Article

Zinc content and yield of biofortified cowpea beans

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Abstract

Zinc (Zn) is an essential trace element for plants, animals and humans; consequently, their deficiency affects their growth and development. It is estimated that between 15 and 30% of the human population in the world exhibit Zn deficiencies. The objective was to study the effect of biofortification with Zn^{2+} on the mineral content and yield of cowpea beans. The experiment was developed under a completely random design in two production cycles. The Zn was applied as zinc sulphate (T1: 0 $\mu M\ L^{-1}$, T2: 25 $\mu M\ L^{-1}$, T3: 50 $\mu M\ L^{-1}$ and T4: 100 $\mu M\ L^{-1}$) and as zinc chelate (T5: 0 $\mu M\ L^{-1}$, T6: 25 $\mu M\ L^{-1}$, T7: 50 $\mu M\ L^{-1}$ and T8: 100 $\mu M\ L^{-1}$). The applications of 25 $\mu M\ L^{-1}$ of $ZnSO_4$ and 50 $\mu M\ L^{-1}$ of Zn-EDTA for both production cycles were the most effective in increasing the content of this element in the bean seed cowpea, determining 1.14 and 0.93 times more zinc respectively, compared to the control. The yield, in cycle 1, was decreased by 53.8 and 20.3% by applying 50 $\mu M\ L^{-1}$ of $ZnSO_4$ and 25 $\mu M\ L^{-1}$ of Zn-EDTA, respectively. The addition of 50 $\mu M\ L^{-1}$ of $ZnSO_4$ and 25 $\mu M\ L^{-1}$ of Zn-EDTA, in cycle 2, increased the yield by 16.7 and 37.3%, respectively, compared to the control. The best treatments to biofortify cowpea beans were 25 $\mu M\ L^{-1}$ of $ZnSO_4$ and 50 $\mu M\ L^{-1}$ of Zn-EDTA for both production cycles.

Keywords: *Vigna unguiculata subsp. unguiculata*, functional food, iron, zinc.

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Introduction

The cowpea bean *Vigna unguiculata* (L.) Walp. Subsp. *unguiculata* is a legume that occurs in tropical and subtropical regions, humans can consume the leaves, roots, grains and immature pods (Lim, 2012) also, is a source of protein, dietary fiber, carbohydrates, vitamins, essential nutrients and phytochemicals in the human diet (Awika and Duodu, 2017). The zinc (Zn) content in the seed is often low (7.3 mg kg^{-1}, Espinosa-Moreno *et al*., 2013), especially when it is produced in soils with microelement deficiencies (Alloway, 2008). However, values of 43 mg kg^{-1} to 65 mg kg^{-1} of Zn have been reported in cowpea beans biofortified with iron (Márquez-Quiroz *et al*., 2015). On the other hand, per capita consumption worldwide is 3.89 kg year^{-1}, while in Mexico it is 1.89 kg year^{-1} (FAOSTAT, 2017).

The Zn is an essential component of various dehydrogenases, proteases and peptidases (Fageria and Baligar, 2005). In this sense, the deficiency of this microelement constitutes a public health problem (Pereira *et al*., 2014). To correct it, strategies have been implemented to increase Zn content in legumes (Praharaj *et al*., 2016). In this sense, the biofortification of the crop with Zn fertilizers has increased the content of this element in legumes by 74.6%, it has increased the antioxidant capacity of the grain 60% (Sida-Arreola *et al*., 2017), and has reduced the content of antinutrients (Sharma *et al*., 2017); likewise, it has been observed that when increasing the dose of Zn^{2+}, the P content tends to decrease (Cakmak *et al*., 2010). In the seeds of various crops, most of the Zn is associated with proteins, peptides (Persson *et al*., 2016), enzymes (Broadley *et al*., 2007) and phytic acid (Broadley *et al*., 2012).

Several studies have shown that biofortification with zinc fertilizers increased the content of the microelement in potato *Solanum tuberosum* (L.) (White *et al*., 2017), rice *Oryza sativa* (L.) (Tuyogon *et al*., 2016), wheat *Triticum aestivum* (L.) (Cakmak, 2008; Zhao *et al*., 2014), maize *Zea mays* (L.) (Potarzycki *et al*., 2015), onions *Allium cepa* (L.) (Almendros *et al*., 2015; Manna and Maity, 2016), common bean *Phaseolus vulgaris* (L.) (Ram *et al*., 2016) and safflower *Carthamus tinctorius* (L.) (Movahhedy-Dehnavy *et al*., 2009). In general, there is little literature on the biofortification of legumes of the genus *Vigna* and the results obtained from other works are mainly focused on the production of biofortified cereals, so the objective of this research was to study the effect of biofortification with Zn^{2+} on the mineral content and yield of cowpea beans.

Materials and methods

Production cycles

The work was carried out in the Academic Division of Agricultural Sciences of the Universidad Juarez Autonoma of Tabasco, geolocated at 17° 47’ north latitude, 92° 57’ west longitude and 29 meters above sea level. The variety of cowpea bean “De Castilla” was used, of indeterminate growth habit.

Production cycle autumn-winter 2013 and spring-summer 2014

The seeds were planted in black polyethylene pots of 25 cm x 30 cm, using inert substrate tepetzil. During the autumn-winter cycle the average temperature was 30 °C, with relative humidity of 86 to 94%, while the spring-summer cycle registered an average temperature of 37 °C, with relative humidity of 80 to 94%.

In both production cycles, the pots were established in a 200 m^2 tropical Megavent protected system, with lateral cover of anti-aphid mesh and Grown Cover mesh in the ground. The plants were arranged in a double row, with a separation of 30 cm between plants and 90 cm between rows, for a planting density of 44 444 plants per hectare. The plants were guided vertically with raffia wire, and phytosanitary management was carried out with applications of Karate® (lambda cyalotrine) and Sulfacob 25® (copper sulfate pentahydrate).

Fertilization and treatments

To the plant of each pot was applied irrigation with nutrient solution (Hoagland and Arnon, 1950), which contained 14 mM of NO_3^-, 1 mM of $H_2PO_4^-$, 2 mM of SO_4^{2-}, 6 mM of K^+, 4 mM of Ca^{2+} and 2 mM of Mg^{2+}. The microelements of the nutrient solution were supplied with the TradeCorp AZ®. product. The nutrient solution was adapted according to the stages of development of the crop at 50 and 100%, at 10-30, 31-100 days after sowing (DDS), respectively, the pH of the solution was maintained between 5.5 and 6, using sulfuric acid. To the ten DDS, 0.25 L of solution was applied per pot day^{-1}, at 31 DDS it was increased to 0.5 L day^{-1} and 1 L day^{-1} was applied after 61 DDS.

The treatments consisted in the addition of Zn + 2 in the form of zinc sulphate ($ZnSO_4 \cdot 7H_2O$ reactive grade, 21% Zn, 0 µM L^{-1}, 25 µM L^{-1}, 50 µM L^{-1} and 100 µM L^{-1}) and as zinc chelate (Zn-EDTA, TradeCorp Zn®, 14% Zn, 0 µM L^{-1}, 25 µM L^{-1}, 50 µM L^{-1} and 100 µM L^{-1}) (Table 1). Both compounds were dissolved in distilled water, and applied from day 31 to day 100 DS, every third day. The total of treatments was eight for each production cycle, with four repetitions. A completely random design was used.

Table 1. Treatments used in the biofortification of cowpea beans with zinc in two production cycles.

Treatment	Fertilizer	Doses of zinc (µM L^{-1})
T1	$ZnSO_4$ $7H_2O$	0
T2	$ZnSO_4$ $7H_2O$	25
T3	$ZnSO_4$ $7H_2O$	50
T4	$ZnSO_4$ $7H_2O$	100
T5	Zn-EDTA	0
T6	Zn-EDTA	25
T7	Zn-EDTA	50
T8	Zn-EDTA	100

Variables evaluated

The variables evaluated were: mineral content and yield of cowpea beans obtained. The harvest of the pods began at 70 DDS and ended at 100 DDS. The harvested pods were placed in labeled brown paper bags, to be later weighed. On the other hand, the nitrogen content (N) in the seed was determined with the Dumas method (1831), the total crude protein was calculated by multiplying the N content by the factor 6.25, the phosphorus (P), potassium (K), iron (Fe) and zinc (Zn) of the seed were determined by optical emission spectrometry with inductively coupled plasma (ICP-OES ICAP® 7200 Duo, Thermo Fisher Scientific), according to the procedure 984.27 (Horwitz, 2002) and previous wet digestion.

Statistical analysis

The data obtained were subjected to an analysis of variance. For the difference between means of the treatments, the orthogonal contrasts test ($p \leq 0.05$) was used (SAS Institute, 2013).

Results and discussion

Mineral content of biofortified beans

The results of the mineral content of biofortified cowpea beans with different doses of zinc fertilizers in the two production cycles are shown in Figure 1.

In cycle 1, the addition of 25 and 100 μM L^{-1} of $ZnSO_4$, and 50 μM L^{-1} of Zn-EDTA increased the total crude protein content and N in 3.7, 3.4 and 7.3%, respectively, compared to the addition of 0 μM L^{-1} of the chemical compound (Figure 1A and Figure 1B). On the other hand, when adding 25 μM L^{-1} of Zn-EDTA, the content of total crude protein and N decreased by 1.9%, respectively, compared to the addition of 0 μM L^{-1} of the chemical compound. On the other hand, the addition of 25 and 50 μM L^{-1} of $ZnSO_4$, and 25 and 100 μM L^{-1} of Zn-EDTA decreased the P content by 21.3, 8.2, 11.5, and 27.9%, respectively, compared to the addition of 0 μM L^{-1} of the chemical compound (Figure 1C). The content of K was decreased by 15.3, 12.4, 9.5, 12.4, 14.6 and 6.6% when adding 25, 50 and 100 μM L^{-1} of $ZnSO_4$, and 25, 50 and 100 μM L^{-1} of Zn-EDTA, respectively (Figure 1D).

At the same time, the application of 25 μM L^{-1} of $ZnSO_4$, and 25, 50 and 100 μM L^{-1} of Zn-EDTA decreased the Fe content by 11.8, 8.9, 3.9 and 10.4%, respectively, compared with the application of 0 μM L^{-1} of the chemical compound (Figure 1E). Similarly, the addition of 50 and 100 μM L^{-1} of $ZnSO_4$ and 25, 50 and 100 μM L^{-1} of Zn-EDTA and a decrease in the content of Zn in 18.2, 18.9, 26.7, 20.6 and 26.5%, respectively, compared to the application of 0 μM L^{-1} of the chemical compound (Figure 1F).

In cycle 2, the addition of 25 μM L^{-1} of $ZnSO_4$, and 50 μM L^{-1} of Zn-EDTA decreased the total crude protein content and N in 4.6 and 1.4%, respectively, compared to the addition of 0 μM L^{-1} of the chemical compound (Figure 1A and Figure 1B). In contrast, when adding 50 μM L^{-1} of $ZnSO_4$, and 25 and 100 μM L^{-1} of Zn-EDTA, the total crude protein content and N decreased by 8.6, 3.7 and 3.1%, respectively, compared to the addition of 0 μM L^{-1} of the chemical compound. On the other hand, the addition of 25 μM L^{-1} of $ZnSO_4$ and 50 μM L^{-1} of Zn-EDTA increased the P content by 18.88 and 6.3%, respectively, compared to the addition of 0 μM L^{-1} of the compound chemical (Figure 1C). However, adding 100 μM L^{-1} of $ZnSO_4$ and 25 and 100 μM L^{-1} of Zn-EDTA decreased the P content by 6.3, 12.5 and 9.4%, respectively, compared to the application of 0 μM L^{-1} of the chemical compound.

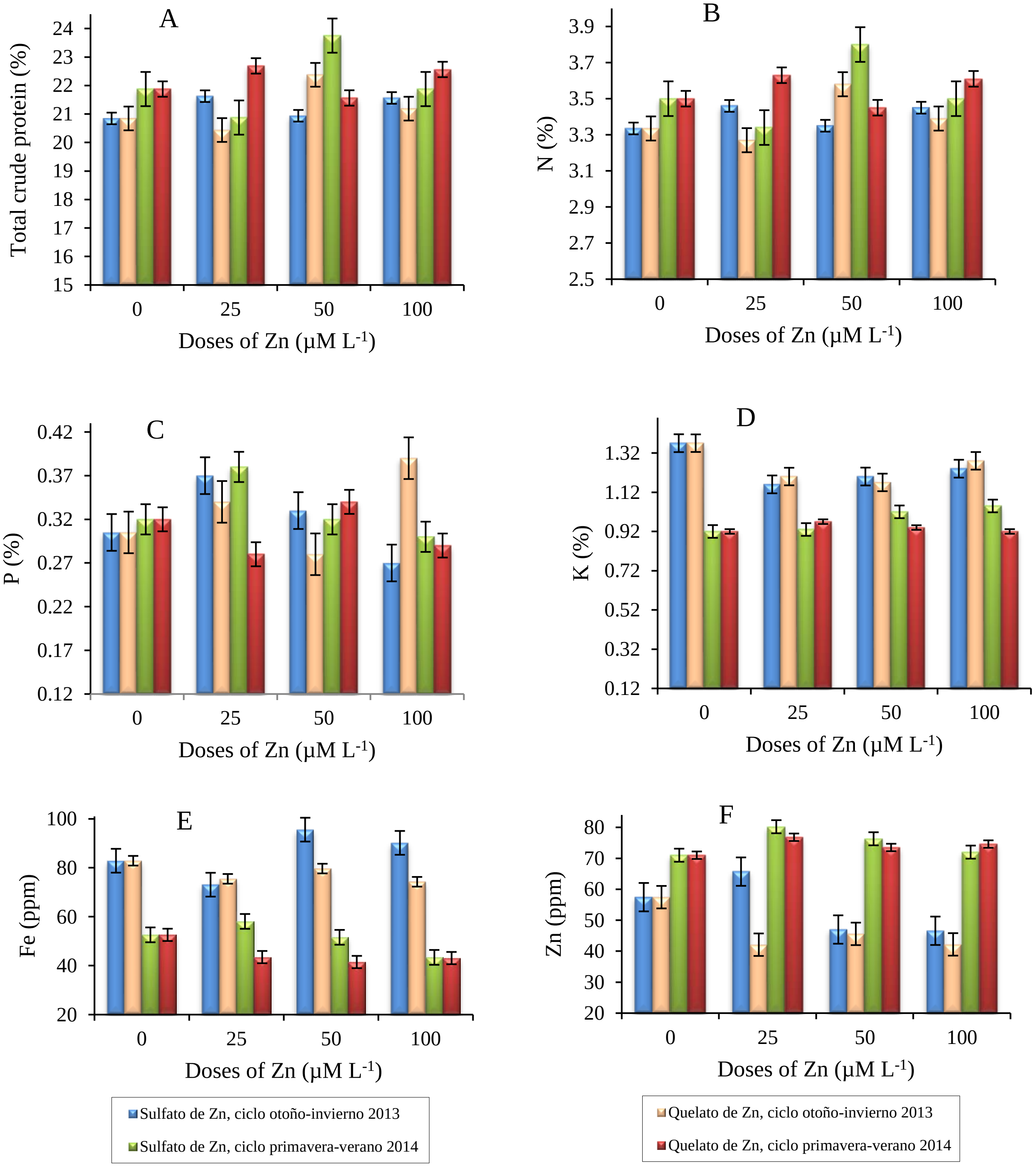

Figure 1. Content of total crude protein (A), nitrogen (B) phosphorus (C), potassium (D), iron (E) and zinc (F) of biofortified cowpea beans in two production cycles. Mean values ± standard error.

The content of K was increased by 1.1, 10.9, 14.1, 5.4 and 2.2% by adding 25, 50 and 100 μM L^{-1} of $ZnSO_4$, and 25 and 50 M L^{-1} of Zn-EDTA, respectively (Figure 1D). At the same time, the application of 50 and 100 μM L^{-1} of $ZnSO_4$ and 25, 50 and 100 μM L^{-1} of Zn-EDTA lowered the Fe content in 1.9, 17.5, 17.3, 21.1 and 18.1%, respectively, in comparison with the application of 0 μM L^{-1} of the chemical compound (Figure 1E). However, the addition of 25, 50 and 100 μM L^{-1} of $ZnSO_4$ and 25, 50 and 100 μM L^{-1} of Zn-EDTA increased the Zn content by 13, 7.5, 1.4, 8.2, 3.5 and 5.1%, respectively, compared to the application of 0 μM L^{-1} of the chemical compound (Figure 1F).

The value obtained in the content of total crude protein and N are similar to those reported in the literature, which range between 16 and 30% and 2.5 and 4.8%, respectively (Carvalho *et al*., 2012). In the present study, the concentration of macroelements in the seed contrast with the results reported by Espinosa-Moreno *et al*. (2013), who reported the total crude protein content, N, P and K in the seeds of 21.9, 3.5, 0.35 and 1.52%, respectively. On the other hand, the content of Fe and Zn showed values that are frequently reported in the literature (Carvalho *et al*., 2012; Kalidass and Mohan, 2012). Consequently, the content of Zn in the biofortified cowpea bean seed is not considered toxic because it does not exceed values of 150 ppm Zn (Mengel *et al*., 2001).

In addition to the above, the increase in the dose of Zn^{2+} will not always allow a greater accumulation in the seed, since it depends on the mobilization of the microelement as a free ion or chelated from the stems to the seed (Olsen *et al*., 2016), which suggests that the retranslocation, via phloem, of the microelements deposited in the stems have an important role in the accumulation of Fe and Zn in the seed (Cakmak *et al*., 2010). In this sense, the cowpea plants of the present experiment could exhibit different capacities to absorb and mobilize the nutriment, due to a lower or higher rate of transpiration during the production cycles (White, 2012).

Similarly, it has been reported that the application of high doses of Zn^{2+} interferes with the absorption and translocation of P, calcium and Fe, besides causing cytological disorders in the plant (Cakmak, 2000; Khudsar *et al*., 2008). Likewise, a decrease in the content of Fe in the wheat grain (Shekari *et al*., 2015) and root protein in the common bean has been reported when applying high doses of Zn^{2+} due to a decrease in the enzymatic activity of nitrate reductase (Chaoui *et al*., 1997).

Performance

In cycle 1, the weight of 100 seeds ranged between 12. 1 and 20.5 g, which is consistent with the data reported in the literature (Giami, 2005). In the present study, the application of $ZnSO_4$ had a negative effect on the number of pods per plant, number of seeds per plant and seed yield per plant, with decreases of up to 37.4, 54.9 and 53.8%, respectively, compared to the addition of 0 μM L^{-1} of $ZnSO_4$. On the other hand, not all the responses of the plants were negative, adding 100 μM L^{-1} of Zn-EDTA increased the number of pods per plant, the number of seeds per plant and the yield per plant in 14.0, 20.3 and 17.2 %, respectively, with respect to the application of 0 μM L^{-1} of Zn-EDTA.

In contrast, plants grown with 50 μM L^{-1} of Zn-EDTA showed an increase of 2.6 and 1.6% in the number of seeds per plant and yield per plant, compared to the application of 0 μM L^{-1} of the chemical compound (Cuadro2). As part of the comparison, by means of orthogonal contrasts, among the fertilizers of Zn, it was determined that the use of Zn-EDTA presented significant differences in the comparisons of T3 *versus* T7, and T4 *versus* T8.

Table 2. Performance components of cowpea bean biofortified with zinc, autumn-winter 2013 agricultural cycle.

Treatment	Doses (μM L^{-1})	Weight of 100 seeds (g)	Num. pod per plant	Num. seeds per plant	Seed yield (g $plant^{-1}$)
T1) $ZnSO_4$	0	13.2	9	144	16
T2) $ZnSO_4$	25	20.5	7	89	9.8
T3) $ZnSO_4$	50	16.2	6	65	7.4
T4) $ZnSO_4$	100	12.3	8	144	10.8
T5) Zn-EDTA	0	12.4	9	118	12.8
T6) Zn-EDTA	25	12.1	7	91	10.2
T7) Zn-EDTA	50	13.1	9	121	13
T8) Zn-EDTA	100	13.8	10	142	15
	Orthogonal contrasts (values of *p*)				
T1 *vs* T2 + T3 + T4		0.3815	0.9607	0.9607	0.9216
T2 *vs* T3 + T4		0.0743	0.0162	0.0001	0.0001
T2 *vs* T6		0.0001	0.9165	0.0425	0.6309
T3 *vs* T7		0.0016	0.0027	0.0001	0.0001
T4 *vs* T8		0.0848	0.0677	0.1057	0.0001
T5 *vs* T6 + T7 + T8		0.0002	0.0076	0.0001	0.0001
T6 *vs* T7 + T8		0.0001	0.6273	0.0001	0.3369

In cycle 2, biofortification with $ZnSO_4$ and Zn-EDTA had a positive effect on the number of pods per plant, number of seeds per plant and yield per plant. In this sense, the addition of 25, 50 and 100 μM L^{-1} of $ZnSO_4$ caused an increase in the number of pods of 25, 5 and 21.6%, respectively, in the number of seeds per plant of 31.3, 35.3 and 58.4%, respectively, and in the yield of 7.2, 16.7 and 33.3%, respectively, compared to the addition of 0 mM L^{-1} of the chemical compound (Table 3). Similarly, the number of pods of 28.3, 35.9 and 32.9% was increased by adding 25, 50 and 100 μM L^{-1} of Zn-EDTA, respectively, compared to the addition of 0 mM L^{-1} of the chemical compound.

Moreover, the number of seeds per plant and yield per plant increased by 65.1, 63.9 and 53.1% and 37.4, 43.4 and 19.3%, respectively, when adding 25, 50 and 100 μM L^{-1} of Zn-EDTA, in comparison with the addition of 0 mM L^{-1} of the chemical compound. As part of the comparison, by means of orthogonal contrasts, among Zn fertilizers, it was determined that the use of $ZnSO_4$ and Zn-EDTA did not present significant differences in the comparisons of T2 *versus* T6, T3 *versus* T7, and T4 *versus* T8.

Table 3. Performance components of cowpea beans biofortified with zinc, spring-summer 2014 agricultural cycle.

Treatment	Doses (μM L^{-1})	Weight of 100 seeds (g)	Num. pod per plant	Num. seeds per plant	Seed yield (g $plant^{-1}$)
T1) $ZnSO_4$	0	13.4	6	62	9.6
T2) $ZnSO_4$	25	11.7	8	81	10.3
T3) $ZnSO_4$	50	13.5	6	84	11.2
T4) $ZnSO_4$	100	13.1	7	98	12.8
T5) Zn-EDTA	0	13.4	5	54	8.3
T6) Zn-EDTA	25	13	7	88	11.4
T7) Zn-EDTA	50	13.4	7	88	11.9
T8) Zn-EDTA	100	12	7	82	9.9
Orthogonal contrasts (values of *p*)					
T1 *vs* T2 + T3 + T4		0.3467	0.0346	0.0159	0.1602
T2 *vs* T3 + T4		0.6538	0.8408	0.7866	0.8332
T2 *vs* T6		0.0976	0.4897	0.6243	0.6543
T3 *vs* T7		0.8967	0.4897	0.7717	0.7472
T4 *vs* T8		0.166	0.4897	0.3018	0.2326
T5 *vs* T6 + T7 + T8		0.3132	0.1025	0.048	0.3545
T6 *vs* T7 + T8		0.0242	0.5489	0.4658	0.4134

The yield obtained in the present study is greater than the 7 g of seed per plant reported by Apaez-Barrios *et al*. (2011), but less than 13 g of seed per plant obtained by the producers of cowpea beans of the state (SIAP, 2013). In this regard, Cakmak *et al*. (2010), reported that the addition of $ZnSO_4$ via fertigation increased significantly the yield and Zn content in the wheat grain, however they recommend that foliar application be combined with the application via fertigation to increase these variables by 200%. In this regard, Prasad *et al*. (2014), report an increase of 69% in the Zn content of the rice when making foliar applications instead of the application to the soil. Likewise, Das and Green (2016), reported that the application of Zn^{2+} improved the performance and nutraceutical quality of the potato.

Conclusions

The applications of 25 μM L^{-1} of $ZnSO_4$ and 50 μM L^{-1} of Zn-EDTA for both production cycles were the most effective in increasing the content of this element in the bean seed cowpea, determining 1.14 and 0.93 times more zinc respectively, compared to the control. The yield, in cycle 1, was reduced 53.8 and 20.3% by applying 50 μM L^{-1} of $ZnSO_4$ and 25 μM L^{-1} of Zn-EDTA, respectively. The addition of 50 μM L^{-1} of $ZnSO_4$ and 25 μM L^{-1} of Zn-EDTA, in cycle 2, increased the yield by 16.7 and 37.3%, respectively, compared to the control. Considering the set of responses, the best treatments to biofortify cowpea beans with Zn^{2+} were 25 μM L^{-1} of $ZnSO_4$ and 50 μM L^{-1} of Zn-EDTA for both production cycles.

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