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Phosphorus and zinc uptake and protein, lysine and tryptophane contents in quality protein maize in relation to phosphorus and zinc fertilization in mollisol

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Abstract

High quality protein maize hybrid HQPM-1 grown with different doses of phosphorus (0, 30, 60 and 90 kg/ha) and zinc (0, 5, 10 and 15 kg/ha) to evaluate the effect of P and Zn on their uptake, plant growth, grain yield and protein quality during *kharif* season 2012 on zinc deficient silty clay loam soil. Application of P and Zn significantly increased the available P in soil however; available Zn reduced with increment of P. There was gradual increase in plant height, ear height, number of leaves and test weight with increase in P and Zn doses however, root dry weight decreased beyond 60 kg P and 10 kg Zn/ha. Application of P up to 60 kg/ha and Zn up to 15 kg/ha reduced the anthesis silking interval. Phosphorus and zinc uptake also increased with increasing P and Zn level upto 60 kg/ha and 15 kg/ha, respectively. The grain and stover yields were significantly affected by P and Zn however; grain yield did not significantly influence due to P and Zn interaction. Phosphorus and zinc application had synergistic impact on protein, lysine and tryptophan content of grain where maximum contents were found with the highest doses of P and Zn.

Key words: quality protein maize, phosphorus and zinc fertilization, uptake, quality—parameters, mollisol

Maize (*Zea mays* L.) is a major cereal crop for human nutrition, worldwide and keeps third place in cereal cultivation in India. It is a fast growing and high nutrients requiring crop. Nitrogen and phosphorus play chief role to produce good maize yields however, researchers in various locations have observed that maize crop could lead to high response to micro-nutrients and the most widely deficient micronutrient for maize production is zinc (Aduloju and Malik, 2013). Zinc is one of the important essential micronutrients for plants and needed in small but if the amount available is not adequate, plants suffer from physiological stress brought about by the dysfunction of several enzyme systems and other metabolic functions. Zinc deficiency seems to be more acute for rice-wheat growing areas. Continuous and indiscriminate use of high analysis fertilizers without any micronutrient addition under intensified cropping for the more than last three decades and neglect of on-farm inputs like organic manures, crop residues, *etc.* in lowland rice growing area of *tarai* especially that are near neutral to alkaline in reaction manifested the occurrence of widespread zinc deficiency (Jaishy *et al.*, 1989) it resulted in considerable decrease in crops yield. Zinc availability to the crops is greatly affected by a number of factors such as soil pH, organic matter and soil textural conditions however; phosphorus is the important element that interferes in Zn availability and uptake, as Zn uptake by plants becomes slower or inadequate with higher levels of P in soil owing to antagonistic relation (Mengel and Kirkby, 1982). Several studies have confirmed that Zn and P imbalance in the plant is due to excessive accumulation of P resulting in Zn imposed deficiency. Similarly, excessive zinc can cause phosphorus deficiency, though this is not as common phenomenon.

Normal maize represents the main source of calories and minerals however, very poor in protein and micronutrients content, especially zinc. In quality protein maize (QPM), the opaque2 (o2) mutation alters the amino acid profile and composition of the endosperm protein, resulting in 2-3-fold increase in levels of lysine and tryptophan in comparison with non-QPM maize. Besides having better protein quality, QPM also showed higher concentration of kernel micronutrients, especially Zn (Chakraborti *et al.*, 2009b). Therefore, keeping in view of high residual P and deficient Zn levels in *tarai* soil, the present study was aimed to know the Zn and P applications effects on their uptake, yield and quality traits of QPM maize.

Materials and Methods

The experiment was carried out in zinc deficient silty clay loam soil at the Norman E. Borlaug Crop Research Centre of G. B. Pant university of Agriculture and Technology, Pantnagar, Uttarakhand (29° N, 79.5° E and 243.8 m above msl) during *kharif* season 2012. The experiment had 16 treatments in a factorial combination of four levels of phosphorus (0, 30, 60 and 90 kg/ha) and four levels of zinc (0, 5, 10 and 15 kg/ha). Based on the treatments, the required amount of phosphorus and zinc were added to the soil before sowing through di ammonium phosphate and zinc sulphate ($ZnSO_4$. 7 H_2O), respectively. Half doses of N (recommended dose 120 kg/ha) through DAP and urea and full dose of K (40 kg/ha) through muriate of potash were also applied in all plots at the time of sowing. Remaining half dose of N was applied in two equal splits at knee high (30 DAS) and prior to tassel emergence stages as top dressing. Maize seeds were sown at 5 cm depth on well prepared seedbed on 02 July with a spacing of 60×25 cm. All other standard agronomic practices were followed uniformly in all the treatments during growth period. The gross and net plot size was 15 and 8.55 m^2, respectively.

Plant growth study was made with the five randomly selected plants in three centre rows excluding two plants closest to the alley at both ends of the rows. The height of the plants and ears was measured from soil surface to the top of the tassel and to base node of the cob, respectively, at dry silk stage. Days from planting to anthesis and silking were estimated by regular observation up to the initiation of anthesis and emergence of silk in at least 50% of plants in each plot. Anthesis-silking interval (ASI) was calculated as the difference between the number of days to 50% silking and 50% anthesis. Dry root biomass was measured by using destructive sampling method in which all part of the root system of randomly selected plants was removed and washed thoroughly with water passed through 0.5 mm sieve and dried at 65°C to constant weight. Stover yield at harvest from one square meter area was oven dried at 65°C till constant weight and grain yields at 15% moisture content after harvest.

The collected plant and grain samples from each treatment at harvest were oven dried at 65°C till constant weight then milled for analysis of phosphorus and zinc contents and quality parameters. The milled samples were digested with nitric and perchloric acid mixture. Nitrogen in digest was determined by Kjeldahl method, P by the vanadomolybdate yellow method using spectrophotometer 20 and Zn by using the atomic absorption spectrophotometer.

Among the quality parameters, protein content in grain was determined by multiplying per cent N in grain with factor 6.25. Tryptophan was estimated by papain hydrolysis method (Hernandez and Bates, 1969) while lysine by increasing the value of tryptophan by 4 times.

Soil samples collected from 0-15 cm depth before sowing and after harvest were air-dried and analyzed on the < 2 mm fraction. Soil pH and electrical conductivity (ECe) were measured at a 1:2.5 soil/water ratio. Organic carbon in the soil was determined according to Walkley and Black's method. Available N was determined using Kjeldahl digestion followed by distillation and titration. Available soil P was measured by Olsen method. Available Zn in the soil was first extracted by diethylenetriaminepentaacetic acid

(DTPA) and then was read by atomic absorption spectrophotometer.

Results and Discussion

Physical and chemical properties of the soil

The results of the physical and chemical properties of the top soil (0-15 cm) depth before the sowing of crop and post harvest soil are present in Table 1 and 4, respectively. At the time of sowing, the soil of the experimental site was slightly alkaline (pH 7.21) in reaction and low in electrical conductivity, available N and Zn; medium in organic carbon and available K while high in phosphorus. The high phosphorus and below the critical level of Zn contents in soil (Table 1) imply the necessity of Zn application in soil studied to supplement this element. In the post harvest soil, no significant influence of P and Zn application was observed on soil pH but increase in electrical conductivity values were intensified when P and Zn levels increased. The significant and highest organic carbon (0.77 and 0.79%) of soil was recorded with the application of 90 kg P/ha and 10 kg Zn/ha, respectively. Available N was found to be increased up to 60 kg P and 10 kg Zn/ha but thereafter decreased non significantly and significantly, respectively.

Table 1. Physical and chemical properties of the experimental soil

Properties	Value
pH 7.21	
Organic carbon (%)	0.87
Electrical conductivity (dS/m)	0.340
Available N (kg/ha)	198.31
Available P (kg/ha)	31.27
Available K (kg/ha)	151.76
Available Zn (ppm)	0.487
Soil texture	Silty clay loam
Bulk density (Mg/m^3)	1.35

The minimal available P was found in control (P0 and Zn0) and with increase in dose of P and Zn; a significant increment in available P was noted. Similar findings were also reported by Singh *et al.*, 2011. Available Zn status reduced significantly to 18.1% with increment of P from 30 to 90 kg/ha. This finding confirmed results obtained by Neue and Marmaril (1985) and Goh *et al.*, (1995) who reported that P application depressed the availability of Zn, presumably due to the formation of organic-metal-phosphate complexes. Whereas available Zn increased to 16.4% when Zn level was increased from 5 to 15 kg/ha (Harrel, 2005).

Table 2. Effect of phosphorus and zinc supplies on growth parameters of quality protein maize

P and Zinc dose	Plant height (cm)	Ear height (cm)	Number of leaves	Dry root biomass (g)/plant
Phosphorus levels (kg/ha)				
0	180.5	69.15	12.21	9.33
30	187.7	71.32	13.37	9.70
60	201.2	76.88	13.78	11.53
90	203.6	77.25	14.16	10.81
SEm±	2.25	0.82	0.18	0.19
CD (P = 0.05)	6.50	2.38	0.53	0.55
Zinc levels (kg/ha)				
0	185.0	69.79	12.81	9.47
5	189.3	71.68	12.99	9.74
10	196.1	75.19	13.90	11.37
15	202.5	77.95	13.82	10.79
SEm±	2.25	0.82	0.18	0.19
CD (P = 0.05)	6.50	2.38	0.53	0.55
P×Zn	NS	NS	NS	**

* $P \leq 0.05$; **$P \leq 0.01$; NS, not significant

Growth attributes

Phosphorus and zinc levels had significant and their interaction had no significant effect on plant height, ear height and number of leaves (Table 2). The application of P and Zn significantly increased the plant height form 7.22-23.14 cm and 4.32-17.43 cm, respectively, over the control. Increase in plant height in response to higher P and Zn levels has been confirmed by Bukvić *et al.*, (2003) and Asif *et al.*, (2013). Inclusion of 60 kg P resulted a significant response for plant height over 30 kg however, was statistically at par with 90 kg P level. The same effect was obtained for 10 kg Zn dose. Phosphorus @ 90 kg/ha and Zn 15 kg/ha levels gave the tallest plant *i.e.* 203.60 and 202.45 cm, respectively. An increasing trend in ear height was also observed with increase in plant height. Plants fertilized with 90 kg P and 10 kg Zn/ha showed greater number of leaves but were at par with 60 kg P and 15 kg Zn/ha, respectively. Root dry mass was affected significantly with P and Zn levels and their interactions. Root dry weight was not affected significantly with addition of 30 kg P and 5 kg Zn/ha over control but increased significantly at 60 kg P and 10 kg Zn however, further increasing P and Zn supply induced a reduction of root dry weight. Results similar to this had earlier been reported by Gianquinto *et al.*, (2009).

Phosphorus and zinc uptake

Sole application of P and Zn on uptake of P and Zn, respectively, by grain, stover and total had significant effect and uptake increased with increase in their levels (Table 5) but at high level of P *i.e.* 90 kg P/ha crop responded to decrease in P uptake. It may be due to higher original level of soil P therefore, crop responded to application of P up to 60 kg/ha. Incase of Zn uptake, 30 and 60 kg P/ha gave at par total uptake while increment of P over 60 kg/ha tended to significant reducing (23%) the uptake of Zn by crop (Prabhakarannair and Babu, 1975 and Oseni, 2009). However, under the condition of deficit original soil Zn, increase in amount of Zn levels up to 15 kg/ha produced increase in total P uptake. The decreased Zn uptake due to P application at the highest level of application of 90 kg/ha may probably be due to the formation of insoluble Zn-PO_4 compounds in the soil as reported by Sahu *et al.*, (1994) and Oseni (2009).

Table 3. Effect of phosphorus and zinc supplies on yield and yield attributes of quality protein maize

P and Zinc dose	Days to 50% tasseling	Days to 50% silking	Days to anthesis silking interval	Cob length (cm)	100 grain weight (g)	Grain yield (q/ha)	Stover yield (q/ha)
Phosphorus levels (kg/ha)							
0	65.92	61.54	4.38	12.80	29.14	27.91	68.84
30	63.83	60.38	3.46	13.17	30.27	33.38	81.86
60	62.25	59.21	3.04	13.52	30.92	40.43	89.48
90	65.00	61.67	3.33	13.29	31.26	35.76	83.71
SEm±	0.30	0.33	0.13	0.25	0.44	0.54	1.15
CD (P = 0.05)	0.86	0.96	0.39	NS	1.28	1.56	3.32
Zinc levels (kg/ha)							
0	65.75	61.42	4.33	12.99	29.85	30.54	72.05
5	64.50	60.71	3.79	13.02	30.07	32.50	79.65
10	64.08	60.71	3.38	13.54	30.73	35.82	83.99
15	62.67	59.96	2.71	13.23	30.94	38.61	88.19
SEm±	0.30	0.33	0.13	0.25	0.44	0.54	1.15
CD (P = 0.05)	0.86	0.96	0.39	NS	NS	1.56	3.32
P×Zn	**	*	NS	NS	NS	NS	**

* $P \leq 0.05$; **$P \leq 0.01$; NS, not significant

Yield and yield attributes

Phosphorus and zinc levels alone and their interaction had significant influence on time taken to 50% tasseling and silking however, interaction effect was non significant on anthesis silking interval (ASI) (Table 3). Maximum days to 50% tassel and silk emergence and ASI was observed in plots where no P and Zn were applied against minimum with P 60 kg/ha (59.2, 62.3 and 3.04 days, respectively) and Zn 15 kg/ ha (59.96, 62.7 and 2.71 days, respectively). These decreased gradually with increase in P and Zn levels however increased again beyond the 60 kg P/ha. These results are in consonance with the findings of Rasheed *et al.*, (2004) who reported that high level of P enhanced days to silking and zinc application reduced number of days to tasseling as well as silking (Asif *et al.*, 2013). Emergence of 50% tassels, silk and ASI reduced significantly from 1.2-2.3, 0.9-3.8 and 0.9-1.3 days with application of P and 0.7-1.5, 1.3-3.1 and 0.5-1.6 days with the application of Zn, respectively, over no P and Zn plots. Weight of 100 seeds was significantly affected by P levels but Zn alone and P and Zn interaction had no significant effect. The increase in seed weight due to increase in doses of P may be due to increase in available P during initial stage of growth when it is most needed. Sharma and Bhardwaj (1998) and Mishra and Abidi (2010) obtained the similar results. Application of 90 kg P/ha and 15 kg Zn/ha increased 100 grain weight by 7.9 and 3.7% higher as compared to no P and Zn, respectively. Grain and stover yields were also significantly affected by sole P and Zn levels and their interaction. Grain and stover yields recorded a significant increase upto the levels of 60 kg P/ha and 15 kg P/ha beyond 60 kg P/ha, these tended to decrease (Fig. 1a and 1b). Application of 30, 60 and 90 kg P increased the grain yields by 19.6, 44.9 and 28.1% whereas 5, 10 and 15 kg Zn/ha by 6.4, 17.3 and 26.4% over control, respectively. The results are in close conformity with the findings of Kaya *et al.* (2009). This increased grain yield due to P application might be attributed to stimulation of root development and increased root surface area which might have resulted in proper uptake and utilization of all other nutrients by plant while reduction in grain and stover yields beyond 60 kg P/ha might be due to formation of insoluble zinc phosphate by antagonistic effect between P and Zn which reduced the root surface area by affecting root development.

Quality parameters

Protein content and amino acid composition such as tryptophan and lysine in grain were significantly affected by the P and Zn doses and lysine content by interaction between P and Zn. Each increase in P and Zn levels linearly increased grain protein (Fig. 2a and 2b) however, 90 kg P/ ha and 10 and 15 kg Zn/ha gave significantly higher protein (10.5, 6.2 and 7.6%, respectively) over control. The results are in agreement with the findings of Jhaw (1991) and Mishra (2012). The concomitant increase in the protein content in grain with increase in dosage of phosphorus might be due to the higher phosphorus concentration in soil which is required for absorption

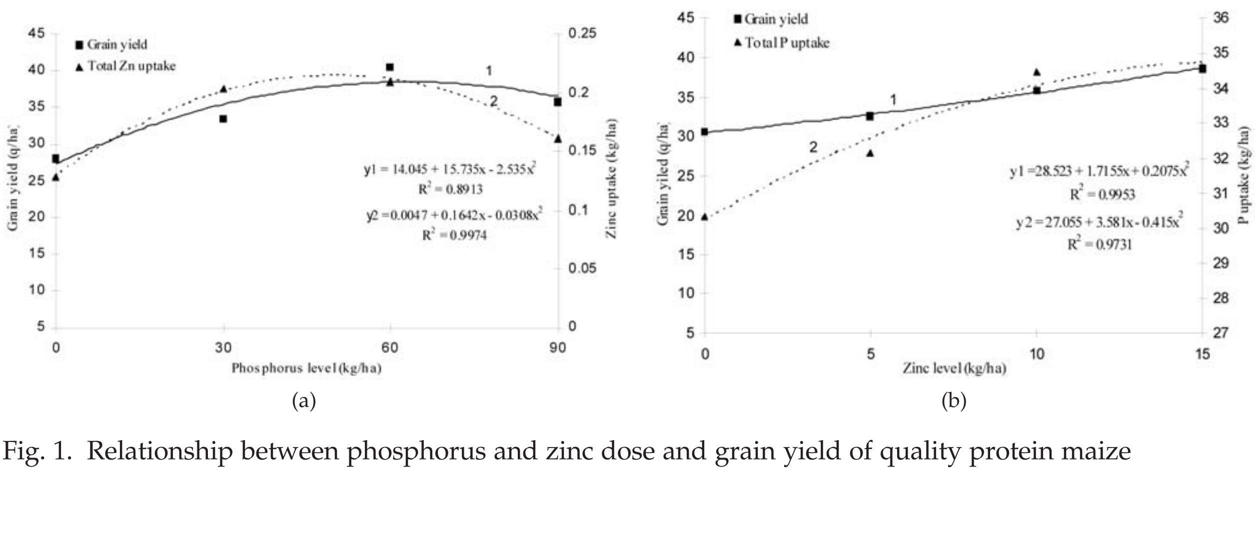
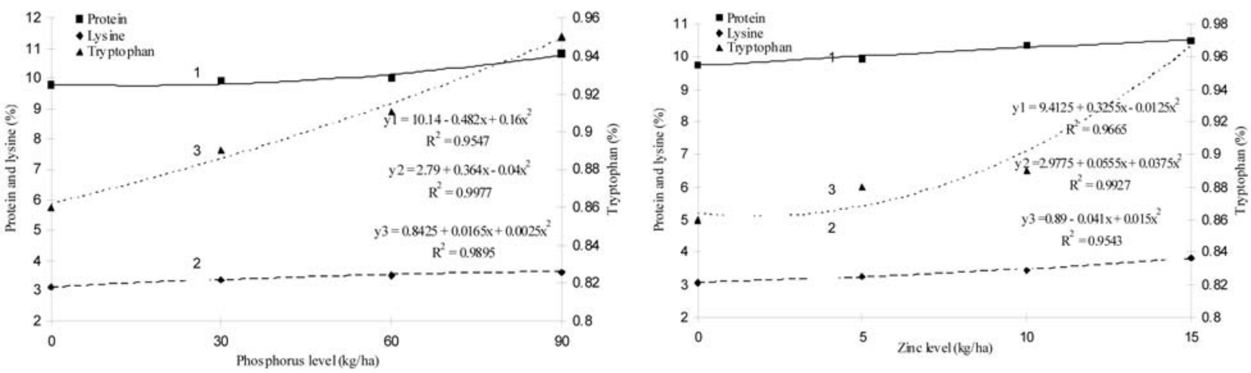


Fig. 1. Relationship between phosphorus and zinc dose and grain yield of quality protein maize

and assimilation of 'N' as it is directly controlled by the plant's capacity to take up and transfer nitrogen from roots and leaves to the seed while zinc is actively involved in protein synthesis in the form of zinc motifs. The synergistic action between phosphorus and zinc may be the reason behind the increasing trend of protein content. Application of zinc led to synergistic action with phosphorus in the zinc deficient soil (Webb and Loneragan, 1990). Tryptophan and lysine were also continued to increase with increasing P and Zn levels (Fig. 2a and 2b). The increasing amounts of tryptophan and lysine contents may be ascribed to the fact that both these nutrients play a vital role in protein synthesis during the grain filling and indirectly enhance the metabolism of nitrogen in the plants. Thus, increased efficiency of the protein synthesis is manifested in the increase in its structural components which include the tryptophan and lysine. Similar findings were reported by Lindsay (1972) and Asif *et al.*, (2013). The maximum amount of tryptophan and lysine was recorded with higher dose of P which was statistically at par with 60



(a) (b)

Fig. 2. Relationship between phosphorus and zinc dose and protein, lysine and tryptophan contents of grain of quality protein maize

Table 4. Effect of phosphorus and zinc supplies on post harvest soil properties

P and Zinc dose	Soil pH	Electrical conductivity (dS/m)	Soil organic carbon (%)	Available N (kg/ha)	Available P (kg/ha)	Available Zn (ppm)
Phosphorus levels (kg/ha)						
0	6.85	0.188	0.75	267.36	33.24	1.65
30	7.16	0.202	0.74	253.25	41.71	2.32
60	6.95	0.203	0.73	261.67	43.02	2.26
90	6.99	0.212	0.77	259.87	43.21	1.90
SEm±	0.13	0.004	0.012	3.65	0.77	0.10
CD (P = 0.05)	NS	0.011	NS	NS	2.22	0.29
Zinc levels (kg/ha)						
0	7.12	0.194	0.72	267.21	40.90	0.53
5	6.96	0.193	0.71	259.44	41.25	2.32
10	6.94	0.212	0.79	266.75	42.66	2.58
15	6.93	0.205	0.77	248.75	43.54	2.70
SEm±	0.13	0.004	0.012	3.65	0.77	0.10
CD (P = 0.05)	NS	0.011	0.035	10.54	2.22	0.29
P×Zn	NS	**	NS	**	**	**

* $P \leq 0.05$; ** $P \leq 0.01$; NS, not significant

Table 5. Effect of phosphorus and zinc supplies on nutrients uptake and quality parameters of quality protein maize

P and Zinc dose	P uptake (kg/ha)			Zn uptake (kg/ha)			Protein (%)	Tryptophan (%)	Lysine (%)
	Grain	Stover	Total	Grain	Stover	Total			
Phosphorus levels (kg/ha)									
0	10.05	16.30	26.36	0.047	0.081	0.128	9.78	0.86	3.11
30	11.28	21.58	32.86	0.073	0.131	0.203	9.93	0.89	3.37
60	14.70	22.81	37.51	0.088	0.122	0.209	10.02	0.91	3.51
90	13.49	21.36	34.85	0.073	0.089	0.161	10.81	0.95	3.61
SEm±	0.26	0.57	0.64	0.002	0.002	0.003	0.14	0.015	0.03
CD (P = 0.05)	0.74	1.65	1.84	0.006	0.006	0.013	0.41	0.043	0.09
Zinc levels (kg/ha)									
0	11.35	19.01	30.35	0.047	0.0667	0.110	9.75	0.86	3.06
5	11.68	20.49	32.17	0.067	0.1004	0.168	9.94	0.88	3.27
10	13.48	20.97	34.45	0.079	0.1265	0.205	10.35	0.89	3.45
15	13.01	21.60	34.61	0.089	0.1295	0.218	10.49	0.97	3.81
SEm±	0.26	0.57	0.64	0.002	0.002	0.003	0.14	0.015	0.03
CD (P = 0.05)	0.74	1.65	1.84	0.006	0.006	0.013	0.41	0.043	0.09
P×Zn	NS	*	*	**	**	**	NS	NS	**

* $P \leq 0.05$; ** $P \leq 0.01$; NS, not significant

kg P/ha while 15 kg Zn/ha gave significantly highest tryptophan and lysine over other Zn levels.

In conclusion, application of P up to 60 kg/ha and Zn up to 10 kg/ha in general, increased the P and Zn uptake by grain and stover, available P in soil and grain and stover yields therefore, combination of P 60kg/ha and Zn 10 kg/ha appears to be the best for obtaining the higher maize productivity and maintaining the fertility. Phosphorus application at higher level (90 kg/ha) did harm the Zn status in soil as well as in plant in Zn deficient soil which clearly proved the commonly held belief that high P availability reduces the Zn uptake in maize, however Zn @ 15kg/ha found to be beneficial and can be applied. Although quality assessing parameters viz., protein, tryptophan and lysine contents were found to be increased significantly with increase in P and Zn levels but further study is needed to elucidate the optimum doses of P and Zn and their interaction for getting optimum contents of quality parameters in quality protein maize under *tarai* condition.

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