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In vitro and *In vivo* K, Zn, Mn and Cu Status of *Vigna sinensis* L. Plants Grown under Reduced Matric Water Potential

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Abstract: The contents of water soluble K, Zn, Mn and Cu elements were determined in *Vigna sinensis* plants grown in hydroponic solutions. These solutions have particular matric water potentials Ψ (0–1.0 MPa) and amended with K, K-Mn and K-Cu nutrients. The results indicated that water stress showed effects on the contents, relative distribution in different organs and shoot/root ratios of nutrient contents in the investigated plants. Nutrient (Nr) has a dominant role in affecting changes in the contents of micro-nutrients, whereas K content was greatly affected by Ψ m x Nr interaction. Also, many significant correlations were recorded between various nutrients at different Ψ m and nutrient treatments. The evaluation and importance of K accompanied with each micronutrient for some physiological aspects of stressed plants were discussed.

key words: Matric water potential, micronutrients, *Vigna sinensis*, allocation, potassium.

Introduction

Drought and deficiency of nutrients are important ecological factors associated with biochemical processes. Potassium is one of the essential nutrients for plant survival in arid and semi-arid regions, as it has beneficial effects in metabolic processes and osmotic adjustment. The early activation of system providing the driving force for uptake of K^+ and other osmotically active solutes is an early component of defence mechanism of the plant against severe osmotic conditions (Zingarelli *et al.*, 1999).

Deficient micronutrient conditions may reduce metabolic activity due to the requirement of zinc and manganese in physiological processes (Welch and Norvell, 1993). Also, both micronutrients are key components of essential enzymes within the plant (Pearson and Rengel, 1995). In this respect the ribulose 1,5 biphosphate carboxylase plays a crucial role because of its high affinity to Cu and its sharp increase in the expanding leaf (Morita, 1980).

Internal concentration of a particular nutrient frequently influences the rate of uptake of that nutrient (Rengel and Graham, 1996). K is important for high sink activity (Cakmak *et al.*, 1994). Hence, two mechanisms for K^+ uptake have been proposed: a high affinity saturable transport system subjected to feedback regulation from K^+ in the roots and a low affinity non-saturating transport system (Kochian and Lucas, 1982). Meanwhile, root absorption of Cu take place by an active energy-dependant process (Bowen, 1981). The uptake of Mn and Cu by plants have been determined by competition between Zn and these ions for a putative common carrier (Rengel and Graham, 1996). Moreover, Mn and Zn absorption showed antagonism with Cu (Lastra *et al.*, 1988). The response of plants to several simultaneous stresses are usually not predictable by single-factor analysis and a combination of different environmental stress factors can result in intensification, overlapping or antagonistic effects (Osmond *et al.*, 1986). The aim of this work was to evaluate and investigate the effect of matric water potential, Ψ m; K singly or accompanied with Zn Mn or Cu in the incubation medium and their interaction on the contents of K, Zn, Mn and Cu within *Vigna sinensis* plants. The allocation and ratio of these ions in different organs (root, stem and leaf) were studied in plants grown under identical root medium conditions.

Materials and Methods

Mature seeds of cowpea (*Vigna sinensis* L.) plants were germinated under natural room conditions. After seven days

of seed sowing, the healthy seedlings were transferred to plastic pots (10 cm diameter and 13cm length) filled with hydroponic Hoagland solutions. The cultures were constantly aerated where the young seedlings were grown under light conditions (10 hours) and the daily temperature were ranging between 18/33°C. Four weeks old plants were treated with test solutions.

Culture Conditions: A set of culture media consisting of solutions of polythene glycol, PEG-6000 (Union Carbide Corp. USA), in concentrations that give particular matric water potential (Ψ m = 0.0, -0.2, -0.5 and -1.0 MPa) were prepared (El-Sharkawi and Springuel, 1977). An other set of cultures having the same levels of water potential were amended with potassium (singly) or K accompanied with micronutrient (Zn, Mn, or Cu) supplied as: KNO_3 , $ZnSO_4$, Mn SO_4 and Cu SO_4 in different ratios. The treatment solutions were: control (deficient of nutrients), K (singly, 500ppm) K-Zn (500:2ppm), K-Mn (500:2ppm) and K-Cu (500:1 ppm).

Assays of K, Zn, Mn, and Cu in plants: Three replicates were assigned at random to each treatment combination (Five K-micronutrients, Nr treatments at four matric water potential levels, Ψ m). The plants were maintained under the effect of treatment solutions. After ten days of treatment, roots, stems, and leaves were excised, thoroughly washed with distilled water, blotted dry between filter papers, weighed, homogenized in 10 ml ice-cold water and the supernatants were kept in deep freeze until the time of assay. Soluble K, Zn, Mn, and Cu were measured in extracts using an atomic absorption spectrophotometer (*Shimadzu model AA-630-02, Shimadzu Corp, Kyoto, Japan*) as described by Williams and Twine (1960).

Statistical analysis: The relative role of single factors and their interaction has been evaluated by suitable inferences including analysis of variance (F-values), coefficient of determination η^2 , (El Sharkawi and Springuel, 1977) and simple linear correlation coefficient, r (Ostle, 1963).

Results

The total contents of K, Zn, Mn and Cu in the whole plants and their relative distribution (as %) in different plant organs (leaves, stems, and roots) treated with K or K accompanied with Zn, Mn, or Cu under different matric water potentials, Ψ m are shown in Fig. 1-4.

Farghali and Saleh: K, Zn, Mn and Cu status of *Vigna sinensis*

Table 1A: Mean values of total contents and shoot/root ratios of K, Zn, Mn, and Cu in *Vigna sinensis* plants at various treatments of K with micronutrients in the root medium.

Ions treatment	K ⁺		Zn ⁺²		Mn ⁺²		Cu ⁺²	
	Content (mg)	Ratio	Content (ppm)	Ratio	Content (ppm)	Ratio	Content (ppm)	Ratio
Control	3.4	2.4	66.1	3.2	161.8	8.6	12.1	2.9
K	4.5	3.7	68.5	5.1	109.9	5.3	8.4	2.7
K-Zn	5.6	3.4	128.3	3.0	95.9	7.0	16.3	3.1
K-Mn	5.3	4.8	65.4	4.9	152.5	9.2	22.9	4.0
K-Cu	4.2	4.9	60.9	4.7	112.8	11.5	39.5	1.6

Table 1B: At different Ψ_m levels (-MPa) in the root medium

Ψ_m (-Mpa)	K ⁺		Zn ⁺²		Mn ⁺²		Cu ⁺²	
	Content (mg)	Ratio	Content (ppm)	Ratio	Content (ppm)	Ratio	Content (ppm)	Ratio
- 0.0	4.3	4.6	51.8	3.3	91.4	7.3	15.4	2.0
- 0.2	4.5	3.8	78.2	4.4	127.6	6.6	15.7	2.8
- 0.5	5.4	3.9	88.5	5.2	142.7	9.4	24.6	3.0
- 1.0	4.3	4.1	86.8	3.9	144.6	10.0	23.6	3.1

Table 2: ANOVA (F and ζ^2 values) for the effect of matric water potential (Ψ_m), potassium with micronutrients (Nr) and their interaction on the concentrations of K, Zn, Mn, and Cu in *Vigna sinensis* plants.

Ions factors	K ⁺		Zn ⁺²		Mn ⁺²		Cu ⁺²	
	F	ζ^2	F	ζ^2	F	ζ^2	F	ζ^2
Ψ_m	3.11*	0.13	2.33	0.17	5.43**	0.33	1.76	0.12
Nr	7.69**	0.43	8.25**	0.80	5.93**	0.48	8.68**	0.78
$\Psi_m \times Nr$	2.66*	0.44	0.53	0.03	0.76	0.19	0.37	0.10

* Significant at P<0.05 level

** Significant at P<0.01 level

Table 3: Correlation coefficient (r) between K and investigated micronutrients in *Vigna sinensis* plants at various treatments of K⁺ with Zn⁺², Mn⁺² and Cu⁺² under different Ψ_m levels (-MPa).

Correlation Ψ_m treatment	K x Zn				K x Mn				K x Cu			
	0.0	-0.2	-0.5	-1.0	0	-0.2	-0.5	-1.0	0	-0.2	-0.5	-1.0
Control	0.941	-0.996**	0.530	-0.711	0.952*	0.608	-0.310	-0.381	0.826	-0.488	0.485	-0.167
K	0.997**	0.851	-0.922	0.887	0.875	0.908	-0.656	0.199	0.091	1.000**	-0.821	-0.739
K-zn	-0.530	0.325	-0.825	0.768	-0.729	-0.099	-0.608	0.909	0.122	0.995**	-0.587	1.000**
K-mu	0.065	0.664	-0.052	-0.831	0.563	0.951*	0.102	1.000**	0.952*	-0.347	-0.373	0.879
K-cu	0.882	0.426	-0.610	-0.110	0.947	0.844	-0.926	-0.547	0.605	-0.781	-0.952*	0.341
Control	0.976*	-0.607	0.643	0.922	0.781	0.496	0.999**	0.808	0.902	0.389	0.682	0.973*
K	0.887	0.522	0.897	0.831	0.102	0.812	0.977*	-0.570	0.549	0.857	0.970*	-0.813
K-zn	0.967*	0.907	0.956*	0.290	0.779	0.303	-0.022	0.754	0.595	-0.125	-0.314	0.847
K-mn	0.860	0.862	0.988*	-0.834	0.362	0.471	0.945	-0.465	0.787	-0.041	0.884	0.876
K-cu	0.697	0.843	0.860	0.896	0.906	-0.891	0.362	0.900	0.333	-0.996**	0.790	0.613

* Significant at P<0.05 level

** Significant at P<0.01 level

Potassium content: It was noticed that , K accumulation in cowpea plants was affected by the external K status and Ψ_m (Fig. 1 and Table 1). An increase in contents took place at moderate Ψ_m level (-0.5 MPa) in different nutrient treatments. Exceptionally the concentration of K was slightly changed in K-Zn treated plants under tested Ψ_m levels which yielded a maximum value. However, the contents markedly decreased at extreme Ψ_m levels, except in case of K-treatment, the decrease in free K started at $\Psi_m = -0.2$ MPa. The effect of single factors Ψ_m , nutrients, Nr and their interaction was significant (Table 2) on K content in the experimental plants. Both Nr and ($\Psi_m \times Nr$) interaction had an equal share in their predominant effect ($\eta^2 = 0.44$), whereas Ψ_m had a subsidiary role.

Allocation of water soluble -K in organs of investigated plants indicate that shoots gained a higher K content than roots, particularly in K-Mn and K-Cu treatments (ratios = 4.8 and 4.9, respectively). In stems the accumulation of K was shown in K-Cu treatment also in the control (no nutrients). The presence of K with Mn accelerate K⁺ to allocate in the leaves at a wide range of Ψ_m levels. The K content in roots decreased in K-Mn treatment under water stress and vice versa in the rest of plants.

Zinc content: With K-Zn treatment, the total Zn⁺² content in plants was relatively higher over a wide range of Ψ_m levels (-0.2 to -1.0 MPa), which reflects the response of internal

Zn⁺² to the external nutrient status (Table 1 and Fig. 2). This was also judged by F value and coefficient of determination , ζ^2 (Table 2) where Nr has a highly significant and a predominant role on the concentration of Zn⁺² in plants. In various treatments (and control) , Zn⁺² concentration greatly increased with decreasing matric water potential.

Shoot / root ratio of Zn⁺² refers to a higher values in the presence of K or K accompanied with micro-nutrients at moderate Ψ_m levels (Table 1). An increase in Zn⁺² of leaves was detected in most of the treated plants under water stress, whereas the roots gained a lower Zn in K-Mn and K-Cu treatments at moderate Ψ_m levels.

Mn content: Total Mn content in plants increased progressively with decreasing Ψ_m (Fig. 3 and Table 1), particularly in K-Mn treatment . The presence of K-Zn nutrients in the incubation media influenced Mn status which decreases at relatively high water stress. The effect of single factors (Ψ_m and Nr) on Mn content were highly significant , but non-significant for their interaction. Meanwhile, Nr had a dominant role on the total Mn content , the Ψ_m role was subdominant ($\zeta^2 = 0.48$ and 0.33, respectively).

Both K-Mn and K-Cu treatments increased the shoot/root ratio of Mn as compared to that in case of K-Zn , K and untreated plants. Likewise, the increasing Mn ratios existed under water stress (Table 1). The leaves considerably gained a higher Mn content than stems or roots. Roots , however had

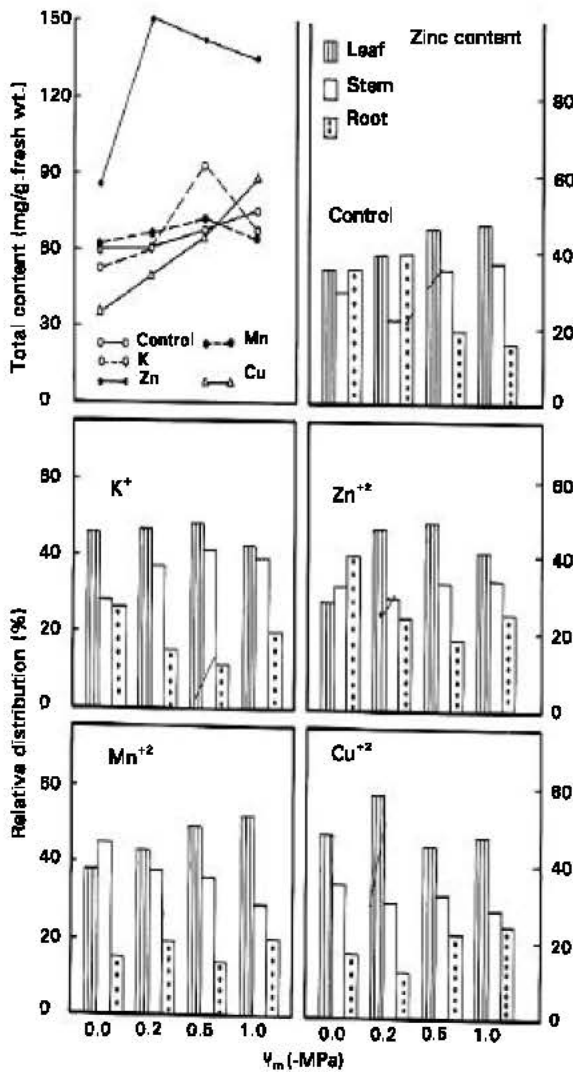


Fig. 1: The total content ($\text{mg}\cdot\text{g}^{-1}$ fresh wt) and relative distribution (%) of potassium, K^+ in different organs of *Vigna sinensis* plants grown in K, Zn, Mn, or Cu nutrients at different Ψ_m levels.

a minor Mn content except in K-treatment in absence of water stress. Under high Ψ_m (0 to -0.2 MPa), stems accumulate Mn^{+2} in solutions amended with K^+ .

Cu content As in Zn^{+2} and Mn^{+2} , the Cu^{+2} content within plants was affected by the presence of Cu^{+2} in the root medium. At low matric water potential, increased Cu^{+2} content was observed particularly in K-Cu treatment compared to untreated plants (control) where Cu decreases. A lower concentration of Cu^{+2} than both Zn^{+2} and Mn^{+2} reflected the sensitivity of plants to Cu^{+2} toxicity. Nutrients had a highly significant effect and a predominant role ($p^2 = 0.78$) on the Cu^{+2} content in whole plants under investigation (Table 2), whereas Ψ_m and its interaction with Nr had no significant effect.

Allocation of Cu^{+2} in shoots, hitherto showed a high ratio (4.0)

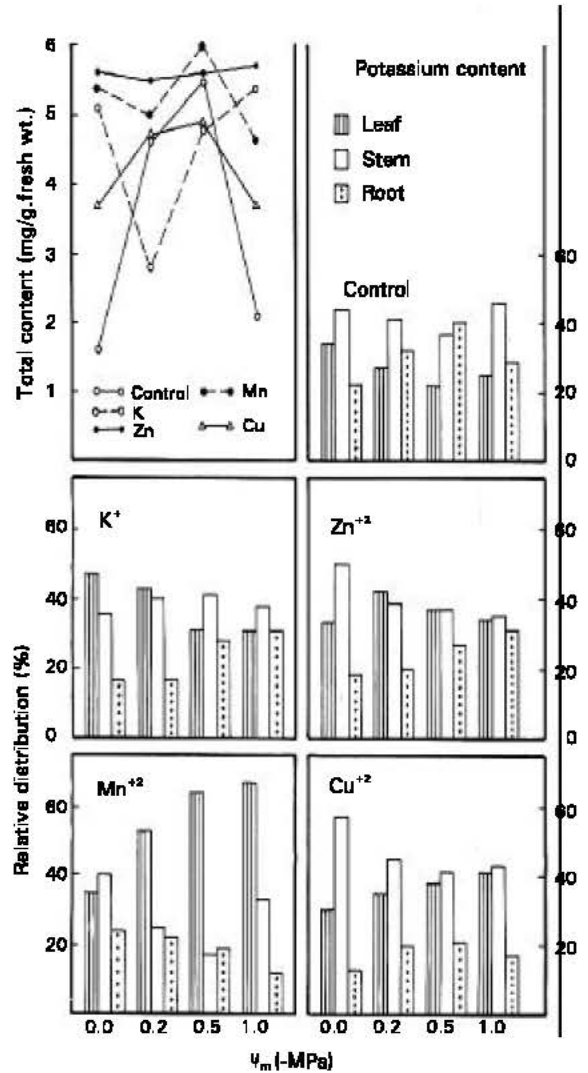


Fig. 2: The total content ($\mu\text{g}\cdot\text{g}^{-1}$ fresh wt.) and relative distribution (%) of Zinc, Zn in different organs of *Vigna sinensis* plants grown in K, Zn, Mn or Cu nutrients under different Ψ_m levels

pronounced an accumulation of Cu^{+2} in roots. However, at in K-Mn treatment, and K-Cu yielded a low ratio (1.6) moderate and low Ψ_m levels the aerial parts / root ratio of Cu was high (3.1, Table 1).

Relative distribution of Cu^{+2} in plant organs differs at different treatments and Ψ_m levels (Fig. 4). In K, and K-Zn treatments a higher Cu^{+2} content in roots were found at high water potentials and vice versa in K-Mn treatment. With K-Cu slight changes in Cu^{+2} content existed in roots, regardless the Ψ_m levels. The Cu^{+2} content in roots of untreated plants decreased under high water stress, whereas it increased in leaves. The same is true in K treatment at relatively low water stress. However, stems accumulated a high Cu at K-Zn and K-Mn, particularly under low Ψ_m .

Correlation Coefficient, r between water soluble ions: Under

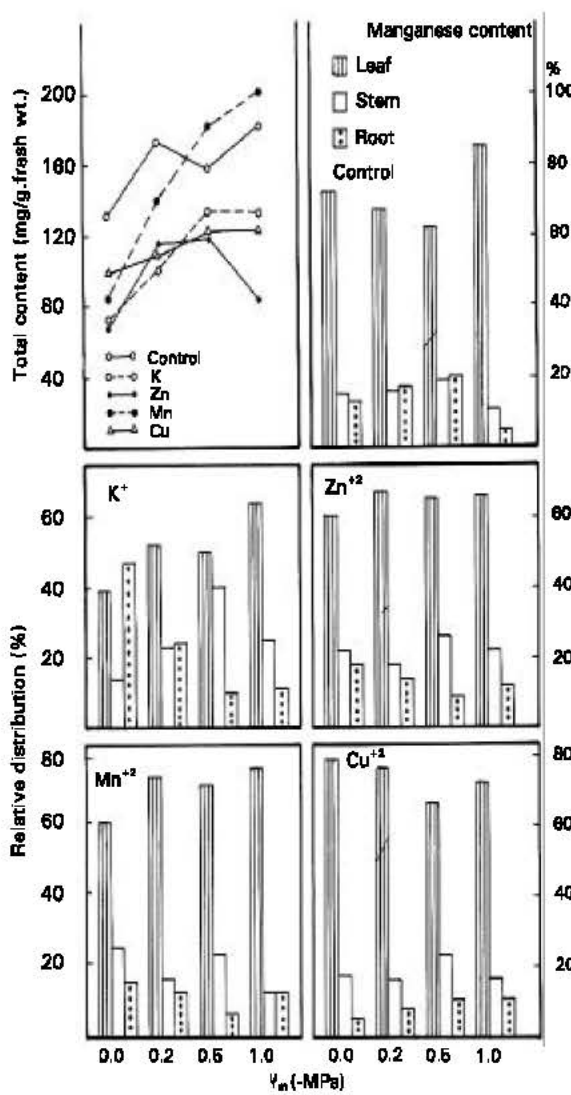


Fig. 3: The total content ($\mu\text{g g}^{-1}$ fresh wt.) and relative distribution (%) of manganese, Mn^{+2} in different organs of *Vigna sinensis* plants grown in K, Zn, Mn or Cu nutrients under different Ψ_m levels.

deficiency or presence of K^+ and micronutrients, *in vivo* relationships between K^+ , Zn^{+2} , Mn^{+2} and Cu^{+2} concentrations were variable at different Ψ_m levels (Table 3). With K-Cu treatment, there was a significant negative correlation between Cu^{+2} and both K^+ and Mn^{+2} contents at $\Psi_m = -0.2$ MPa. The same correlation was found in case of K^+ with Zn^{+2} in untreated plants.

However, a significant positive correlation existed between K^+ and both Zn^{+2} and Cu^{+2} in K-treatment at high Ψ_m levels. Likewise, K^+ positively correlated with Mn^{+2} and Cu^{+2} in both K-Mn and K-Zn treatments of water stress in root media. In K-treated plants, Cu^{+2} had a significant positive correlation with both Zn^{+2} and Mn^{+2} at low water potentials and also in case of untreated plants. The absence of nutrients yielded the same correlation of Mn^{+2} with both K^+ and Zn^{+2} of unstressed plants. Similarly, Zn^{+2}

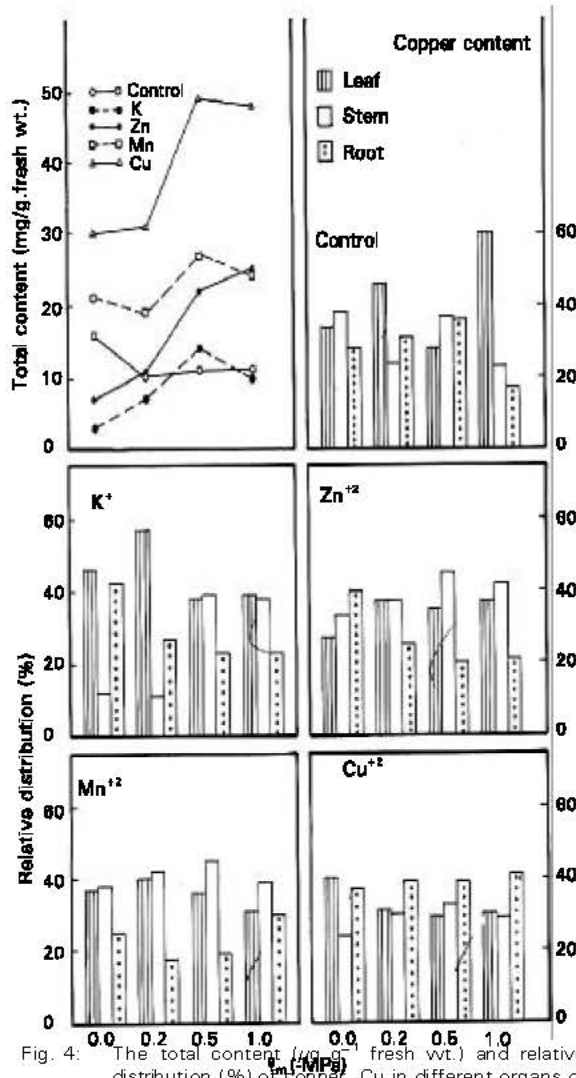


Fig. 4: The total content ($\mu\text{g g}^{-1}$ fresh wt.) and relative distribution (%) of copper, Cu in different organs of *Vigna sinensis* plants grown in K, Zn, Mn, or Cu nutrients under different Ψ_m levels.

significantly correlated with Mn^{+2} in K-Zn treatment. Likewise, under moderate water stress, the correlation was true between both ions in treated K-Zn and K-Mn plants.

Discussion

Decreased matric water potential, Ψ_m affected the contents of potassium, zinc, manganese and copper in *Vigna sinensis* plants. The increase in nutrient concentrations is mostly an adaptational response of the plants to absorb water. Similarly, the micronutrients increased in response to presence of that nutrient in the root medium. Statistically, Zn, Mn and Cu were greatly affected by the external nutrient factor, Nr (judged by χ^2 , Table 2). However, the major element, K is not only affected by Nr but also by (Nr \times Ψ_m) interaction. Net uptake of K^+ was dependant on K^+ concentration in the root medium and on K^+ status of the root (Botella *et al.*, 1997). This indicates that K nutrition under water stress conditions may have beneficial effects in the osmo-ionic adjustments of

plants. The same fact was recorded by Farghali and El-Sharkawi (1988) in cotton seedlings. They concluded that, the higher contents of potassium in aerial parts may be the serving sites of intense metabolism. K^+ status, however was enhanced in treated K-Zn and K-Mn plants which refers to a synergistic effects of both Zn^{+2} and Mn^{+2} on K^+ uptake.

Different shoot/root ratios of tested micronutrients yielded higher values under low matric water potentials. While, in case of K^+ , this took place at extreme Ψ_m levels as well as in K-Mn and K-Cu treatments. The decreased ratio of Cu^{+2} , particularly in the presence of external K-Cu elements may be due to its activity in developing roots (Lastra et al., 1988) and deleterious effects on the photosynthetic system (Boszynski et al., 1982). Whereas, a maximum Cu^{+2} ratio was detected in K-Mn medium, a higher shoot/root ratio of Mn^{+2} (11.5) existed in K-Cu treatment (Table 1). The shoot concentration of Mn^{+2} and Cu^{+2} were higher when plants were grown at deficient rather than at sufficient Zn^{+2} . It is concluded that, uptake of Mn^{+2} and Cu^{+2} by plants have been determined by competition between Zn^{+2} and these ions for a putative common carrier (Rengel and Graham, 1996). A maximum value of shoot/root Zn^{+2} ratio was found in K treated plants, whereas K-Zn treated plants have a lower ratio. This indicates that more excessive Zn^{+2} is transported to the shoot when the transport of Zn into the vacuole of root cells is saturated (Harmens et al., 1993 and Gonzalez et al., 1999).

In water stressed roots, markedly increased K^+ was found in plants incubated in K solution. This increase of K is transported to the leaves of K-Mn treated plants. In stems the same was true in K-Cu treatment which refers to the associations of external Cu^{+2} in K^+ translocation. Stems also accumulate Zn^{+2} and Mn^{+2} in plants treated with K^+ , meanwhile zinc content increases in roots incubated in K-Zn treatment of non stressed plants. The Zn^{+2} storage within the roots may not occur because Zn^{+2} can be remobilized from various pools within the plant (Lastra, 1988). Whereas the addition of K^+ increased Mn^{+2} uptake in roots and stems, leaves the site of Mn have a higher content under different treatments and Ψ_m levels. This mention to the association of Mn^{+2} in the photosynthetic capacity and chlorophyll contents in leaves (Gonzalez and Lynch, 1997). In contrast, deleterious effect of Cu^{+2} on the photosynthetic system was restricted in the leaves, particularly with K-treatment. Therefore, excessive Cu^{+2} in the root medium leads to a higher Cu^{+2} content within the roots. In water stressed plants, the restricted Cu^{+2} translocation to shoots existed at different nutrient treatments.

The correlation of K^+ with both Mn^{+2} and Cu^{+2} within plants was improved in the presence of Mn^{+2} or Zn^{+2} added to K^+ in the root media. The same relationship was true in case of Zn^{+2} with Mn^{+2} . Likewise, in potassium treated plants the Cu^{+2} positively correlated with Zn^{+2} and Mn^{+2} under water stress conditions. Whereas, at high Ψ_m levels K^+ synergistically correlated with Zn^{+2} and Cu^{+2} in K^+ treated plants, the antagonistic correlation was found between Cu^{+2} and both K^+ and Mn^{+2} in plants incubated in K-Cu solution.

Potassium functions, however can improve the osmoregulation of guard cells (Talbot and Zeiger, 1998) of cultivated plants in arid and semi-arid areas by addition of micronutrients. The deficiency of K should result in accumulation of photosynthates in source leaves and leading to severe limitation in root growth (Kakmak, 1994). Likewise, in shoots K is the main limiting factor for stressed plants and low K treatment (Botella et al., 1997). The role of K^+ is extending to yield a beneficial correlation between micronutrients which have a vital role in the photosynthetic apparatus, enzyme activity and growth regulators. Hence, solute accumulation leads to increased water retention and turgor

maintenance under drought stress.

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References

- Bazynski, T., M. Krol, Z. Krupa, M. Ruzkowska, U. Wajcieszka and D. Wolinska, 1982. Photosynthetic apparatus of spinach exposed to excess copper. *Z. Pflanzenphysiol.*, 108: 385-395.
- Botella, M.A. Martinez, S. V. Pardines and A. Cerda, 1997. Salinity induced potassium deficiency in maize plants: *J. Pl. Physiol.*, 150: 200-205.
- Bowen, J.E., 1981. Kinetic of uptake of boron, zinc, copper and manganese in barley and sugarcane. *J. Pl. Nut.*, 3:215-223.
- El-Sharkawi, H.M. and I.V. Springuel, 1977. Germination of some crop plant seeds under reduced water potential. *Seed Sci. Technol.*, 5:677-688.
- Farghali, K.A. and H.M. El-Sharkawi, 1988. Interactive effects of NPK nutrients, water potential and irradiance on contents of potassium and phosphate in cotton seedlings. *Phyton*, 28:171-181.
- Gonzalez, A. and J.P. Lynch, 1997. Effect of manganese toxicity on leaf CO_2 assimilation of contrasting common bean genotypes. *Physiologia Plantarum*, 101: 872-880.
- Gonzalez, A., V.K. Kov and G.J. Wagner, 1999. A comparison of Zn, Mn, Cd and Ca transport mechanisms in oat root tonoplast vesicles. *Physiologia Plantarum*, 106:203-209.
- Harmens, H., N.G.G.P.B. Gusmao, P.R. Den Hartog, J.A.C. Verkiej and W. O. Ernst, 1993. Uptake and transport of zinc in zinc sensitive and zinc tolerant *Silene vulgaris*. *J. Pl. physiol.*, 141: 309-315.
- Kakmak, I., C. Hengeler and H. Marschner, 1994. Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *J. Exp. Bot.*, 45: 1245-1250.
- Kochian, L.V. and W.J. Lucas, 1982. Potassium transport in corn roots. Y. Resolution of kinetics into a saturable and linear component. *Pl. Physiol.*, 70:1723-1731.
- Lastra, O., A. Chueca, M. Lechica and J.L. Gorge, 1988. Root uptake and partition of copper, iron, manganese, and zinc in *Pinus radiata* seedlings grown under different copper supplies. *J. Pl. Physiol.*, 132: 16-22.
- Morita, K., 1980. Release of nitrogen from chloroplast during leaf senescence in rice (*Oryza sativa* L.). *Annals of Bot.*, 46: 297-302.
- Osmond, C.B., M.P. Austin, J.A. Berry, W.D. Billings, J.S. Boyer, W.J.H. Dacey, P.S. Nobel, S.D. Smith and W.E. Winner, 1986. Stress physiology and the distribution of plants. *BioSci.*, 37:38-48.
- Ostle, B., 1963. *Statistics in Research - The Iowa State Univ. Press*, Ames.
- Pearson, J.N. and Z. Rengel, 1995. Uptake and distribution of Zn and Mn in wheat grown at sufficient and deficient levels of Zn and Mn. 1- During vegetative growth. *J. Exp. Bot.*, 46: 833-839.
- Rengel, Z. and R.D. Graham, 1996. Uptake of zinc from chelate-buffered nutrient solutions by wheat genotypes differing in zinc efficiency. *J. Exp. Bot.*, 47: 217-226.
- Talbot, L.D. and E. Zeiger, 1998. The role of sucrose in guard cell osmoregulation. *J. Exp. Bot.*, 49: 329-337.
- Welch, R.M. and W.A. Norvell, 1993. Growth and nutrient uptake by Barley (*Hordeum vulgare* L. Cu. Herta): Studies using an N-(2-Hydroxyethyl)ethylenedinitrioltriacetic acid-buffered nutrient solution technique. *Pl. Physiol.*, 101: 627-631.
- Williams, C.H. and J.R. Twine, 1960. Flame photometric method for sodium, potassium and calcium-In: Paech K. Tracey, M.V. (eds.). *Modern methods of Plant Analysis*. Vol 5 Pp 3-5. Springer-Verlag, Berlin-Göttingen-Heidelberg.
- Zingarelli, L., M.T. Marre, F. Massardi and P. Lado, 1999. Effects of hyper-osmotic stress on K^+ fluxes H^+ extrusion transmembrane electric potential difference and comparison with the effects of fusicoccin. *Physiologia Plantarum*, 106: 287-295.