Effects of form and level of orthophosphate on growth, uptake and distribution of some elements in maize (*Zea mays* L.cv.ksc700)

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Abstract

In this study, the plants of maize (*Zea mays* L.cv.ksc700) were grown in Hoagland solution containing 1, 3 and 5 mM KH_2PO_4 or K_2HPO_4 under controlled conditions. At similar levels of mono and diphosphate, plants supplied with monophosphate have produced higher content of dry matter and characterized with higher total chlorophyll content and longer and well proliferated root system than those treated with similar levels of diphosphate. Plants fed with monophosphate exported more than 50% of the total produced substances toward root system whereas, for the plant supplied with diphosphate, it was less than 20% approximately. In addition, there were a positive and significant correlation between the rate of the uptake and the content of the measured elements such as P, N, Ca^{+2}, Mg^{+2}, K^+, Na^+ and monophosphate level; however, the same elements except for K^+ negatively correlated with diphosphate. In both groups, the major proportion of the absorbed elements was more or less accumulated in leaves in relation to that of other parts of the plants. At the end of experiment, while pH decreased in the root medium supplied with monophosphate, an increase was observed in pH of those containing diphosphate. Differences between means values related to the same parameters in most of the cases were significant at $P<0.05$.

Keyword: Zea mays; growth; macroelement; absorption; distribution; pH

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Introduction

Phosphorous as a macronutrient plays a great role in the metabolism of all living organisms. It takes part in molecular structure of some key organic substances such as phospholipids, nucleoproteins, nucleotides and nucleic acids. Consequently, it involves in cell division, energy transformation and transfer, protein synthesis, and the transfer of inheritance characteristics (Marshner, 1969).The plants suffering from phosphorous deficiency were characterized usually with low rate of the photosynthesis and growth (Yong-fu et al., 2006; Foyer and Spencer, 1996; Jacob and Lowler, 1991) and delayed flower initiation (Roositer, 1978). Phosphorous is supplied to the plants mostly in two forms of orthophosphate such as $H_2PO_4^-$ and HPO_4^{2-}.

In some soils, in spite of the presence of an adequate content of inorganic phosphate, plants suffer from shortage of this element. The availability of inorganic phosphate for the plants

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and its specifications depends on the pH of root medium and its variations (Lindsay, 1979; Ullrich-Eberius et al., 1984; Frihata et al., 1992). High and low pH and also the presence of the hydroxides of some metals such as Ca^{+2} and Mg^{+2} in alkaline soils, Al^{+3} and Fe^{+3} in the acidic soils cause phosphate adsorption and precipitation (Holford, 1997; Lindsay et al., 1989). In anaerobic conditions, phosphates are more soluble than in aerated soils (Khalid et al., 1979). Cold and wet weather affects phosphorous uptake rate negatively (Sutton, 1969). Phosphorous uptake is enhanced by ammonium compared to $NO3^-$ nitrogen (Hoffmann et al., 2007). Root architecture is sensible to the internal status of phosphorous so that a deficit in phosphorous of root medium induces some morphological, biochemical and physiological variations as adaptive mechanisms to overcome phosphorous deficiency (Neumann et al., 2000; Neumann and Marinara 2002; Rouached et al., 2010). As for phosphate uptake, in two last decades, a number of proteins were isolated from some plants species (Smith et al., 1999; Mudge et al., 2002) and yeast (Romans et al., 1977), identified as phosphate transporters with different affinity for phosphate and their activity caused the depolarization of plasma membrane and acidification of cytosol (Ullrich and Novacky, 1990).

The principal aims of this study were to quantify and qualify the effects of various levels of two forms of orthophosphates on some growth parameters, rate of the uptake and distribution of the elements and pH variation of nutritive solutions.

Materials and Methods

Culture method

Seeds of *Zea mays* L. cv KSC 700 were provided from Seed and Tree Improvement Center, Karaj, Iran. Three hundred seeds of approximately the same size and weight were selected, sterilized with 1% sodium hypochlorite for 10 minutes and washed with sufficient quantity of distilled water, then cultured on water moistened filter paper in Petri dish and kept at room temperature. After five days, 48 seedlings of morphologically the same size were selected and transferred to black plastic pots- 3 seedlings per pot- containing sand free of any impurity, watered with Hoagland nutrient solution with the following chemical composition: KNO_3, 6mM; $Ca(NO_3)2.4H_2O$, 4 mM; $MgSO_4.7H_2O$, 1 mM; $MnCl_2.4H_2O$, 0.009 mM; H_3BO_3, 0.046 mM; $ZnSO_4.7H_2O$, 0.0008 mM; $CuSO_4.5H_2O$., 0.0003 mM; H_2MoO_4, 0.0001 mM ; NaFe DTPA, 0.075 mM. per liter. Plants were distributed in two experimental groups (I and II) , each one including three treatments (1, 3 and 5 mM KH_2PO_4 and K_2HPO_4 , respectively) with three replications of three plants .The plants were kept under the following conditions: light and dark periods,12h-12h; day and night temperature ,25-28C° ; light intensity at top of the plants, 10000 lux and relative humidity, 70% for 26 days. Twenty six day old plants were harvested and their growth parameters including stem and root length, stem diameter, dry and fresh weight of leaves, stem and root, chlorophyll (a, b) and the content of P, N, K^+, Na^+, Ca^{+2}, and Mg^{+2} of the above mentioned organs were determined.

The content of chlorophyll (chl a, chl b, and total chl) of the leaf were determined by solving chlorophyll of three discs of 9 mm in diameter detached from leaves of all treated plants and weighed, then immersed in 5 ml of 80% acetone, kept in dark at 4° C until they were depigmented completely. Density optic of all prepared chlorophyll solutions were recorded at λ=663 nm and 645 nm. Total content of chlorophyll and related fraction of chl a and chl b were calculated using the following equations and expressed by mg $/gr^{-1}$ fresh weigh (Arnon, 1949):

$$\text{Chl a} = 12.7(A)663_{nm} - 2.69(B)645_{nm} = C_{mg.L}{}^{-1}$$
$$\text{Chl b} = 22.9(A)645_{nm} - 4.68(B)663_{nm} = D_{mg.L}{}^{-1}$$
$$\text{Total Chl} = 2.20(A)645_{nm} + 8.02(B)663_{nm} = E_{mg.L}{}^{-1}$$

Nitrogen, phosphorous, potassium, sodium, calcium and magnesium of leaf, stem and root of the plants were measured separately according to the following methods and expressed by $mg.g^{-1}$ of dry weight: calcium and magnesium content by complexometry, potassium and sodium by flame photometry,

Table 1
Effects of various levels of KH_2PO_4 and K_2HPO_4 on some growth parameters of maize(*Zea mays*, CV. KSC700) and pH

Growth Parameters	Root Fresh Weight (g)	Stem Fresh Weight (g)	Leaf Fresh Weight (g)	Root Dry Weight (g)	Stem Dry Weight (g)	Leaf Dry Weight (g)
KH_2PO_4 (mM)						
1	2.87±0.25a	3.42±0.46a	4.81±0.23 ab	0.49±0.05 a	0.21±0.07ab	0.32±0.04ab
3	3.05±0.15a	3.75±0.39a	4.84±0.42 ab	0.5±0.05a	0.24±0.05a	0.36±0.08ab
5	2.58±0.38a	3.87±0.3a	5.15±0.55a	0.51±0.04a	0.22±0.12ab	0.4±0.01a
K_2HPO_4 (mM)						
1	1.19±0.19b	2.58±0.49b	4.25±0.26bc	0.16±0.1b	0.12±0.02ab	0.33±0.03ab
3	1.07±0.12b	3.23±0.25ab	3.95±0.24c	0.15±0.05b	0.11±0.04ab	0.3±0.01b
5	1.02±0.41b	4±0.5a	3.68±0.3c	0.13±0.06b	0.1±0.01b	0.28±0.07b

Growth Parameters	Total Dry Weight (g)	Root Length (cm)	Stem Length (cm)	Shoot Length (cm)	pH Initial	pH Final
KH_2PO_4 (mM)						
1	1.03±0.05a	21.33±0.58a	15.93±0.51a	62.5±0.87ab	6.5	6.5
3	1.10±0.11a	21.23±0.55a	15.88±0.45a	62.53±0.68ab	6	5.8
5	1.17±0.13a	20.5±0.5a	15.53±0.5a	62.67±0.76a	6.5	5.8
K_2HPO_4 (mM)	0.61±0.06 b	16.83±1.53b	15.2±0.36a	61.17±0.29c	7.5	7.7
1						
3	0.56±0.03b	16.5±1b	15.82±0.2a	61.5±0.5bc	7.5	7.9
5	0.52±0.07b	16.2±0.5b	15.8±0.2a	61.33±0.29c	7.5	8.3

In each column, difference between means with similar letter is not significant at $p<0.05$

Table 2
Effects of various level of KH_2PO_4 and K_2HPO_4 on chl a, chl b and total chl content of maize, *Zea mays*, CV. KSC700 (mg/g FW)

	Chl a	Chl b	Chl (a+b)
KH_2PO_4 (mM)			
1	1.72± 0.53 b	0.49± 0.38 b	2.21± 0.87 b
3	1.97± 0.72 ab	0.55± 0.31 b	2.52± 1.02 b
K_2HPO_4 (mM)			
5	2.83± 0.6 a	0.98 ± 0.16 a	3.81± 0.75 a
1	1.56± 0.27 b	0.51± 0.16 b	2.07± 0.42 b
3	1.56± 0.45 b	0.38± 0.07 b	1.94± 0.38 b
5	1.29± 0.22 b	0.49± 0.16 b	1.78± 0.06 b

In each column, difference between means with similar letter is not significant at $P<0.05$

total nitrogen by Kejeldal method-steam distillation (Amin and Flowers, 2004) and phosphorous content by molybdate-vanadate method (Kennedy, 1984). Two above mentioned experiments were laid down using a complete factorial design with one factor, differed from each other only by form of supplied orthophosphate. Consequently, all collected data were submitted to one way analysis of variance (ANOVA) and means were compared by Duncan's Multiple Range Test.

Results

On the basis of the amount of biomass per plant values as reported in Tables 1, the levels of mono and diphosphate used in the experiments were not limiting for the growth of the studied genotype of maize. The growth of plants was affected much more by the forms of orthophosphate than their levels in root medium and the plants supplied with mono phosphate were differentiated from those treated with similar levels of diphosphate by higher biomass quantity, longer and well proliferated root system, and higher total chlorophyll content (Table 2). Difference between means values corresponding the same parameters in plants

Table 3
Effects of various level of KH_2PO_4 and K_2HPO_4 on rate of uptake per plant per period of growth as unit of time in whole plant of maize (*Zea mays*,CV. KSC700)

	N	P	Mg^{2+}	Ca^{2+}	K^+	Na^+
KH_2PO_4 (mM)	14.7±0.16 c	5.9±0.18c	7.8±0.23c	12±0.04c	23.6±0.15c	8.6±0.13 b
1						
3	16.6±0.25 b	8.3±0.39 b	9.2±0.16 b	12.5±0.17 b	27.79±0.22 b	10.9±0.01 a
5	18±0.16a	9.5±0.09 a	11.3±0.2 a	14.4±0.06a	32.4±0.28a	11.04±0.16a
K_2HPO_4 (mM)						
1	8.5±0.05 d	2.1±0.03 d	4±0.09 d	6.4±0.04d	16.9±0.11 e	5.2±0.16c
3	7.7±0.14e	1.4±0.14e	3.5±0.39e	5.1±0.17e	21.8±0.22d	4.2±0.13d
5	6.7±0.23f	1.2±0.09 e	3±0.1f	4.8±0.11f	21.9±0.09d	3±0.005e

In each column, difference between means with similar letter is not significant at P<0.05

Table 4
Effects of various levels of KH_2PO_4 and K_2HPO_4 on content of some elements in stem of maize, *Zea mays*,CV. KSC700 **(**mg/g DW)

	N	P	Mg^{2+}	Ca^{2+}	K^+	Na^+
KH_2PO_4 (mM)	14.54±0.18 bc	5.24±0.05 c	8.57±0.08 cd	14.28±0.23 a	23.47±0.14 f	5.35±0.32 b
1						
3	15.4±0.45 a	6.78±0.3 b	10.25±0.05 b	12.5±0.27 b	24.17±0.29 e	6.75±0.22 a
5	15.65±0.36 a	7.99±0.15 a	12.63±1.91 a	12.73±0.25 b	31.61±0.45 c	6.75±0.25 a
K_2HPO_4 (mM)						
1	14.34±0.21 bc	3.33±0.11d	10±0.29 bc	11.67±0.11 c	26.91±0.12 d	3.75±0.11 c
3	14.66±0.29 b	2.83±0.04e	9.8±0.2 bcd	10.9±0.09 d	48.36±0.21 b	3.02±0.47 d
5	14.03±0.05 c	2.24±0.15f	8.4±0.23 d	10±0.05 e	57.08±0.07 a	3.15±0.07 d

In each column, difference between means with similar letter is not significant at P<0.05

supplied with similar levels of both forms of orthophosphate were significant at P<0.05. In plants fed with mono –phosphate, half of total biomass was exported into root system, while in plants supplied with diphosphate it did not exceed 20% of their total biomass.

As for the rate of the uptake of P, N, Ca^{+2}, Mg^{+2}, K^+ and Na^+, on the basis of the amount of each element per plant per period of growth as time unit, it appears that the rate of uptake of the elements increased according to monophosphate level and decreased significantly with the increase in diphosphate level, except for K^+ (Table 3). The rate of K^+ uptake increased with increasing its content in root medium but its relative rate of the uptake was higher in plants supplied with monophosphate. In both groups, rate of the uptake of other elements followed that of the uptake of phosphate. Difference between means of each element within each group and among two groups were significant at P<0.05 as shown in Tables 3 and 4. In both groups of the plants, the content of the elements in leaf was more or less higher than that of other parts of the plants, except for Mg^{+2} and Ca^{+2} in plants fed with monophosphate (Tables 4 ,5 and 6).

The pH of nutrition solutions enriched with monophosphate decreased half of a unit pH and increased the same level of diphosphate in relation to the corresponding initial pH (Table 1).

Discussion

The plants of the studied cultivar of maize (*Zea mays* L.cv.ksc 700) supplied with monophosphate have produced about twice more biomass than those treated with similar levels of diphosphate. High amount of biomass per plant, high total content of chlorophyll and especially that of phosphorous in leaves and whole plant could be considered as rational index representing high rate of the photosynthesis in the plants as shown in Tables 1, 2, and 4). There is a close and positive correlation between increasing leaf phosphate content and the rate of

photosynthesis (Usuda and Shimogawara, 1991; Susanne et al 2006). High photosynthetic activity of the plants fed with monophosphate needed to have a balanced internal nutrient status by way of enhancing the growth rate of root system through stimulating rhizogenic activity of the pericycle that resulted in highly proliferated root system formation. Such a proliferated root system formation has been previously reported in some plant species grown in fertile soils (Christie and Moorby, 1975) and considered as a characteristic of the plants that compete effectively in high nutrient environments (Chapin, 1980). Root proliferation process facilitates acquisition of ions by increasing contact surface of the root with different ions in the root medium. Exporting 50% of total photosynthetic assimilates into root provides not only energy requirements but also supplies necessary substances for root elongation and lateral root formation (Table 1). Significant difference between means values related to the same growth parameters of the plants treated by similar levels of two forms of orthophosphate and high value of the means concerning those of the plants fed with monophosphate confirm the suitability of monophosphate under our experimental conditions (Table 1).

As for the rate of uptake of the measured elements such as P, N, Ca^{+2}, Mg^{+2}, K^{+} and Na^{+}, there were a positive and significant correlation between the rate of absorption of the elements and monophosphate levels in root medium; however, such a correlation was significantly negative even for phosphate except for K^{+} in plants treated with diphosphates as shown in Table 3. In both groups, the rate of uptake of nitrate followed the rate of orthophosphate uptake (Table 3). High content of nitrogen in plants fed with monophosphate might be considered as consequence of high rate of nitrate reductase activity in root stimulated by increasing phosphate uptake rate presented in Tables 3. Such a relation between nitrate and phosphate has been previously reported (Gniazdowska and Rychter, 2000; Gniadoweska et al., 1998). The

Table 5
Effects of various levels of KH_2PO_4 and K_2HPO_4 on content of some elements in leaf of maize, *Zea mays*,CV. KSC700 (mg/g DW)

	N	P	Mg^{2+}	Ca^{2+}	K^{+}	Na^{+}
KH_2PO_4 (mM)						
1	18.25±0.18 b	7.55±0.11 c	6.75±0.23 c	11.33±0.1bc	31.34±0.23 e	14.84±0.18 c
3	18.43±0.28 b	10.28±0.19 b	7.6±0.15 b	11.5±0.09 b	35.2±0.07 d	17.5±0.45 a
5	19.23±0.21 a	11.07±0.22 a	9.09±0.07 a	15.15±0.05 a	36.75±0.1c	16.15±0.1 b
K_2HPO_4 (mM)						
1	17.22±0.2 c	4.39±0.16 d	6.67±0.32 c	11.11±0.03 c	40.83±0.14 b	14.73±0.05 c
3	16.56±0.38 d	3.67±0.09 e	6.3±0.42 c	10.71±0.09 d	49±0.01 a	13.5±0.05 d
5	16.19±0.14 d	3.3±0.28 e	6.43±0.02 c	9.97±0.26 e	49.19±0.35 a	10.12±0.06 e

In each column, difference between means with similar letter is not significant at P<0.05

Table 6
Effects of various levels of KH_2PO_4 and K_2HPO_4 on content of some elements in root of ***Zea mays***,CV. KSC700 **(**mg/g DW)

	N	P	Mg^{2+}	Ca^{2+}	K^{+}	Na^{+}
KH_2PO_4 (mM)						
1	10.47±0.19c	4.48±0.32 c	7.5±0.07 a	9.8±0.24b	14.5±0.51 f	5.14±0.06c
3	11.5±0.01 b	5.36±0.28 b	7.2±0.09 a	10.14±0.05ab	16.53±0.4d	5.4±0.28 c
5	12.74±0.27a	5.94±0.42a	7.38±0.12a	10.23±0.2a	17.63±0.32c	7.32±0.22 a
K_2HPO_4 (mM)						
1	10.39±0.46c	2.49±0.06d	3.06±0.06b	8.75±0.22 c	15.47±0.18e	7.03±0.05a
3	10±0.1 c	1.19±0.06e	2.8±0.11 bc	5.51±0.32 e	19.33±0.12b	6.3±0.08 b
5	9.31±0.24 d	1.38±0.05e	2.7±0.33c	8±0.1 d	22.5±0.07 a	4.51±0.32 d

In each column, difference between means with similar letter is not significant at P<0.05

content of the elements in leaves of both groups were more or less higher than that of other parts of the plants. High content of elements has been previously reported in leaves for nitrogenous solutes under high rate of the transpiration by Pate (1980) and phosphate (Mimura et al., 1996). Recent molecular genetics studies on Arabidopsis have shown that phosphate concentration in the leaf tissue is regulated genetically (Poirier et al., 1991; Delhaize and Randall, 1995). Accordingly, the plant cells usually benefit a negative electrochemical potential that allows the cations to be absorbed passively. In contrast, it appears as a barrier for the anions uptake. Consequently, anion uptake become active and demands a supply of energy and the intervention of H^+/anion co$^-$ transporters. The function of the co-transporter requires acidulation of root medium by involving H^+/pumps activity and or ion exchange across epidermal cell membrane. The epidermis cells and those of peripheral layers of root cortex of maize have the ability to decrease apoplasm pH as a response to the increase in K^+, Ca^{+2} level and high pH of root medium (Felle, 1998).

As for the uptake of the related forms of orthophosphate, it has been suggested that two kinds of co-transporters such as H^+/ $H_2PO_4^-$ and $2H^+/HPO_4^{2-}$ take part in mono and diphosphate uptake respectively. The decrease in the pH of the root medium containing monophosphate is a good evidence showing that the root of the plants have excreted much more H^+ than those treated with diphosphate and used less H^+ as companion ion for monophosphate uptake therefore, protected the polarization status of plasma membrane. By contrast, the root medium of the plants fed with diphosphate had been more alkaline. This could be considered as a sign of depolarization of the plasma membrane resulted from the insufficiency of H^+ produced by proton pumps of plasma membrane to provide necessary H^+ to the co-transporters $2H^+/ HPO_4^{2-}$ responsible for the transport of diphosphate across cell membrane and compensate the effect of NO_3^- uptake in alkalization of root medium. In consequence, it might have lowered the solubility of some of the salts in root medium and, as a result, affected their uptake rate negatively as shown in Tables (3, 4). In the end, the results of this study allow us to conclude as follow:

- On the basis of the amount of biomass per plant and some other studied growth parameters and internal nutritional status of the plants, it seems that the studied cultivar of maize is highly sensible to the form of orthophosphate than its administrated levels and prefers to be supplied with monophosphate rather than diphosphate.
- Uptake of diphosphate leads the pH of root medium to be more alkaline. Consequently, it decrease not only its proper uptake rate but also that of other elements by lowering their solubility.
- Low rate of diphosphate uptake due to the inability of root system of the plants in acidification of rhizosphere might be related to the insufficiency of proton excretion activity of root and low affinity of co-transporters for diphosphate and / or low density of the related co-transporters in the root system.

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