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PHOSPHORUS-ZINC INTERACTION

I. SITES OF IMMOBILIZATION OF ZINC IN MAIZE AT A HIGH LEVEL OF PHOSPHORUS

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SUMMARY

Pot experiments, using Zn^{65} were conducted on maize (Ganga-5) with different treatment combinations of phosphorus and zinc in sand and soil culture for two consecutive years (1972 and 1973). The deficiency of zinc was induced and a significant reduction in yield was recorded at a high level of P application. In the zinc-deficient plants, the concentration of zinc significantly increased in the roots and the nodes and decreased in the leaves and the internodes. Contrarily, in healthy plants the distribution pattern of the level of zinc and phosphorus in the roots, nodes, internodes, and leaves was recorded to be acropetal and basipetal respectively. In zinc-deficient plants, a significant rise in the pH of leaves and a decrease in the zinc uptake were also recorded. The autoradiographs and radiochemical analysis showed that a high level of P rendered the applied zinc unavailable to the leaves by immobilizing almost 40 per cent or more of the total absorbed zinc in the roots and 20 per cent or more at the nodes of the stem than that with 12 and 6 per cent in the roots and the nodes respectively at the normal dose of P supply (30 ppm P).

INTRODUCTION

The high dosages of phosphorus in the nutrient media have been reported to accentuate zinc deficiency [3, 12, 14, 19] and lead to reduced growth and to a reduced yield potential of crops. The site of phosphorus–zinc interaction has been recognized in the root zone [13, 17, 18] and the zinc deficiency has been attributed to the immobilization of zinc owing to the increase in the concentrations of phosphorus in

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the roots above the threshold values [4]. Since the concentration of phosphorus in the shoots is raised manifold by giving high phosphorus treatment [4 14 20]. Therefore zinc immobilization could be expected somewhere in the shoot too, but little work in this direction has been done so far. Furthermore, nothing is known about the biochemical causes for zinc immobilization because of the high phosphorus content in the plants. In the present investigations, an attempt has been made to examine the effect of different doses of phosphorus on the uptake, translocation and distribution of zinc in plants by using radioactive ^{65}Zn in sand and soil culture conditions. The pH of the plant cell-sap, which is an important indicator of the organic-acid metabolism and is a regulator of the activities of enzymes involved in the physiological and biochemical processes has also been determined.

MATERIAL AND METHODS

Pot experiments were conducted in sand and soil culture during 1972 and 1973 by growing maize (Ganga-5 variety). For the removal of contaminants, the sand was washed with dilute HCl (5 per cent), followed by deionized water and sterilized before filling it in one-kg-capacity polythene pots. In sand-culture experiments, sodium bicarbonate-extractable phosphorus and DTPA-extractable zinc were built up to levels of 5 and 0.2 ppm respectively by adding KH_2PO_4 and $ZnSO_4$, as a basal dose in each pot. This was done with a view to avoiding the appearance of P and zinc deficiency at P_0Zn_5 and Zn_0P_{30} treatments respectively. A basal dose of 100, 30, 15, 12, 10, 2, 1, 1, and 0.5 ppm N, K, Ca, Mg, Fe, Mn, Cu, B, and Mo was applied to each pot. The treatments consisted of different combinations of 0, 30, 60 and 120 ppm phosphorus and 0, 5 and 10 ppm zinc. The zinc was added in the form of radioactive $Zn^{65}SO_4$.

In soil culture study, the loamy sand soil (pH 8.2) was filled in 3-kg capacity earthenware pots lined with polythene. The soil contained 1.6 per cent calcium carbonate, 0.3 per cent organic carbon and 40, 1.4, 7.9, 23 and 0.62 ppm P_2O_5, copper, manganese, iron and zinc respectively. Basal doses of 100 and 30 ppm N and K respectively were applied to each pot. Treatment combinations of P and Zn in soil culture were the same as in sand culture. Each treatment was replicated four times in a completely randomized-block design. A separate experiment by adding untagged zinc sulphate was also performed. The pots were irrigated with deionized water.

Two seedlings in each pot of soil culture and one in sand culture were maintained and allowed to grow up to 60 days. The dry-matter production of plants was recorded. Autoradiographs of plants uprooted from the sand-culture experiment at the age of 60 days were taken by following the technique described by Overman and Clark [16]. The plants were divided into roots, stem, node, internode, and leaf + leaf sheath and the activity of Zn^{65} was recorded with a gamma counter. The concentration of Zn in these parts was determined with the Techtron AA-120 atomic absorption spectrometer. The phosphorus concentration in plant tissues was estimated by employing chloromolybdic acid-reductant method. [10].

From non-radioactive experiments, the pH of the cell-sap of leaves was determined in case of plants at 7, 14, 21, 28 and 42 days old. All the green leaves of the plant were cut into small pieces and homogenized. One gram of leaf pieces was crushed in 20 ml of distilled water (pH 6.7) and the pH of the extract was measured with a pH meter. The sampling time of leaves at all the growth stages of the plant was strictly kept the same. This observation was recorded during 1973 only.

RESULTS AND DISCUSSION

It is evident from Fig. 1 that the maximum production of dry matter was recorded in the P_{30}, Zn_5 treatment. A significant reduction in the production of dry matter of maize, and the zinc-deficiency symptoms were recorded in $P_{60}Zn_0$, $P_{120}Zn_0$ and $P_{120}Zn_5$ treatments in soil culture. In sand culture, the deficiency symptoms were recorded at $P_{120}Zn_0$ and $P_{120}Zn_5$ treatments. But for this variation, the results of sand and soil culture were quite similar. The appearance of the symptoms of zinc deficiency at a relatively low level of P application in soil culture (60 ppm P) was due to the initial high P concentration (40 ppm P_2O_5) in the soil used for the pot-culture experiment. The appearance of deficiency symptoms at a high level of P can be attributed to the inhibitory effect of P in zinc translocation (Fig. 3) to the sites of active plant metabolism. A similar hypothesis has also been proposed by by Adriano *et al.*[1] and Boawn and Brown [2]. The reduction in yield owing to low zinc concentration in the leaves may be attributed to the reduction in the activity of the CO_2-fixing enzymes, ribulose 1–5 diphosphate carboxylase and carbonic anhydrase during the process of photosynthesis [7 8 11].

In the treatment with a high level of phosphorus, where zinc deficiency was recorded, the pH of the cell-sap of 14 and 21-day-old leaves was significantly higher than that of the healthy plants. The difference was more pronounced at 21 days (Fig. 2). In such plants, the concentration of phosphorus increased (Tables 1 and 2), whereas the concentration and the uptake of zinc decreased significantly (Fig. 1). The rise in the pH of leaves indicates that the synthesis of

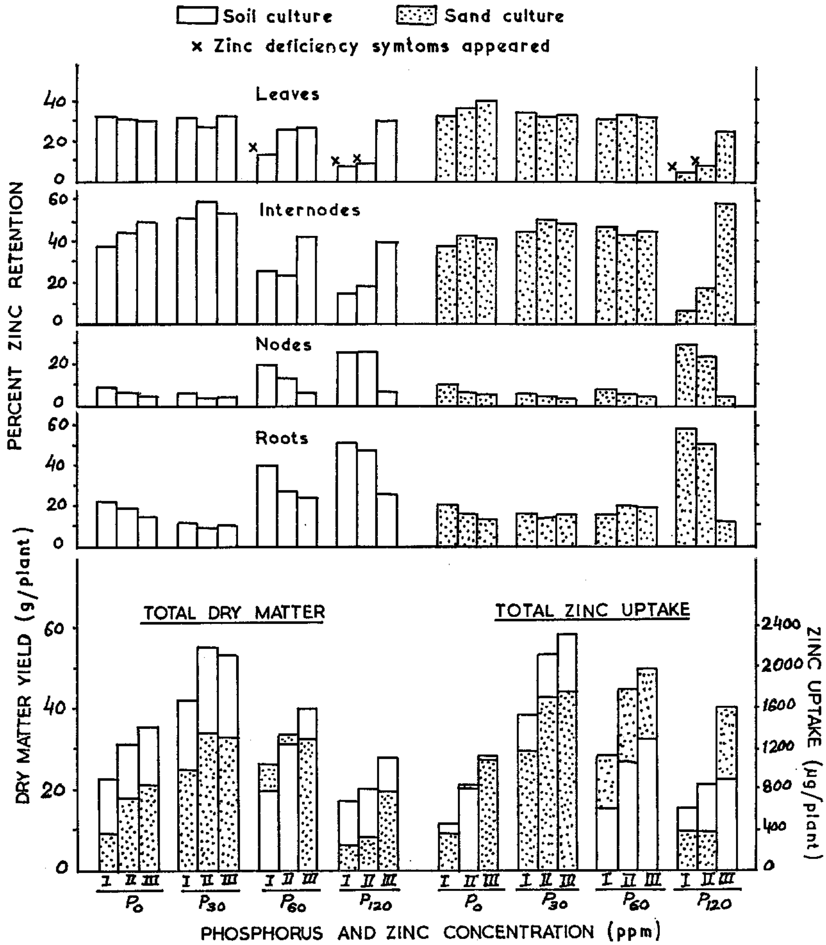

Fig. 1. Dry matter production, zinc uptake and percent retention of zinc in different plant parts at the age of 60 days at the various levels of phosphorus and zinc (av. values of 1972 and 1973). (C.D. at 5% (for combined analysis) for Total dry wt. = 2.1, Total zinc uptake = 10.1). I = 0, II = 5, III = 10 ppm Zn.

organic acid is retarded in zinc-deficient plants. The rise in the pH with a higher uptake of anions than that of cations is well known [5 9]. However, from these data, it can be speculated that some organic-acid complex may be involved as a carrier for the absorption of zinc whose synthesis is reduced at a high phosphorus level and as a result of which the uptake of zinc is decreased significantly.

A detailed study of P/Zn ratio in the different parts of maize at

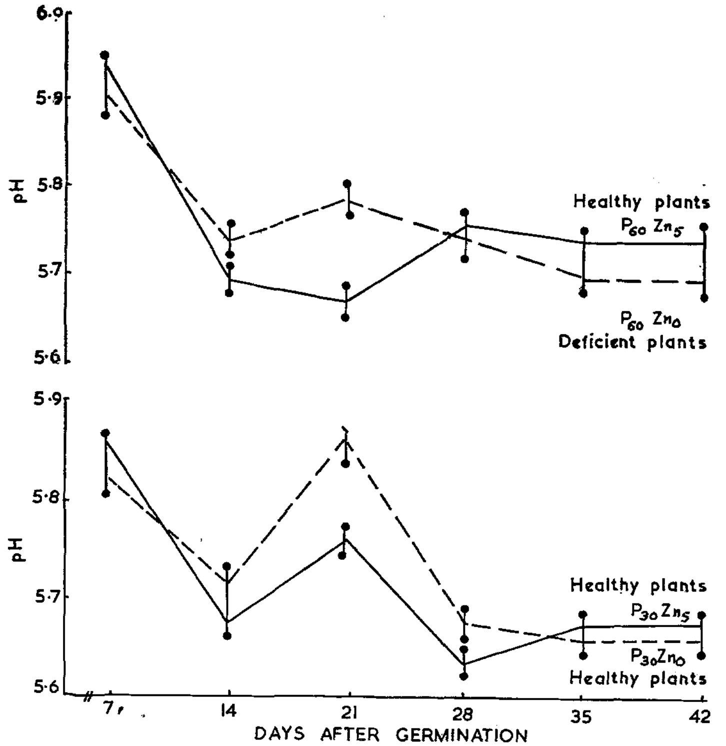

Fig. 2. pH of the cell sap of leaves at different P and Zn levels (ppm).

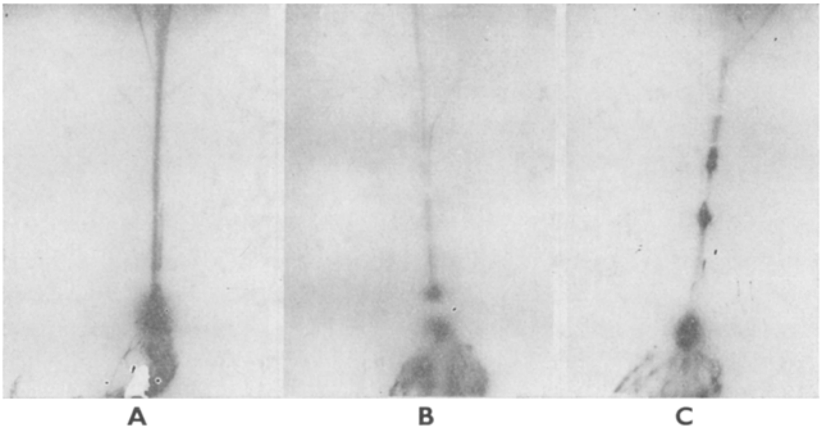

Fig. 3. Autoradiographs showing the distribution of zinc in the different parts of maize at various levels of phosphorus and zinc in sand culture. A – $P_{30}Zn_0$, B – $P_{120}Zn_0$, C – $P_{120}Zn_5$

TABLE 1

Effect of different doses of phosphorus and zinc on P and Zn content (ppm) and P/Zn ratio in the different parts of maize at the age of 15 days (Average values of 1972 and 1973)

Treatment (ppm)	Sand culture						Soil culture					
	Roots		Stem		Leaves		Roots		Stem		Leaves	
	P	Zn	P	Zn	P	Zn	P	Zn	P	Zn	P	Zn
P_0Zn_0	1600	80	3250	65	3016	58	980	60	1688	40.2	1296	38.4
	(20)		(50)		(52)		(16)		(42)		(40)	
P_0Zn_5	2090	110	3082	67	2820	60	1021	70	2052	45.6	1680	40.7
	(19)		(46)		(47)		(14)		(45)		(42)	
P_0Zn_{10}	1920	120	3640	80	3120	65	1100	75	2982	50.0	1820	42.0
	(16)		(48)		(48)		(15)		(41)		(43)	
$P_{30}Zn_0$	1900	96	2108	68	2400	60	1120	75	2640	55.0	2500	50.0
	(20)		(31)		(40)		(15)		(48)		(50)	
$P_{30}Zn_5$	2820	141	3096	86	3120	65	1400	70	3088	60.0	2840	52.6
	(20)		(36)		(48)		(20)		(48)		(54)	
$P_{30}Zn_{10}$	2016	112	2944	92	3375	75	1440	80	3100	70.0	2870	60.0
	(18)		(32)		(54)		(18)		(41)		(48)	
$P_{60}Zn_0$	2721	131	3080	112	3360	42	2300	115	4240	80.0	4681	15.1
	(21)		(28)		(80)		(20)		(53)		(310)	
$P_{60}Zn_5$	3600	120	4000	160	4128	86	1575	105	2000	80.0	1600	32.0
	(20)		(25)		(48)		(15)		(25)		(50)	
$P_{60}Zn_{10}$	2300	230	2975	175	3200	80	1688	80	2300	70.0	2400	60.0
	(20)		(17)		(40)		(21)		(31)		(40)	
$P_{120}Zn_0$	5200	260	6050	275	7800	15	3212	212	4660	218.0	5000	10.1
	(20)		(22)		(520)		(15)		(20)		(500)	
$P_{120}Zn_5$	6000	300	7550	285	9000	18	2681	200	3421	190.0	4060	13.1
	(20)		(30)		(500)		(13)		(17)		(312)	
$P_{120}Zn_{10}$	4240	212	5000	200	5168	76	1278	80	2782	65.0	2012	40.0
	(20)		(25)		(68)		(17)		(43)		(50)	

( ) Values in parenthesis indicate P/Zn ratio.
C.D. at 5% (for combined analysis).
for zinc = 3.7 and P = 5.7 (Soil culture).
for zinc = 2.9 and P = 4.8 (Sand culture).

15 and 60* days of growth (Tables 1 and 2) reveals that the P/Zn ratio of leaves at both the growth stages and that of the internodes also at 60 days of plant growth plays a significant role in understanding P–Zn interaction and its consequent effect on crop production. The ratio of phosphorus to zinc in zinc-deficient plants was more than 310 in the leaves at 15 days' growth, whereas it was more than 260 at 60 days' age. This is in line with some earlier reports[4 15 20].

* P/Zn ratio at 60 days could be calculated from Table 2.

TABLE 2

Dry matter production (g/plant) of different plant parts of maize and their phosphorus and zinc concentration (ppm) at the age of 60 days (average values of 1972 and 1973)

Treatments (ppm)	Attributes	Soil culture				Sand culture			
		Roots	Nodes	Internodes	Leaves	Roots	Nodes	Internodes	Leaves
P_0Zn_0	Dry wt.	2.5	2.0	10.0	8.0	1.5	1.0	3.6	3.0
	P	450	570	534	564	968	976	985	995
	Zn	40	18	18	18	50	38	38	39
P_0Zn_5	Dry wt.	3.0	2.0	16.0	10.5	2.0	1.2	8.0	6.8
	P	780	840	765	749	1800	2100	2640	2150
	Zn	50	22	22	23	60	42	44	43
P_0Zn_{10}	Dry wt.	3.1	2.0	18.0	12.0	2.1	1.2	10.0	8.0
	P	720	600	620	645	1750	1196	1350	1888
	Zn	52	30	31	28	70	46	45	41
$P_{30}Zn_0$	Dry wt.	3.5	2.0	20.0	15.5	2.5	1.4	11.2	10.0
	P	862	1404	1369	1382	1120	1410	1426	1680
	Zn	50	42	40	30	70	46	45	41
$P_{30}Zn_5$	Dry wt.	3.5	2.1	31.0	18.0	2.8	1.5	17.6	12.2
	P	922	924	1128	1532	968	1000	1029	1320
	Zn	55	42	41	32	80	50	49	44
$P_{30}Zn_{10}$	Dry wt.	3.4	2.0	30.0	18.0	2.8	1.0	17.0	12.2
	P	900	1128	1478	1537	1140	1248	1500	1680
	Zn	70	48	41	41	90	48	50	48
$P_{60}Zn_0$	Dry wt.	3.0	2.0	9.0	5.8	2.1	1.5	12.2	10.3
	P	1020	1400	1657	3124	1040	1500	1500	1564
	Zn	80	60	18	12	80	50	43	34
$P_{60}Zn_5$	Dry wt.	3.6	2.1	14.5	10.8	3.0	1.6	15.8	13.1
	P	1124	1190	1240	1415	200	2352	2400	2700 2
	Zn	80	70	25	24	110	56	48	45
$P_{60}Zn_{10}$	Dry wt.	3.6	2.2	20.6	14.0	3.1	1.5	16.2	11.2
	P	1170	1324	1426	1735	1080	1160	1210	1344
	Zn	85	34	27	25	120	58	55	56
$P_{120}Zn_0$	Dry wt.	3.0	1.4	8.0	4.5	1.6	0.8	2.1	1.5
	P	1561	1213	3021	3676	2800	2615	4550	5170
	Zn	100	110	11	10	140	145	13	11
$P_{120}Zn_5$	Dry wt.	3.2	1.8	9.5	5.2	1.5	1.0	3.2	2.4
	P	1201	1440	2861	3844	2640	2914	4140	4940
	Zn	121	118	15	14	132	94	20	13
$P_{120}Zn_{10}$	Dry wt.	3.1	2.0	12.4	10.4	1.8	1.2	9.2	6.8
	P	1024	1514	1602	1916	1100	1440	2422	2900
	Zn	70	24	28	25	110	60	64	58

C.D. at 5% (for combined analysis).
for zinc = 2.8 and P = 7.8 (Soil culture).
for zinc = 2.1 and P = 6.2 (Sand culture).

However, when the zinc supply is increased (Zn_{10}ppm), the P/Zn ratio drops and plant do not exhibit deficiency symptoms even under the treatment with the highest level of P.

It is interesting to note that the concentrations of zinc and phosphorus were found in acropetal and basipetal positions respectively in plants under the $P_{30}Zn_5$ treatment (normal fertility level) (Tables 1 and 2). But this trend changed, when the plant became deficient in zinc. It contained a very low concentration of zinc (9–15 ppm) in the leaves, but a very high concentration in the roots and the nodes of the stem (56–218 ppm). Under such conditions, 40 and 20 per cent or even more of the total absorbed zinc was retained in the roots and the nodes respectively (Fig. 1). At the normal phosphorus level (P_{30} ppm) the percentage retention of the total zinc uptake in the roots and the nodes did not exceed 15 and 6 respectively. This result is in agreement with those of Carroll and Loneregan [6] who reported that the legumes retained 35 per cent of the total absorbed zinc in the roots when the growth was limited by its deficiency as compared with 18% under the optimum supply of zinc. In the present experiment at severe deficiency, the concentration of zinc in the nodes was found to increase considerably, and became equal to that of the roots and sometimes even higher than it (Fig. 3). The incorporation of radioactive zinc into the different parts of the zinc-deficient maize plant also gave the same trend (Table 3). The results thus suggest that the sites of immobilization of zinc at the high phosphorus level are not only the roots but also the nodes of the stem.

In the phosphorus-induced zinc deficiency, in plants, the concentration of P decreased in the node, whereas it increased in the internodal region. There is a possibility that in such plants, the P-constituted organic substances are synthesized and accumulated at the internodes. This accumulation impairs the translocation of zinc and when the zinc status increases, it competes with synthesized organic complex and moves upwards. This may be the reason for the accumulation of zinc at the nodes (Fig. 3), where the concentration of phosphorus is low (Table 1 and 2). A detailed investigation is needed for the identification of P-constituted organic complex or other constituents which might be synthesized and accumulated and hinder the translocation of zinc in the plants.

TABLE 3

Effect of phosphorus on the incorporation of $Zinc^{65}$ (counts/200 seconds) in different parts of maize at 60 days growth (Average value of year 1972 and 1973)

Treatments (ppm)	Soil culture				Sand culture			
	Roots	Nodes	Internodes	Leaves	Roots	Nodes	Internodes	Leaves
P_0Zn_0	1000	980	970	970	1200	1180	1160	1180
P_0Zn_5	1200	1000	1060	1080	1410	1220	1250	1270
P_0Zn_{10}	1225	1160	1130	1180	1430	1320	1330	1360
$P_{30}Zn_0$	1100	1040	1060	1080	1320	1250	1260	1270
$P_{30}Zn_5$	1620	1380	1300	1340	1810	1560	1510	1530
$P_{30}Zn_{10}$	1660	1400	1340	1380	1850	1620	1530	1560
$P_{60}Zn_0$	1160	1100	300	210	1380	1320	980	1020
$P_{60}Zn_5$	1900	1600	800	760	2090	1810	1060	1110
$P_{60}Zn_{10}$	1300	900	890	900	1520	1120	1090	1100
$P_{120}Zn_0$	1200	1800	390	120	1410	2010	400	200
$P_{120}Zn_5$	2000	2500	670	440	2210	2710	510	310
$P_{120}Zn_{10}$	2500	2300	920	870	2710	2560	1140	1070

C.D. at 5% (for combined analysis) 80.4 (Soil culture); 60.2 (Sand culture).

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