Australian Journal of Crop Science AJCS

AJCS 13(01):11-16 (2019) ISSN:1835-2707
doi: 10.21475/ajcs.19.13.01.p783

Zinc biofortification strategies in food-type soybean cultivars

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Abstract

Reducing the risk of Zn-deficiency in humans by diversifying human diet and through agricultural interventions, such as agronomic biofortification, is still a major challenge. This greenhouse study used a number of biofortification strategies to investigate the effect of Zn fertilization on soybean grain cultivars intended for human consumption. A completely randomized 5×3 factorial design with four replications was used. The design included five different Zn application strategies as the main plot. These were (1) soil application; (2) foliar application at the vegetative growth stage 4 (V4) growth stage; (3) foliar application at vegetative growth stage 8 (V8); (4) foliar application at reproductive growth stage 4 (R4); and (5) a control (without Zn). Three soybean cultivars (BRS 213, BRSMG 790A, and BRS Favorita RR®) were assigned as sub-plots. Zinc foliar fertilization at stage R4 was found to be the most efficient method for soybean cultivation when Zn availability in the soil was low. The cultivars had different Zn accumulation responses, and the cultivar BRSMG showed the highest Zn increase. Zinc fertilization increased the protein content in soybean grain, plant height, and the number of grains per plant.

Keywords: *Glycine max* (L); green soybean; malnutrition; micronutrient; plant nutrition.
Abbreviations: 100DM _100-grain dry matter; 100FW_100-grain fresh weight; CARBO_carbohydrate; FPI_insertion height of the first pod; FPW_fresh pod weight; NGPl_number of grains per plant; NGPo_number of grains per pod; NPP_number of pods per plant; PH_plant height; R4_reproductive growth stage 4; R6_reproductive growth stage 6; RR_Roundup Ready; V4_vegetative growth stage 4; V8_vegetative growth stage 8.

Introduction

Currently, despite some progress in reducing the prevalence of hunger in the world, malnutrition is still a continuing problem, and more than 795 million people are unable to meet their daily nutritional requirements for healthy living. Furthermore, the food quality may not always meet the nutritional requirements for vitamins and minerals, Therefore, populations around the globe continue to suffer from deficiencies of essential nutrients, such as zinc (Zn) (FAO, 2015).

The continued expansion of the world population, along with climate change, urban sprawl, and declining soil fertility, mean that major agricultural impacts can be expected, increasing the challenge of providing nutritionally adequate diets to the population (Miller and Welch, 2013).

About half of the soils across the globe are Zn deficient. Therefore, the cultivation of cereals for human consumption on these soils leads to low Zn concentrations in the produced foods (Das and Green, 2013). The incidences of micronutrient deficiencies in crops have increased markedly in recent years because of intensive cultivation, loss of topsoil layers by erosion, liming of acidic soils, declining proportions of use of farmyard manure compared with that of chemical fertilizers, increased chemical fertilizer purity, and use of marginal lands for crop production (Almeida Júnior et al., 2007; Fageria et al., 2012). As a consequence, plants grown in Zn deficient soils generally have low tissue concentrations of this nutrient, which can reduce crop yields. Furthermore, food products made from these crops make a smaller Zn contribution to human diet. An increase in the Zn concentration in the staple foods of humans could provide substantial benefits in terms of Zn intake through diet (Alloway, 2008).

A possible solution to correct Zn deficiency in human nutrition is the biofortification of crops with essential elements. This technique has been proposed as a public health strategy to deal with mineral deficiencies in humans (Cakmak, 2008; White and Broadley, 2009; Zimmermann, 2013; Joy et al., 2015). Biofortification can be achieved through the use of two different techniques, namely, agronomic (fertilization) or genetic (breeding) biofortification (Cakmak, 2008). Applying Zn fertilizer to crop leaves or the soil can increase the Zn content in food crops (Joy et al., 2015). Similarly, plant breeding can increase Zn levels through the development of new cultivars that can accumulate more Zn and more efficiently translocate it to edible plant parts (Raboy, 2009).

The form in which Zn is applied to plants has a considerable effect on the absorption of this nutrient (Correia et al., 2008). Han et al. (2011) studied Zn availability during soybean growth and found that Zn application as a seed coating and through foliar sprays was more efficient than Zn application to the soil (as side dressing or incorporation) in boosting yield responses and use efficiency. However, few studies have addressed grain biofortification rather than soybean plant growth and yield following Zn application.

Soybean [*Glycine max* (L.) Merrill] is one of the most important plant sources of high quality protein and other nutrients, particularly in diets with animal-product restrictions. It is a nutritionally rich food, which helps maintain the health of the population (Penha et al., 2007; Keatinge et al., 2011). A limiting factor in soybean consumption is an "off-flavor," which is caused by the instability and oxidation of fatty acids. Therefore, soybean cultivars that are lipoxygenase-free or contain reduced levels of the enzymes responsible for this characteristic are being developed to improve grain flavor, which should boost soybean consumption (Esteves et al., 2010). An interesting option for the use of soybean in human foods is in the form of green grains, known as green soybean, vegetable soybean, or edamame (vegetables cooked in water and salt) (Mendonça and Carrão-Panizzi, 2003). Although different soybean cultivars are planted for green soybean production, the technique for cultivation is the same as that for common soybean until the reproductive growth stage R6, when the completely filled and green pods are harvested. The aim of this study was to evaluate the effects of different forms of Zn fertilization on the biofortification of soybean cultivars recommended for human consumption.

Results and Discussion

Traits affected by Zn fertilization and genetic variation

The effect of interaction between Zn fertilization methods and soybean cultivars was significant on Zn, lipid, and protein content of grains (Table 1). The agronomic parameters of first pod insertion height, number of pods per plant, number of grains per pod, fresh pod weight, 100-grain fresh weight, 100-grain dry matter, and grain ash content varied among cultivars. Plant height, number of grains, and carbohydrate content were influenced by the Zn fertilization method adapted and the cultivar type. There were no significant differences in the fiber content, which accounted for 6.76% of the grain composition, on average, under the treatments applied.

Grain Zn and other metabolite levels are differentially affected by Zn fertilization methods and vary among cultivars

All the application treatments altered the Zn content in the grain of the cultivars studied compared with that of the control. The grain Zn content varied from 23.93 to 49.17 mg Zn kg^{-1} (Table 1). The highest levels were found when Zn was applied to soybean leaves at the R4 stage. This stage is characterized by fast pod growth and the beginning of grain development. It precedes a period of fast and constant dry matter accumulation, which increases the nutritional demand of the plant. In an evaluation of Mn accumulation in soybean grains, Carvalho et al. (2015) observed a greater accumulation when Mn was applied at R3 instead of R1. This may be related to the period when grain reserves accumulate, because at R3, i.e., the beginning of pod formation, the application was closer to grain filling than at R1, i.e., the beginning of flowering.

Cultivar BRSMG 790A accumulated the most Zn in the grain (49.17 mg kg^{-1}) when Zn was applied as a foliar application at R4 (Table 1). Zinc foliar fertilization at R4 increased the grain Zn content by 56% in cultivar BRS 213, 105% in BRSMG 790A, and 36% in BRS Favorita RR® compared with the contents in the control grain. This suggests that the studied soybean cultivars differed in Zn uptake capacity and Zn translocation to grains, but responded positively to the application strategies used for grain biofortification. Cultivar BRSMG 790A, which is intended for human consumption, had the highest Zn uptake and Zn translocation to grains, and therefore, the highest grain Zn content. It was followed by BRS 213. Oliveira et al. (2016) reported a mean content of 40 mg kg^{-1} when they assessed genotypic variation in relation to the grain Zn content of 24 soybean cultivars.

The higher grain Zn availability resulting from foliar application rather than soil fertilization demonstrates that this form of biofortification is an attractive and useful strategy that could be used to solve health problems related to Zn deficiency (Cakmak, 2008). Joy et al. (2015) suggest that the cost-benefit of Zn foliar applications to wheat seems to be equivalent to the fortification of flours, a common stage in the production process. Previous research has shown that foliar Zn applications to wheat were more effective than soil applications of Zn, and resulted in a maximum grain Zn content of 30 mg kg^{-1} (Mao et al., 2014).

Only the lipid content in cultivar BRSMG 790A differed significantly in response to Zn application. The lipid levels were the highest when no Zn was applied (18.30%) and when the nutrient was applied to the soil (18.12%) (Table 1). Zn application did not significantly change the lipid content in cultivars BRS 213 and BRS Favorita RR®, because the mean grain lipid content remained at 14.89 and 17.08%, respectively.

Foliar Zn application at R4 increased the protein grain content compared with that in the control. Cultivar BRS 213 had the highest protein content (46.11%), followed by BRS Favorita RR® (42.32%). These cultivars differed from BRSMG 790A, which contained 38.55% protein after Zn was applied at R4 (Table 1).

Zinc is involved in plant protein synthesis. This may have contributed to the positive results for grain protein content in response to Zn application. The ash and carbohydrate content differed significantly among the cultivars (Table 2). The highest ash content was found in cultivar BRS 213 (5.98%), which differed from that of cultivars BRSMG 790A (5.76%) and BRS Favorita RR® (5.56%). Cultivars BRS 213 and BRSMG 790A had higher carbohydrate content (25.89 and 26.75%, respectively), while BRS Favorita RR® contained 23.87% carbohydrate, which was not affected by Zn fertilization.

Carbohydrate content was similar after the different treatments, viz., 27.75% for the control, 25.48% for soil Zn fertilization, 26.29% for foliar Zn fertilization at V4, and 25.66% for foliar Zn fertilization at V8. However, carbohydrate content was the lowest when Zn was applied to the leaves at R4 (22.34%) (Table 3). In a study on the centesimal composition of six food-type soybean cultivars, Vieira et al. (1999) found that all cultivars studied had a

Table 1. Mean values for the zinc, lipid, protein, and fiber grain contents of food-type soybean cultivars after they were subjected to different zinc biofortification strategies. Growing season 2013/14, Lavras, MG, Brazil.

Cultivar	Control	Soil	Foliar V4	Foliar V8	Foliar R4
			Zinc (mg kg^{-1})		
BRS 213	27.29 b D	36.31 b B	30.11 c C	36.13 a B	42.56 b A
BRSMG 790A	23.93 c D	35.13 b B	33.15 b C	33.75 b B	49.17 a A
BRS Favorita RR®	31.86 a D	39.74 a B	35.05 a C	35.71 a C	43.44 b A
CV(%)			3.97		
			Lipids (%)		
BRS 213	13.61 b A	15.87 b A	14.30 b A	15.48 a A	15.33 a A
BRSMG 790A	18.30 a A	18.12 a A	15.70 b B	13.05 b C	16.40 a B
BRS Favorita RR®	16.87 a A	16.87 b A	17.20 a A	17.06 a A	17.40 a A
CV(%)			5.51		
			Protein (%)		
BRS 213	38.78 a B	39.16 a B	39.71 a B	39.22 a B	46.11 a A
BRSMG 790A	35.14 a B	35.18 b B	37.77 a A	40.97 a A	38.55 b A
BRS Favorita RR®	37.57 a B	41.17 a A	40.50 a A	40.67 a A	42.32 b A
CV(%)			3.65		

Means followed by the same lowercase letter along a column and the same uppercase letter along a row are not significantly different at the 5% probability level according to the Scott-Knott test.

Table 2. Mean plant height (PH), first pod insertion height (FPI), number of pods per plant (NPP); number of grains per pod (NGPo), fresh pod weight (FPW), 100-grain fresh weight (100FW), 100-grain dry matter weight (100DM), carbohydrate content in the grain (CARBO), and ash content (ASH) values for food-type soybean cultivars after they were subjected to different zinc biofortification strategies. Growing season 2013/14, Lavras, MG, Brazil

	BRS 213	BRSMG 790A	BRS Favorita RR®	CV (%)
PH (cm)	41.93 c	45.72 b	55.56 a	10.71
AFP(cm)	8.51 c	14.00 b	15.45 a	21.79
NPP (un)	26.82 a	26.72 a	22.00 b	26.92
NGPo (un)	2.29 b	2.09 b	2.51 a	23.10
FPM (g)	27.27 a	22.60 b	29.10 a	32.97
100FM (g)	29.34 b	28.28 b	31.09 a	11.38
100DM (g)	13.22 b	14.34 a	13.17 b	16.34
CARBO (%)	25.89 a	26.75 a	23.87 b	7.19
ASH (%)	5.98 a	5.76 b	5.56 b	3.75

Means followed by the same lowercase letter in the column are not significantly different at the 5% probability level according to the Scott-Knott test.

Table 3. Mean plant height (PH), number of grains per plant (NGP), and carbohydrate content (CARBO) values of soybean cultivars used for human consumption after they were subjected to different Zn fertilization strategies. Growing season 2013/14, Lavras, MG, Brazil.

	PH (cm)	NGP (un)	CARBO (%)
Control	44.0 b	48.62 b	27.75 a
Soil	48.9 a	58.66 a	25.48 a
Foliar V4	48.0 a	59.66 a	26.29 a
Foliar V8	49.7 a	55.08 a	25.66 a
Foliar R4	48.0 a	56.41 a	22.34 b
CV (%)	10.71	21.43	7.19

Means followed by the same lowercase letter in the column are not significantly different at the 5% probability level according to the Scott-Knott test.

typical grain-type centesimal composition, with a mean dry base content of 39.52% protein, 23.04% oil, 5.41% ash, 5.75% fiber, and 32.01% total carbohydrates. Ciabotti et al. (2006) observed no difference in the centesimal compositions of cultivar BRS 133, a grain-type soybean, and the lipoxygenase-free cultivar BRS 213 (food-type) The cultivar BRS 133 had a mean content of 32.7% protein, 15.7% lipid, and 30.0% carbohydrate, whereas the corresponding values in BRS 213 were 33.2%, 15.3%, and 31.1%, respectively.

Plant nutrient availability may influence the chemical composition of the grains. Therefore, an adequate nutrient supply favors plant development and stimulates the production of metabolites required for grain or seed development (Veiga et al., 2010).

Strategies such as fertilization can immediately and effectively raise Zn concentrations in grains and possibly increase yields, particularly in Zn-poor soils. A Zn foliar application could be combined with other management measures, such as the application of single agrochemicals or even herbicide, fungicide, or insecticide combinations, which would reduce time and costs (Velu et al., 2013).

Growth traits and yield

BRS Favorita RR® was taller (55.56 cm) than BRSMG 790A (45.72 cm) and BRS 213 (41.93 cm) (Table 2). The plants in all the Zn treatments had similar plant heights and they differed from the mean of the control treatment. First pod insertion height also had a similar pattern (Table 3). Cultivar BRS Favorita RR® had the highest insertion height (15.45 cm), followed by BRSMG 790A (14.0 cm) and BRS 213 (8.51 cm) (Table 2). BRS Favorita RR® had the lowest number of pods per plant (22.00), and the means for cultivars BRS 213 and BRSMG 790A were similar (26.82 and 26.72 pods per plant).

There were no significant differences between the soybean cultivars for the number of grains per plant. However, applying Zn to the soil or leaves produced similar increases in the number of grains per plant compared with that of the control treatment (Table 3). The variation ranged from 48.62 (control) to 59.66 (foliar fertilization in V4) grains per plant. Santos et al. (2013) evaluated a number of soybean genotypes and reported similar values, with means of 40–67 grains per plant. Cultivar BRS Favorita RR® had the largest number of grains per pod (2.51), which was statistically different from BRS 213 (2.29) and BRSMG 790A (2.09). BRS Favorita RR® had a higher 100-grain fresh weight (31.09 g) than the other cultivars, while the values for the cultivars BRS 213 and BRSMG 790A (29.34 g and 28.28 g, respectively) were statistically not different. Charlo et al. (2011) suggests that a higher grain weight is a desirable factor in green-soybean genotypes because it indicates that the plant material will have higher green grain yields. However, Smiderle et al. (2011) studied the grain yield of eight soybean cultivars and found means of up to 95 g for 100-grain fresh weight. In this study, cultivar BRSMG 790A had the highest mean for 100-grain dry matter (14.34 g).

The results related to Zn functions in the plant were as expected. Zinc is necessary for the production of tryptophan, an amino acid precursor of the plant growth hormone indole acetic acid, which is also involved in nitrogen metabolism and helps maintain cell membrane integrity (Malavolta, 2006). Zinc fertilizers applied to the soil or leaves increased plant height and number of grains per plant compared with that in the control treatment, which showed the importance of applying Zn fertilizers to Zn-poor soils (Table 3). In a study on strategies to increase Zn contents in soybean, Inocêncio et al. (2012) showed that the Zn dynamics and stocks in the soil-plant system significantly impacted soybean productivity even in soils with a Zn content above the critical level. Therefore, the restitution of exported nutrients must not be overlooked in fertilization programs.

The production capacity of different genotypes and their influence on nutrient concentrations in the edible plant parts are extremely important when the objective is biofortification. Oliveira et al. (2016) found that yield, plant height, first pod insertion height, and iron, zinc, and phosphorus grain contents varied among 24 soybean cultivars that were being investigated for identifying genotypes that could facilitate biofortification. Furthermore, foliar Zn fertilization increases some soybean agronomic characteristics, which promises to improve biofortification. The correction of Zn deficiency in plants increases yields and contributes to an improvement in the nutritional quality of food crops, which leads to nutritional benefits (Das and Green, 2013).

Fertilization to increase the Zn content in cereals (agronomic biofortification) requires modifications to the quantity and frequency of Zn application. In addition, soil nutrient concentrations must be monitored to avoid excessive accumulation and damage to soil biology, which may occur before phytotoxic effects on crops become visible. If soil Zn concentrations are monitored, then foliar fertilization with micronutrients, such as Zn, are less likely to exceed the maximum safe and permissible concentrations (Alloway, 2008).

Materials and methods

Plant material

The experiment was carried out in a greenhouse at the Crop Science Department, Federal University of Lavras, Minas Gerais, Brazil (21°14’S latitude, 45°00’W longitude; 918 m asl). Pots with a 5 dm^3 capacity were filled with soil samples from the 0–20 cm layer of a medium texture Red Latosol, which had the following chemical properties: pH in water: 5.4, K: 20 mg dm^{-3}, P (Mehlich-1): 0.84 mg dm^{-3}, S: 1.56 mg dm^{-3}, Ca: 0.32 $cmol_c$ dm^{-3}, Mg: 0.10 $cmol_c$ dm^{-3}, Al: 0.10 $cmol_c$ dm^{-3}, potential acidity (H+Al): 2.90 $cmol_c$ dm^{-3}, organic matter (OM): 2.36 dag kg^{-1}, Fe: 22.53 mg dm^{-3}, Mn: 3.88 mg dm^{-3}, Cu: 1.88 mg dm^{-3}, and B: 0.13 mg dm^{-3}. The total Zn content in this soil was 0.33 mg dm^{-3}, which means that it is classified as being in the low Zn content range for soybean cultivation according to thresholds established by Galrão (2002).

Experimental design and Zn strategies

The experiment was arranged in a completely randomized 5×3 factorial design, with four replications. Five Zn fertilization strategies were evaluated. These were application to the soil, foliar application at vegetative stage V4 (four fully developed trifoliolate leaves), foliar application at V8 (eight fully developed trifoliolate leaves), foliar application at reproductive stage R4 (full pod), and a control without Zn fertilization. Three soybean cultivars (BRS 213, BRSMG 790A, and BRS Favorita RR®) were tested and there were four replicates. Each experimental unit consisted of a pot with two plants. Cultivars BRS 213 and BRSMG 790A are recommended for human consumption (food-type), and BRS Favorita RR® is an industrial-use cultivar.

The Ca and Mg soil content were increased using dolomitic limestone following soil chemical analysis. The need for soil correction was defined using the base saturation method where 70% base saturation was considered to be the ideal level. After 30 days, the soil was fertilized according to Malavolta (1980). A total of 200 mg P dm^{-3} (single superphosphate), 150 mg K dm^{-3} in two applications (potassium chloride), and 50 mg S dm^{-3} (single superphosphate) were applied. Micronutrients, except for Zn, were also applied shortly before planting. These were 7 mg Mn, 3 mg Cu, 0.5 mg B, and 0.2 mg Mo per dm^3 soil volume in the form of manganese chloride, copper sulfate, boric acid, and ammonium molybdate.

Exactly 3 mg Zn per dm^3 soil was applied according to Sfredo (2008). The Zn source was zinc sulfate (23% Zn). In the soil fertilization treatment, the Zn solution was applied at planting and 1.82 mg L^{-1} was applied to the leaves in the foliar fertilization treatments. Prior to treatment, the application of the correct amount of solution required to wet the plant completely, but without leaching into the soil, was estimated. The volume applied varied because it is based on the plant size at each development stage. The solution was applied to each pot separately with a manual pressure sprayer.

Experimental methodology

In the greenhouse, six seeds per pot were sown, and the seedlings were thinned to two plants per pot after the first trifolium appeared (V1). During cultivation, irrigation water was applied with a micro sprinkler to maintain the soil moisture level at 60% field capacity. When necessary, pest and disease control measures were adapted using the insecticide teflubenzurom applied at a rate of 0.05 L ha^{-1} and the fungicide azoxystrobin + ciproconazole applied at a rate of 0.3 L ha^{-1} plus an oil-based adjuvant (0.5% Nimbus). Weed control was performed manually.

Traits measured

Data collection was performed when the plants had reached stage R6 (pod containing green seeds that fill the pod cavity at one of the four uppermost nodes on the main stem with a fully developed leaf). The following agronomic and productive parameters were determined: plant height and 1st pod insertion height (cm); fresh weight of pods per plant (g); number of pods per plant; number of grains per plant; number of grains per pod; 100-grain fresh weight (beans with a mean moisture content of 53%); and 100-grain dry matter weight (mean moisture of 13%). The Zn content and grain compositions were determined using samples that had been previously oven-dried with forced air circulation at 65°C to a constant weight and then ground in a knife mill. The methodology described by Malavolta et al. (1997) was used to determine the total Zn content (mg kg^{-1}). The centesimal composition of the grains, based on the quantification of moisture, ash, protein, lipid, crude fiber, and carbohydrate content, was determined according to AOAC (2006).

Statistical analysis

The data were subjected to analysis of variance and when the F test detected significant differences, the means were grouped by the Scott-Knott test ($p < 0.05$) using SISVAR software (Ferreira, 2011).

Conclusion

Under the studied conditions, Zn fertilizer applied to soybean leaves at growth stage R4 was the most effective way to improve grain Zn levels when the soil availability of this nutrient is low. The cultivars had variable Zn accumulation responses, and the Zn grain content increased most in cultivar BRSMG 790A. Furthermore, Zn fertilization increased the soybean grain protein content, plant height, and number of grains per plant.

Acknowledgments

The authors thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for their advice and financial support. We also thank FAPEMIG (Fundação de Amparo à Pesquisa de MG) for supplying the soybeans and Dr. Li Li (Robert W. Holley Center for Agriculture and Health – USDA/Cornell University-USA) for proof reading the manuscript.

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