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DIFFERENTIAL SUSCEPTIBILITY OF TWO VARIETIES OF COWPEA (*VIGNA UNGUICULATA* (L) WALP) TO PHOSPHORUS-INDUCED ZINC DEFICIENCY

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SUMMARY

In a soil pot culture experiment conducted under greenhouse conditions, two varieties of cowpea (*Vigna unguiculata* (L) Walp) were found to be differentially susceptible to phosphorus-induced zinc deficiency. Although phosphate application, in the absence of applied zinc, caused growth disorder in both the varieties, the symptoms were more virulent, appeared early and were induced with both 25 and 75 ppm phosphorus in variety HFC–42–1, as against FOS–1, in which case zinc deficiency symptoms appeared under $P_{75}Zn_0$ treatment only. Contrary to the expectation the more susceptible variety was found to have higher zinc concentration in its shoots and thus the differential susceptibility of these varieties of cowpea could not be related to their zinc absorption characteristics. High phosphate application caused efflux of zinc during 16–32 day growth in both the varieties. However, higher sensitivity to phosphorus-induced zinc disorder in variety HFC–42–1, appeared to be due to nearly 2 times higher P concentration in this variety as against FOS–1. The results indicate that variety HFC–42–1 has a greater need for zinc because of its inherent capacity to accumulate more phosphorus. Dry matter yield and phosphorus and zinc content in the varieties were also differentially affected by the applications of both phosphate as well as zinc.

INTRODUCTION

Differences in the 'feeding power' among varieties of a plant species have, in certain cases, been found to be greater than those existing between related genera [9]. These differences can be attributed to genetic variation and may presumably arise from differences in the

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efficiency of absorption and utilization of nutrient constituents of the soil[7]. Hence, the use of genetic variability in plant breeding to 'tailor' plants to fit problem soils has been regarded by Brown *et al.*[4] as a way of accelerating the natural evolutionary process. It is also known that phosphate fertilization under certain soil environmental conditions induces or accentuates zinc deficiency in plants[11]. However the degree of susceptibility to phosphorus-induced zinc deficiency may also vary in different varieties of a species. Cowpea being an important pulse crop of tropical countries, and grown for both grain and fodder purposes, requires much attention as regards its phosphate fertilization. Since a large area of cultivable land in Haryana is zinc deficient[14] it was considered essential to study the relationship of phosphorus and zinc nutrition in some improved varieties of cowpea.

MATERIALS AND METHODS

Two fodder varieties of cowpea (*Vigna unguiculata* (L) Walp), namely FOS-1 and HFC-42-1, were raised under greenhouse conditions in polythene lined, undrained culture vessels, holding 1.5 kg of pulverised and airdried soil. The soil used was light brown loamy sand (pH 8.4, Olsen's P 7.5 ppm, and 0.1 *N* HCl-soluble Zn 0.67 ppm) collected from Rawalwas, Haryana.

The treatments included all possible combinations of three levels of phosphorus (0, 25, 75 ppm) and two levels of zinc (0, 10 ppm) with six replications for each treatment. All the pots received a basal dose of N, K, Mg, Fe and Cu at a rate of 50, 50, 10, 5 and 1.25 ppm, respectively. The nutrient treatments and the basal applications were provided to the pots one week prior to sowing of seed, and mixed throughly with the entire bulk of the soil. During this period pots were irrigated to approximately 0.1 b suction twice, with alternate drying. Ten seeds of uniform size were sown in each pot. On the fourth day of emergence, seedlings were thinned to four per pot. The soil cultures received deionized water for irrigation throughout the growth of the plants Three replicates selected at random from each treatment were harvested 16 days after sowing and the rest after 32 days.

Harvested plants were weighed immediately for their fresh weight and then washed with deionized and double distilled water. Plant samples were dried in a ventilated oven at 70°C for 48 hours and their dry weights were recorded. During the entire period of growth, the plants were regularly checked for the appearance of phosphorus and zinc deficiency symptoms. On the day of each harvest, shoot lengths were also recorded. Since there was considerable leaf shedding in plants showing nutrient disorder, each fallen leaf was collected, washed, dried and then included with the corresponding sample. Plant samples were digested in HNO_3:$HClO_4$ (5:1) mixture. Zinc was determined on

'Varian Techtron' atomic absorption spectrophotometer and phosphorus by Chlorostannous molybdophosphoric blue method as outlined by Johnson and Ulrich[8].

RESULTS

Visual symptoms, growth and forage yield

i. Symptoms. Visual response to phosphate application was evident as early as 7 to 9 days after sowing, in both the varieties of cowpea. However, there was a distinct varietal difference in the type of phosphorus deficiency symptoms: plants of FOS-1 variety were pale and yellowish, where as those of HFC-42-1 exhibited a pronounced bluish dark green color. This difference was over and above the normal color difference that exists in the foliage of these two varieties under conditions of healthy growth. Application of zinc in the absence of added phosphate accentuated these symptoms in the plants.

Phosphate application in the absence of applied zinc caused the appearance of zinc deficiency symptoms in both the varieties (Fig. 1). Detailed characteristics of these symptoms have been reported by Safaya *et al.*[15] In variety HFC-42-1, symptoms were induced with both 25 and 75 ppm phosphorus (in the absence of zinc) and started appearing as early as 9 days after sowing. In $P_{75}Zn_0$ plants of this variety, scattered dark brown irregular spots appeared first on the primary leaves and two days later on the first trifoliate. However, in $P_{25}Zn_0$ plants the trifoliate leaf was the first affected and only then the symptoms appeared on the primary leaves. In variety FOS-1 zinc deficiency symptoms appeared 14 days after sowing, and on 75 ppm phosphorus-supplied plants only. The symptoms were more virulent in variety HFC-42-1 as compared to FOS-1 suggesting a higher susceptibility of the former variety to phosphorus-induced metabolic disorder. In either case, the affected leaves dropped from the plant causing a premature loss of green fodder.

ii. Shoot length. The effects of phosphorus and zinc treatments on shoot length were not significantly manifest during early growth of plant, except in variety FOS-1, which showed a response to phosphorus alone. By the second harvest, shoot length of cowpea varieties was visibly affected by both phosphorus and zinc but the phosphorus × zinc effect was significant for variety HFC-42-1 only.

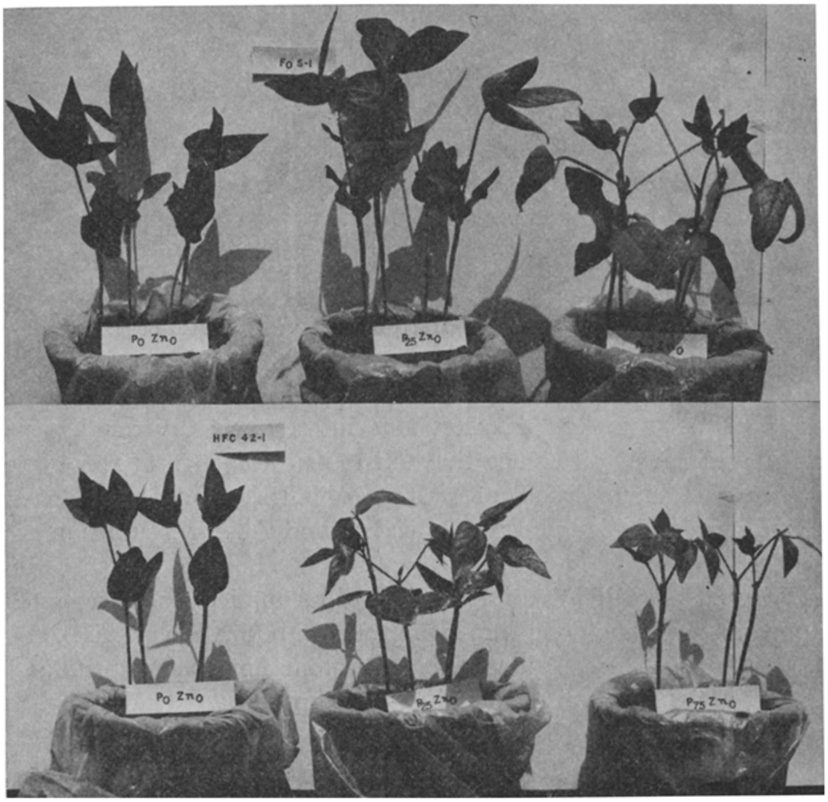

Fig. 1. Response of FOS-1 (*above*) and HFC 42-1 (*below*) varieties of cowpea to increasing levels of phosphorus in the absence of applied zinc.

Maximum shoot length was attained by plants under $P_{25}Zn_{10}$ treatment. Due to severe retardation in growth as well as premature shedding of the leaves, the shoot length values of P_0Zn_{10} and $P_{75}Zn_0$ plants at 32 days growth were lower than those recorded at 16 days.

iii. Forage yield. Growth response of cowpea varieties to phosphate application was evinced only in the presence of applied zinc; where as in the absence of the latter, plants were adversely affected as the phosphorus level was increased to 75 ppm (Fig. 1, 2). On an average (irrespective of variety and zinc level) dry weight of shoot increased to a maximum with 25 ppm phosphorus dose (Table 1). Examination of fresh and dry weights of plants at 16 and 32 day growth stages indicated a severe retardation in the growth rate of

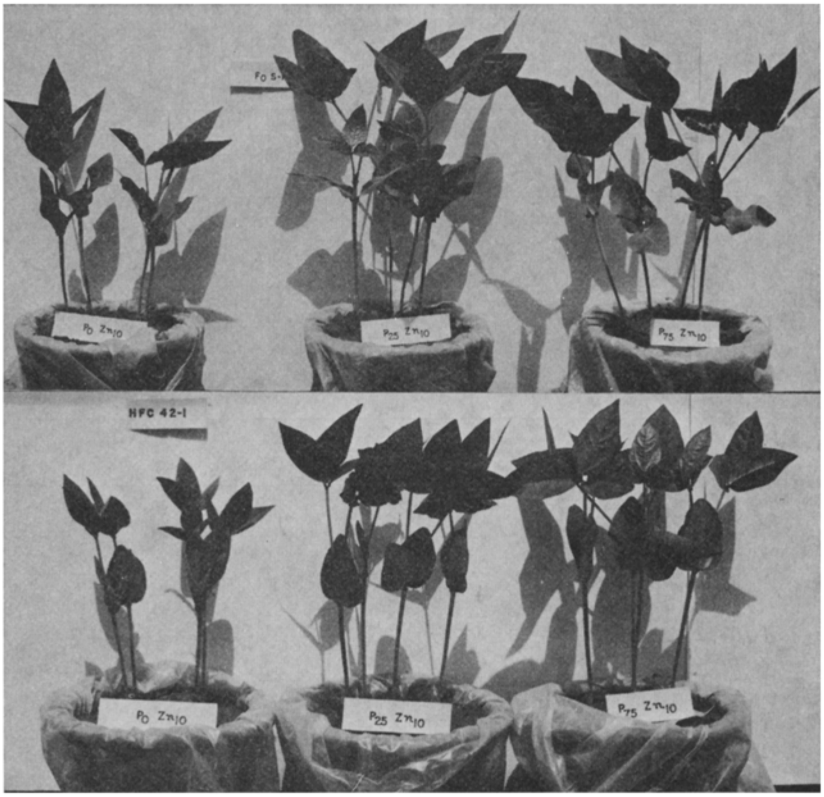

Fig. 2. Response of FOS-1 (*above*) and HFC 42-1 (*below*) varieties of cowpea to increasing levels of phosphorus in the presence of 10 ppm applied zinc.

$P_{75}Zn_0$ plants, especially those of variety HFC-42-1. A decrease in fresh weight values with time in $P_{75}Zn_0$ plants was obviously a consequence of premature defoliation, which has been recorded[15] as one of the symptoms of zinc deficiency in cowpea.

Dry matter accumulation in the two varieties differed significantly (Table 1), being lower in case of HFC-42-1. During early growth a highly significant differential response to both phosphorus as well as zinc was evident, but at 32 day stage it was significant in relation to zinc alone. Dry weight of plants was slightly reduced with zinc when applied without phosphorus. However, in the presence of the latter, variety HFC-42-1 gave consistently much higher response to zinc as compared to variety FOS-1. At 16 day growth stage the response to

TABLE 1

Effect of phosphorus and zinc application on the dry matter yield of shoots (mg/plant) of cowpea varieties at two stages of growth

Treatment (ppm)		Day 16		Day 32	
P	Zn	FOS-1	HFC-42-1	FOS-1	HFC-42-1
0	0	408	326	621	420
	10	406	320 P$\bar{x}$ 365	566	384 P$\bar{x}$ 498
25	0	572	315	720	502
	10	643	576 P$\bar{x}$ 526	1102	1198 P$\bar{x}$ 880
75	0	444	305	582	327
	10	588	602 P$\bar{x}$ 486	987	865 P$\bar{x}$ 690
Variety $\bar{x}$		510	407	763	616
$Zn_0\bar{x}$		395	$Zn_{10}\bar{x}$ 522	$Zn_0\bar{x}$ 528	$Zn_{10}\bar{x}$ 850

F test (** $P \leqslant .01$, * $.01 < P \leqslant .05$, N.S. $P > .05$)

	Day 16	Day 32
P	**	**
Zn	**	**
V	**	**
P × Zn	**	**
P × V	**	N.S.
Zn × V	**	*
P × Zn × V	**	N.S.

zinc evinced at 25 and 75 ppm phosphorus levels respectively, was 12 and 32 percent for variety FOS-1, and 83 to 97 percent for variety HFC-42-1. At 32 days growth the magnitude of response to zinc application was observed to have increased for both the varieties, being 53 to 70 percent for FOS-1 and 139 to 165 for HFC-42-1. The interaction effect of phosphorus and zinc on the forage yield of HFC-42-1 plants was equally high. By increasing phosphorus level from 25 to 75 ppm the yield of non-zinc and zinc-supplied plants of variety FOS 1 decreased by about 19 and 10 percent, and those of variety HFC-42-1 by 35 and 28 percent respectively. However under adequate zinc supply the latter variety gave higher response to phosphate application as compared to the former.

Nutrient content in plants

i. Zinc. The two varieties of cowpea differed significantly ($P < 0.01$) in their shoot-zinc concentration. Variety HFC-42-1 had higher concentration of zinc under almost all the treatments as against

TABLE 2

Zinc concentration in shoots of cowpea varieties (ppm dry wt) as affected by different combinations of phosphorus and zinc

Treatment (ppm)		Day 16			Day 32		
P	Zn	FOS-1	HFC-42-1		FOS-1	HFC-42-1	
0	0	10.4	13.9		7.7	9.7	
	10	28.9	46.2	P$\bar{x}$ 24.8	33.5	43.5	P$\bar{x}$ 23.5
25	0	6.6	12.1		6.2	12.2	
	10	28.2	36.5	P$\bar{x}$ 20.8	24.5	39.7	P$\bar{x}$ 20.7
75	0	10.1	13.4		6.9	14.1	
	10	33.2	32.0	P$\bar{x}$ 22.2	26.2	40.6	P$\bar{x}$ 21.9
Variety $\bar{x}$		19.5	25.7		17.5	26.6	
$Zn_0\bar{x}$		11.1	$Zn_{10}\bar{x}$ 34.2		$Zn_0\bar{x}$ 9.5	$Zn_{10}\bar{x}$ 34.6	

F test (** $P \leqslant .01$, * $.01 < P \leqslant .05$, N.S. $P > .05$)	Day 16	Day 32
P	*	*
Zn	**	**
V	**	**
P × Zn	N.S.	*
P × V	*	N.S.
Zn × V	*	*
P × Zn × V	*	N.S.

variety FOS-1 (Table 2). Application of zinc increased the tissue-zinc concentration in both the varieties but on an average the increase in variety FOS-1 was of a higher magnitude. Phosphate application in general, decreased zinc concentration, but this effect was not obvious in non zinc-supplied plants. At 16 days growth, zinc concentration in varieties showed a significant differential response to phosphate but the same was lost with the age of plants. Total uptake of zinc in the two varieties at the first harvesting stage was almost similar but as the growth proceeded, variety HFC-42-1 was found to accumulate significantly higher amounts of zinc. Uptake of zinc was found to increase with both zinc and phosphate applications in either variety at both stages of growth. However, at 32 days growth there was a significant reduction in zinc accumulation as the level of phosphorus was increased from 25 to 75 ppm. Though phosphorus × zinc effect on zinc concentration was manifest at 32 days growth only, the effect of this interaction on zinc uptake was highly significant throughout the growth period. Zinc uptake in the varie-

TABLE 3

Phosphorus concentration in shoots of cowpea varieties (% dry wt.) as affected by different combination of phosphorus and zinc

Treatment (ppm)		Day 16		Day 32	
P	Zn	FOS-1	HFC-42-1	FOS-1	HFC-42-1
0	0	0.12	0.16	0.10	0.33
	10	0.11	0.18 P$\bar{x}$ 0.14	0.12	0.20 P$\bar{x}$ 0.19
25	0	0.32	0.83	0.69	1.07
	10	0.24	0.45 P$\bar{x}$ 0.46	0.27	0.40 P$\bar{x}$ 0.60
75	0	1.24	2.53	1.60	2.28
	10	0.87	1.13 P$\bar{x}$ 1.44	0.68	1.02 P$\bar{x}$ 1.39
Variety $\bar{x}$		0.48	0.88	0.57	0.88
$Zn_0\bar{x}$		0.87	$Zn_{10}\bar{x}$ 0.49	$Zn_0\bar{x}$ 1.01	$Zn_{10}\bar{x}$ 0.44

F test (** $P \leqslant .01$, * $.01 < P \leqslant .05$, N.S. $P > .05$)

	Day 16	Day 32
P	**	**
Zn	**	**
V	**	**
P × Zn	**	**
P × V	**	**
Zn × V	**	**
P × Zn × V	**	N.S.

ties was also differentially affected by phosphorus as well as zinc; the latter interaction (Zn × v) being significant at 32 days growth only.

ii. Phosphorus. Concentration of phosphorus in the shoots of variety HFC-42-1 was consistently higher as compared to that of FOS-1 (Table 3). Phosphate application increased phosphorus concentration in both the varieties but zinc caused a severe depression. The phosphorus × zinc effect on phosphorus concentration was highly significant at both the stages of growth but that of phosphorus × zinc × variety at 16 day stage only. Phosphorus concentration in the two varieties showed a highly differential response to both phosphate as well as zinc application throughout the growth period. During early growth, variety HFC-42-1 accumulated significantly higher amount of phosphorus as compared to FOS-1. However, by the second sampling stage this difference ceased. Application of phosphate increased phosphorus uptake but the two varieties exhibited differential response in this respect at 16 days growth. Although the overall effect of zinc on phosphorus uptake was non-significant yet its depressing effect on phosphate absorption by variety FOS-1 under 25 and 75 ppm phosphorus treatments was quite evident.

DISCUSSION

The higher susceptibility of variety HFC-42-1 to phosphorus-induced zinc deficiency was apparent by the early appearance of symptoms, their intensity and significant growth response to zinc application. Further evidence was provided when zinc deficiency symptoms in variety HFC-42-1 appeared under both $P_{75}Zn_0$ as well as $P_{25}Zn_0$ treatments and in variety FOS-1 in $P_{75}Zn_0$ plants only. In an earlier study on navy beans Ellis[6] had observed 'Sanilac' variety to be more susceptible to zinc deficiency than 'Saginaw.' While examining the cause of differential susceptibility of these varieties, Ambler and Brown[1] found that the tops of 'Sanilac' contained less zinc but more phosphorus and iron than 'Saginaw.' They concluded that higher susceptibility of 'sanilac' variety to zinc deficiency was due to high concentration of phosphorus and iron in its tops.

Of particular interest in this study is that the more susceptible variety, HFC-42-1, contained significantly higher proportion of relative as well as total zinc content in its shoot tissues than the less susceptible FOS-1. Obviously, tissue-zinc concentration or zinc uptake characteristics of these varieties could not be related to their differential susceptibility to phosphorus-induced zinc deficiency. A critical examination of phosphorus concentration and uptake data would, however, reveal variety HFC-42-1 to be highly efficient in phosphate absorption. On an average this variety had nearly twice as much phosphorus concentration in its tops as in FOS-1. A varietal difference in phosphorus content was also reported for 'Chief' and 'Lincoln' soybeans by Paulsen and Rotimi[12] but the differences were not as pronounced as exhibited by the cowpea varieties under study. Since high tissue-phosphorus concentration renders plant susceptible to zinc deficiency it is possible that the higher sensitivity of variety HFC-42-1 to phosphorus induced zinc deficiency symptoms was due to its inherent capacity to accumulate more phosphorus. This may also be the reason for the higher concentration of zinc in this variety; as it would serve to create a favorable phosphorus: zinc balance essential for normal growth. The unusually high concentration of phosphorus in the tops of variety HFC-42-1 under $P_{25}Zn_0$ and $P_{75}Zn_0$ treatments and that of FOS-1 under $P_{75}Zn_0$ treatment, further substantiate the above inference as a reason of their differential susceptibility.

Since increasing phosphate levels in soil caused no depression in the zinc concentration of non zinc-supplied plants, it may be argued as to how phosphorus could have induced zinc deficiency. In fact zinc concentration was rather found to increase with phosphate application in 32-day old HFC-42-1 plants receiving no external supply of zinc. In many studies[2 3 10] phosphate application was found to induce zinc deficiency symptoms and growth disorder without causing any depression in plant-zinc concentration. Such observations have led to the belief that imbalance in phosphorus/zinc ratios rather than plant-zinc status alone is the main cause for the appearance of zinc deficiency symptoms as induced by high phosphate applications. However, a critical examination of data pertaining to the total accumulation of zinc in whole plants (shoot + root) with time (Table 4), revealed that by increasing the level of phosphate application the absorption rate of zinc was reduced. Moreover, there were indications of efflux of zinc in $P_{75}Zn_0$ plants of either variety during 16–32 days growth. In zinc supplied plants of variety FOS-1 the rate of zinc uptake during 16–32 days growth decreased uniformly with the increase in phosphate level. On the contrary, in variety HFC-42-1 zinc uptake rate under $P_{25}Zn_{10}$ treatment was 9-times higher as com-

TABLE 4

Total zinc accumulation in 16 and 32-day old cowpea plants (shoot + root), and the mean rates of zinc uptake during 16–32 days growth, as affected by different phosphorus and zinc applications

Treatment	FOS-1			HFC-42-1		
	Day 16	Day 32	Mean uptake rate μg Zn/day	Day 16	Day 32	Mean uptake rate μg Zn/day
	μg Zn/whole plant			μg Zn/whole plant		
P_0Zn_0	7.1	9.3	0.14	6.3	11.4	0.32
P_0Zn_{10}	18.4	45.5	1.69	22.9	28.4	0.34
$P_{25}Zn_0$	6.4	8.9	0.16	5.6	9.1	0.22
$P_{25}Zn_{10}$	27.6	50.3	1.42	31.5	81.1	3.10
$P_{75}Zn_0$	7.5	6.6	−0.06	6.2	4.8	−0.09
$P_{75}Zn_{10}$	35.6	49.0	0.84	34.8	61.5	1.67

pared to P_0Zn_{10}; but with further increase in phosphorus level to 75 ppm it decreased by about one-half. It is interesting to note that while zinc application to phosphate deficient plants of FOS-1 variety increased their zinc uptake rate nearly 12-fold, in HFC-42-1 plants the uptake rate remained unchanged, and was about one-fifth of the former variety. It is therefore evident that for adequate zinc absorption variety HFC-42-1 needs some optimum balance in the external supply of zinc and phosphorus. This may also explain the highest responses exhibited by this variety to both of these nutrients.

Working with corn seedlings, Edwards and Kamprath[5] have recently observed that for maintaining adequate rate of zinc uptake the presence of phosphate in external solution is essential. While, partly this inference appears to be justified, as also indicated by the behavior of HFC-42-1 variety, the fact remains that high levels of phosphate have an adverse effect on the absorption of zinc, which might not be expressed in terms of concentration differences on account of accompanying differences in the growth rate of plants. These results further support our previous finding[13] that phosphorus induces zinc stress by reducing zinc flux through roots, thus imposing restriction on the functional requirements of zinc by plants.

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