



Effects of kinetin on growth, grain yield and some mineral elements in wheat plants growing under excess salinity and oxygen deficiency

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Abstract

Wheat plants, 22d. old, were exposed to wide range of soil water osmotic potential ($\Psi_s = 0$ to -1.2 MPa) induced by NaCl and CaCl₂ treatments in combination with roots maintained under aerobic (drained at field capacity) or nonaerobic (flooded) conditions in the soil, and sprayed with 10 mg L⁻¹ kinetin solution. In drained plants, not receiving kinetin, increased soil salinity resulted in appreciable inhibition of shoot growth and reduction in chlorophyll (Chl.), soluble sugars (SS) contents and grain yield. Shoot growth, Chl. content, soluble sugars and grain yield were significantly lower for flooded plants than unflooded analogues over the entire Ψ_s range. Both salinity and waterlogging synergize to increase Na⁺, Ca²⁺ and Cl⁻ accumulation in shoot tissues and to decrease the stability of leaf membranes to either dehydration (40% polyethylene glycol 6000) or heat (51 °C) stress. The ratio of K⁺/Na⁺ transported to shoots under aerobic and anaerobic conditions decreased progressively on salinization. The association between the internal mineral element concentrations was largely affected by kinetin treatment. Kinetin application ameliorated the deleterious effects of salinity and oxygen deficiency. It reduced Na⁺, Ca²⁺ and Cl⁻ accumulation and improved K⁺ uptake under salinity and waterlogging stresses. Increased K⁺/Na⁺ ratio helped the plants to avoid Na⁺ toxicity and enhanced shoot growth and grain yield. Kinetin also reduced membrane injury by dehydration and heat stresses and improved the water status of plants under both aerobic and anaerobic conditions. The effects of single factors (Soil salinity ' Ψ_s ', soil waterlogging 'WL' and Kinetin 'Kin') and their interactions ($\Psi_s \times$ WL, $\Psi_s \times$ Kin, WL \times Kin and $\Psi_s \times$ WL \times Kin) were shown by analysis of variance to be statistically significant for most parameters tested. Calculation of the coefficient of determination (η^2) led to three important findings. (1) Salinity (Ψ_s) was dominant in affecting leaf relative water content (RWC), shoot dry mass, grain yield, stability of leaf membranes to dehydration stress and the contents of Na⁺, Ca²⁺, Mg²⁺ and Cl⁻. (2) Kinetin (Kin) had a dominant effect on the stability of leaf membranes to heat stress as well as on chlorophyll and soluble sugars contents. (3) The share of waterlogging (WL) was dominant for K⁺ content. It can be concluded that kinetin application helped wheat plants to grow successfully in the areas subjected to combined effects of salinity and oxygen deficiency, such as in salt marshes.

Abbreviations: PEG – polyethylene glycol, WL – waterlogging, Kin – kinetin, RWC – relative water content, MPa – mega pascal, Ψ_s – osmotic potential, η^2 – coefficient of determination, Chl – chlorophyll. SS – soluble sugars. RH – relative humidity, VPD – vapour pressure deficits, SAR – Sodium adsorption ratio

1. Introduction

Excess soil salinity is a major concern in agriculture in arid and semi-arid regions where evapotranspiration causes salts originating from irrigation water

(or sometimes naturally from the soil) to become concentrated in the rooting zone. Under conditions of soil salinity, rapid reduction in net photosynthesis [7], inhibition of growth [22], disturbance of anatomical structure [41, 23], alterations in cell mem-

brane structure [10] and K^+ deficiency [6] have been reported.

Soil waterlogging is a major limiting factor for the growth of vascular plants grown in humid regions [17]. Irrigation, especially where it is frequent or on poorly drained land, also can give rise to soil oxygen deficiency in the rooting zone [43]. Soil waterlogging and the consequent oxygen depletion result in substantial soil chemical change and reduction of photosynthesis [44, 3], shoot and root growth [28, 20] and final yield [15]. Waterlogging can also decrease absorption of water [18, 39].

Both soil salinity and waterlogging alter root and shoot hormone relations e.g. decreases cytokinins and gibberellins and increases abscisic acid contents [8, 55]. Recently, plant growth regulators have been applied to counteract the deleterious effects of adverse environmental stresses. In waterlogged plants, a variety of plant growth regulators have been considered to be of crucial importance in the process of adventitious root formation [47]. Kinetin, is one of the cytokinins known to improve the growth of many crop plants grown under salinity [40], soil acidity [19], soil waterlogging [20] and soil pollution [21].

Since soil salinity and oxygen deficiency alone is harmful to the growth of most dry land species, the combined effects of excess salt and oxygen shortage would be expected to be especially damaging. Under natural conditions, as in salt marshes, plants are subjected to salinity stress and waterlogging, and only a highly specialized vegetation is adapted to tolerate such diverse conditions [13, 48]. However, the effects of these two stressors in combination on plant growth and metabolism are poorly understood [46, 33].

The interactions between soil salinity and soil waterlogging in combination with exogenous kinetin as a three-factorial treatment combination has not been studied. Often their effects on the growth and metabolic activities of the plants have been studied separately (as single factorial treatments). Since, in natural habitats, multifactorial effects and interactions are the rule rather than the exception, therefore, single factorial laboratory experiments concerning the effects of environmental stresses on the growth and metabolic activities in plants are not good simulations of the natural conditions. Accordingly, in the present paper, the possibility of a salinity \times waterlogging \times kinetin interaction affecting growth and grain yield in wheat plants was studied under natural field conditions. In addition, the possible dual role for the effect of the three factors and their interactions on the sta-

bility of leaf membranes, leaf relative water content, chlorophyll, soluble sugars and some mineral element content (Na^+ , K^+ , Ca^{2+} , Mg^{2+} and Cl^- and their association) were also investigated.

2. Materials and methods

2.1 Plant cultivation

Wheat plants (*Triticum aestivum* sacha 8) were grown in plastic pots containing 1400 g air dry soil (sand/clay 2: 1 v/v) under field conditions at the experimental farm of the Faculty of Science, Assiut University as described previously [19]. The roots of wheat plants grown for 22 day in soil, the water content of which was maintained at field capacity, were irrigated with solutions having NaCl and $CaCl_2$ in combination to result in different equivalent osmotic potentials (Ψ_s) in the range from 0 to -1.2 MPa with a fixed sodium adsorption ratio (SAR) of 1/8 see [22]. The plants were either exposed to oxygen deficiency (waterlogging) or maintained under aerobic conditions (watered periodically to field capacity). In waterlogging treatment, the plants were flooded by maintaining the water (unstressed $\Psi_s = 0$) or saline solution levels (salt-stressed $\Psi_s = -0.3$ to -1.2 MPa) 1 to 2 cm above the soil surface [49] by periodically adding water or saline solutions.

One week after salinity and waterlogging treatment, the growing shoots were sprayed three times at 3d intervals with 10 mg L^{-1} kinetin. Kinetin was purchased from Sigma (St. Louis, Mo., U.S.A.). The control plants were sprayed with distilled water. Each treatment combination at every stress level had three replicates which were arranged in a completely randomized design. On day 7, after the last kinetin application, the plants were analyzed for the stability of leaf membranes, leaf relative water content, chlorophyll content and some mineral element contents. Ears were harvested after grain ripening, oven dried and the grain yield and dry mass production were determined.

2.2 Membrane stability test

The stability of leaf membranes was assessed by determining electrolyte leakage from leaf segments exposed to dehydration (40% polyethylene glycol, PEG) and heat (51°C) stress. A method used by Blum and Ebercon [4] on wheat was adopted.

The degree of membrane injury (based on electrolyte leakage) was calculated according to the following formula:

$$\% \text{ injury} = 1 - [1 - (T_1/T_2)/1 - (C_1/C_2)] \times 100,$$

where T_1 and T_2 represent the first and second conductance measurements on the treatment samples and C_1 and C_2 the first and second measurements on the control.

2.3 Chlorophyll and soluble sugar contents

Chlorophyll a and b and soluble sugar contents were measured spectrophotometrically (Bausch and Lomb Spectronic 2000) according to Tood and Basler [45] and Buysse and Merckx [9], respectively.

2.4 Leaf relative water content

For measuring leaf relative water content the method of Weatherley and Barrs [50] was adopted. Relative water content (RWC) was calculated as follows:

$$\text{RWC}\% = \frac{\text{fresh weight} - \text{dry weight}}{\text{saturated weight} - \text{dry weight}} \times 100.$$

Diurnal pattern of leaf relative water content, and fluctuation in air temperature, vapour pressure deficits (VPD) and relative humidity (R.H.) were measured at 3h intervals from 7 a.m. to 7 p.m.

2.5 Mineral elements

Ca^{2+} and Mg^{2+} were analyzed by an atomic absorption spectrophotometer (Shimadzu, Model AA – 630-O₂). Na^+ and K^+ were analyzed by the flame emission technique. In this respect, a flame photometer M7D was used. Content of chloride (Cl^-) was determined according to Johnson and Ulrich [30].

The relative role (shares) of single factors and their interactions on the parameters tested and association between the mineral ions were evaluated by analysis of variance (F values), coefficient of determination (η^2) and simple linear correlation coefficient (r), respectively [35]. The coefficient of determination (η^2) has been devised to evaluate the relative effect of each single factor and interaction in contributing to total response. In that case:

$$(\eta^2) = \frac{\text{sum of squares due to the factor}}{\text{total sum of squares due to the treatment combination}}.$$

3. Results

3.1 Stability of leaf membranes

Under aerobic conditions, solution of 40% PEG (Table 1) caused 6.69% injury in the membranes of unstressed plants ($\Psi_s = 0$). The percent of membrane injury increased to 47% on salinization at $\Psi_s = -1.2$ MPa, which is about 8-fold the injury at $\Psi_s = 0$. Leaf membranes of waterlogged plants were less stable (more injured) than plants growing at field capacity over the entire Ψ_s levels.

Exposure of leaf segments taken from unflooded salt-unstressed plants to heat stress (51 °C) caused 30.8% injury in the membranes. Lowering of osmotic water potential in the root environment in combination with oxygen deficiency enhanced the percentage of injury by heat. The highest injury (78.5%) was noticed in plants flooded with -1.2 MPa saline solution.

Kinetin application decreased the membrane injury by either PEG dehydration or heat stress compared with their kinetin untreated analogues (unstressed-flooded plants were an exception). The reduction of membrane injury by kinetin was more pronounced at higher stress levels, especially under flooding conditions.

Soil salinity (Ψ_s), waterlogging (WL) and kinetin (Kin) as well as their interactions (Table 2) had highly significant effects on the stability of leaf membranes, except the interaction $\Psi_s \times \text{Kin}$ for membrane stability to heat. Based on the calculated coefficient of determination (η^2), the role of Ψ_s in affecting membrane stability to PEG dehydration was dominant, but that of kinetin was subsidiary. Under heat stress the reverse held true.

3.2 Chlorophyll content

Chlorophyll a and b contents (Table 1) progressively decreased with increasing salinity. The magnitude of the salinity effect varied with aeration of the root environment. Chl reduction by salinity was much greater under anaerobic conditions than in plants grown in drained soil. For example, Chl a and b contents at $\Psi_s = -1.2$ MPa were about 20 and 23% (under anaerobic) and 50 and 51% (aerobic condition) that of the unstressed plants.

Supplying kinetin to waterlogged and drained plants, whether salt-stressed or not, increased the content of chlorophyll compared with kinetin-untreated plants. The kinetin effect was more pronounced in plants flooded with saline solutions. For example, Chl

Table 1. Changes in the stability of leaf membranes (measured as percent of injury %), chlorophyll content (Chl.) and growth (dry matter production and grain yield) of wheat plants grown under excess soil salinity (Ψ_s), aerobic (drained) and waterlogging (WL) conditions in the presence or absence of kinetin (Kin) solution

Kin Mg L ⁻¹	Parameters		Membrane injury %		Chl. content (mg·g ⁻¹ lf.f.wt)		Growth		Soluble sugars
	Soil aeration	Ψ_s (MPa)	PEG (40%)	Heat (51 °C)	a	b	Dry matter (g)	Grain yield (g)	(mg·g ⁻¹ D.W.)
0	Drained	0.0	6.69	30.81	2.32	1.57	20.77	22.49	22.32
		-0.3	15.13	50.81	2.28	1.36	17.31	20.31	21.10
		-0.6	27.70	57.73	2.07	1.35	15.23	18.07	18.32
		-0.9	31.79	63.27	1.54	1.14	8.88	9.27	15.21
		-1.2	47.03	70.25	1.18	0.80	7.32	2.74	10.32
	WL	0.0	20.23	44.29	1.22	1.02	15.52	19.70	15.32
		-0.3	27.49	69.57	1.23	0.94	10.37	14.64	14.33
		-0.6	38.54	74.85	0.53	0.43	9.25	8.01	12.58
		-0.9	64.79	75.79	0.40	0.41	5.27	–	10.48
		-1.2	72.42	78.52	0.25	0.24	2.94	–	8.73
10	Drained	0.0	5.27	19.53	2.80	1.82	17.36	29.74	35.36
		-0.3	6.67	30.21	3.54	2.16	19.35	27.98	38.26
		-0.6	13.77	46.07	2.96	2.01	17.73	18.74	30.64
		-0.9	20.59	48.45	2.53	1.70	16.52	17.66	25.13
		-1.2	8.96	44.78	2.73	1.78	9.10	7.34	20.39
	WL	0.0	10.92	48.27	1.99	1.25	17.61	25.98	32.75
		-0.3	16.73	49.81	2.09	1.50	18.88	20.65	30.36
		-0.6	2.99	21.73	2.69	1.71	14.47	18.72	27.94
		-0.9	36.07	40.29	2.09	1.31	11.45	8.62	24.36
		-1.2	40.79	56.73	2.18	1.46	8.52	4.32	23.33

content of plants flooded at -1.2 MPa and receiving kinetin was about 10-fold that of kinetin untreated plants at the same water potential. Furthermore, supplying kinetin under anaerobic conditions increased Chl contents of salt-stressed plants over that of the unstressed plants ($\Psi_s = 0$ and flooded with H_2O).

The effects of Ψ_s , WL, Kin and their interactions on Chl a and b contents were statistically significant ($\Psi_s \times$ WL interaction was an exception for Chl b). Kinetin had a dominant role in affecting Chl content, but the role of waterlogging was subdominant.

3.3 Relative water content (RWC)

Diurnal fluctuation in leaf relative water (RWC) content are shown in Table 3. In the absence of kinetin, under both aerobic and nonaerobic conditions, salt unstressed plants ($\Psi_s = 0$) showed a decrease of RWC in the early morning toward a minimum at 1 p.m. However, they tended to regain turgidity early in the

afternoon. Salinity caused some changes in the diurnal pattern of leaf RWC where plants at most salinity levels ($\Psi_s = -0.6$ to -1.2 MPa) showed a minimum RWC at 4p.m. In the presence of kinetin, the reverse held true. Leaf RWC fluctuated during the late part of the day (4–7 p.m.) and ended up at a lower or higher level at sunset relative to its value at sunrise.

Mean daily leaf relative water content (Table 3) was generally lower in salt-stressed plants, grown under aerobic conditions, than the unstressed plants. Combination of salinity with oxygen deficiency resulted in a greater reduction in leaf water content. In plants flooded with -0.3 , -0.6 , -0.9 and -1.2 MPa solutions, the RWC was 9%, 2%, 4% and 8% lower than unflooded plants irrigated with the same saline solutions.

Kinetin treatment improved the water status of wheat plants. The response of RWC in highly stressed plants (-1.2 MPa) to kinetin was higher than that grown at lower stress levels (-0.3 MPa) where RWC

Table 2. F and (η^2) values for the effects of salinity (Ψ_s), waterlogging (WL), kinetin (Kin) and their interactions on the stability of leaf membranes, chlorophyll content (Ch1), leaf relative water content (RWC), soluble sugars (S.S.), dry mass production (d.m.) and grain yield in wheat plants

Source of Variance	Membrane stability				Ch1. Content								Production			
	Dehydration		Heat		Ch1.a		Ch1.b		SS		RWC		d.m.		Grain	
	(40% PEG)		(51 °C)													
	F	η^2	F	η^2	F	η^2	F	η^2	F	η^2	F	η^2	F	η^2	F	η^2
Ψ_s	104.5**	0.42	96.7**	0.23	33.9**	0.10	100.0**	0.10	334.2**	0.24	158.5**	0.53	131.1**	0.61	603.5**	0.75
WL	144.2**	0.15	142.3**	0.08	388.2**	0.28	1100.0**	0.27	292.5**	0.05	30.3**	0.03	87.6**	0.10	318.9**	0.10
Kin	245.1**	0.25	654.5**	0.38	721.2**	0.52	2085.0**	0.52	966.9**	0.66	283.8**	0.24	144.1**	0.17	349.2**	0.11
$\Psi_s \times WL$	18.4**	0.08	114.3**	0.27	3.3	0.01	5.0*	–	37.5**	0.03	18.96**	0.06	2.9	0.01	9.3**	0.01
$\Psi_s \times Kin$	18.5**	0.08	3.99	0.01	17.0**	0.05	82.5**	0.08	11.9**	0.01	35.11**	0.12	15.8**	0.08	10.04**	0.01
WL \times Kin	13.2**	0.01	31.9**	0.02	23.9**	0.02	32.5**	0.01	35.4**	0.01	6.19*	0.01	20.6**	0.03	0.73	–
$\Psi_s \times WL \times Kin$	4.2*	0.02	5.5*	0.01	8.18**	0.02	20.0**	0.02	2.1	–	4.06*	0.01	3.3	0.02	15.3**	0.02

* Significant at 5% confidence level.

** Significant at 1% confidence level.

Table 3. Diurnal pattern and average daily leaf relative water content of wheat plants grown under excess of salinity (Ψ_s), aerobic (drained) and waterlogging (WL) conditions in the presence and absence of kinetin (Kin) solution and the prevailing climatic factors, air temperature ($^{\circ}\text{C}$), Relative Humidity (RH%) and vapour pressure deficit (VPD)

Kin Mg L^{-1}	Soil aeration	Ψ_s (MPa)	Diurnal Change in Leaf RWC (%)					Mean daily Values
			7 a.m.	10 a.m.	1 p.m.	4 p.m.	7 p.m.	
0	Drained	0.0	91.66	87.96	75.45	85.87	88.88	85.97
		-0.3	86.32	84.43	70.88	73.30	78.32	79.04
		-0.6	82.19	80.97	72.31	67.50	73.73	75.34
		-0.9	80.40	75.03	74.60	62.55	69.58	72.43
		-1.2	77.27	67.07	59.94	58.17	64.37	65.36
	WL	0.0	95.72	89.88	82.06	88.65	92.05	89.97
		-0.3	86.06	81.34	67.55	64.20	54.91	70.82
		-0.6	81.62	78.08	77.42	69.76	62.16	73.81
		-0.9	73.32	70.92	68.45	64.56	59.81	67.41
		-1.2	62.35	58.43	54.31	58.34	52.30	57.15
	Drained	0.0	95.02	89.93	85.33	73.71	81.15	85.07
		-0.3	87.36	85.83	74.81	81.90	87.30	83.44
		-0.6	84.52	76.74	67.29	83.22	88.22	80.00
		-0.9	81.12	82.89	79.87	83.42	84.93	82.45
		-1.2	82.32	70.28	76.02	81.38	87.27	81.26
10	WL	0.0	94.53	87.08	92.64	80.21	86.88	88.27
		-0.3	89.75	81.78	77.60	81.22	90.76	84.22
		-0.6	85.82	89.46	72.43	72.44	80.33	80.10
		-0.9	81.52	87.85	70.35	79.10	86.83	81.13
		-1.2	66.21	64.59	61.59	65.73	65.03	76.26
	Air Temp ($^{\circ}\text{C}$)		14	25	36	30	18	
	R.H. (%)		52	38	25	29	50	
	V.P.D. (mmHg)		6.28	15.52	33.16	23.51	8.53	

of plants stressed at -1.2 MPa and received kinetin were 16 and 22% higher than kinetin untreated analogues; which contrasts to 4 and 14% increases at lower stress level (-0.3 MPa) in drained and flooded plants, respectively.

Salinity, waterlogging, kinetin and their interactions had significant effects on the leaf RWC. The role of salinity was dominant, but that of kinetin was subdominant.

3.4 Soluble sugars

In the absence of kinetin (Table 1), unflooded-salt-stressed plants had lower soluble sugar contents in their shoots than the unstressed plants. Soil waterlogging caused marked reduction in the content of shoot soluble sugar over the entire Ψ_s range. Unstressed and salt stressed plants receiving kinetin solution had much more soluble sugar in their shoots under both anaerobic and aerobic conditions compared to their kinetin untreated analogues.

Statistical significant effects for Kin, WL, Ψ_s and their interactions on SS was detected by F values ($\Psi_s \times \text{Kin} \times \text{WL}$ interaction was an exception). The role of kinetin in affecting shoot SS was dominant followed by Ψ_s and waterlogging.

3.5 Mineral elements

The contents of Na^+ , K^+ , Ca^{2+} and Cl^- in shoot tissues of wheat plants (Table 4) increased with increasing salinity under aerobic conditions. Ion accumulation was much greater with treatment combination of salinity and oxygen deficiency. The ratio of K^+/Na^+ was greatly modified by addition of saline solutions and soil flooding, decreasing more than 3-fold and 7-fold between $\Psi_s = 0$ and -1.2 MPa in drained and flooded plants, respectively.

Shoot K^+ , Mg^{2+} , Ca^{2+} and Cl^- contents were generally higher but the contents of Na^+ were lower in wheat plants grown in drained soil and receiving kinetin solution than in those sprayed with distilled

Table 4. Changes in internal shoot elements contents of wheat plants grown under excess salinity (Ψ_s), aerobic (drained) and waterlogging (WL) conditions in the presence or absence of kinetin solution

Kin Mg L ⁻¹	Soil aeration	Ψ_s (MPa)	Na ⁺	K ⁺	K ⁺ /Na ⁺	Ca ²⁺	Mg ²⁺	Cl ⁻
0	Drained	0.0	12.67	18.45	1.50	11.85	14.40	35.05
		-0.3	22.00	23.86	1.10	40.43	19.74	83.91
		-0.6	31.33	23.46	0.75	52.27	16.52	113.00
		-0.9	41.00	24.37	0.51	55.46	18.29	119.03
		-1.2	45.00	20.75	0.46	68.17	19.29	168.37
	WL	0.0	8.33	27.47	3.63	26.67	17.71	49.60
		-0.3	32.67	34.05	1.05	48.14	17.50	129.20
		-0.6	45.00	36.07	0.81	60.66	16.48	136.17
		-0.9	48.33	36.38	0.76	64.40	15.49	152.77
		-1.2	64.00	33.47	0.53	75.65	19.27	184.03
10	Drained	0.0	9.17	18.02	1.98	21.00	14.37	58.17
		-0.3	22.33	18.37	1.27	38.09	22.33	123.07
		-0.6	21.67	27.28	1.26	62.91	18.33	101.08
		-0.9	20.00	26.70	1.34	78.44	21.27	186.10
		-1.2	31.00	28.61	0.92	85.34	22.24	185.20
	WL	0.0	5.83	29.30	5.18	26.84	14.61	79.93
		-0.3	11.00	38.64	6.11	28.29	15.33	71.40
		-0.6	11.67	35.98	3.20	35.54	15.38	101.00
		-0.9	25.00	48.93	1.96	51.95	22.03	130.40
		-1.2	28.33	46.07	1.63	72.51	22.29	115.03

water (−0.6 MPa for Cl[−] was an exception). Under oxygen deficiency, kinetin treatment reduced the contents of Ca²⁺, Na⁺ and Cl[−] over the entire Ψ_s range ($\Psi_s = 0$ was an exception for Cl[−]), but increased accumulation of K⁺ at all stress levels and Mg²⁺ at higher stress levels only. K⁺/Na⁺ ratio was higher for kinetin – treated plants than in the untreated analogues under both anaerobic and aerobic conditions.

Data in Table 5 indicate that single factors and their interactions significantly affected the internal mineral elements (Kin effect for Ca²⁺ and $\Psi_s \times$ Kin and $\Psi_s \times$ WL \times Kin interactions for K⁺ were exceptions). The role of salinity in affecting Na⁺, Ca²⁺, Mg²⁺ and Cl[−] was dominant. In the case of K⁺ the dominant role was occupied by WL effect followed by Ψ_s .

In plants not receiving kinetin solution (Figure 1), under both aerobic and anaerobic conditions, Na⁺ was positively correlated ($P < 0.05$) with Cl[−] and Ca²⁺. Also, significant positive correlation was found between Ca²⁺ and Cl[−]. In the presence of kinetin, the significant correlations were somewhat different. The most pronounced effect for kinetin under aerobic condition was the significant positive correlations between Mg²⁺ and each of Na⁺ and Cl[−], and the lack of the

significant correlation existed between Na⁺ and each of Ca²⁺ and Cl[−] and between Cl[−] and Ca²⁺.

In flooded plants, Kinetin application resulted in significant positive correlations between Mg²⁺ and each of Na⁺, Cl[−] and Ca²⁺. Also positive significant correlations were found between Ca²⁺ and Mg²⁺ and between Na⁺ and K⁺ as a result of kinetin treatment.

3.6 Growth and grain yield

Shoot growth (dry mass production) and finally grain yield were reduced by salinity. Flooding by either distilled water ($\Psi_s = 0$) or saline solutions (salt-stressed plants) caused further reduction in shoot biomass and grain yield than their drained analogues. Furthermore, plants without kinetin and flooded with −1.2 MPa solutions died within 4 weeks of treatment.

Kinetin application during salinity and oxygen deficiency improved shoot growth and grain yield. The most clear effect was noticed in plants flooded with −1.2 MPa solution where kinetin helped the plants to grow successfully and produced grains.

Single factors as well as their interactions significantly affected dry mass production and grain yield,

Table 5. F and (η^2) values for the effects of salinity (Ψ_s), waterlogging (WL), kinetin (Kin) and their interactions on some mineral elements contents of wheat plants

Source of variance	Na ⁺		K ⁺		Ca ²⁺		Mg ²⁺		Cl ⁻	
	F	η^2	F	η^2	F	η^2	F	η^2	F	η^2
Ψ_s	176.6**	0.53	13.1**	0.08	93.6**	0.83	42.7**	0.50	1696.9**	0.78
WL	7.6**	0.01	505.5**	0.75	14.8**	0.01	12.81**	0.04	497.2**	0.01
Kin	384.8**	0.29	7.1*	0.05	0.3	—	20.48**	0.06	462.8**	0.01
$\Psi_s \times WL$	6.4*	0.02	6.3**	0.04	34.3**	0.03	12.5**	0.15	322.9**	0.04
$\Psi_s \times Kin$	24.1**	0.07	0.2	0.01	36.6**	0.03	14.2**	0.17	473.8**	0.06
WL \times Kin	66.0**	0.05	33.8**	0.05	371.8**	0.08	5.8*	0.01	520.3**	0.02
$\Psi_s \times WL \times Kin$	10.2**	0.03	3.9	0.02	18.2**	0.02	6.1**	0.07	692.2**	0.08

* Significant at 5% confidence level.

** Significant at 1% confidence level.

except the $\Psi_s \times WL$ and $\Psi_s \times WL \times Kin$ interactions (for dry mass) and WL \times Kin interaction (for grain yield). The role of salinity (Ψ_s) was dominant followed by the role of kinetin.

4. Discussion

Under aerobic conditions, lowering of soil water osmotic potential decreased the stability of leaf membranes to either dehydration (40% PEG) or heat stress (51 °C) where leaf segments taken from salt-stressed plants being more injured than those taken from salt-unstressed plants. This could be due to the alteration in cell membranes structure and properties under salinity [10]. It is clear that membranes of salt-stressed plants were less injured by dehydration stress (percent of injury was 47% at -1.2 MPa) than by heat stress (70% injury at -1.2 MPa). The lower injury caused by dehydration stress in salt-stressed plants could be explained by the protecting effect of salt accumulated inside the cell [44]. Data in Table 4 on the accumulation of some mineral ions support this view.

Exposure of wheat roots to waterlogging enhanced membrane injury by either heat or PEG dehydration in both unstressed and salt-stressed plants. This means that membrane resistant to solute leakage in cereal plants was decreased in response to waterlogging. The differences in the permeability of cell membranes under anaerobic conditions may result from change in the degree of saturation of fatty acids in the phospholipids of cell membranes [52].

Soluble sugars were markedly declined with reduced Ψ_s under aerobic conditions, probably through inhibition of carbon metabolism and pho-

tosynthetic activity [7] and reduction of chlorophyll content (Table 1). The deleterious effect of salinity on shoot soluble sugars was enhanced by soil waterlogging. This means that both salinity and waterlogging synergize in their impact on chlorophyll contents and photosynthetic activities and accumulation of its products.

Generally, salt-stressed plants maintained lower leaf relative water content than salt-unstressed plants. This could be due to reduction of water supply to the leaves, probably through effects of salinity on the anatomical structure and disorganization of the vascular cylinder [41, 23], and decreased the conductance of root membranes or plasmodesmata [54]. Exposure of wheat roots to oxygen deficiency results in further reduction in leaf relative water content. Such reduction could be due to decreased water absorption under anaerobic conditions [18, 39].

Under combination of salt stress and flooding conditions (as in salt marshes), mutual effects of elements on their absorption are of particular interest. Elements at high concentration in the root medium are taken up at higher rates which may lead to excessive accumulation in plant tissues. In general, salt-stressed wheat plants were higher in contents of Na⁺, Ca²⁺ and Cl⁻ in their shoot tissues than were the unstressed plants ($\Psi_s = 0$). The effect was most pronounced under combination of salinity and anaerobic conditions. This could be due to the large increase in their activity in the external solutions. Accumulation of such inorganic ions, which contribute to osmoregulation, under saline condition is considered as one of the most important adaptive mechanisms of water deficits [38].

The high concentration to which Na⁺ in preference to K⁺ can be accumulated in the shoot, espe-

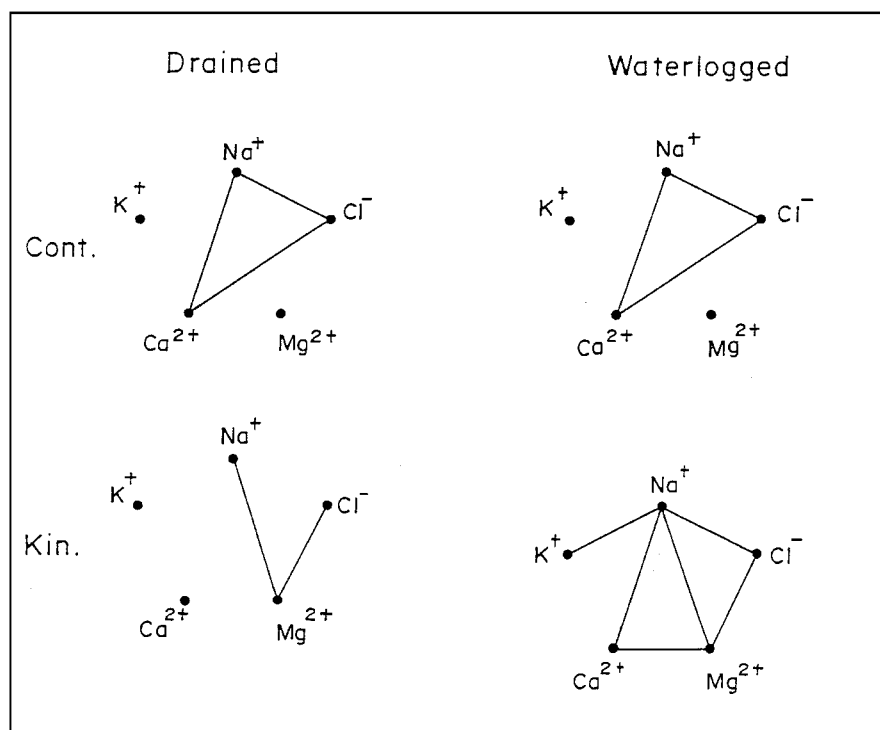


Figure 1. Significant positive correlation ($P < 0.05$) between ions in shoot of wheat plants grown under excess salinity under aerobic (drained) and anaerobic (waterlogged) conditions in the absence (Cont.) and presence of kinetin (Kin.).

cially under salinity stress, is an interesting feature. In this study, the K^+/Na^+ ratio in the shoots of wheat decreased steadily with increasing salt addition. This is in agreement with previous findings of He and Cramer [27] and Gadallah [22]. It is accepted that competition exists between Na^+ and K^+ leading to a reduced level of internal K^+ at a high external NaCl concentration [24, 5, 6]. Data in this study (Table 4) indicate that the internal K^+ level was not reduced by salinity, but sometimes it increased. In our study, soil salinity was induced by the NaCl and $CaCl_2$ combination. Sodium-calcium interaction under salinity has been recently reviewed [14]. Therefore, it can be concluded that Ca^{2+} is important for the maintenance of K^+ transport in the presence of Na^+ . A high selectivity of cereal roots for transport of K^+ [29] could be an alternative explanation for increased K^+ content in this study.

It has been known that anoxia eliminates or strongly inhibits active ion transport [11], so that any outward transport of Na^+ that contributes to avoidance of high internal concentration is lost, while K^+ influx is simultaneously arrested. This result is a passive influx of Na^+ with little K^+ influx, so that the

K^+/Na^+ ratio reaching the shoot is decreased many times under flooding conditions. However, Zhang et al. [57] reported greater loss of K^+ than that of Na^+ during anoxia. Our results did not coincide with this study where K^+/Na^+ ratio in flooded wheat plants was generally higher than in the unflooded analogues. This could be due to improvement of K^+ uptake under anaerobic conditions by addition of Ca^{2+} to the external medium.

Shoot dry mass and finally grain yield were strongly reduced by soil salinization under aerobic conditions. The underlying causes are complex and can range from partial stomatal closure [7], detrimental effects on the photosynthetic enzymes [53] and ion toxicity or nutrient imbalance [31]. Generally, shoot dry mass and grain yield were significantly lower for waterlogged plants than drained plants. In addition, senescence and death of old leaves and sometimes the whole plant (e.g. at $\Psi_s = -1.2$ MPa without kinetin) is well documented. Possible reasons for this include lack of nutrients which come from roots, depletion of phytohormones such as cytokinins [8], lack of photosynthate because of ABA accumulation and stomatal closure [55], and ion toxicity due to

accumulation of much Na^+ and Cl^- under flooding conditions (Table 4).

Adaptation of wheat plants to salinity and waterlogging, in terms of ion control and growth, was accelerated by exogenously added kinetin. Generally, kinetin treatment protected leaf membranes against either heat or dehydration stress injury compared to untreated parallels ($0 \text{ mg L}^{-1} \text{ Kin}$). Such effect could be due to the effects of kinetin on cell membrane permeability [51]. Membrane protection by kinetin can improve the tolerance of wheat plants to salinity and saline-anaerobic conditions where the extent of membrane damage is commonly used as a measure of tolerance to various stresses in plants [4, 20, 21] and adjustment to solute transport capabilities across membranes are of paramount importance to salinity adaptation [34].

Kinetin application reduced the harmful effects of saline-anaerobic treatment combination through reduction of inorganic ions (Na^+ , Ca^{2+} and Cl^-) accumulation (Table 4). Such an effect may help the plants to avoid ions toxicity. Also, kinetin improved K^+ uptake under salinity and waterlogging stresses, which effectively increased the K^+/Na^+ ratio in the tissues. This effect is considered to be important in salt tolerance where maintenance of high cytoplasmic level of K^+ is essential for survival in saline habitats [12] and the characteristic of K^+ and Na^+ transport are determinant of the NaCl tolerance in plants [2].

Kinetin application increased soluble sugar accumulation in wheat plants growing under aerobic and anaerobic conditions. In recent years, increasing attention is being paid to a possible role of carbohydrates availability and utilization in conferring anoxia tolerance [32, 1, 26, 37]. However, the presence of soluble sugar may allow the operation of anaerobic pathways for utilization of carbohydrates under a condition of energy shortage [16, 1]. Also, some authors reported data indicating that carbohydrates supplied exogenously enhance anoxia tolerance in plant tissues [36, 57, 56]. Therefore, it can be concluded that enhancement of wheat growth under anaerobic conditions by kinetin treatment in the present study is partially attributed to increases in soluble sugar which has been reported to play a role in anoxia tolerance [37, 25].

Despite the different external treatment combination in this study, the results indicated significant interactions between soil salinity, soil waterlogging and kinetin on the parameters tested. This means that in natural habitats the plants not only respond to the

environmental factors as single factors, but were also affected by their interactions.

Finally, it can be concluded that application of kinetin increase the ability of anoxia-intolerant wheat to grow successfully under severe saline-anaerobic conditions. The effects of kinetin in this respect varied from reduction in inorganic ion accumulation and increasing membranes stability and K^+/Na^+ ratio to enhancement of chlorophyll formation and soluble sugar accumulation. The facts mentioned above make it possible to recommend the treatment of plants grown under conditions of soil salinity and oxygen shortage (as in salt marshes) with solution of kinetin.

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