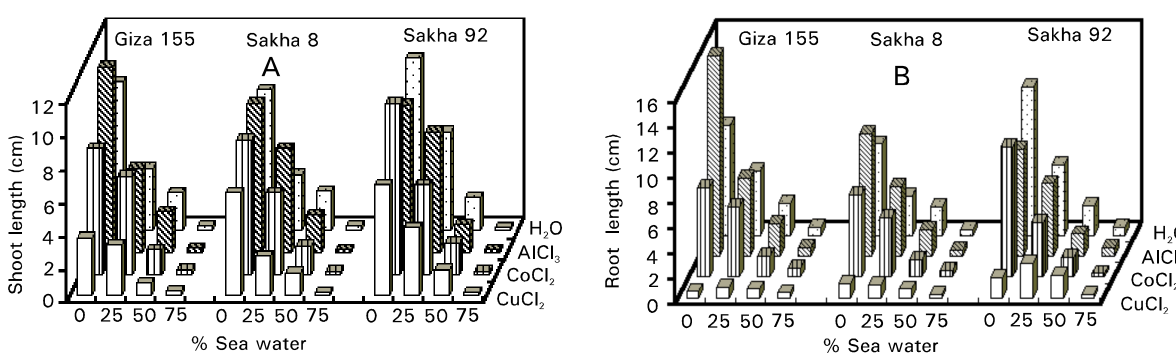

Fig. 1: The length of shoot and root of Giza 155, Sakha 8 and Sakha 92 wheat cultivars under the effect of pretreatments with trace elements and sea water stress treatments.

Table 4: The contents of major nutrient elements (mg/g d. wt) in Giza 155, Sakha 8 and Sakha 92 wheat cultivars under the treatment by sea water stress and $AlCl_3$, $CoCl_2$ and $CuCl_2$ pretreatments

		Cultivars																	
		Giza 155						Sakha 8						Sakha 92					
		Shoot			Root			Shoot			Root			Shoot			Root		
Treatments		0 %	25 %	50 %	0 %	25 %	50 %	0 %	25 %	50 %	0 %	25 %	50 %	0 %	25 %	50 %	0 %	25 %	50 %
Na^{+}	H_2O	0.129	0.242	0.618	0.446	0.482	0.126	0.112	0.112	0.254	0.285	0.263	0.576	0.417	0.224	0.138	0.062	0.137	0.400
	$AlCl_3$	0.122	0.167	0.100	0.101	0.101	0.245	0.062	0.132	0.251	0.082	0.208	0.329	0.051	0.183	0.281	0.072	0.165	0.234
	$CoCl_2$	0.034	0.124	0.100	0.031	0.255	0.200	0.134	0.194	0.146	0.053	0.100	0.200	0.095	0.428	1.084	0.121	0.591	0.763
	$CuCl_2$	0.184	0.151	0.322	0.501	0.301	0.421	0.054	0.201	0.572	0.067	0.534	0.601	0.227	0.151	0.221	0.529	0.211	0.100
Mg^{+2}	H_2O	1.482	1.630	3.230	2.892	2.669	1.706	0.889	0.942	1.285	1.774	2.147	2.960	2.144	1.578	1.526	0.856	2.022	2.621
	$AlCl_3$	1.238	0.954	0.852	1.092	1.272	1.724	0.840	0.841	1.973	0.806	1.685	2.276	0.618	1.215	2.464	0.711	1.110	1.954
	$CoCl_2$	0.191	0.870	0.862	0.411	2.106	1.671	0.523	1.227	1.315	0.480	1.008	2.170	1.256	1.801	4.784	1.842	3.331	4.270
	$CuCl_2$	2.712	2.594	2.939	3.595	3.320	3.729	0.466	1.434	2.696	1.695	4.668	4.398	1.277	1.272	2.763	2.918	5.125	1.971
Fe^{+3}	H_2O	0.323	0.535	1.022	0.089	0.147	0.242	0.054	0.108	0.044	0.174	1.283	0.162	0.341	0.125	0.162	0.270	0.206	0.144
	$AlCl_3$	0.216	1.103	0.185	0.051	0.176	0.431	0.108	0.109	0.216	0.080	0.224	0.323	0.023	0.190	0.194	0.300	0.139	0.484
	$CoCl_2$	0.044	0.075	0.404	0.050	0.206	0.139	0.072	0.087	0.294	0.114	0.377	0.388	0.126	0.206	0.254	0.108	0.613	0.404
	$CuCl_2$	0.108	0.162	0.259	0.243	0.968	1.614	0.050	1.896	0.185	0.108	0.269	0.269	0.087	0.161	0.517	0.507	0.549	0.243
Cu^{+2}	H_2O	0.002	0.010	0.192	0.168	0.153	0.159	0.045	0.090	0.016	0.036	0.058	0.001	0.135	0.098	0.159	0.096	0.304	0.179
	$AlCl_3$	0.146	0.039	0.033	0.049	0.095	0.205	0.045	0.072	0.020	0.029	0.089	0.198	0.042	0.034	0.093	0.041	0.033	0.116
	$CoCl_2$	0.069	0.010	0.085	0.010	0.042	0.017	0.032	0.062	0.168	0.075	0.079	0.185	0.032	0.345	0.594	0.123	0.495	0.331
	$CuCl_2$	0.096	0.116	1.610	1.121	1.208	0.759	0.045	0.087	0.427	0.633	0.269	0.518	0.123	0.106	0.092	0.789	0.644	0.187
Mn^{+2}	H_2O	0.068	0.005	0.153	0.128	0.110	0.004	0.020	0.039	0.084	0.123	0.036	0.108	0.062	0.025	0.029	0.053	0.073	0.016
	$AlCl_3$	0.131	0.003	0.029	0.082	0.052	0.080	0.039	0.022	0.034	0.154	0.029	0.098	0.021	0.017	0.058	0.021	0.035	0.086
	$CoCl_2$	0.027	0.023	0.022	0.038	0.060	0.074	0.058	0.081	0.058	0.061	0.022	0.098	0.040	0.115	0.117	0.065	0.175	0.147
	$CuCl_2$	0.029	0.004	0.126	0.336	0.222	0.092	0.042	0.018	0.115	0.129	0.134	0.229	0.086	0.024	0.098	0.103	0.138	0.047

Table 5: Statistical analysis (ANOVA) probabilities of significance for variations in length, dry weight and major nutrient elements in Giza 155, Sakha 8 and Sakha 92 wheat cultivars under the treatment by sea water stress and $AlCl_3$, $CoCl_2$ and $CuCl_2$ pretreatments

	Shoot length	Root length	Dry weight
Giza 155			
Sea water	0.0001	0.0001	0.0001
Pretreatment	0.0001	0.0001	0.0001
Interaction	0.0001	0.0001	0.0001
LSD at 0.05	0.161	0.137	0.080
Sakha 8			
Sea water	0.0001	0.0001	0.0001
Pretreatment	0.0001	0.0001	0.0001
Interaction	0.0001	0.0001	0.0001
LSD at 0.05	0.090	0.104	0.103
Sakha92			
Sea water	0.0001	0.0001	0.0001
Pretreatment	0.0001	0.0001	0.0001
Interaction	0.0001	0.0001	0.0001
LSD at 0.05	0.130	0.147	0.104

	Nutrient elements									
	Na^{+}		Mg^{+2}		Fe^{+3}		Cu^{+2}		Mn^{+2}	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Giza 155										
Sea water	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Pretreatment	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Interaction	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LSD at 0.05	0.0002	0.001	0.001	0.001	0.0025	0.110	0.0008	0.0009	0.0009	0.0009
Sakha 8										
Sea water	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Pretreatment	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Interaction	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LSD at 0.05	0.0009	0.0008	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Sakha92										
Sea water	0.0001	0.0001	0.0001	0.0001	0.0001	0.3815	0.0001	0.0001	0.0001	0.0001
Pretreatment	0.0001	0.0001	0.0001	0.0001	0.0001	0.0691	0.0001	0.0001	0.0001	0.0001
Interaction	0.0001	0.0001	0.0001	0.0001	0.0001	0.0401	0.0001	0.0001	0.0001	0.0001
LSD at 0.05	0.0001	0.0001	0.0001	0.0001	0.001	0.257	0.0001	0.0001	0.0001	0.0001

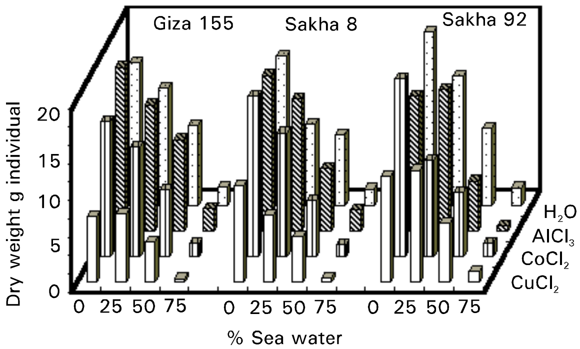

Fig. 2: The dry weight/individual of Giza 155, Sakha 8 and Sakha 92 wheat cultivars under the effect of pretreatments of trace elements and sea water stress treatments.

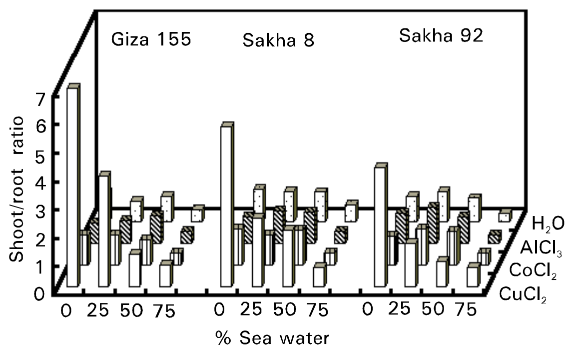

Fig. 3: Shoot/root ratio of Giza 155, Sakha 8 and Sakha 92 wheat cultivars under the effect of pretreatments of trace elements and sea water stress treatments.

treatment) and pretreatment of grains of the three cultivars with $AlCl_3$, or $CoCl_2$ increased the dry weight of their growing plants and even the decreases by $AlCl_3$ pretreatment in Sakha 92 and by $CoCl_2$ in Giza 155 were non-significant.
Generally the remarkable decrease in the length of shoot compared with that of root by the used sea water treatments reduced the shoot/root ratio under different sea water treatments (Fig. 3). The present data also showed a remarkable increase in the shoot/root ratio under all sea water treatments after grains pretreatment by $CuCl_2$ as compared with other pretreatments ($AlCl_3$ and $CoCl_2$) which had little effects on the ratios of three cultivars of wheat under sea water treatments. Also, the ratios for Giza 155 were greater under all treatments as compared with the other cultivars.

Tolerance index: Tolerance index of the three cultivars was deteriorated in response to sea water treatments (Table 3). The effect was remarkable in Sakha 8 compared to other cultivars. The reduction in tolerance index, in the three cultivars of wheat, was enlarged greatly with the pretreatment of their grains with $CuCl_2$. Pretreatment with $AlCl_3$ on the opposite, increased the tolerance index of Giza 155 and Sakha 8 cultivars. The increase in Sakha 92 was only for 25% sea water treatment. The pretreatment with $CoCl_2$ increased the tolerance index of both Sakha 8 and Sakha 92 under control conditions, while it reduced the occurred reduction in the ratio by effect of 25% sea water treatment in Sakha 8 cultivar.

Major soluble elements: Generally, the major soluble elements (Na^+, Mg^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}) were reduced significantly with the pretreatment of trace elements in the three cultivar shoots, except Mg^{2+} and Cu^{2+} in both shoot and root and Fe^{3+} and Mn^{2+} in the root under pretreatment of $CuCl_2$ (Tables 4 and 5). Most elements were accumulated in the three cultivar roots with values in some cases many times of the control value with the grains pretreatments with $CuCl_2$. There was also a considerable accumulation of the elements in root with the pretreatment of $CoCl_2$. There was a responsive accumulation of Mn^{2+} to $AlCl_3$ pretreatment in the shoot and root of Giza 155 and Sakha 8. Sea water treatments enhanced the accumulation of soluble elements with remarkable values in the shoot system of Giza 155 and Sakha 8 when compared to control. The increase in elements of shoot was a result of similar increase in the root system of Sakha 8 except Fe^{3+}, while in Giza 155 the increase was only in root contents of Na^+ and Fe^{3+}, but the other elements (Mg^{2+}, Cu^{2+} and Mn^{2+}) decreased in root by sea water treatment. The contents of Na^+, Mg^{2+}, Fe^{3+}, Cu^{2+} and Mn^{2+} were decreased greatly in the shoot of Sakha 92 and accumulated in the root, except Fe which decreased in both organs by sea water treatments. The accumulation in root reached to 6, 3 and 2 times the control values for Na^+, Mg^{2+} and Cu^{2+} respectively under 50% sea water treatment (Table 4).
The three wheat cultivars acquired nutrients content in both shoot and root which differed by the pretreatment with trace elements in combination with the sea water treatments. Whereas in Giza 155 the pretreatment with $AlCl_3$ enhanced the reduction of the measured nutrients in the shoot system when combined with all sea water treatments, except for Fe^{3+} at 25% which was increased. The contents of the root, on the contrary, increased especially under 50% sea water treatment. The combination of sea water treatments with the pretreatment by $CoCl_2$ increased the nutrients in Giza 155 shoot and root except Mn^{2+} in the shoot. Although the pretreatment of the previous cultivars with $CuCl_2$ increased the nutrients in the shoot under all sea water treatments, it increased only Mg^{2+} and Fe^{3+} in the root.
The contents of Na^+ and Mg^{2+} in both shoot and root of Sakha 8 cultivar increased in response to the combination of sea water with all pretreatments (Table 4). However, there was a gradual increase of Fe^{3+} and Cu^{2+} in response to sea water after pretreatment with all the used trace elements, except the Cu^{2+} element in the shoot of this cultivar after pretreatment with $AlCl_3$. Although pretreatment of this cultivar with $AlCl_3$ decreased Mn^{2+} in both shoot and root, the pretreatment with $CoCl_2$ increased the element at sea water of 25% in the shoot and of 50% in the root. The pretreatment with $CuCl_2$ increased shoot Mn^{2+} content only at 50% sea water treatment, but it gradually increased the element in the root with the increase in sea water concentrations.
When concerning Sakha 92, the pretreatment with $AlCl_3$ and $CoCl_2$ trace elements led to an accumulation of the measured elements in both shoot and root and an accelerating effect was in response to sea water treatments. The pretreatment by $CuCl_2$ reduced the accumulation of Na^+ in both shoot and root under all sea water treatments, but the reduction was more sharp in root than in shoot. The pretreatment with $CuCl_2$ increased also the amounts of Mg^{2+}, Fe^{3+} and Mn^{2+} in shoot under all sea water treatments, but it decreased them in root. The Cu^{2+} content in this cultivar was reduced in both shoot and root systems in response to sea water under pretreatment with $CuCl_2$.
In general, pretreatment with $CuCl_2$ increased the root contents of the measured elements over those of shoot under the highest sea water treatment, the response was clear in Sakha 92 compared with other cultivars. The $CuCl_2$ pretreatment had also led to the increase in most elements in the shoot system under 25% sea water treatment. On the other hand, the greatest effect was by the pretreatment with $CuCl_2$, while the lowest was by $AlCl_3$, also the order of accumulation of most elements in shoot and root was as Sakha 8 > Sakha 92 > Giza 155.

In order to evaluate the effect of chlorine ion present in $AlCl_3$, $CoCl_2$ and $CuCl_2$, the contents of the measured elements, which were increased by these trace elements pretreatment under all conditions, were summed and correlated with the chlorine contents, 79.76, 54.62 and 52.74 which are in $AlCl_3$, $CoCl_2$ and $CuCl_2$ respectively (Chlorine weight/molecule weight, g/g). The results showed that there were 67, 46 and 33 records for the measured elements which were increased by three cultivars seed pretreatment by $CuCl_2$, $CoCl_2$ and $AlCl_3$ respectively, regardless the sea water treated or not. However, this indicated that the decrease in number of records was found to coincide with the increase in content of chlorine in the used compounds.

Discussion

The excessive concentrations of heavy metals are highly toxic to many organisms including higher plants (Woolhouse, 1983; Verkleij and Schat, 1990). Therefore Shkolnik and Bozhenko (1974) and Shkolnik *et al.* (1970) used Al, Co and Cu with a limited concentrations to increase the resistance of plants to drought, under experimental or natural conditions (in arid regions). However, in the present study Al, Co and Cu were used in a hardening regime to propagate resistance of some wheat cultivars to sea water stress. The data confirmed a progressive reduction in germination, dry weight/individual, both shoot and root length and shoot/root ratio for the three wheat cultivars in response to sea water stress. This finding also agreed with the results of Glenn (1987) and Conner (1994). The severity of reduction in germination was noticeable by all the salinity levels. Even the lowest sea water treatment (25%) exhibited greater reduction than the upper salinity limit, for long-term irrigation of even the most salt tolerant crops, such as the date palms, which was studied by Glenn *et al.* (1998).

Pretreatment grains of the three cultivars by $AlCl_3$ and $CoCl_2$ increased their germination percentage under sea water concentration up to 75% in both Giza 155 and Sakha 92. The improvement in the percentage of germination in the two cultivars proved the success of using $AlCl_3$ and $CoCl_2$ as pretreatment of grains in order to reduce the inhibitory effect of salinity for their germination. The maximum percentage of germination and the least time for plumule and radicle emergence and their mean inhibition (MI) indicated a remarkable counteraction for the sea water inhibition to germination of the three cultivar grains by $AlCl_3$ and $CoCl_2$ pretreatments. The best effect was for $CoCl_2$ pretreatment and Giza 155 that showed the lowest response to the pretreatment, while Sakha 8 and 92 showed the highest. Between the two Sakha cultivars, it was found that Sakha 8 acquired the best improvement in its germination percentage but the time for maximum percentage of germination was longer than that for Sakha 92. When considering MI Sakha 92 was found to be the most improved cultivar by the pretreatment, especially under the highest sea water treatment (75% sea water). Even pretreatment of Sakha 92 grains with $CoCl_2$ has led to 100% grains germination under 50% sea water. The pretreatment with the used trace elements in addition to its improvement for seed germination, it decreased the time for germination which is beneficial for germination of wheat in the desert areas having short rainy season, as in the northern part of Egypt. From these findings, one can suggest the use of 25% sea water for irrigation of wheat and if there is a need to increase percentage of sea water, then it is necessary to use the suitable cultivars in one hand and suitable pretreatment of the cultivated wheat grains on the other. The study recommended using $CoCl_2$ and $AlCl_3$ with the used concentration and it showed the danger of using $CuCl_2$ or any other Cu source for pretreatment or in general as a fertilizer during the germination stage of wheat cultivars.

The growth of Giza 155 and Sakha 8 cultivars showed the greatest response for low sea water concentrations combined with grains pretreatments by both $AlCl_3$ and $CoCl_2$, where the length of their shoot and root and the whole plant biomass were increased especially in Giza 155. The pretreatment with $CuCl_2$ on the opposite inhibited the growth in length for both shoot and root and hence decreased the plant biomass of the three wheat cultivars under sea water salinity treatments compared with control. The pretreatment with the chlorides of Al, Co, Cu did not improve the growth of Sakha 92 in terms of root and shoot lengths, fresh and dry weights which decreased due to all the used sea water treatments except the low salinity level (25%) combined with pretreatment by $AlCl_3$. However this remarkable inhibition in growth parameters of the three wheat cultivars could be due to the used concentration of $CuCl_2$, as Nagoor and Vias (1997) found concentrations above 50 mg/ml of the heavy metals inhibits growth. Pretreatment by $CuCl_2$ as compared with control or pretreatment by both $AlCl_3$ and $CoCl_2$ caused more inhibition in growth of root than in that of shoot. Therefore, there was a remarkable increase in shoot/root ratio in response to the pretreatment by $CuCl_2$. The pretreatment by $CuCl_2$ may inhibit the wheat cultivars stress adaptation as it is well established that tolerant plants acquire smaller shoot/root (Bartels and Nelson, 1994). Meanwhile, the other trace elements have not any remarkable effect on the ratio of the three wheat cultivars. The obtained difference between cultivars is due to the difference in their tolerance as reported by El-Shourbagy and Missak (1975) and Migahid and Sadek (1994). The Cultivar Giza 155 acquired more response to $AlCl_3$ under control treatments, while Sakha 8 is more responsive for $AlCl_3$ under sea water salinity stress. The state of pretreatment effect was summarized in a tolerance index under different sea water treatments which indicated a general increase in the tolerance index that was achieved by pretreatment with $AlCl_3$ and $CoCl_2$ especially under control conditions (without sea water). Pretreatment with $CuCl_2$, on the contrary, reduced the tolerance index in the three cultivars of wheat. Pretreatment with $AlCl_3$ diminished the inhibition of most sea water treatments (25 and 50%) in Giza 155 cultivar and only of the lowest treatment (25%) in the other cultivars. Pretreatment with $CoCl_2$ did the same effect but only under low sea water treatment in cultivars Sakha 8.

Most of the measured soluble nutrient elements were reduced after pretreatment grains of Giza 155 cultivar by most of trance elements, while the reduction in Sakha 92 cultivar was only after $AlCl_3$ and $CoCl_2$ pretreatment under the control conditions. Meanwhile, in Sakha 8 there was unclear role for the used trace elements on the nutrient elements. Sea water stress in the present study accumulated Na ion in both shoot and root of the three cultivars of wheat. According to current theory, Na^+ and other excess cations were accumulated in the vacuole under saline stress (Wyn-Jones, 1981). Also, the more tolerant plant contains considerable amount of Na^+, whereas the most sensitive one tended to exclude Na^+ (Hayward and Wadleigh, 1949 and Bernstein 1962). Janes (1966) detected rapid accumulation of Na^+ and Cl^- in pepper leaves and of Cl^- in bean leaves as the concentration of NaCl increased in the nutrient solution. Also Glenn (1987) recorded Na^+ accumulation with salinity in several plant species to use Na^+ in osmotic adjustment.

The accumulation of Mg^{+2} in both shoot and root was remarkable in response to sea water stress with a few exceptions of the three cultivars of wheat. Pretreatment with $AlCl_3$ prevented Mg^{+2} accumulation under most sea water treatments in the three wheat cultivars. Rengel (1990) reported Al^{+3} competitive inhibition for net Mg^{+2} uptake by intact rye-grass plants. It is also important to note that Na^+ and Mg^{+2} were accumulated in root than in shoot of the three cultivars by sea water treatments combined with pretreatment by all the used trace elements. This may indicate that translocation of the two elements up to shoot was suppressed by high salinity and this may be for their accumulation in root due to their importance in osmotic adjustment (Elhaak and Wegmann, 1993).

The three cultivars of wheat accumulated Fe^{+2} in their shoot and root in response to sea water treatments combined with or without pretreatment with the used trace elements. Sakha 92 shifted from this and did not accumulate Fe^{+2} when subjected to sea water treatment without pretreatment with trace elements. These findings increase the importance of using trace elements in pretreatment of plant grains. The content of Cu in the three cultivars of wheat did not show clear response to sea water treatments combined with or without the pretreatment by $AlCl_3$ or $CoCl_2$, but with the pretreatment of $CuCl_2$, Cu accumulated in both Giza 155 and Sakha 8 cultivars shoots on the expense of the contents of their roots. In Sakha 92 Cu was decreased in response to sea water treatments in both shoot and root. This may indicate an enhancement of Cu translocation towards shoot by $CuCl_2$ pretreatment.

The content of Mn was increased by sea water in the shoot of both Giza 155 and Sakha 8 cultivars as a result of its translocation from root which showed a decreased content. Clear inhibition in Mn uptake in Sakha 92 was observed from the element decrease in both shoot and root by the sea water treatments. This inhibition was attenuated by the pretreatment with the chlorides of three trace elements (Al, Co, Cu).

From the present study it may be concluded that cultivars Giza 155 and Sakha 8 were more responsive to the pretreatment of the used trace elements than cultivars Sakha 92 especially $AlCl_3$ and $CoCl_2$. However, Migahid and Sadek (1994) found also Giza 155 the most responsive to the hardening by the same trace elements especially $AlCl_3$. The present study showed that hardening effect for the three used elements could have the following order $AlCl_3 > CoCl_2 > CuCl_2$ in the three wheat cultivars. The excess Na accumulation could be a good indicator for enhancing salinity tolerance by the used trace elements in the three wheat cultivars. Recently Sharma and Kumar (1999) have shown that the excessive accumulation of ions is toxic to development of seedling under salinity stress. Therefore, the developing concept that salt tolerance in glycophytes is related to the ability of a plant species to avoid accumulating excess monovalent cations in leaves (Jeschke, 1984; Lauchli, 1984 and Luttge, 1983). This fact perhaps seemed to be applicable in Giza 155 and Sakha 8 cultivars more than in Sakha 92 cultivar because the former cultivars by pretreatment with Al and Co chlorides exhibited low accumulations of Na under salinity treatments by the sea water. It is also important to note that the present study showed that adjustment of the content of chlorine during the pretreatment of grains was important because the increase in chlorine content inhibited germination of the treated grains and affect greatly their growth nutrient element balance.

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