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RESEARCH PAPER

Chemical composition of corn and sorghum grains cultivated in Oxisol with different application methods and doses of zinc

Aline P. Puga¹, Renato de Mello Prado², Ben-Hur Mattiuz², Diego Wyllyam do Vale³, and Ivana M. Fonseca⁴

¹Instituto Agronômico de Campinas (IAC), Avenida Barão de Itapura, 1481- Campinas (SP), Brazil. ²Faculdade de Ciências Agrárias e Veterinárias (FCAV), Universidade Paulista Júlio de Mesquita Filho (UNESP). Via de acesso Prof. Paulo Donato Castellane, s/n. - Jaboticabal (SP), Brazil.

³Escola Superior de Agricultura "Luiz de Oueiroz" (ESALO), Universidade de São Paulo (USP), Avenida Pádua Dias, 11 - Piracicaba (SP), Brazil.

⁴Embrapa Cocais, Avenida São Luís Rei de França, 4 Qd 11 - São Luís (MA), Brazil.

Abstract

A.P. Puga, R.M. Prado, B. Mattiuz, D.W. Vale, and I.M. Fonseca. 2013. Chemical composition of corn and sorghum grains cultivated in Oxisol according to different application methods and doses of zinc. Cien. Inv. Agr. 40(1):97-108. In general, tropical soils present low concentrations of zinc (Zn), and the deficiency of Zn is recognized as a world nutritional problem for cereal production and human beings. Therefore, the main goal of this study was to assess the effects of different methods of Zn application on the quality of corn and sorghum grains grown in Oxisol. Two experiments were set up in the experimental area of UNESP (campus of Jaboticabal, Brazil). The following nine treatments were applied: three doses of Zn by banded application (seed furrows), three doses of Zn by incorporation into soil (0-20 cm depth), foliar application, seed application, and control (no Zn applied). The treatments were arranged in randomized blocks with four replicates. The contents of Zn, carbohydrates and proteins were determined for corn and sorghum grains. Regardless of the method, Zn application promoted higher contents of this micronutrient in corn and sorghum grains. The banded application method of Zn in soil promoted greater contents of total carbohydrates, starch and protein in both cultures. The incorporation of Zn into the soil method provided higher contents of soluble carbohydrates in both corn and sorghum grains.

Key words: Carbohydrate, protein, Sorghum bicolor, Zea mays L., Zn.

Introduction

Cereals are grasses mostly cultivated for their edible grains, and they represent important source of minerals and proteins in the developing world. Grains are used as basic food stock in most countries (Cakmak, 2008).

In developing countries, the diet is often based on cereal consumption, which has low Zn (Zn) content and bioavailability (Cakmak et al., 2010). The most frequent deficiencies in humans are those of iron, Zn, iodine and vitamin A, and these deficiencies occur especially among women and children in developing countries (WHO, 2002). Zn deficiency in humans is common, and it is estimated to affect 25% of the world population (Maret and Sandstead,

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2006), especially in several regions with soils deficient in Zn, including India, Pakistan, China, Iran and Turkey (Cakmak *et al.*, 1999, Hotz and Brown, 2004). The lack of this micronutrient in human beings is responsible for severe health complications, including problems in physical growth; damage to the immune system; damage to learning ability; and increased risk of infections, DNA damage and cancer development (Gibson, 2006; Hotz and Brown, 2004; Prasad, 2007).

It is possible that low quality cereal grains occur because the main goal of plant breeding programs has been to increase yield during the last decades (Peleg *et al.*, 2008). However, the nutritional composition is equally important and has been largely neglected in such programs, especially concerning micronutrient contents (Cakmak, 2002). Thus, future studies assessing more efficient Zn application methods to promote Zn uptake in plants and to maximize the accumulation of this micronutrient in grains must be developed (Camak, 2008).

In soils with slight Zn deficiency, the yields and quality can be affected without any evident symptoms (Alloway, 2009), which can result in food production with comprised nutritional value. Therefore, the enrichment of such cereals with Zn is an important global challenge and of great priority in research (Cakmak *et al.*, 2010).

Cereals often present low contents of proteins and micronutrients, including iron and Zn (Newell, 2008). According to Peck *et al.* (2008), no studies have been conducted to evaluate the effect of Zn on the quality of grains or to assess if the increase of available Zn can raise protein concentration in grains (Hemantaranjan and Garg, 1988) because Zn deficiency results in low protein content in plants (Moinuddin and Imas, 2010). Such decrement is due to decreased RNA as well as the deformation and reduction of ribosomes (Brown *et al.*, 1993).

In addition, a few field studies on wheat have suggested that the application of fertilizer containing Zn increases the productivity and concentration of Zn in grains by approximately 3.5-fold by soil and foliar application methods in calcareous soils (Yilmaz *et al.*, 1997).

Studies on Zn with different crops have also been conducted in other locations as follows: beans in Germany (Cakmak *et al.*, 1989), potatoes in Iran (Mousavi *et al.*, 2007) and wheat in Turkey (Ozturka *et al.*, 2006). Nevertheless, there is a lack of studies concerning this micronutrient in Brazil, especially in field conditions. Therefore, agronomic techniques suitable to elevate Zn, protein and carbohydrate contents both quantitatively and qualitatively in grains are of great importance and should be extended to other cultures and in different edaphoclimatic conditions.

Thus, the main goal of this study was to assess the effects of different Zn application methods and doses on grain quality for corn and sorghum grown in Oxisols.

Materials and methods

The experiment was conducted at FCAV/UNESP (21°15'22" S and 48°18'58" W; altitude of 575 m) in Oxisol with a clay texture according to EMBRAPA (2006). Based on the international classification of Köppen, the climate of the region is Cwa. The soil chemical analysis (0 to 20 cm soil layer) was performed according the methods described by Raij et al. (2001) and had the following properties: pH in $CaCl_{2} = 5.5$; organic matter (OM) = 1.6%; P (resin) = 31 mgkg; $K = 0.11 \text{ cmol}_{a} \text{ kg}^{-1}$; $Ca = 2.5 \text{ cmol}_{a} \text{ kg}^{-1}$; Mg = 1.2 cmol, kg⁻¹; H + Al = 2.2 cmol, kg⁻¹; sum of bases (SB) = $3.8 \text{ cmol}_{\text{kg}^{-1}}$; CEC = $6.0 \text{ cmol}_{\text{s}}$ dm^{-3} ; V% = 63; S = 1.0 mg kg⁻¹; B = 0.30 mg kg⁻¹; $Cu = 1.0 \text{ mg kg}^{-1}$; Fe = 13.0 mg kg $^{-1}$; Mn = 16.1 mg kg⁻¹; and Zn = 0.5 mg kg⁻¹. According to the interpretation of Raij et al. (1997), the content of Zn in this area is considered low, therefore, a deficient condition.

The plots were composed of four lines that were 5 m in length. The two central lines were used for sowing, and the other lines were used as borders with a spacing between lines of 0.9 m for corn and 0.45 m for sorghum. A simple hybrid of corn (Impacto) and simple hybrid of sorghum (Dow822) were used. Sowing was carried out in December 2008, and the medium precipitation during the

experimental period from sowing to harvest was 996 mm. The average temperature of the period was 23.8 °C. No irrigation system was used.

At the time of sowing, basic fertilization was applied uniformly in all treatments with the following composition: 30 kg ha⁻¹ N, 50 kg ha⁻¹ P_2O_5 and 50 kg ha⁻¹ of K_2O in the form of urea and NPK fertilizer (02-20-20).

The following nine treatments were applied: three doses of Zn (2, 4 and 8 kg of Zn ha⁻¹) by banded application (seed furrows), three doses of Zn (6, 12 and 24 kg of Zn ha⁻¹) incorporated into soil (0-20 cm depth), foliar application (0.4 kg of Zn ha⁻¹), seed application (40 g of Zn kg⁻¹ of seeds), and control (no Zn application). The treatments based on soil and foliar application received Zn in the form of Zn sulfate (22.7% Zn and 11% S), and treatments applied to seeds used Zn oxide (79% Zn). The first foliar application was performed 15 days after emergence, and the second application was performed 30 days after emergence. Before the seed application, a sugar solution was applied to the seeds to ensure higher adhesion of fertilizer. The experimental design included randomized blocks with four replicates.

Harvest was performed when grains of both cultures had an approximate humidity of 13%, which was approximately 150 days after emergence of plants. The grains were then oven-dried with forced air circulation (65-70 °C) and pulverized. The material was digested by the nitric/perchloric acid procedure according to Bataglia *et al.* (1983), and Zn content was determined by atomic absorption spectrometry.

The carbohydrate content in grains was determined according the method described by Nelson (1944) and adapted by Somogyi (1952). To analyze the starch content, the samples were previously subjected to acid hydrolysis and determined by the previously cited method.

Samples were analyzed for crude protein (CP) by the Dumas combustion method using a nitrogen AutoAnalyzer (LECO®, model FP-528) (Wiles *et al.*, 1998).

The data from the studied variables were subjected to variance analysis by the F-test and using the degrees of freedom from treatments in orthogonal contrasts. Moreover, a polynomial regression was performed for doses pertaining to the Zn application methods via soil. More experimental details are presented in Puga (2011).

Results and discussion

Treatment effects on nutrient contents in grains

With regard to the Zn content in grains from the corn culture, it was noted that the Zn application promoted higher Zn content (24 to 28 mg kg⁻¹) compared to the control (19 mg kg⁻¹) (Table 1). When studying the correlation of Zn deficiency in corn, Galrão (1994) observed that the contents of this micronutrient in grains varies little among treatments (Zn application methods), which was similar to the present findings. Ferreira et al. (2001) reported that the Zn content in grains increases by 7% due to the application in seed furrows, but the increase reached 32% in the present study. Similarly, when studying doses of Zn applied in soil for corn culture. Kanwal et al. (2010) noted significant increments in the content of this micronutrient in corn grains (21.8 to 30.7 mg kg⁻¹).

According to Welch (2002), increasing the Zn application in soil significantly increases its concentration in edible parts of plants from food cultures. Relatively greater Zn contents in corn

$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Tine andication	Z	d	Л	Č	Ma	U	a	5	Бa	Ми	7.0
Sol, bandled, $k_{\rm g}$ hu ² 10 11 3 2 34 95 11 3 2 34 95 95 95 95 95 95 95 95 95 95 95 95 95 95 96 95 95 96 <th< td=""><td></td><td>15.0</td><td>-</td><td>4</td><td>00</td><td>9m</td><td>2</td><td></td><td>20</td><td>21</td><td>TIM</td><td>20</td></th<>		15.0	-	4	00	9m	2		20	21	TIM	20
	Soll, banded, 2 kg ha ⁻¹	15.0	3.4	4.9	0.2	1.6	1.1	6	2	24	L	25
	Soil, banded, 4 kg ha ⁻¹	15.1	3.2	4.6	0.2	1.5	1.1	3	2	23	9	24
	Soil, banded, 8 kg ha ⁻¹	15.2	3.3	4.9	0.2	1.5	1.1	ю	2	27	9	26
	Soil, incorporated, 6 kg ha ⁻¹	15.0	3.1	4.6	0.3	1.5	1.1	4	2	22	9	26
	Soil, incorporated, 12 kg ha ⁻¹	15.1	3.2	4.8	0.2	1.6	1.2	3	2	23	9	26
	Soil, incorporated, 24 kg ha ⁻¹	15.0	3.1	4.6	0.2	1.4	1.1	3	2	24	9	28
	Foliar, 0.4 kg ha ⁻¹	15.1	3.2	4.8	0.2	1.5	1.1	4	3	26	L	24
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Seed, 40 g kg ⁻¹	15.1	3.0	4.2	0.3	1.4	1.1	3	2	27	9	24
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Control	14.9	3.1	4.6	0.2	1.5	1.1	4	2	22	9	19
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$							F-test					
	Control vs. others	0.513 ns	0.085 ns	$0.124\mathrm{ns}$	0.035 ns	0.036 ns	$0.054\mathrm{ns}$	1.284ns	0.117 ns	$1.930\mathrm{ns}$	0.767 ns	21.051**
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Soil vs. (foliar + seed)	$0.092\mathrm{ns}$	0.906 ns	1.485 ns	0.106 ns	0.583 ns	0.018 ns	$0.079\mathrm{ns}$	$0.352\mathrm{ns}$	3.608 ns	0.159 ns	2.767 ns
	Foliar vs. seed	$0.000 \mathrm{ns}$	$0.602\mathrm{ns}$	2.507 ns	2.859 ns	1.284 ns	0.216 ns	2.122 ns	$0.000\mathrm{ns}$	$0.192\mathrm{ns}$	$0.306\mathrm{ns}$	$0.000 \mathrm{ns}$
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Soil, incorporated vs. soil banded	$0.246\mathrm{ns}$	1.016 ns	$0.548\mathrm{ns}$	0.106 ns	$0.048\mathrm{ns}$	0.288 ns	$0.079\mathrm{ns}$	$0.157\mathrm{ns}$	1.299 ns	0.637 ns	3.416 ns
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Doses in banded	0.232 ns	0.106 ns	$0.396\mathrm{ns}$	$0.000\mathrm{ns}$	$0.073\mathrm{ns}$	$0.000 \mathrm{ns}$	$0.000\mathrm{ns}$	$0.200\mathrm{ns}$	3.972 ns	0.529 ns	$1.280\mathrm{ns}$
$CV (\phi_0)$ 2.5 11.6 10.1 24.6 12.6 6.9 21.7 21.4 13.2 20.4 Soil, banded, $2kgha^{+1}$ 17.4 5.6 7.1 0.2 3.7 1.2 8 12 141 34 Soil, banded, $4kgha^{+1}$ 17.3 5.5 7.1 0.2 3.7 1.2 7 11 137 30 Soil, banded, $4kgha^{+1}$ 17.3 5.5 7.1 0.4 3.4 1.2 7 11 144 34 Soil, incorporated, $24kgha^{+1}$ 16.6 5.6 7.1 0.3 3.6 1.2 7 1.47 29 Soil, incorporated, $24kgha^{+1}$ 16.9 5.6 7.1 0.3 3.2 1.2 1.2 21 29 Soil, incorporated, $24kgha^{-1}$ 16.9 5.6 7.1 0.3 3.2 1.2 21 21 22 26.6 23.7 1.2	Doses in incorporated	0.056 ns	$0.064\mathrm{ns}$	0.113 ns	2.739 ns	0.548 ns	$3.000\mathrm{ns}$	0.724ns	$1.000\mathrm{ns}$	0.111 ns	$0.130\mathrm{ns}$	0.843 ns
Soil, banded, 2 kg harl17.45.67.10.23.71.281.21.13.4Soil, banded, 4 kg harl16.95.16.50.33.11.271113730Soil, banded, 4 kg harl16.95.16.50.33.11.271114434Soil, incorporated, 0 kg harl16.65.56.60.33.41.271114434Soil, incorporated, 12 kg harl16.95.67.10.33.41.271214729Soil, incorporated, 12 kg harl16.95.67.10.33.41.271114434Soil, incorporated, 12 kg harl17.24.86.00.33.41.271214729Soil, incorporated, 12 kg harl17.25.67.10.33.41.271114228Soil, incorporated, 24 kg harl17.25.67.10.33.41.271114228Seed, 40 kg ¹ 61161.161.11.271114228Control5.67.10.33.41.271114228Seed, 40 kg ²¹ 5.67.10.33.41.271112628Control5.67.10.33.41.271112656Control	CV (%)	2.5	11.6	10.1	24.6	12.6	6.9	21.7	21.4	13.2	20.4	8.9
Soil, banded, 4 kg harl[695.1 6.5 0.3 3.1 1.2 7 11 137 30 Soil, banded, 8 kg harl 17.3 5.5 7.1 0.4 3.4 1.2 7 11 144 34 Soil, incorporated, 6 kg harl 16.6 5.5 6.6 0.3 3.4 1.2 7 11 144 34 Soil, incorporated, 12 kg harl 16.9 5.6 7.1 0.3 3.5 1.2 7 11 142 29 Soil, incorporated, 24 kg harl 17.2 5.5 6.4 0.4 3.2 1.2 7 12 167 29 Soil, incorporated, 24 kg harl 17.2 5.6 7.1 0.3 3.6 1.1 6 11 142 29 Seed, 40 g kg^1 6.9 5.6 7.1 0.3 3.4 1.2 7 11 142 28 Seed, 40 g kg^1 6.0 0.3 3.4 1.2 7 11 142 28 Seed, 40 g kg^1 6.0 0.3 3.4 1.2 7 11 142 28 Control 5.6 7.1 0.3 3.4 1.2 7 11 142 28 Seed, 40 g kg^1 5.6 7.1 0.3 3.4 1.2 7 11 142 28 Control 5.6 7.1 0.3 3.3 1.2 7 11 142 28 Control 0.100 0.100	Soil, banded, 2 kg ha ⁻¹	17.4	5.6	7.1	0.2	3.7	1.2	8	12	141	34	37
Soil, banded, 8 kg harl17.35.57.10.43.41.271114434Soil, incorporated, 6 kg harl16.65.56.60.33.41.281214729Soil, incorporated, 12 kg harl16.95.67.10.33.51.271215029Soil, incorporated, 12 kg harl17.25.26.40.43.51.271215029Soil, incorporated, 24 kg harl17.25.26.40.43.21.161114228Seed, 40 g kg ⁻¹ 17.25.67.10.33.41.271216930Seed, 40 g kg ⁻¹ 16.95.67.10.33.41.271114228Control23.31.161.114228Seed, 40 g kg ⁻¹ 5.67.10.33.41.271216930Seed, 40 g kg ⁻¹ 5.67.10.33.41.271114228Control26.60.33.31.1271113030Seed, 40 g kg ⁻¹ 5.67.10.33.31.271113030Control26.60.33.31.27111303030Seed, 40 g kg ⁻¹ 5.60.10ns0.20ns0.003ns1.087ns0.002ns0.653	Soil, banded, 4 kg ha ⁻¹	16.9	5.1	6.5	0.3	3.1	1.2	7	11	137	30	36
Soil, incorporated, 6 kg ha ¹ 16.65.56.60.33.41.281214729Soil, incorporated, 12 kg ha ¹ 16.95.67.10.33.51.271215029Soil, incorporated, 12 kg ha ¹ 17.25.26.40.43.21.261112629Foliar, 0.4 kg ha ¹ 17.25.26.40.43.21.261112629Foliar, 0.4 kg ha ¹ 17.24.86.00.33.01.161114228Seed, 40 g kg ¹ 16.95.67.10.33.41.271216332Control200.33.31.16111422828Seed, 40 g kg ¹ 5.56.60.33.31.271216330Control5.67.10.33.31.271113030Seed, 40 g kg ¹ 5.26.60.33.31.271113030Control5.55.60.10ns0.00ns0.02ns0.002ns0.038ns1.833ns0.03Control vs. others0.014ns0.178ns0.100ns0.29ns0.027ns0.002ns0.368ns1.563ns0.00Soil vs. (foliar + seed)0.28ns0.29ns0.179ns0.29ns0.179ns3.200ns0.174ns1.563ns0.00Soil vs. (foliar + seed) <td>Soil, banded, 8 kg ha⁻¹</td> <td>17.3</td> <td>5.5</td> <td>7.1</td> <td>0.4</td> <td>3.4</td> <td>1.2</td> <td>L</td> <td>11</td> <td>144</td> <td>34</td> <td>37</td>	Soil, banded, 8 kg ha ⁻¹	17.3	5.5	7.1	0.4	3.4	1.2	L	11	144	34	37
Soil, incorporated, 12 kg harl16.95.67.10.33.51.271215.029Soil, incorporated, 24 kg harl17.25.26.40.43.21.261112629Foliar, 0.4 kg harl17.25.26.40.43.21.161114228Seed, 40 g kg ¹ 17.25.67.10.33.41.271114228Control5.67.10.33.41.271113030Control5.67.10.33.31.271113030Control5.67.10.33.31.271113030Control5.60.10ns0.10ns0.03ns1.087ns0.002ns0.368ns1.853ns0.005Control vs. others0.014ns0.178ns0.100ns0.025ns0.003ns1.087ns0.000ns2.133ns0.00Soil vs. (foliar + seed)0.014ns0.178ns0.299ns0.076ns0.229ns1.174ns1.563ns0.0002.133ns0.10Field0.12ns2.069ns2.701ns0.229ns1.179ns3.521ns3.000ns2.133ns0.10Field0.12ns0.013ns0.076ns0.014ns1.174ns1.563ns0.000ns2.133ns0.10For tororected1.437ns0.013ns0.076ns0.214ns1.179ns3.520ns1.565ns0.000ns <td>Soil, incorporated, 6 kg ha⁻¹</td> <td>16.6</td> <td>5.5</td> <td>6.6</td> <td>0.3</td> <td>3.4</td> <td>1.2</td> <td>8</td> <td>12</td> <td>147</td> <td>29</td> <td>35</td>	Soil, incorporated, 6 kg ha ⁻¹	16.6	5.5	6.6	0.3	3.4	1.2	8	12	147	29	35
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Soil, incorporated, 12 kg ha ⁻¹	16.9	5.6	7.1	0.3	3.5	1.2	7	12	150	29	39
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Soil, incorporated, 24 kg ha ⁻¹	17.2	5.2	6.4	0.4	3.2	1.2	9	11	126	29	40
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Foliar, 0.4 kg ha ⁻¹	17.2	4.8	6.0	0.3	3.0	1.1	9	11	142	28	38
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Seed, 40 g kg ⁻¹	16.9	5.6	7.1	0.3	3.4	1.2	7	12	163	32	35
Control vs. others 0.014 ms 0.178 ms 0.100 ms 0.025 ms 0.003 ms 1.087 ms 0.002 ms 0.368 ms 1.853 ms 0.002 Soil vs. (foliar + seed) 0.028 ms 0.620 ms 0.025 ms 0.003 ms 1.087 ms 0.002 ms 0.368 ms 1.853 ms 0.002 Foliar vs. seed 0.028 ms 0.620 ms 0.290 ms 0.076 ms 0.025 ms 1.174 ms 1.563 ms 0.368 ms 1.353 ms 0.100 Foliar vs. seed 0.712 ms 2.069 ms 2.701 ms 0.229 ms 1.179 ms 3.522 ms 3.000 ms 0.224 ms 1.565 Soil, incorporated vs. soil banded 1.437 ms 0.013 ms 0.076 ms 0.014 ms 1.174 ms 1.361 ms 0.276 ms 3.602 Doses in banded 0.487 ms 0.840 ms 0.541 ms 2.280 ms 1.656 ms 1.656 ms 2.053 ms 0.214 ms 1.080 Doses in incorporated 1.151 ms 0.690 ms 3.857 ms 0.392 ms 1.000 ms 0.123 ms 1.080 Doses in incorporated 1.151 ms 0.669 ms 3.857 ms 0.392 ms 1.000 ms 0.123 ms 1.289 ms 0.021 ms	Control Sorg	17.1	5.2	6.6	0.3	3.3	1.2 E taat	٢	11	130	30	32
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Solitys, (total + seed) 0.042018 0.02018 0.02018 0.02018 0.02018 0.00018 2.00018 1.565 Soil, incorporated vs. soil banded 1.437 ns 0.013 ns 0.310 ns 0.076 ns 0.014 ns 1.174 ns 1.361 ns 0.276 ns 0.003 ns 3.602 Doses in banded 1.437 ns 0.840 ns 0.541 ns 2.280 ns 1.656 ns 1.600 ns 2.053 ns 0.214 ns 1.080 Doses in incorporated 1.151 ns 0.660 ns 3.857 ns 0.392 ns 1.000 ns 0.704 ns 0.123 ns 1.289 ns 0.024		CIT LT0.0	SIL0/1.0	SILUUT.U	SII 020.0	SII COV.0	сп / 0.0.1 1 174 - 2	сн 200.0	SH 000 0	сп ссо.т	0 100	0.461
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Soil, incorporated vs. soil banded 1.437 ns 0.013 ns 0.076 ns 0.014 ns 1.174 ns 1.361 ns 0.276 ns 0.003 ns 3.603 Doses in banded 0.487 ns 0.840 ns 0.541 ns 2.280 ns 1.656 ns 1.656 ns 1.600 ns 2.053 ns 0.500 ns 0.214 ns 1.080 Doses in incorporated 1.151 ns 0.656 ns 0.690 ns 3.857 ns 0.392 ns 1.000 ns 0.704 ns 0.123 ns 1.289 ns 0.026	Foliar vs. seed	0.712 ns	2.069 ns	2.701 ns	0.229 ns	1.179 ns	3.522ns	$3.000\mathrm{ns}$	0.828 ns	2.249 ns	1.569 ns	0.759 ns
Doses in banded 0.487 ns 0.840 ns 0.541 ns 2.280 ns 1.656 ns 1.800 ns 2.053 ns 0.500 ns 0.214 ns 1.080 Doses in incorporated 1.151 ns 0.656 ns 0.690 ns 3.857 ns 0.392 ns 1.000 ns 0.704 ns 0.123 ns 1.289 ns 0.026	Soil, incorporated vs. soil banded	1.437 ns	0.013 ns	0.310 ns	0.076 ns	0.014 ns	1.174ns	1.361 ns	0.276ns	0.003 ns	3.603 ns	0.732 ns
Doses in incorporated 1.151 ns 0.656 ns 0.690 ns 3.857 ns 0.392 ns 1.000 ns 0.704 ns 0.123 ns 1.289 ns 0.026	Doses in banded	$0.487\mathrm{ns}$	$0.840\mathrm{ns}$	0.541 ns	2.280 ns	1.656ns	$1.800\mathrm{ns}$	2.053 ns	$0.500\mathrm{ns}$	$0.214\mathrm{ns}$	1.080 ns	$0.050\mathrm{ns}$
	Doses in incorporated	1.151 ns	0.656 ns	0.690 ns	3.857 ns	$0.392\mathrm{ns}$	$1.000\mathrm{ns}$	$0.704\mathrm{ns}$	$0.123 \mathrm{ns}$	1.289 ns	$0.026\mathrm{ns}$	2.082 ns
CV (%) 3.2 13.3 14.8 25.1 15.6 4.8 17.6 10.3 13.4 14.8	CV (%)	3.2	13.3	14.8	25.1	15.6	4.8	17.6	10.3	13.4	14.8	11.1

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grains are vital to human nutrition and are thus essential to the biofortification of basic food crops (Graham *et al.*, 1992). However, accumulation of Zn in grains or seeds is a complex and intricate process that comprises a series of steps from its translocation from roots to shoot and finally to the phloem unloading for grain development (Welch, 1986).

For the nutrient content in sorghum grains, there were no significant differences for the macro- and micronutrients assessed, except for Zn (Table 1), in all tested treatments. The application of Zn resulted in higher Zn content (35 to 40 mg kg⁻¹) in grains compared to the control (32 mg kg⁻¹).

Similar to the corn culture, there was only differences in the first comparison group (Table 1) in which the application of Zn resulted in higher Zn content compared to the control. When studying foliar application of Zn in wheat, Ozturka *et al.* (2006) found that the greater accumulation of Zn during grain development occurs in the initial stage of grain formation (milk grain stage) suggesting that the foliar application of Zn during the final stage of wheat growth can be an effective way of increasing the Zn concentration in grains.

Effects of treatments on carbohydrate contents in grains

For the corn culture, in the first comparison group tested (control vs. other treatments) (Table 2), there were no differences in all the variables analyzed (soluble carbohydrates, starch and total carbohydrates).

Moreover, there were no significant differences between the soil application and plant application treatments (Table 2) as well as between the soil application and seed application. However, Mousavi *et al.* (2007) studied foliar application of Zn sulfate in potato crops and demonstrated that higher doses of this element increases starch content in potatoes. With regard to the Zn applications in soil, the banded application provided higher contents of soluble carbohydrates, starch and total carbohydrates. According to Dechen and Nachtigall (2006), plants have low starch contents when deficient for Zn.

When Zn was applied to the soil, differences were observed among doses. The banded application of Zn promoted a decrease with linear adjustment in soluble carbohydrate content (Figure 1a) and a quadratic adjustment in the starch content (Figure 1b) (maximum point of 3.7 kg ha⁻¹) and total carbohydrate content (Figure 1c) (maximum point of 3.6 kg ha⁻¹) in corn grains.



Figure 1. Contents of soluble carbohydrates (a), starch (b) and total carbohydrates (c) in corn grains according to the banded application of Zn in soil.

Zinc application	Soluble Carbohydrates	Starch	Total Carbohydrates
Corn		– g glucose per100 g —	
Soil, banded, 2 kg ha-1	8.20	55.77	63.97
Soil, banded, 4 kg ha-1	8.17	55.88	64.05
Soil, banded, 8 kg ha-1	6.56	49.46	56.02
Soil, incorporated, 6 kg ha-1	7.78	47.15	54.94
Soil, incorporated, 12 kg ha-1	7.28	46.97	54.25
Soil, incorporated, 24 kg ha-1	8.96	50.22	59.18
Foliar, 0.4 kg ha-1	10.38	49.56	59.93
Seed, 40 g kg ⁻¹	5.68	51.55	57.23
Control	8.67	59.74	68.41
		F-test	
Control vs. others	0.026 ns	0.001 ns	0.000 ns
Soil vs. (foliar + seed)	0.440 ns	0.113 ns	0.023 ns
Foliar vs. seed	76.208 ns	1.208 ns	2.487 ns
Soil, incorporated vs. soil banded	1.358**	28.378**	27.925**
Doses in banded	5.865*	9.890**	15.334**
Doses in incorporated	5.382*	5.989*	10.434**
CV (%)	9.7	5.1	4.1
Sorghum		– g glucose per 100 g –	
Soil, banded, 2 kg ha-1	9.13	58.74	67.87
Soil, banded, 4 kg ha-1	11.56	66.39	77.95
Soil, banded, 8 kg ha-1	10.77	41.66	52.43
Soil, incorporated, 6 kg ha-1	11.74	45.42	57.16
Soil, incorporated, 12 kg ha ⁻¹	11.23	45.32	56.55
Soil, incorporated, 24 kg ha-1	11.63	48.29	59.92
Foliar, 0.4 kg ha ⁻¹	11.37	61.70	73.07
Seed, 40 g kg ⁻¹	9.49	48.17	57.65
Control	10.62	60.02	70.64
		F-test	
Control vs. others	3.630*	0.177 ns	0.869 ns
Soil vs. (foliar + seed)	3.736*	19.038**	10.501**
Foliar vs. seed	13.173**	73.969**	72.718**
Soil, incorporated vs. soil banded	12.165**	103.681**	61.819**
Doses in banded	3.497 NS	116.923**	67.686**
Doses in incorporated	5.049*	21.293**	20.432**
CV (%)	6.8	4.3	4.1

Table 2. Summary of the variance analysis regarding starch, soluble and total carbohydrate contents in corn and sorghum crops according to different Zn application methods.

According to the F-test, ** denotes significance at 1% probability, and ns denotes not significant.

However, when Zn was incorporated into the soil, a quadratic adjustment existed for the soluble carbohydrate content (Figure 2a) (minimum point of 8.7 kg ha⁻¹), starch content (Figure 2b) (minimum point of 12.3 kg ha⁻¹) and total carbohydrate content (Figure 2c) (minimum point of 11.6 kg ha⁻¹). This trend may have occurred due to the use of higher doses in these treatments, thereby increasing the bioavailability of Zn and its uptake by roots. In an experiment with cassava, Souza *et al.* (1991) verified that the Zn dosage of 4 kg ha⁻¹ in soil does not cause differences in the starch content.

With regard to the sorghum crop in the first contrast tested (control vs. other treatments (Table 2), differences occurred only for the soluble carbohydrates. Studies have demonstrated that the role of Zn in carbohydrate metabolism (Sharma *et al.*, 1990) is to inhibit the transport of photoassimilates (Samarakoon and Rauser, 1979). However, little is known about the effects of Zn concerning the carbohydrate content in plants (Ghnaya *et al.*, 2010).

When comparing the soil application to the plant application, the soil application resulted in a higher content of soluble carbohydrates in corn grains, and the plant application promoted higher contents of starch and total carbohydrates (Table 2).

When comparing the applications of Zn to plants, foliar application promoted higher contents of soluble carbohydrates, starch and total carbohydrates.

When comparing the applications of Zn to soil, the banded application provided higher contents of starch and total carbohydrates, but the incorporation of Zn into soil increased the contents of soluble carbohydrates.

Zn application demonstrated differences among doses of Zn applied to the soil, except for the soluble



Figure 2. Contents of soluble carbohydrates (a), starch (b) and total carbohydrates (c) in corn grains according to Zn application in soil by incorporation.

carbohydrate content. The banded application of Zn promoted a quadratic adjustment in starch content (Figure 3a) (maximum point of 3.5 kg ha⁻¹) and total carbohydrates (Figure 3b) (maximum point of 3.4 kg ha⁻¹) in sorghum grains. Incorporation of Zn into soil promoted an increase with linear adjustment in soluble carbohydrates (Figure 4a) and a quadratic adjustment in starch content (Figure 4b) (minimum point of 13.5 kg ha⁻¹) and total carbohydrate content (Figure 4c) (minimum point of 13.8 kg ha⁻¹).



Figure 3. Contents of starch (a) and total carbohydrates (b) in sorghum grains according to the banded application of Zn in soil.

Effects of treatments on protein content in grains

As presented in Table 3, the first contrast (control vs. other treatments) for corn showed that the application of Zn promoted a higher content of protein in corn grains when compared to the control regardless of the application method. Zn is the micronutrient that affects protein synthesis the most in plants (Obata *et al.*, 1999). There are over 300 enzymes that require Zn for structural integrity and biological function (Marschner, 1995). According to Sharma *et al.* (1982), the protein synthesis rate and content in plants with Zn deficiency are drastically reduced. Such decrement is attributed to decreased RNA as well as the deformation and reduction of ribosomes (Brown *et al.*, 1993).

No differences were observed when Zn applications in soil and plants were compared. Nevertheless, comparison of the Zn treatment via leaves with the treatment via seeds indicated that foliar application provided a higher content of protein in grains.



Figure 4. Contents of soluble carbohydrates (a), starch (b) and total carbohydrates (c) in sorghum grains according to Zn application in soil by incorporation.

Comparison of the application of this micronutrient in soil demonstrated that the banded application of Zn promoted greater increases in protein content than the incorporated treatment.

Zn application resulted in differences among doses when incorporated in soil. Zn applied in such manner promoted increases with linear adjustment for the protein content of corn grains (y = 0.029x + 7.726; R² = 0.72). When studying the effect of Zn in a nutrient solution on beans, Cakmak *et al.* (1989) Zn observed that the Zn

Zinc application	Corn	Sorghum
	g kg	g ⁻¹
Soil, banded, 2 kg ha-1	8.69	9.81
Soil, banded, 4 kg ha-1	8.45	10.03
Soil, banded, 8 kg ha-1	8.69	9.72
Soil, incorporated, 6 kg ha-1	7.71	9.04
Soil, incorporated, 12 kg ha-1	7.95	9.44
Soil, incorporated, 24 kg ha ⁻¹	8.53	10.07
Foliar, 0.4 kg ha-1	8.37	9.95
Seed, 40 g kg ⁻¹	7.92	9.20
Control	7.93	9.61
	F-te	est
Control vs. others	6.258**	0.077 ns
Soil vs. (foliar + seed)	3.020 ns	0.850 ns
Foliar vs. seed	5.786*	12.783**
Soil, incorporated vs. soil banded	24.405**	7.804**
Doses in banded	0.943 ns	6.640*
Doses in Incorporated	5.735*	13.090**
CV (%)	3.3	3.1

 Table 3. Summary of the variance analysis regarding the protein content in corn and sorghum grains according to different Zn application methods.

According to the F-test, ** and * denote significance at 1 and 5% probability, and ns denotes not significant.

supply increases the protein content of leaves from this culture as compared to plants that did not receive Zn. Such increment in protein content can be attributed to Zn application increasing plant Zn uptake (Table 3), which is reflected by protein synthesis because Zn catalyzes RNA polymerase, inhibits RNAse and is a part of the ribosome structure (Prado, 2008).

The first contrast (control vs. other treatments) for sorghum showed that there were no differences in the protein content of sorghum grains in all tested treatments (Table 3), and no differences were found when comparing the addition of Zn to soil with the addition to plants. However, Zn deficiency disrupts protein synthesis (RNA) and nitrate reduction, thereby promoting a decrease in RNA levels, which results in less protein synthesis and difficulties in cell division. These effects can be explained by the fact that Zn inhibits RNAse (RNA disintegrator) and is a part of the RNA polymerase, which synthesizes RNA (Malavolta, 2006).

Compared to seed application of Zn, Znfoliar application of Zn promoted higher protein content in grains. Mousavi *et al.* (2007) studied foliar application of Zn sulfate in potato crops and showed that higher doses of this element promotes increased protein content in this culture.

Compared to incorporation of Zn, banded application of Zn caused greater increases of protein content.

Zn application via soil resulted in different protein contents in grains. The banded application of Zn caused an increase with quadratic adjustment of the protein content in grains ($y = -0.0205x^2 +$ 0.1815x + 9.5879; R² = 0.94), and the 4.4 kg ha⁻¹ dose provided the highest content. Moreover, incorporated application of Zn resulted in a quadratic adjustment ($y = 0.0038x^2 - 0.0662x + 9.526$; $R^2 = 0.84$) (minimum point of 8.7 kg ha⁻¹).

In Zn deficiency conditions, several metabolic processes in plants, such as RNA metabolism and protein synthesis, are compromised (Kitagishi; Obata, 1986).

Resumen

A.P. Puga, R.M. Prado, B. Mattiuz, D.W. Vale v I.M. Fonseca. 2013. Efecto de diferentes métodos de aplicación y dosis de zinc sobre la composición química de los granos de maíz v sorgo, cultivados en un suelo Oxisol. Cien. Inv. Agr. 40(1):97-108. Los suelos tropicales, en general, tienen una baja concentración de zinc (Zn), y la deficiencia de este micronutriente es reconocida como un problema nutricional mundial para la producción de cereales y para los seres humanos. Así, el objetivo de este estudio fue evaluar los efectos de diferentes métodos de aplicación de Zn sobre la calidad del grano de maíz y sorgo. Para esto, se realizaron dos experimentos en el área experimental de FCAV/UNESP, Jaboticabal, Brasil. Se aplicaron nueve tratamientos: tres dosis de Zn aplicadas en forma localizada en los surcos de las semillas, tres dosis de Zn aplicadas en forma incorporada al suelo (0-20 cm de profundidad), una dosis aplicada en forma foliar, y el tratamiento control (sin aplicación de Zn); dispuestos en un diseño de bloques completos al azar con cuatro repeticiones. Las variables medidas fueron: contenido de Zn, proteínas y carbohidratos en los granos de maíz v sorgo. La adición de Zn, independientemente del modo, promovió mavores niveles de este micronutriente en los granos de maíz y sorgo. El modo de aplicación de Zn en el suelo de una forma localizada mostró mayores niveles de carbohidratos, almidón y proteína en las semillas de ambos cultivos. La aplicación de Zn en el suelo de modo incorporado resultó en altos niveles de carbohidratos solubles en los granos de maíz y sorgo.

Palabras clave: Hidratos de carbono, proteínas, Sorghum bicolor, Zea mays, Zn.

References

- Alloway, B.J. 2009. Soil factors associated with zinc deficiency in crops and humans. Environmental Geochemistry and Health 31:537–548.
- Bataglia, O.C., A.M.C. Furlani, J.P.F. Teixeira, P.R. Furlani, and J.R. Gallo. 1983. Métodos de análise química de plantas. Boletim Técnico 78. Instituto Agronômico. Campinas. 48 pp.
- Brown, P.H., I. Cakmak, and Q. Zhang. 1993. Form and function of zinc in plants. *In*: Zinc in Soils and Plants. Robson, A.D. (ed.). The Netherlands: Kluwer Academic Publishers - Dordrecht.
- Cakmak, I., H. Marschner, and F. Bangerth. 1989. Effect of zinc nutritional status on growth, protein metabolism and levels of indole-3-acetic acid and other phytohormones in bean (*Phaseolus vulgaris* L.). Journal of Experimental Botany 40: 405-412.
- Cakmak, I., M. Kalayci, H. Ekiz, H.J. Braun, and A. Yilmaz. 1999. Zinc deficiency as an actual problem in plant and human nutrition in Turkey: A NATO-Science for Stability Project, Field Crops Research 60:175–188.
- Cakmak, I., R. Graham, and R.M. Welch. 2002. Agricultural and molecular genetic approaches to improving nutrition and preventing micronutrient

malnutrition globally. *In*: Cakmak, I., and R.M. Welch (eds.). Encyclopedia of Life Support Systems. Eolss, Oxford. p. 1075–1099.

- Cakmak, I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant Soil 308: 1-17.
- Cakmak, I., M. Kalayci, Y. Kaya, Y. Torun, A.A. Aydin, N. Wang, Y. Arisoy, Z. Erdem, H. Yazici, A. Gokmen, O. Ozturk, and W.J. Horst. 2010. Biofortification and localization of zinc in wheat grain. Journal of Agricultural and Food Chemistry 58: 9092–9102.
- Dechen, A.R, and G.R. Nachtigall. Micronutrientes. 2006. *In*: Fernandes, M. S. (ed.) Nutrição Mineral de Plantas. Viçosa: Sociedade Brasileira de Ciência do Solo. p. 327-354.
- Embrapa. 2006. Centro Nacional e Pesquisa em Solos. Sistema Brasileiro de Classificação de Solos. Brasilia: Embrapa-SPI, Rio de Janeiro: Embrapa-Solos. 306 pp.
- Ferreira, A.C.B., G.A.A. Araújo, P.R.G. Pereira, and A.A. Cardoso. 2001. Características agronômicas e nutricionais do milho adubado com nitrogênio, molibdênio e zinco. Scientia Agricola 58:131-138.
- Galrão, E.Z. 1994. Métodos de correção da deficiência de zinco para o cultivo do milho num Latossolo Vermelho-Escuro argiloso sob cerrado. Revista Brasileira de Ciência do Solo 18:229-233.
- Gibson, R.S. 2006. Zinc: the missing link in combating micronutrient malnutrition in developing countries. Proceedings of the Nutrition Society 65:51–60.
- Ghnaya, A.B., A. Hourmant, S. Cerantola, N. Kervarec, J.Y. Cabon, M. Branchard, and G. Charles. 2010. Influence of zinc on soluble carbohydrate and free amino acid levels in rapeseed plants regenerated in vitro in the presence of zinc. Plant Cell, Tissue and Organ Culture 102:191–197.
- Graham, R.D., J.S. Ascher, and S.C. Hynes. 1992. Selecting zinc-efficient varieties for soils of low zinc status. Plant Soil 146:241–250.
- Hotz, C., and K.H. Brown. 2004. Assessment of the risk of zinc deficiency in populations and options for its control. Food and Nutrition Bulletin 25:S91-S204.

- Hemantaranjan, A., and O.K. Garg. 1988. Iron and zinc fertilization with reference to the grain quality of *Triticum aestivum* L. Journal of Plant Nutrition 11:1439–1450.
- Kanwal, S., A.M.R. Rahmatullah, and R. Rashid. 2010. Zinc partitioning in maize grain after soil fertilization with zinc sulfate. International Journal of Agriculture & Biology 12:299-302.
- Kitagishi, K., and H. Obata. 1986. Effects of zinc deficiency on the nitrogen metabolism of meristematic tissues of rice plants with reference to protein synthesis. Soil Science and Plant Nutrition 32:397-405.
- Malavolta, E. 2006. Manual de nutrição mineral de plantas. São Paulo: Ceres. 638 pp.
- Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. London: Academic Press. 889 pp.
- Maret, W., and H.H. Sandstead. 2006. Zinc requirements and the risks and benefits of zinc supplementation. Journal of Trace Elements in Medicine and Biology 20:3–18.
- Moinuddin, P.I. 2010. Effect of zinc nutrition on growth, yield, and quality of forage sorghum in respect with increasing potassium application rates. Journal of Plant Nutrition 33:2062–2081.
- Mousavi, S.R., M. Galavi, and G. Ahmadvand. 2007. Effect of zinc and manganese foliar application on yield, quality and enrichment on potato (*Solanum tuberosum* L.). Asian Journal of Plant Sciences: 8:1256-1260.
- Nelson, W.L., and A. Mehlich. 1953. The Development, evaluation, and use of soil tests for phosphorus availability. Agronomy 4:153-188.
- Newell, M.M. 2008. Nutritionally improved agricultural crops. Plant Physiology 147:939-953.
- Obata, H., S. Kawamura, K. Senoo, and A. Tanaka. 1999. Changes in the level of protein and activity of Cu/Zn-superoxide dismutase in zinc deficient rice plant, *Oryza sativa* L. Soil Science and Plant Nutrition 45:891–896.
- Ozturka, L., M.A. Yazicia, C. Yucelb, A. Torunb, C. Cekicc, A. Bagcid, H. Ozkanb, H. Braune, Z. Sayersa, and I. Icakmaka. 2006. Concentration and localization of zinc during seed development and germination in wheat. Physiologia Plantarum: 128:144–152.

- Peck, A.W., G.K. Mcdonald, and R.D. Graham. 2008. Zinc nutrition influences the protein composition of flour in bread wheat (*Triticum aestivum* L.). Journal of Cereal Science 47:266–274.
- Peleg, Z., Y. Saranga, A. Yazici, T. Fahima, L. Ozturk, and I. Cakmak. 2008. Grain zinc, iron and protein concentrations and zinc-efficiency in wild emmer wheat under contrasting irrigation regimes. Plant and Soil 306:57–67.
- Prado, R.M., L.M. Romualdo, D.E. Rozane, A.A. Vidal, and A.V. Marcelo. 2008. Modos de aplicação de zinco na nutrição e na produção de matéria seca do milho BRS 1001. Bioscience Journal 24:67-74.
- Prasad, A.S. 2007. Zinc: Mechanisms of host defense. Journal of Nutrition 137:1345–1349.
- Puga, A.P. 2011. Modos de aplicação de zinco em milho e sorgo cultivados em Latossolo Vermelho distrófico. Jaboticabal. 101 pp.
- Raij, B. van, J.A. Quaggio, H. Cantarella and C.A. Abreu. 1997. Interpretação de resultados de análises de solo. *In*: Raij, B. van., H. Cantarella, J.A. Quaggio, and A.M.C. Furlani (eds.). Recomendações de adubação e calagem para o estado de São Paulo. 2 ed. rev. Campinas: Instituto Agronômico. p. 8-13.
- Raij, B. van., J.C. Andrade, H. Cantarella, and J.A. Quaggio (eds.). 2001. Análise química para avaliação da fertilidade do solo: Instituto Agronômico. Campinas. 285 pp.
- Samarakoon, A.B., and W. Rauser. 1979. Carbohydrate level and photoassimilate export from leaves of *Phaseolus vulgaris* exposed to excess cobalt, nickel and zinc. Plant Physiology 63:1165-1169.

- Sharma, C.P., P.N. Sharma, S.S. Bisht, and B.D. Nautiyal. 1982. Zinc deficiency induced changes in cabbage. In: Proceedings of the Ninth Plant Nutrition Colloquium, Warwick, England, A. Scaife (eds.). Commonwealth Agricultural Bureau, Farnham Royal Bucks.
- Sharma, P.N., C. Chatterjee, S.C. Agarwala, and C.P. Sharma. 1990. Zinc deficiency and pollen fertility in maize (*Zea mays*). Plant Soil 124:221-225.
- Somogyi, M. 1952. Notes on sugar determination. Journal of Biological Chemistry 195:19-23.
- Souza, L.D., J.D. Gomes, and R.C. Caldas. 1991. Interação calagem, fósforo e zinco para a cultura da mandioca em Sinop (MT). Embrapa. 21 pp.
- Welch, R.M. 1986. Effects of nutrient deficiencies on seed production and quality. Advances in Plant Nutrition 2:205–247.
- Welch, R.M. 2002. The impact of mineral nutrients in food crops on global human health. Plant Soil 247: 83–90.
- WHO. 2002. Reducing Risks, Promoting Healthy Life. Methods Summaries for Risk Factors assessed in Chapter 4. WHO (World Health Organization). Geneva, Switzerland. 33 pp.
- Wiles, P.G., I.K. Gray, and R.C. Kissling. 1998. Routine analysis of protein by Kjeldahl and Dumas methods: review and interlaboratory study using dairy products. Journal of AOAC International 81: 620-632.
- Yilmaz, A., H. Ekiz, B. Torun, I. Gultekin, S. Karanlik, S.A. Bagci, and I. Camak. 1997. Effect of different zinc application methods on grain yield and zinc concentration in wheat grown on zinc-deficient calcareous soils in Central Anatolia. Journal of Plant Nutrition 20:461-471.