Legume Res., **37** (1) : 55 - 61, 2014
10.5958/j.0976-0571.37.1.008

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
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EFFECTS OF ZINC AND NITROGEN FERTILIZATIONS ON GRAIN YIELD AND SOME PARAMETERS EFFECTING ZINC BIOAVAILABILITY IN LENTIL SEEDS

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Received: 04-04-2012 Accepted: 17-03-2013

ABSTRACT

This study aimed to investigate the effect of Zinc (Zn) and Nitrogen (N) fertilizations on grain zinc (Zn) phosphorus (P) and phytic acid (PA) concentrations, PA/Zn molar ratio, yield, phytase activity and protein content of lentil varieties. Zinc fertilization led to decrease in grain P concentrations and related parameters. Phytic acid/Zn molar ratios decreased with the increment of Zn and protein concentrations in grains, but increased with the increase of grain P levels. While there were positive relations amongst the grain P concentrations and P-related parameters, there was a close negative relation with Zn generally. Although, protein concentrations negatively affected by grain P and PA concentrations, there was a significant positive relation with N fertilization. Increase of both Zn and P concentrations in grains negatively affected phytase activity, but a positive correlation was seen between the protein and phytase. Grain yield increased with both Zn and N fertilization.

Key words: Lentil, Nitrogen, Phytic acid, Phytic acid to zinc ratio, Phytase Activity, Protein and Zinc.

INTRODUCTION

Phytic acid is found in most cereal grains, legumes, nuts, oilseeds, tubers, pollen, spores, and organic soils. 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 *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). A clear major role for phytate is as a store of inositol, phosphate, K, Mg, Ca, Mn, Fe and Zn for use by the seedling (Lott and Buttrose, 1978 ; Chen and Lott, 1992). These are released to developing seedlings by the action of phytase enzymes (Chen and Pan, 1977). Phytic acid, by binding nutritionally important minerals such as Zn, impairs their biological utilization. Phytic acid is also able to form complexes with proteins, and thus impairs digestibility and bioavailability of proteins in seeds (Reddy *et al.*, 1982). In most cases, PA to Zn molar ratios in foods are considered a predictor of Zn bioavailability rather than P, PA and Zn concentrations. Ratios above 20 to 30 have been reported to reduce Zn absorption and growth of animals (Oberleas and Harland, 1981). In the literature, it was documented that genetic variation effected plant P and PA concentrations (Raboy *et al.*, 1991; Erdal *et al.*, 2002; Kaya *et al.*, 2009). 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. Containing about 25% protein, 56% carbohydrate, and 1.0% fat in seeds, lentil is one of the best and cheapest sources of vegetable protein. Phytate content in legumes has been involved in reducing the bioavailability of minerals and inhibiting the activity of several enzymes (Knuckles *et al.*, 1989).

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One of the most effective way to decrease PA/Zn molar ratio is increasing the Zn concentration in grains especially under Zn deficient conditions. Also the attempts on increasing the activity of phytate-degrading enzymes (i.e. phytase) can play a key role to increase Zn bioavailability.

The present study was carried out to study the effects of Zn and N fertilizations on the levels of grain P, PA, Zn, and PA to Zn molar ratios, phytase activity and protein content of lentil cultivars. It was aimed to decrease PA/Zn molar ratio by increasing Zn in the grains depending on Zn fertilization. It was also tried to increase the bio-available protein content apart from phytate-bounded by making N fertilization. Also the other aim of the study is to compare lentil varieties in terms of examined parameters and thus Zn- bioavailability.

MATERIALS AND METHODS

This field experiment was conducted at Suleyman Demirel University research and experimental farm during 2009-2010 growing season. The experiment was set up in a randomized blocks design with 3 replications. The soil used was clay-loam with pH 8.3 (1:2.5); $CaCO_3$ 18%; organic matter 1.8%; 0.5 M $NaHCO_3$-extractable P is 30 Kg ha^{-1} (Olsen *et al.*, 1954); NH_4OAC-exchangable K is 600 Kg ha^{-1} (Jackson, 1967); DTPA-extractable Zn is 0.44 mg kg^{-1} (Lindsay and Norvell, 1978). Officially registered 3 lentil varieties (Emre 20, Sultan1, Alidayý) were used in the experiment. Sowing was done in 7.2 m^2 (1.2 x 6 m) plots by hand and the row space was 20 x 5 cm. Three Zn (0, 7 and 14 kg Zn ha^{-1} as $ZnSO_4.7H_2O$) and 3 N (0, 30 and 60 kg N ha^{-1} as NH_4SO_4) levels were applied to the soil 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 yields were calculated. After drying at 65 ^{0}C and grinding, seed samples were ashed at 550 ^{0}C 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 PA is based on precipitation of ferric phytate and measurement of iron (Fe) remaining in the supernatant (Haug and Lantzsch, 1983). For this, 0.5 g grinded seed sample was put in 25 ml. 0.2 N HCl for 3 h for the extraction of PA. 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 PA to Zn was calculated by dividing millimole of PA with millimole of Zn. Phytase activity was measured as described by Scheuermann *et al.* (1988). One gram grinded seed sample was incubated in 10 ml 50 mM sodium (Na) citrate buffer (pH = 5.3) and 50 mM Na-phytate at 37 ^{0}C for 1 h. After incubation, solutions were centrifuged and supernatants were brought up to 50 ml and analyzed for inorganic P. The N concentrations of samples were determined according to a Kjeldahl method (Bremler 1965). For this purpose, 0.5 g of grinded sample was placed in a digesting tube to which 6 mL of concentrated sulfuric acid (H_2SO_4) and 5 g of catalyst (potassium sulfate + copper sulfate) were added. Samples were digested using a block digesting system (KB 8 S Kjeldatherm, Gerhardt). After sodium hydroxide (40% w/w) was added, the sample was distilled using an automated unit (VAP20, Gerhardt). The ammonium N was fixed in 2% boric acid and titrated with 0.1 molar H_2SO_4 in the presence of an indicator (bromocresol green and methyl red in 95% ethanol). Protein content of grains was determined by multiplying total nitrogen by a conversion factor of 6.25

RESULTS AND DISCUSSION

Increasing levels of Zn significantly increased the grain Zn concentrations in all lentil cultivars. The highest Zn concentrations were reached at the highest Zn level in all cultivars. Comparing the control (-Zn), increment rate of Zn concentrations for Emre 20, Sultan 1 and Alidayý cultivars were about 46%, 37% and 45%, respectively. The general mean values showed that grain Zn concentration under control conditions was 27 mg kg^{-1}, but this level significantly increased up to 33 and 38 mg kg^{-1} with Zn_7 and Zn_{14} treatments, respectively Variety differences significantly affected grain Zn concentrations as well. Zinc concentration in the grains of Alidayý cultivars was higher than both Emre 20 and Sultan 1. Nitrogen

fertilization had no significant effect on Zn concentrations (Table 1 and Table 2). Under Zn deficiency, average P concentrations in seeds ranged from 3404 to 4088 mg kg^{-1} (Table 1) with a mean value of 3682 mg kg^{-1}. But for all cultivars, P concentrations significantly decreased with Zn fertilization leading to Zn increase in grains. Depending on the means, decrease rates were between 4% - 17% for Emre 20, 6% - 14 % for Sultan 1 and 15% -19% for Alidayý, respectively. Effect of N fertilization on grain P concentrations showed differences with the lentil varieties. Nitrogen fertilization led to decrease of grain P concentration generally. Looking at NxP interaction, the lowest P concentrations were determined with $N_{30}xZn_{14}$ application, generally. The highest levels were with Zn_0xN_0 (Sultan 1) and Zn_0xN_{30} (Emre 20 and Alidayý) applications. Under N_0 conditions, grain P concentrations were higher comparing the N_7 and N_{14} treatments (Table 1 and Table 2). Individual effect of Zn, N and variety and their interactions significantly affected grain PA concentration (Pd"0.05). Under Zn deficiency, PA concentrations were 9.7 mg g^{-1} (Emre 20) 7.7 mg g^{-1} (Sultan 1) and

TABLE 1: Effect of Zn and N fertilization on Zn, P and PA concentrations, PA/Zn molar ratio, phytat-P and phytat-P in total P in grains of lentil cultivars

Varieties	N levels (kg N ha^{-1})	Zn levels (kg Zn ha^{-1})											
		Zn_0	Zn_7	Zn_{14}	Mean	Zn_0	Zn_7	Zn_{14}	Mean	Zn_0	Zn_7	Zn_{14}	Mean
		Zn (mg kg^{-1})				P (mg kg^{-1})				PA (mg g^{-1})			
Emre 20 (V_E)	N_0	23	34	36	31	3593	3374	3326	3431	10.2	9.7	6.6	8.8
	N_{30}	24	32	36	31	3606	2901	2425	2977	8.1	7.8	7.3	7.8
	N_{60}	24	32	33	30	3465	3167	3128	3253	10.8	6.8	6.5	8.2
	Mean	24	33	35		3555	3147	2960		9.7	8.1	6.8	
Sultan 1 (V_S)	N_0	26	28	38	31	3594	3476	3024	3365	7.5	6.8	5.9	6.7
	N_{30}	28	33	35	32	3257	3007	2807	3023	8.3	5.7	5.2	6.4
	N_{60}	27	35	37	33	3362	3165	2926	3151	7.2	6.6	6.4	6.7
	Mean	27	32	37		3404	3216	2919		7.7	6.4	5.8	
Alidayy (V_A)	N_0	26	35	46	36	4085	3474	3342	3633	9.7	9.0	6.0	8.2
	N_{30}	32	35	41	36	4110	3456	3306	3624	7.7	7.0	6.7	7.1
	N_{60}	28	35	39	34	4070	3465	3249	3594	9.5	8.8	5.2	7.8
	Mean	29	35	42		4088	3465	3299		9.0	8.3	6.0	
		$LSD_{(0.05)}$ V , N, Zn: 0.7; VxNxZn: 2.1				$LSD_{(0.05)}$ V, N, Zn: 62; VxNxZn: 186				$LSD_{(0.05)}$ V, N, Zn: 0.03; VxNxZn: 0.1			
		PA/Zn (molar ratio)				Phytat-P (mg kg^{-1})				Phytat-P in total P (%)			
Emre 20 (V_E)	N_0	45	29	19	31	2873	2733	1860	2489	80	82	56	72
	N_{30}	46	22	18	29	2282	2198	2057	2179	63	76	85	75
	N_{60}	34	25	23	27	3044	1916	1832	2264	88	61	59	69
	Mean	42	25	20		2733	2282	1916		77	73	67	
Sultan 1 (V_S)	N_0	29	25	16	23	2114	1916	1663	1898	58	55	55	56
	N_{30}	30	18	15	21	2339	1606	1465	1803	72	53	52	59
	N_{60}	27	19	18	21	2029	1860	1804	1898	61	59	61	60
	Mean	29	20	16		2161	1794	1644		64	56	56	
Alidayy (V_A)	N_0	38	26	13	26	2734	2536	1691	2320	67	73	51	64
	N_{30}	24	20	17	20	2170	1972	1888	2010	53	57	57	56
	N_{60}	34	26	14	25	2677	2480	1465	2207	65	72	45	61
	Mean	32	25	15		2527	2329	1681		62	67	51	
		$LSD_{(0.05)}$:V, N, Zn: 0.7; VxNxZn: 2.2				$LSD_{(0.05)}$ V, N, Zn: 9.2; VxNxZn: 27.5				$LSD_{(0.05)}$ V, N, Zn: 1; VxNxZn: 3			

9.0 mg g^{-1} (Alidayý), with a mean value of 8.8 mg g^{-1} (Table 1 and Table 2). Fertilization of cultivars with Zn reduced PA concentrations in al cultivars by about 14% at Zn_7 and 30% at Zn_{14} levels. Grain PA concentrations varied with variety. In a study, it was demonstrated that grain PA concentrations in lentil grains were determined as 6.2 mg kg-1 and 8.1 mg g^{-1} and these values differed with variety (Vidal-Valverde *et al.*, 1994) According the general means, the highest PA concentration was determined in the grains of Emre 20 and this was followed by Alidayý and Sultan 1. The decreasing effect of Zn application on PA concentration under Zn-deficient conditions can be attributed to the inhibitory effect of Zn on root uptake and shoot accumulation of P and thus PA. It is well documented that Zn deficient plants possess an enhanced uptake capacity for P, and supply of Zn to Zn-deficient plants reduces uptake and accumulation of P in plants (Loneragan *et al.*, 1982; Cakmak and Marschner, 1986; Rengel and Graham, 1995, Erdal *et al.*, 2002). Therefore, increases in P and PA concentrations in seeds under Zn deficiency can be ascribed to Zn deficiency-enhanced uptake and shoot accumulation of P. It should, however, be kept in mind that increases in PA concentration of seeds by Zn deficiency might also be related to a concentration effect due to Zn deficiency-dependent decreases in grain yield and size. Mean values showed that PA concentrations decreased first with lower N level (N_{30}) in all varieties, but then increased again with N_{60} level. The mean values also gave the same results (Table 1 and Table 2). Decrease of PA concentration under N and Zn applied conditions might also be attributed to dilution effect due to increased grain yield under N and Zn applied conditions. Phytic acid to Zn molar ratios were significantly affected by Zn, N and variety and their interactions ($P \leq 0.05$). Depending on the concentrations of Zn, and PA, there was a large variation in PA to Zn molar ratios in seeds. Phytic acid to Zn molar ratio in the seeds of each lentil variety regularly decreased with the increasing levels of Zn doses. This decreasing tendency was observed at each N level. According to means obtained from Emre 20, Sultan 1 and Alidayý cultivars, PA/Zn molar ratios decreased from 42 to 25 and 20; 29 to 20 and 16 and 32 to 25 and 15, respectively. Depending on the general means, decreasing rates with Zn_7 and Zn_{14} were 32% and 50% respectively (Table 1 and Table 2). Nitrogen fertilization had decreasing effect on PA/Zn generally. Decreasing of PA/Zn ratios can also be depended on dilution of PA with increasing of grain weight under N and Zn applications. Another important factor for lowering PA/Zn molar ratios can strongly be depended on the increase of Zn concentrations in grains with Zn fertilization. As mentioned before, FA/Zn molar ratio is often considered an indicator for bioavailability of Zn in foods. Phytic acid to Zn molar ratios under Zn-deficient conditions were extremely higher than acceptable ratio for Zn bioavailability (Oberleas and Harland, 1981; Wise, 1995; Grüner *et al.*, 1996) But these ratios were drawn down until acceptable values for Zn bioavailability with Zn fertilization. In different studies, it was indicated that Zn fertilization is very effective to reduce PA/Zn molar ratio (Erdal *et al.*, 2002, Kaya *et al.*, 2009). Akay and Ertas (2008) found that PA concentrations and PA/Zn ratios showed variations depending on chickpea varieties and increasing levels of Zn resulted in a decrease of PA/Zn in all varieties. Due to phytate-P is related PA amount, the factors having effect on PA concentrations also effected phytate-P. Zinc fertilization resulted in significant decrease of

TABLE 2: General means of Zn, P, PA, PA/Zn, Phytat-P and Phytate-P in total P values

Treatments	Zn	P	PA	PA/Zn	Phytat-P	Phytat-P in total P
N_0	33	3476	7.9	27	2236	64
N_{30}	33	3208	7.1	23	1997	63
N_{60}	32	3333	7.6	24	2123	63
Zn_0	27	3682	8.8	34	2474	68
Zn_7	33	3276	7.6	23	2135	65
Zn_{14}	38	3059	6.2	17	1747	58
V_E	31	3220	8.2	29	2311	72
V_S	32	3180	6.6	22	1866	58
V_A	35	3617	7.7	24	2179	60

phytate-P with or without N fertilization. The avarage phytate-P decrased 14% (Zn_7) and 29% (Zn_{14}) comparing the Zn0. As in PA concentrations, phytate-P amounts varied with lentil varieties. The highest phytate-P concentration was found in the seeds of Emre 20, but the lowest was found in the seeds of Sultan 1. Nitrogen fertilization had a significant effect on phytate-P. The lowest phytate-P was determined with N application at levels of 30 kg N ha^{-1} comparing N_0 and N_{60} treatments. Depending on the Zn and N application and variety, phytate-P rate in total P varied between 45% and 88% (Table 1 and Table 2). Looking at the means, it could be seen that Zn fertilization negatively affected the rate of phytate-P in total P. Phytate-P rates also varied with the variety. Average values indicated that 72%, 58% and 60% of the total P has been as phytate-P in the seeds of Emre 20, Sultan 1 and Alidayý, respectively. These results are in the accordance with the previous studies (Lolas *et al.*, 1976; Chitra *et al.*, 1995; Erdal *et al.*, 2002; Mate and Radomir, 2002).

The grain yield was significantly affected by individually N, Zn and variety and their interactions. Nitrogen and Zn fertilization increased grain yield for each variety. The lowest yields were determined with the N_0xZn_0 treatments the highest were obtained with $N_{60}xZn_{14}$ for all varieties. According to the means, the grain yields showed regular increase with N and Zn levels, and these increments reached up to 35% for both nutrients. The grain yield showed great variation with the variety. Sultan 1 had lower yield than that of the Emre 20 and Alidayý varieties (Table 3 and Table 4) . The findings of this study showed similarities with the results of Liu *et al.*, (2004) and Zeidan *et al.*, (2006). Phytase activity in grain significantly varied with N, Zn and cultivar and their interactions (Table 3). It was seen that, Zn fertilization had a negative effect on phytase activity. On the contrary, N fertilization led to increase of phytase activity. The highest phytase activity was measured in the grains of variety Sultan 1, whilst the lowest was measured in the seeds of cv. Alidayý (Table 4). Bioavailability of Zn in foods shows a high dependency on the levels of phytase activity. Phytases are enzymes that degrade phytate and permit higher availability of Zn and other mineral nutrients such as P and Fe. In a study using diets containing triticale or corn, better P utilization and growth was found in animals that were supplied with triticale diets (Pointillart *et al.*, 1987). The superiority of triticale diets over the corn diets was attributed to higher phytase activity in triticale than in corn. As found within genotypes of different cereals activity of phytase shows an important genotypic variation (Singh and Sedeh, 1979; Erdal *et al.*, 2002). As the effects of varied Zn supply on phytase activity of the cultivars are not in a same or similar trend, it can be suggested that Zn nutrition has no direct effect on phytase activity. Also in the literature there are controversial results on the effects of Zn on phytase activity: the addition of Zn was found to exert an

TABLE 3: Effect of Zn and N fertilization on yield, phytase activity and protein content in grains of lentil cultivars

Varieties	N levels (kg N ha^{-1})	Zn levels (kg Zn ha^{-1})											
		Zn_0	Zn_7	Zn_{14}	Mean	Zn_0	Zn_7	Zn_{14}	Mean	Zn_0	Zn_7	Zn_{14}	Mean
		Yield (kg/ha)				Phytase Activity (µmol Pi g^{-1} min^{-1})				Protein (%)			
Emre 20	N_0	694	1100	1129	974	4.32	4.29	3.97	4.19	24	25	26	25
	N_{30}	764	1118	1154	1012	4.38	4.32	3.83	4.17	26	27	27	27
	N_{60}	858	1143	1392	1131	4.27	4.15	4.04	4.15	27	28	28	28
	Mean	772	1120	1225		4.32	4.25	3.95		26	27	27	
Sultan 1	N_0	496	556	713	588	4.30	4.40	4.44	4.38	26	27	28	27
	N_{30}	580	626	724	643	4.32	4.40	4.41	4.38	27	27	27	27
	N_{60}	716	733	1053	834	4.23	4.25	4.38	4.29	27	27	29	28
	Mean	597	638	830		4.28	4.35	4.41		27	27	28	
Alidayy	N_0	756	861	886	834	3.68	3.72	3.81	3.74	22	24	24	23
	N_{30}	967	1010	1151	1043	4.07	3.92	3.85	3.95	23	25	25	24
	N_{60}	1228	1269	1316	1271	4.13	4.09	4.03	4.08	27	27	28	27
	Mean	984	1047	1118		3.96	3.91	3.89		24	25	26	
		$LSD_{(0.05)}$: V, N, Zn: 28; VxNxZn: 83				$LSD_{(0.05)}$: V, N, Zn: 7.73; VxNxZn: 23				$LSD_{(0.05)}$: V, N, Zn: 0.27; VxN: 0.47			

TABLE 4: General means of yield, phytase activity and protein values

Treatments	Yield	Phytase Activity	Protein
N_0	800	4.10	25
N_{30}	897	4.17	26
N_{60}	1077	4.17	28
Zn_0	*784*	*4.19*	*26*
Zn_7	*935*	*4.17*	*26*
Zn_{14}	*1058*	*4.08*	*27*
V_E	1039	4.17	27
V_S	688	4.35	27
V_A	1049	3.92	25

inhibitory effect on the activity of purified oat phytase (Greiner and Alminger, 1999), while Davies and Flett (1978) found that Zn ions are required for maximal activity for intestinal phytase in rats. A decrease in phytase activity might be expected under very severe Zn-deficient conditions due to inhibition of protein synthesis in Zn-deficient cells (Cakmak *et al.*, 1989). Grain protein concentrations significantly increased with the increase of N and Zn levels in all cultivars. On average of all cultivars, protein concentrations increased by 12% with N and by 4% with Zn. For all cultivars, the lowest protein concentrations were determined under Zn_0xN_0 conditions, the highest protein levels were determined with $Zn_{14}xN_{60}$ applications (Table 3 and Table 4). Grain protein concentrations ranged between 22% (Alidayý, Zn_0xN_0) to 29% (Sultan 1, $Zn_{14}xN_{60}$). Due to the close relation between N and protein formation, increase of protein concentration with N fertilization is an expected result (Marshner, 1995). Also, protein concentration in plant decreases under Zn deficient conditions (Cakmak *et al.*, 1989), and increase with Zn applications.

CONCLUSIONS

Although, PA concentrations increased with the increasing levels of P in seeds, it showed a decreasing tendency with the increment of seed Zn concentrations in all lentil cultivars. Also PA to Zn molar ratio decreased with Zn fertilization until the acceptable levels for Zn bioavailability. While N fertilization seems to have positive effect on phytase activity, increasing levels of Zn led to decrease in phytase. Lentil cultivars showed a great genotypic variation in terms of seed PA concentration, PA to Zn molar ratio and phytase activity. An important finding in this study is that the variety (Sultan 1) which has the lowest PA (6.6 mg kg^{-1}) and PA/Zn molar ratio has the highest phytase activity (4.35 µ mol Pi min^{-1}). So, it may be said that bioavailability of Zn in the grains of Sultan 1 is higher and this variety can be chosen for growing. But it shouldn't be forgotten that the yield of this variety is quite lower comparing to others. Another important finding in this study is that, Zn concentrations of seed can be increased by Zn fertilization to increase Zn bioavailability. Grain protein concentrations increased with N and Zn fertilization. This result is important for receiving high level of bio-available protein. And this also might be important to prevent a decrease in phytase activity under the scarce protein synthesis (Cakmak *et al.*, 1989). Our findings show that phytase activity is higher in the seeds of cultivars having higher protein content.

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