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Full Length Research Paper

Phytase activity, phytic acid, zinc, phosphorus and protein contents in different chickpea genotypes in relation to nitrogen and zinc fertilization

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Seeds of three chickpea varieties were grown under zinc (Zn) and nitrogen (N) fertilized conditions and levels of zinc, phosphorus, phytic acid and protein concentrations as well as phytase activity were determined. In the field experiment, seed Zn concentrations increased with Zn fertilization in all varieties. Zinc fertilization had a negative effect on seed phosphorus (P) and phytic acid (PA) concentration. Phytic acid to Zn molar ratio decreased with zinc fertilization. Protein content of seeds increased with N and Zn fertilization. Zinc nutrition had no direct effect on phytase activity. Grain Zn, P, PA and protein concentrations varied depending on chickpea variety.

Key words: Zinc, nitrogen, phosphorus, phytic acid, phytase.

INTRODUCTION

Zinc (Zn) is an essential nutrient for all forms of life. Zinc deficiency is a global micronutrient deficiency in humans. Worldwide, about 3 billion people, especially in developing countries, are affected by micronutrient deficiencies including Zn deficiency. Zinc deficiency in human results in a number of health problems, such as impairments in linear growth, sexual maturation, learning ability, immune functions and the central nervous system, susceptibility to infection, impaired wound healing, etc. (Prasad, 1984; Tamura and Goldenberg, 1996; Welch and Graham, 1999, 2004; Brown et al., 2001). Based on national food balance data, approximately 20.5% of the world's population is estimated to be at risk of inadequate Zn intake, with the percentage of individuals at risk highest in South East Asia (33.1%), Sub Saharan Africa (28.2%), South Asia (26.7%) and Latin America and the Caribbean (24.8%) (Wuehler et al., 2005). A high proportion of cereal-based foods in the diet and low intake of animal products were suggested to be one of the major reasons for the widespread occurrence of Zn deficiency in humans especially in developing countries (Cakmak et al., 1999; Paul et al., 1998).

Existence of high concentrations of phytic acid (PA, myo-inositol hexa phosphate) and fiber in diets is a major cause for the occurrence of mineral, especially Zn, deficiency in human beings (Ferguson et al., 1989; Gibson et al., 1997). Cereals, legumes and nuts store phosphorus (P) as PA, which, together with inositol penta phosphate, is one of the main inhibitors of Zn absorption. Phytic acid is the storage form of P and usually accounts for 60-80% in wheat, 66-70% in barley, 71-88% in corn 50-70% in soybeans, 27-87% in lentils and 40 - 95% in chickpeas of total P (Lolas et al., 1976; Chitra et al., 1995; Erdal, 1998; Erdal et al., 2002; Mate and Radomir, 2002). In the studies conducted by different authors on different seeds, PA content varied from 0.39-1.35% in wheat, 0.83-2.2% in corn, 0.50-1.89% in triticale, 0.54-1.46 in rye, 0.74-2.10 in beans and 0.28-1.26 in chickpeas (Reddy et al., 1982; Singh and Reddy, 1977; Lolas et al., 1976; Erdal, 1998).

In many animal experiments, addition of PA to diets reduced bioavailability of Zn and growth of animals and the removal of PA from diets by addition of phytate-degrading enzymes (that is, phytase) improved bioavailability of Zn and growth of animals (Welch et al., 1974; Jie and Zhenying, 1995). Phytic acid is also able to form complexes with proteins and thus impairs digestibility and

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Table 1. Analysis of variance for the results of parameters obtained from the experiment.

Parameter	P	PP	PP (%)	PA	Zn	PA/Zn	Protein	Yield	Phytase
Variety (V)	**	**	**	**	**	**	**	**	**
N	**	**	**	**	**	**	**	**	**
Zn	**	**	**	**	**	**	**	**	**
V x N	**	**	ns	**	**	*	ns	**	**
V x Zn	**	**	**	**	ns	ns	ns	*	**
N x Zn	**	**	**	**	ns	**	ns	**	**
V x N x Zn	**	**	**	**	ns	*	ns	*	**

*: $p < 0.05$, **: $p < 0.01$, ns: non-significant.

bioavailability of proteins in seeds (Reddy et al., 1982). In most cases, PA to Zn molar ratio in foods is considered a predictor of Zn bioavailability. Ratios above 20 to 30 have been reported to reduce Zn absorption and growth of animal (Oberleas and Harland, 1981; Solomons, 1982). As shown previously, the concentration of PA in seeds is highly dependent on the rate of root uptake of P and its translocation from leaves into seeds (Michael et al., 1980; Raboy and Dickinson, 1993). As root uptake and shoot accumulation of P are greatly affected by Zn deficiency (Loneragan et al., 1982; Rengel and Graham, 1995), it can be suggested that varied Zn supply can influence PA concentration of seeds of plants grown under Zn deficient conditions.

Increasing level of PA, decreases protein availability by forming phytate-protein bounds as well (Reddy et al., 1982). Henrik et al. (2002) revealed that PA was stored in protein-christalloid in grains of cereal and legumes and speculated that the synthesizing metabolism of grain PA probably was closely related to the accumulation of protein in rice grains. However no significant correlation between the content of PA and the accumulation of total protein was found in a study on the legume seeds (Morre et al., 1990) and study on rice varieties (Wu et al., 2007). Phytase is an enzyme that can break down the undigestible PA (phytic acid) part found in grains and oil seeds and thus release digestible P, calcium and other nutrients. The enzyme phytase is normally produced (endogenous phytase) in ruminants. Non-ruminants (monogastric animals) like human beings, dogs, birds, etc. do not produce this enzyme. Research in the field of animal nutrition has put forth the idea of supplementing phytase enzyme, exogenously, so as to make available bound nutrients like calcium, phosphorus, other minerals, carbohydrates and proteins.

The study was carried out to determine influence of N and Zn fertilization on levels of PA, P, Zn, protein, PA to Zn molar ratios and phytase activity of three chick pea cultivars grown in Zn-deficient soils.

MATERIALS AND METHODS

This field experiment was conducted at Suleyman Demirel University research and experimental farm. The experiment was set up in a randomized blocks design having 3 replications. The experimental soil was clay loam, non-saline, having pH 8.3 (1:2.5 soil to water ratio), 1.8% organic matter (Walkey and Black method, Jackson, 1973) high level of available $NaHCO_3$ P and 1 N NH_4OAC extractable K (Olsen et al., 1954; Knudsen et al., 1982). The available Zn determined in DTPA extract (Lindsay and Norvell, 1978) on Atomic Absorption Spectrophotometer was 0.44 mg kg^{-1}.

Officially registered 3 chickpea varieties (Akcin 91, Gökce, Aydın 92) were used in the experiment. Sowing was done in 11.4 m^2 (1.8 x 6 m) plots by hand and the row space was 30 x 10 cm. Three different Zn and nitrogen levels were used in the experiment. $ZnSO_4.7H_2O$ was applied at the rates of 0, 7 and 14 kg ha^{-1} before the sowing and NH_4SO_4 were applied at the rates of 0, 30 and 60 kg N ha^{-1} together with the sowing. The experiment was not irrigated. The mid rows of plots were hand-harvested to protect side effects. After the harvest, plants were dried and grains were separated, then grain yield and 100 grains weights were calculated.

After drying at 60^0C and grinding, seed samples were ashed at 550^0C for 8 h. The ash was dissolved in 3.3% HCl (v/v) for Zn determination by an atomic absorption spectrometer and P by the vanadate-molybdate method with spectrophotometer. The assay of phytic acid is based on precipitation of ferric phytate and measurement of iron (Fe) remaining in the supernatant (Haug and Lantzsch, 1983). About 0.5 g ground seed sample was used for extraction of phytic acid in 25 ml. 0.2 N HCl (pH 0.3) for 3 h. The extracts brought up to 50 ml with de-ionized water were centrifuged, and 1 ml of supernatant was treated with a ferric solution (NH_4 Fe $(SO_4)_2.12\ H_2O$) in a boiling water bath for 30 min. After cooling, samples were centrifuged and 1 ml supernatant was treated with a bipyridine solution to measure Fe remaining in the supernatant. Further details of the method are described by Haug and Lantzsch (1983). The molar ratio of phytic acid to Zn was calculated by dividing millimoles of phytic acid with milimoles of Zn. Phytase activity was measured as described by Scheuermann et al. (1988). One gram ground seed sample was incubated in 10 ml 50 mM sodium (Na) citrate buffer (pH = 5.3) and 50 mM Na-phytate at 37^0C for 1 h. After incubation, solutions were centrifuged and supernatants were brought up to 50 ml and analyzed for inorganic P.

RESULTS AND DISCUSSION

Analysis of variance showed that all parameters except for PP (Phytate-P) used in this study were (P, PP, Zn,) significantly affected by ($p < 0.05$) variety, N and Zn fertilization (Table 1). Grain P and PP contents diminished with increasing levels of Zn and N fertilization. This diminishing trend observed for all three chickpea genotypes.

Table 2. Effect of N and Zn applications on concentrations of P, PP and PP (%).

Variety	N level (kg N ha^{-1})	P (mg kg^{-1})				PP (mg kg^{-1})				PP (%)			
		0*	7	14	Mean	0	7	14	Mean	0	7	14	Mean
Akcin 91	0	4453	4256	4018	4243	2367	2060	1910	2112	53.1	48.4	47.6	49.7
	30	4169	4015	3919	4034	2076	1998	1937	2004	49.8	49.8	49.4	49.6
	60	3875	3857	3821	3851	1889	1813	1790	1831	48.8	47.0	46.8	47.5
	mean	4166	4043	3919		2111	1957	1879		50.6	48.4	47.9	
Gökce	0	4220	3944	3838	4001	2086	1939	1819	1948	49.4	49.2	47.4	48.7
	30	3920	3792	3671	3794	1919	1839	1753	1837	48.9	48.5	47.7	48.4
	60	3693	3420	3243	3452	1766	1600	1507	1624	47.8	46.8	46.4	47.0
	mean	3944	3719	3584		1924	1793	1693		48.7	48.1	47.2	
Aydın 92	0	4476	4155	4063	4231	2264	2102	2028	2131	50.6	50.6	49.9	50.3
	30	3909	3832	3785	3842	1973	1924	1893	1930	50.5	50.2	50.0	50.2
	60	3820	3703	3660	3728	1877	1813	1762	1817	49.1	48.9	48.2	48.8
	mean	4068	3897	3836		2038	1946	1895		50.1	49.9	49.3	
LSD (0.05)		G: 19.41; N: 19.41; Zn: 19.41; G x N x Zn: 58.23				G: 12.25; N: 12.25; Zn: 12.45; G x N x Zn: 36.75				G: 0.35; N: 0.35; Zn: 0.35; G x N x Zn: 1.05			

*Zn application (kg Zn ha^{-1}).

Diminishing rate depending on Zn applications was about 6% for Akcin 91 and Aydın 92, 10% for Gökce. Increasing level of N also led to decrease at the rates of 9, 14 and 12% for Akcin 91, Gökce and Aydın 92, respec-tively. Even though, the rates of PP in total P (PP %) were negatively affected by both Zn and N fertilization, these decreases were not significant. The rates deter-mined were around 46.8 to 53.1%, 46.4 to 49.4 % and 48.2 to 50.6% for Akcin 91, Gökce and Aydın 92, respec-tively (Table 2).

Increasing levels of both Zn and N fertilizations resulted in significantly decrease of PA content in all chickpea varieties (Table 3). According to means, PA contents for all varieties were the highest without Zn applications and were the lowest with the higher Zn level. Significant differences were determined among the varieties as well (Table 3).

The decreasing effect of Zn on PP and PA can be attributed to the inhibitory effect of Zn on root uptake and shoot accumulation of soil P. It is well documented that Zn-deficient plants posses an enhanced uptake capacity of P and supply of Zn to plants reduces uptake and accumulation of P in plants (Loneragan et al., 1982; Erdal, 1998; Rengel and Graham, 1995; Erdal et al., 2002). Akay and Ertas (2008) found that PA concentrations and PA/Zn ratios showed variation depending on chickpea varieties and increasing levels of Zn resulted in a decrease of PA/Zn and ratio in all varieties. Results are similar with ours findings. As seen in Table 5, there are significant positive correlations with P concentrations among PP and PA (Raboy et al., 1991), but negative significant correlation with grain Zn concentrations among P, PP and PA. These findings are similar with previous studies (Kacar and Katkat, 2007; Ryan et al., 2008). It should, however, be kept in mind that increase in phytic acid concentrations of seeds by Zn deficiency might also be related to concentration effect due to Zn-deficiency dependent decrease in grain yield and size (Erdal et al., 2002) Decrease of P, PP and PA due to increased N levels may be explained with dilution effect of N on grains (Kacar and Katkat, 2007). Soil Zn applications had a significant positive effect on grain Zn concentrations. While Zn concentration obtained from control plots were 35.2, 37.2 and 33.0 mg kg^{-1}, these values reached up to 40.8, 42.1 and 38.7 mg kg^{-1} with higher Zn levels for Akcin 91, Gokce and Aydın 92 varieties, respectively. Despite grain Zn concentrations of Akcin 91 and Gokce increased with the first N level (30 kg N ha^{-1}), it decreased with the second N level (30 kg N ha^{-1}). Comparing to control treatment, grain Zn concentration of Aydın 92 diminished first with the lower N level, but significantly increased again with higher N level. Phytic acid to Zn molar ratios determined from the whole varieties significiantly decreased with Zn and N applications. According to mean values, PA/Zn ratios changed with varieties. While the highest ratio was calculated at Akcin 91, the lowest was determined at Gokce (Table 3). Increase in Zn concentrations with Zn applications is well documented. As mentioned before, application of Zn had a significantly positive effect on grain Zn concentrations and also on grain yield especially under Zn deficient conditions. These effects varied with the plant variety and species even though they are grown in the same conditions (Erdal, 1998; Torun et al., 2001; Erdal et al., 2002;

Table 3. Effect of N and Zn applications on PA and Zn concentrations and PA/Zn molar ratio.

Variety	N level (kg N ha^{-1})	PA (mg g^{-1})				Zn (mg kg^{-1})				PA/Zn			
		0*	7	14	Mean	0	7	14	Mean	0	7	14	Mean
Akcin 91	0	8.3	7.2	6.7	7.4	33.3	38.7	40.7	37.6	24.9	18.6	16.5	19.7
	30	7.3	7.0	6.8	7.0	37.0	38.7	44.0	39.9	19.7	18.1	15.5	17.5
	60	6.4	6.3	6.3	6.3	35.3	38.0	37.7	37.0	18.1	16.6	16.7	17.0
	mean	7.3	6.8	6.6		35.2	38.4	40.8		20.7	17.7	16.2	
Gökce	0	5.9	5.5	5.1	5.5	38.7	39.0	41.3	39.7	15.2	14.1	12.3	13.9
	30	5.4	5.2	4.9	5.2	37.7	41.0	45.0	41.2	14.3	12.7	10.9	12.6
	60	5.0	4.5	4.2	4.6	35.3	36.3	40.0	37.2	14.2	12.4	10.5	12.4
	mean	5.4	5.1	4.7		37.2	38.8	42.1		14.5	13.1	11.2	
Aydın 92	0	6.4	5.9	5.7	6.0	32.0	36.7	38.3	35.7	20.0	16.1	14.9	16.8
	30	5.6	5.4	5.3	5.4	33.0	34.6	38.7	35.4	17.0	15.6	13.7	15.3
	60	5.3	5.1	5.0	5.1	34.0	37.0	39.0	36.7	15.6	13.8	12.8	13.9
	mean	5.8	5.5	5.3		33.0	36.1	38.7		17.6	15.2	13.7	
LSD (0.05)		G: 0.046; N: 0.046; Zn: 0.046; G x N x Zn: 0.137				G: 1.05; N: 1.05; Zn: 1.05; G x N: 1.81				G: 1.47; N: 1.47; Zn: 1.47; G x N x Zn: 4.41			

*Zn application (kg Zn ha^{-1}).

Table 4. Effect of N and Zn applications on phytase activity, protein content and grain yield.

Variety	N level (kg N ha^{-1})	Phytase activity (U g^{-1})				Protein (%)				Yield (kg ha^{-1})			
		0*	7	14	Mean	0	7	14	Mean	0	7	14	Mean
Akcin 91	0	3.6	4.0	3.7	3.8	16.0	16.8	16.9	16.6	1055	1133	1219	1136
	30	3.8	3.8	3.9	3.8	17.3	17.5	17.8	17.5	1296	1287	1301	1295
	60	4.2	4.4	4.3	4.3	19.0	19.5	20.7	19.7	1309	1340	1359	1336
	mean	3.9	4.1	4.0		17.4	17.9	18.5		1220	1253	1293	
Gökce	0	3.4	3.6	3.6	3.5	14.2	14.6	14.7	14.5	488	492	544	508
	30	3.1	3.9	3.6	3.5	14.7	15.5	15.8	15.3	631	678	693	667
	60	3.6	3.5	3.2	3.4	17.5	17.5	17.6	17.6	996	1013	1041	1017
	mean	3.4	3.7	3.5		15.9	16.0	18.1		705	728	759	
Aydın 92	0	4.3	3.9	3.8	4.0	17.3	17.5	19.4	18.1	936	1136	1159	1077
	30	4.0	3.9	3.9	3.9	17.9	17.8	18.1	17.9	1234	1238	1310	1261
	60	4.3	3.9	3.5	3.9	19.2	19.6	19.7	19.5	1318	1400	1413	1377
	mean	4.2	3.9	3.7		18.1	18.3	19.1		1163	1258	1294	
LSD (0.05)		G: 0.06; N: 0.06; Zn: 0.06; G x N x Zn: 0.179				G: 0.515; N: 0.515; Zn: 0.515				G: 2.11; N: 2.11; Zn: 2.11; G x N x Zn: 6.34			

*Zn application (kg Zn ha^{-1}).

Wei et al., 2007).

Depending on the cultivars, N and Zn fertilizations showed different effect on phytase activity. While N and Zn applications led to increase in phytase activity for a variety, it led to decrease in another. So it was not possible to reach a certain conclusion. Phytase activity varied with chickpea varieties as well. Soil N and Zn fertilizations had a positive effect on grain protein concentrations. While grain protein concentrations were the lowest at control treatments for both fertilizations, the highest protein levels were reached with the highest N and Zn doses (Table 4).

Table 5. Correlations between the dates obtained from the experiment.

	P	PP	Zn	PA/Zn	Protein	Yield	Phytase	PA
P	1.00	0.64**	-0.30**	0.77**	-0.23*	ns	0.25*	0.96**
PP		1.00	-0,42**	0.75**	ns	ns	ns	0.82**
Zn			1.00	-0.81**	-0.25*	ns	-0.27*	-0.37**
PA/Zn				1.00	ns	ns	0.31**	0.83**
Protein					1.00	0.81**	0.55**	ns
Yield						1.00	0.50**	ns
Phytase							1.00	ns
PA								1.00

*: $p < 0.05$, **: $p < 0.01$, ns: non-significant

Bioavailability of Zn in foods is highly depended on phytase activity. Phytases are enzymes that degrade phytate and permit higher availability of Zn and other nutrients such as P and Fe. Phytase activity of the cultivars is are not in the same or similar trend; it can be suggested that Zn nutrition has no direct effect on phytase activity (Erdal, 1998; Erdal et al., 2002). Also in the literature there are different result relating Zn application and phytase activity (Davies and Flett, 1978; Greiner and Alminger, 1999). Because N takes place in protein structure, and Zn plays an important role on protein synthesis, increase in protein concentration with N and Zn fertilization is an expected result (Marschner, 1995).

Both Zn and N fertilization increased grain yield significantly for all varieties. The highest yield was reached with the highest N and Zn levels. The significant differences for grain yield among varieties were determined as well (Table 4). Similar findings were recorded by the authors given above.

The results showed that Zn fertilization had a positive effect for increasing bioavailability of Zn in diets and this effect showed differences depending on the varieties even if they are grown in the same conditions.

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