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Research Article

The Effects of Different Mineral Nutrients on the levels of Cytokinins in Maize (*Zea mays* L.)

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Abstract: In this study, Z (*trans*-Zeatin) and ZR (*trans*-Zeatin Riboside) levels in the roots, stems, leaves, flowers and fruits of maize plants (*Zea mays* L.) were determined using high performance liquid chromatography (HPLC). Plants were grown in media containing macro elements (nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and iron) in optimum, deficient and excess concentrations. According to the average Z values for all periods, the highest Z levels were determined in the roots of plants treated with excessive P (-P); in stems, female flowers treated with excessive K (-K) and in leaves and kernels treated with excessive Ca (-Ca). The highest ZR levels were found in roots and stems treated with excessive Fe (-Fe), S (-S) and in leaves, female flowers and kernels treated with excessive K (-K).

Key Words: Zeatin, Zeatin riboside, Mineral nutrition, HPLC, Maize (*Zea mays* L.)

Mısır Bitkisinde (*Zea mays* L.) Farklı Mineral Besin Maddelerinin Sitokinin Seviyeleri Üzerine Etkileri

Özet: Bu çalışmada, makroelementlerin (azot, fosfor, potasyum, kalsiyum, magnezyum, kükürt ve demir) optimum, eksik ve aşırı konsantrasyonlarının mısır (*Zea mays* L.) bitkisinin kök, gövde, yaprak, dişi çiçek ve meyvelerinde bulunan sitokinin grubu hormonlardan Z (*trans*-Zeatin) ve ZR (*trans*-Zeatin ribozid) seviyeleri üzerine etkileri saptanmıştır. Z ve ZR seviyelerinin belirlenmesinde yüksek basınçlı sıvı kromatografisi (HPLC) kullanıldı. Hormon seviyeleri bitkilerin 1. Peryot (4-5 yapraklı olduğu), 2. Peryot (çiçeklenme) ve 3. Peryot (meyve gelişmesi) olmak üzere 3 peryotta belirlendi. Bütün peryotlardaki ortalama Z ve ZR degerlerine göre En yüksek Z seviyesi aşırı fosfor uygulanan bitkilerin köklerinde, aşırı potasyum uygulanan bitkilerin gövde ve dişi çiçekleri ile, aşırı kalsiyum uygulanan bitkilerin yaprak ve tohumlarinda bulundu. En yüksek ZR seviyeleri ise aşırı demir ve kükürte maruz bırakılan bitkilerin gövdelerinde, aşırı potasyum uygulanan bitkilerin yaprak, dişi çiçek ve meyvelerinde saptandı.

Anahtar Sözcükler: Zeatin, Zeatin ribozid, Mineral madde, HPLC, Maize (*Zea mays* L.)

Introduction

Cytokinins, which stimulate cell division, are synthesised in roots and transferred to the other organs of plant. The mineral composition of the soil influences the synthesis of cytokinins. It has been reported that high levels of nitrogen, phosphorus and potassium decreased the levels of cytokinins in *Coleus blumei* Benth. (Banko & Boe, 1975). Also, low levels of nitrogen, phosphorus and potassium have reduced the cytokinin levels in leaves, buds and roots in *Helianthus annuus* L. (Salama & Wareing, 1979). It has been reported that nitrogen deficiency decreased the levels of derivatives of base and riboside of cytokinins in *Betula pendula* Roth. and *Acer pseudoplatanus* L. (Darral & Wareing, 1981). In the case of *Acer negundo* L. grown on media containing an excessive amount of minerals, cytokinin activity was reduced in roots, but did not change in leaves (Kazaryan et al., 1988). Kazaryan et al. (1988) found similar results in the roots of *Rosa canina* L. Zeatin and zeatin riboside levels of the roots and the shoots of *Plantago major* L. decreased when it was treated with a dilution of optimum nutrient solutions (Kuiper et al., 1989). Excess Ca concentration decreased zeatin and zeatin riboside levels in *Cicer arietinum* L. (Galleago et al., 1991). The interaction between the concentrations of nitrogen and

its different forms and cytokinin levels was observed in *Epidendrum fulgens* Brongn. protocorms (Mercier and Kerbauy, 1991) and *Sorghum* Moench. (Amzallag et al., 1992). Elevated levels of cytokinin in the roots of barley (*Hordeum* L.) were recorded due to an increase in the nitrogen concentrations in the medium (Samuelson and Larson, 1993). In contrast, cytokinin levels in the roots of *Urtica dioica* L. decreased under low nitrogen concentrations. However, no change was observed in the shoots (Wagner and Beck, 1993).

The aim of this study is to determine the relationships between macro elements and cytokinins, which has not been sufficiently investigated. In order to investigate the effects of macro elements on cytokinin levels, maize (*Zea mays* L.) plants were grown in media containing optimum, deficient (-) and excess (+) concentrations of N, P, K, Ca, Mg, S and Fe mineral compounds. Endogen cytokinins were analysed by high performance liquid chromatography (HPLC).

Materials and Methods

Seeds of maize (*Zea mays* L.) were used. Six seeds of maize were planted in plastic pots (6 litres) containing cleaned silica sand (0.8-1.2 mm diameter). The pots were divided into 15 groups, each including 3 pots. These groups were as followings: control (containing nutrition minerals at optimum levels), -N (deficient nitrogen), +N (excessive nitrogen), -P (deficient phosphorus), +P (excessive phosphorus), -K (deficient potassium), +K (excessive potassium), -Ca (deficient calcium), +Ca (excessive calcium), -Mg (deficient magnesium), +Mg (excessive magnesium), -Fe (deficient iron) and +Fe (excessive iron).

The stock solutions were prepared according to Witham et al. (1971, Table 1). The nutritious minerals prepared from the stock solutions were used at optimum, deficient and excessive levels. The excess levels of nutritious minerals were fourfold the optimum levels, as indicated in Table 2. The nutrient solutions, the amounts of which are given in Table 2, were diluted to a volume of 1 litre with distilled water and used on the plants. One hundred and fifty millilitres of the solutions of nutritious elements was added to each pot 2 times per week. Also, each pot was irrigated with 150 ml distilled water per day.

Table 1. The stock solutions

Chemical Compounds	Compounds	Concentrations (g/Litre)
A. Ammonium acid phosphate	$NH_4H_2PO_4$	23
B. Ammonium nitrate	NH_4NO_3	40
C. Calcium nitrate	$Ca(NO_2)_2$	189
D. Calcium chloride	$CaCl_2$	29
E. Magnesium chloride	$MgCl_2.6H_2O$	41
F. Magnesium nitrate	$Mg(NO_3)_2.6H_2O$	51
G. Magnesium sulphate	$MgSO_4.7H_2O$	99
H. Potassium acid phosphate	KH_2PO_4	27
I. Potassium nitrate	KNO_3	121
J. Potassium sulphate	K_2SO_4	87
K. Ferric chloride	$FeCl.6H_2O$	10
L. Microelement Stock Solution elements below mixed together/1 litre	Molarity (x 10^{-2}) distilled water:	
Boric acid	H_3BO_3	0,72
Copper chloride	$CuCl_2.2H_2O$	0,02
Manganese chloride	$MnCl_2.4H_2O$	0,45
Zinc chloride	$ZnCl_2$	0,06
Molybdic acid (85% MoO_4)	$H_2Mo_4.H_2O$	0,01
M. Fe EDTA (iron complex of etilendiametetraacetic acid):		
Dissolve 1340 mg disodium etilendiaminetetraacetate ($Na_2C_{10}H_{10}O_8N_2.2H_2O$) in 500 ml of distilled water and heat. While still hot add 990 mg $FeSO_4.7H_2O$ and stir vigorously.		

Table 2. The Nutrient solutions (ml).

Stock Solutions	Cont.	+N	-N	+P	-P	+K	-K	+Ca	-Ca	+Mg	-Mg	+S	-S	+Fe	-Fe
A	5	20	0	20	0	5	5	5	5	0	0	0	0	5	5
B	0	32	0	1	1	6	6	8	8	6	6	0	0	0	0
C	5	20	0	5	5	5	5	20	0	5	5	5	5	5	5
D	5	21	21	5	5	5	5	20	0	5	5	0	0	5	5
E	0	0	0	0	0	0	0	0	0	20	0	5	5	0	0
F	0	20	0	0	0	0	0	0	0	20	0	5	5	0	0
G	5	5	5	5	5	5	5	5	5	20	0	20	0	5	5
H	0	5	5	20	0	10	0	0	0	5	5	5	5	0	0
I	5	20	0	5	5	10	0	5	5	1	1	5	5	5	5
J	0	5	5	0	0	10	0	0	0	4	4	0	0	0	0
K	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0
L	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
M	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0

- = Absences of the mineral compounds
+ = Excessive concentration of mineral compounds

Two-gram samples from each organ (roots, stems and leaves) of the plants were harvested in the 1st period. Five-gram samples were collected in the 2nd period from the organs (roots, stems, leaves and female flowers) of the plants. Also, 5-g samples were taken in 3rd period from the organs (roots, stems, leaves and kernels) of the plants. Harvested samples were stored at -80°C until extraction. Extraction, purification and HPLC analysis were performed according to the modified method of Kuraishi et al. (1991).

After frozen samples were powdered in liquid nitrogen, cold methanol was added. They were stored at 4°C for 24 hours in the dark after the samples were homogenised in an Ultra Tissue Lysis (Ultrasonic Processor Jenway LTD.). After that, the samples were filtered with filter paper (Whatman No: 1) and the filtrates were obtained. The residues were treated in the same way as mentioned above, and the former and latter filtrates were combined. These filtrates were filtered with PTFE filters (0.45 µm; Cutting, 1991). After evaporation of the samples at 35°C, the extracts were redissolved in 100 $mol.m^{-3}$ KH_2PO_4 (pH 8) buffer solutions and centrifuged at 10,000 g for 1 hour at 4°C. Then, the filtrates were placed in flasks (50 ml), each containing 1 g PVPP, and mixed and filtered with Whatman paper (No: 1; Mooney and Staden, 1984). The filtrates were passed from PVPP and through Sep-Pak C_{18} (Waters) cartridges (Machackova et al., 1993). Cartridge adsorbing hormones were eluated with 80% methanol and the extracts were collected in vials. The isocratic system was used for HPLC analysis. The extracts in the vials were injected into HPLC (Shimadzu, LC-10 AD) equipped with an ultraviolet detector (Unicam) and a µBondapak column using acetonitrile (11.5%; pH 5.5) as the mobile phase. The flow rate, pressure and wavelength were selected to be 2 ml min^{-1}, 2000 psi and 265 nm, respectively (Horgan and Kramers, 1979; Morris et al., 1990; Featonby-Smith and Van Steaden, 1984). Under these conditions, the retention times of Z and ZR were determined to be 5.70 and 7.20 minutes for standard.

Abbreviations used in the text are as follows: Z, *trans*-zeatin; ZR, *trans*-zeatin riboside PVPP, polvinylpolypyrolidone; HPLC, high performance liquid chromatography; -, Absences of mineral compounds; +, Excessive concentration of mineral compounds

Results

The analysis of variance showed a significant relationship between Z and ZR levels in leaves, roots and stems for all periods in terms of mineral concentrations. Similar relations were observed between mineral types and levels of cytokinins in female flowers and kernels. The highest amounts of Z in roots, stems and leaves were found in the samples of the 1st period. The highest amount of ZR was recorded in the samples of roots and stems of the 2nd period, and in leaves in the 1st period (Table 3).

Table 3. Changes in Z and ZR levels in roots, stems and leaves according to period.

	Roots		Stems		Leaves	
Periods	Z	ZR	Z	ZR	Z	ZR
1	3.49[a]	7.85[b]	2.50[a]	5.08[b]	1.81[a]	7.09[a]
2	1.12[b]	18.98[a]	1.35[b]	10.64[a]	0.75[b]	1.86[c]
3	0.85[b]	7.53[b]	0.27[c]	3.08[c]	0.90[b]	2.42[b]

According to the average Z values of all periods, the highest Z level was determined in the roots of plants treated with +P, in the stems and female flowers of plants treated with +K and in the leaves and kernels of plants treated with +Ca. The highest ZR levels were found in roots and stems treated with +Fe and S, and in leaves, female flowers and kernels treated with +K (Table 4).

In the 1st period, the highest and lowest Z levels were observed in the roots of plants grown in the medium containing +Mg and -Fe. However, the highest and lowest ZR levels were determined in the plants grown in the medium containing +S and -P. The highest and lowest Z levels of samples from in the 2nd period were found in the roots of plants grown in the medium containing +P and -Ca and -Fe. However, the highest and lowest ZR levels were determined in the roots of plants grown in the medium containing +Fe and -K. In the 3rd period the highest and lowest Z levels were observed in the roots exposed to +Mg and +S, but the highest and lowest ZR levels were obtained in the roots of the plants treated with +Mg and -N (Figure 1).

The highest and lowest Z and ZR levels in the stems were observed in samples of stems grown in media containing +Fe and –N in the 1st period, respectively. However, the highest Z and ZR levels of 2nd period stems were detected in the control group and +Fe group. However, the lowest Z and ZR levels were observed in the stems of plants grown in -Mg and -K. The highest Z and ZR levels of stems of the 3rd period were found in the control and +Fe groups, but the lowest Z and ZR levels were observed in -Fe and -K (Figure 2).

The highest Z and ZR levels of the leaves of the 1st period were found in +Ca while the lowest Z and ZR levels were found in -Fe. The highest and lowest Z levels of the leaves of the 2nd period were detected in +K and -P. The highest and lowest ZR levels of the leaves of the 2nd period were found in +K and -Ca. The highest Z and ZR levels of leaves of the 3rd period were observed in +K, and the lowest Z and ZR levels were in -N (Figure 3).

Female flowers did not occur in plants grown in -N and -P. The highest and lowest Z levels were observed in female flowers of plants grown in media with +K and -S. However, the highest and lowest ZR levels were found in plants grown in media including +Ca and -Ca (Figure 4).

Kernels did not occur in the plants grown in -N, -P, -K and –S. The highest and lowest Z levels in kernels were observed in media with -Ca and -K. The highest and lowest ZR levels were found in +K and -Ca (Figure 4).

Table 4. The relations of Z and ZR levels of roots, stems, female flowers and kernels with levels of minerals compounds according to the average of all periods

	Roots		Stems		Leaves		Female Flowers		Kernels	
Minerals	Z	ZR	Z	ZR	Z	ZR	Z	ZR	Z	ZR
+N	1.79[bc]	9.27[d]	1.43[bc]	6.92[b]	1.29[bc]	3.79[b]	0.84[ab]	2.20[b]	1.15[ab]	2.12[bc]
+P	2.43[a]	12.42[bc]	1.49[abc]	6.72[b]	1.11[c]	3.57[b]	0.74[ab]	1.68[b]	1.23[ab]	2.21[b]
+K	1.69[c]	10.78[cb]	1.87[a]	5.72[c]	1.44[ab]	4.98[a]	0.96[a]	2.17[b]	0.59[c]	5.05[a]
+Ca	1.77[c]	13.85[ab]	1.60[ab]	6.68[b]	1.59[a]	4.63[a]	0.89[a]	2.00[b]	1.29[a]	2.79[ab]
+Mg	2.17[ab]	14.38[a]	1.84[a]	6.73[b]	1.28[bc]	3.62[b]	0.77[ab]	1.79[b]	0.76[bc]	2.05[c]
+S	2.01[bc]	12.56[b]	1.12[c]	7.67[a]	1.40[ab]	4.63[a]	0.51[b]	1.55[b]	0.85[bc]	2.76[ab]
+Fe	1.71[c]	14.78[a]	1.68[ab]	6.82[b]	1.07[c]	3.30[b]	0.68[ab]	1.76[b]	0.78[bc]	2.29[b]

Means followed by the same letter are not significantly different at P≤0.05 according to Duncan's multiple range test

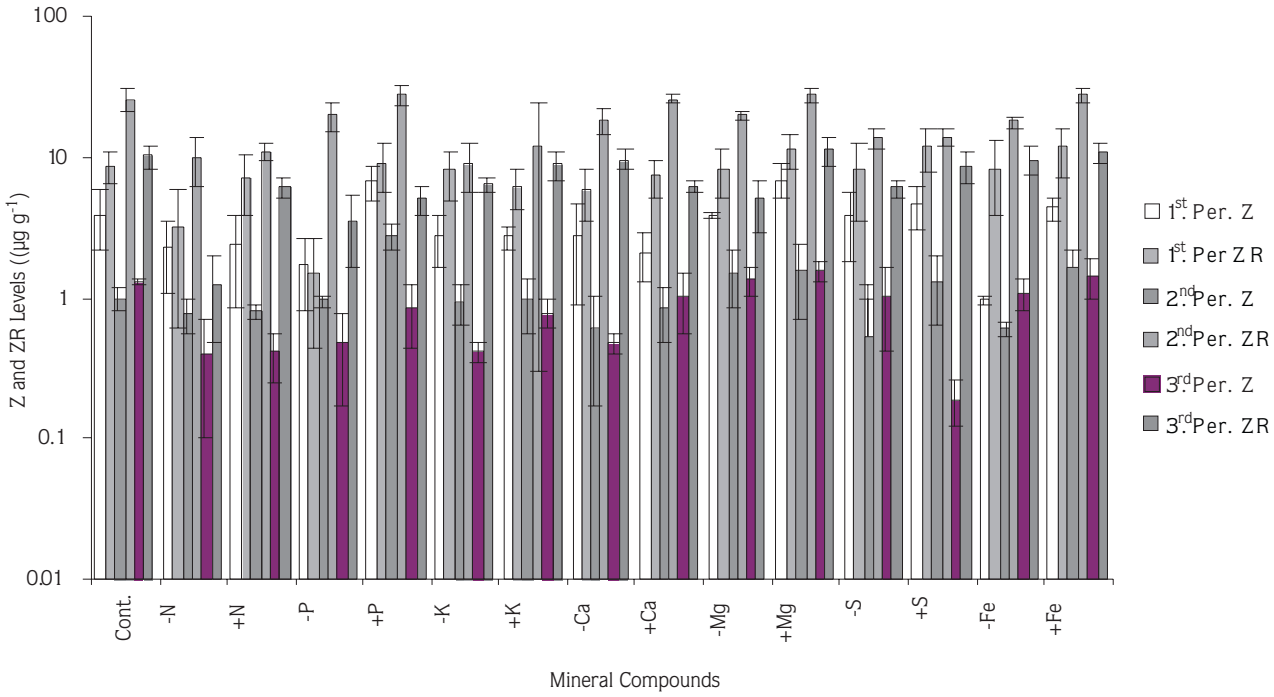

Figure 1. The changes in Z and ZR levels in roots according to kinds of mineral.

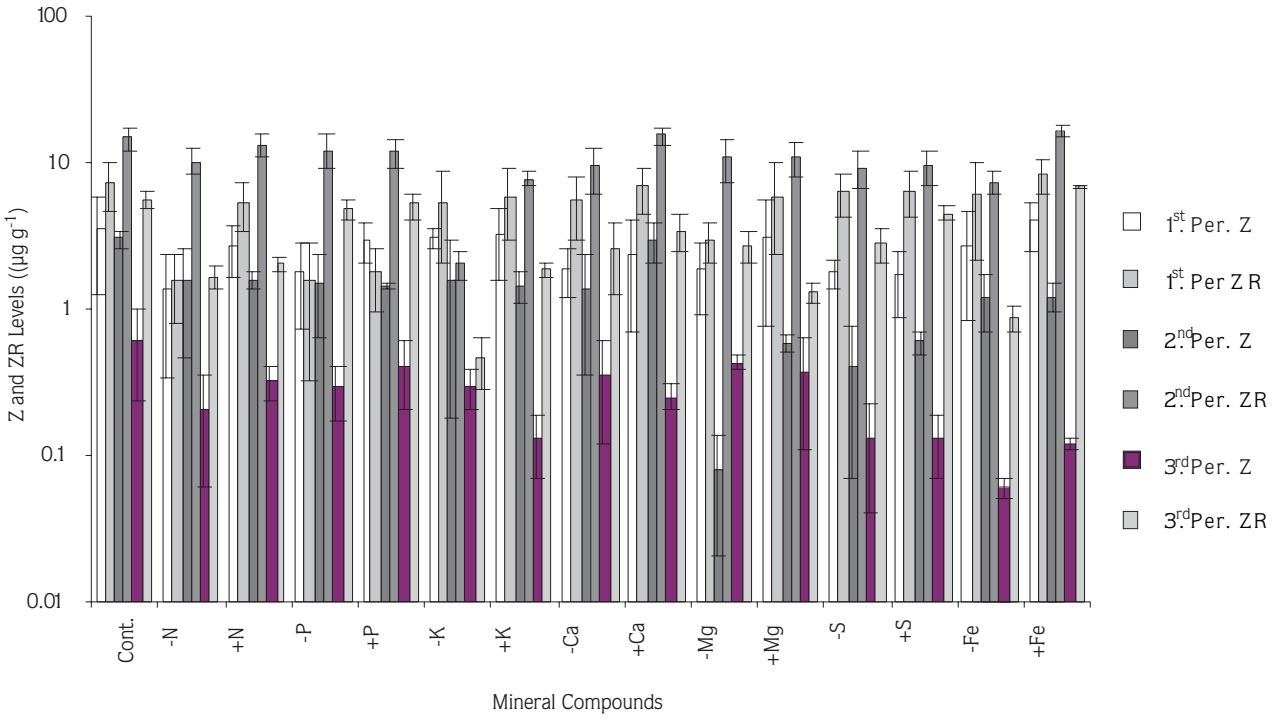

Figure 2. The changes in Z and ZR levels in stems according to kinds of mineral.

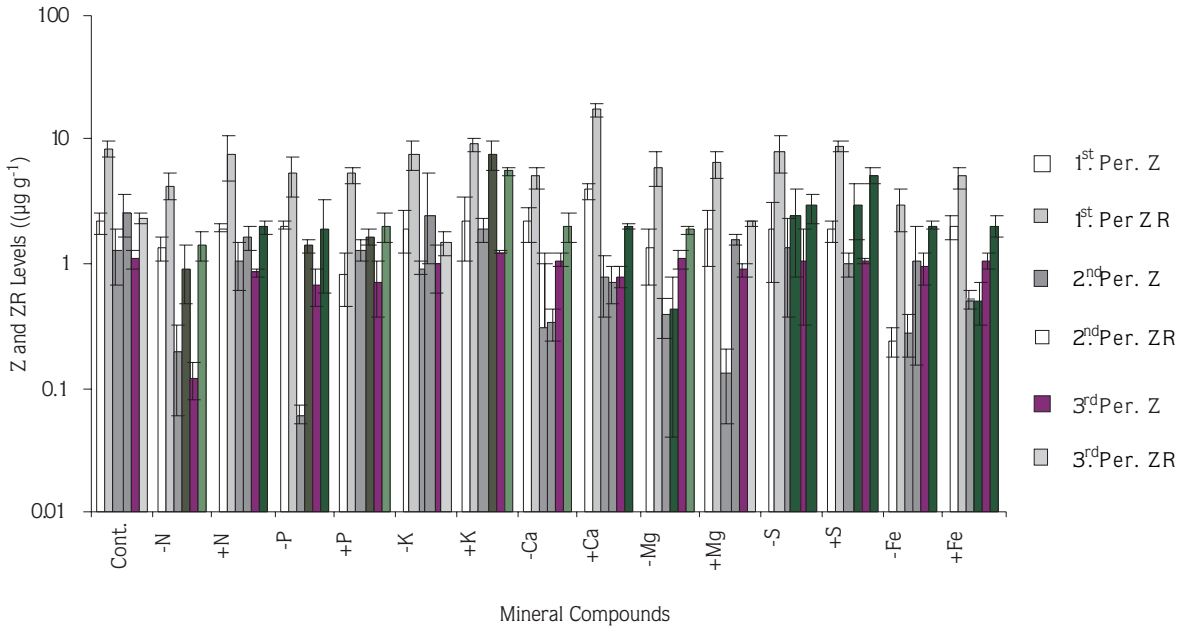

Figure 3. The changes in Z and ZR levels in leaves according to kind of mineral.

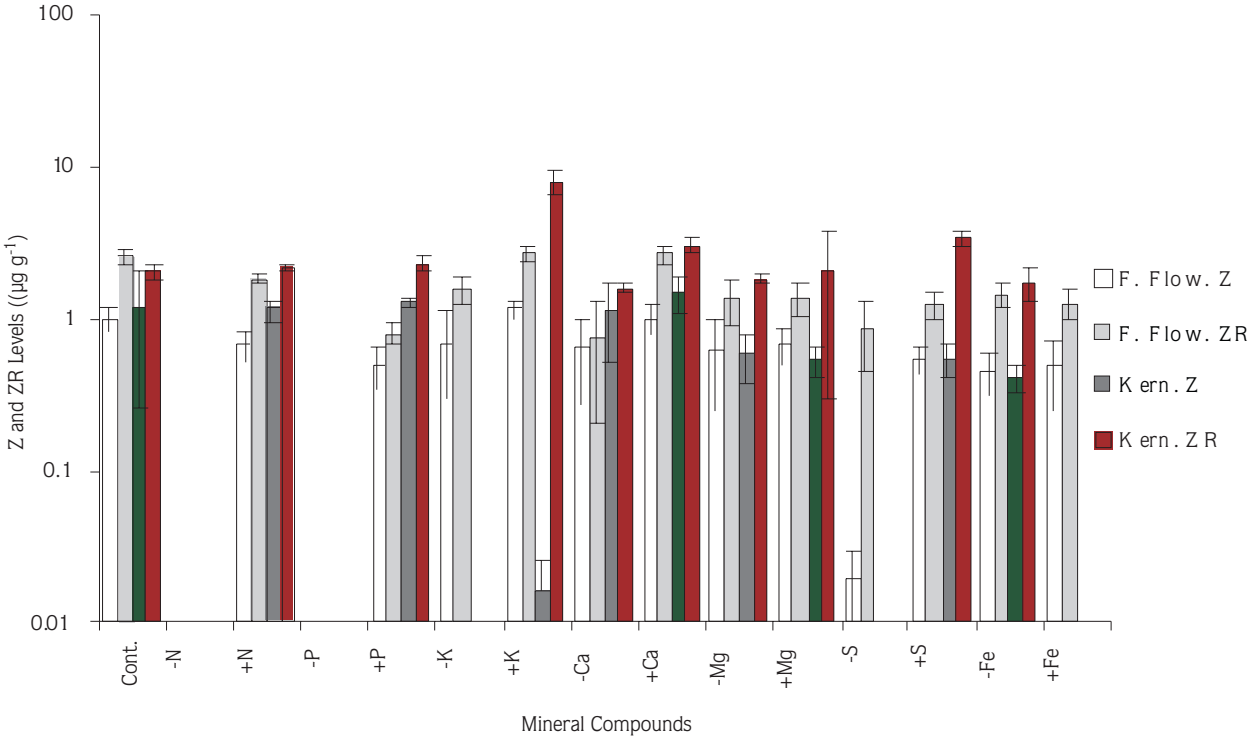

Figure 4. The changes in Z and ZR levels in female flowers and kernels according to kind of mineral.

Discussion

Z and ZR levels in roots were lower in all periods in -N, +N and -P, in comparison to the control plant. These results, obtained from the plants grown in medium -N, were in good agreement with the studies carried out on *Helianthus annuus* (Salama and Wareing, 1979), *Urtica dioica* (Wagner and Beck, 1993) and *Plantago major* (Kuiper et al., 1989). The findings related to -P were similar to the results obtained on the roots of *Helianthus annuus* by Salama and Wareing (1979). The Z levels in the roots treated with +P were found to be twofold those of the control plants in the 1st and 2nd periods. Also, ZR levels were some what higher than in the control. However, in the 3rd period both Z and ZR levels in roots were lower than in the control. The reason for this decrease in Z and ZR levels might be the excessive amount of P, causing toxicity to the plant roots in the long term. Z and ZR levels in the roots of plants treated with -K were lower than in control plants throughout all experimental periods. Salama and Wareing (1979) reported that deficient concentrations of K decreased cytokinin levels in the roots of *Helianthus annuus*. Our findings are similar to their results. Z and ZR levels decreased in the plants grown in +K for all periods. Z and ZR levels in roots decreased remarkably when plants were grown in the medium with -Ca. Similarly, Z and ZR levels declined in all periods when the plants were grown in an excessive concentration of Ca. Our results are in good agreement with the work of Galleago et al. (1991). The Z levels in the plants treated with medium with -Mg showed similar results to those of control plants in all three periods. ZR levels were lower in all three periods than in the control. Z and ZR levels were higher in the plants treated with +Mg in all three periods. The Z and ZR levels in the roots of plants treated with -S decreased. However, Z and ZR levels were higher in the 1st period when the plant was grown in +S. In contrast, Z and ZR levels were lower in the 2nd and 3rd periods. Stout et al. (1951) reported that SO^{-2}_4 ions in tomato roots prevented the absorption MoO^{-2}_4 ions. Moreover, Nicholas and Nason (1954) found that Mo had an important role in nitrate reductase enzyme activity converting nitrate to nitrite. The reason for the low levels of Z and ZR in the roots in the 2nd and 3rd periods could be the indirect effect of both situations, resulting from the increase in S concentrations in the medium, as mentioned in the three studies above. Z and ZR levels in the roots treated with the medium with -Fe were lower in all periods. The levels of these hormones in the roots increased when the plant was grown in +Fe.

Z and ZR levels were significantly lower in the stems in the deficient and the excessive concentrations of N, P, K, Ca, Mg and S in all periods compared to control plants. Our findings from the plant stems grown in +N, +P and +K were similar to the findings of Banko and Boe (1975). Z and ZR levels in the stems were lower in all periods when the plants were treated with the medium containing -Fe. Z levels in the stem were slightly higher in +Fe in the 1st period and lower in the 2nd and 3rd periods, but ZR was higher in all periods.

Z and ZR levels in the leaves were higher in all periods when the plants were treated with medium containing –N and +N compared to the control plants. These findings are in good agreement with the results obtained from *Betula pendula* and *Acer pseudoplatanus* (Darral and Wareing, 1981), and *Helianthus annuus* (Salama and Wareing, 1979). Z and ZR levels in leaves were lower than those of the control plants at -P and +P in all periods. Our findings are similar to the results of Salama and Wareing (1979). -Ca caused reduction in Z and ZR levels in the leaves in all periods. Z and ZR levels in the leaves of plants, grown in +Ca, were higher in the 1st period, and lower in the 2nd and the 3rd periods. Similarly, the levels of Z and ZR were lower in the leaves of the plants treated with –Mg and +Mg in all periods compared to the control. Z and ZR levels in leaves of plants grown in -S were remarkably lower in all periods. Z levels were lower in the leaves of plant treated with +S, while ZR levels were higher in all periods. Z and ZR levels were lower in the leaves of plant treated with –Fe and +Fe.

Z and ZR levels were lower in the female flowers of the plants treated with +N and +P when compared with those of the control. They were lower in female flowers of plants treated with the medium containing -K and -Ca. The levels of Z and ZR were higher in plants grown in +K and +Ca. However, Z and ZR levels in the female flowers were lower in plants grown in excessive and in deficient concentrations of Mg, S and Fe.

ZR levels were higher in the kernels of the plants treated with +N. Z levels were nearly equal to those of the control plant. Z levels were lower in the kernels of the plant treated with +K, but ZR was higher. Z and ZR levels in the kernels were lower in -Ca. In contrast, Z and ZR

were slightly higher in +Ca. The levels of Z and ZR in the kernels of plants treated with -Mg and +Mg. Z levels were lower in the kernels of the plant treated with a +S concentration, while ZR was higher in the same situation.

Both Z and ZR levels were lower in the plants grown in -Fe. Z levels in the plants treated with +Fe were lower, but ZR levels were higher.

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