

Response of Haricot Bean Varieties to Different Levels of Zinc Application in Selected Areas of Ethiopia

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Abstract
Zinc is an essential trace element for plants, animals, and humans, and its deficiency is the fifth most important risk factor associated with illness and death in the developing world. The experiment was conducted in Taba, Halaba, and Butajira soils to evaluate the response of haricot bean varieties to different Zn levels. It was carried out in a quadruple factorial design on farmers' fields and in a greenhouse with soils collected from the aforementioned locations. The two factors were the concentration of zinc fertilizer (0, 0.5, 1, or 1.5%) and the haricot bean variety (Nasir, Ibado, Hawassa Dume, or Sari-1). Zinc sulfate ($ZnSO_4{\cdot}7H_2O$) was sprayed on the leaves, and nitrogen (N) and phosphorus (P) were applied to the soil equally for all treatments just before planting, using urea and triple super phosphate (TSP) respectively. Four haricot bean seeds/pot were sown and, later, two seedlings were thinned. For the field experiment, haricot beans were planted in rows with a spacing of 10 cm between plants and 40 cm between rows. Plant height, number of pods per plant, number of seeds per pot, 1000 seed weight, and biomass and grain yields were determined. Leaves and seeds from each treatment pot and plot were analyzed for their zinc contents. The results from pot and field experiments indicated that haricot bean production varied significantly among varieties both in yield parameters and tissue Zn concentrations, with the highest grain yield being produced by Nasir and Hawassa Dume. Nasir also produced the highest seed Zn; therefore, it was found to be the best variety both in quantity and quality. Haricot bean production also varied significantly among locations, with the highest grain yield at Butajira. The application of increasing levels of zinc fertilizer significantly increased tissue Zn concentrations. The growing season also significantly affected haricot bean production in terms of yield parameters and tissue Zn concentrations. The highest grain yield was observed during the belg (short growing) season, whereas the highest tissue Zn concentrations were recorded during the meher (long growing) season. Therefore, we can conclude that Zn fertilization and the consumption of haricot bean varieties with high levels of Zn could significantly improve the Zn status of people for whom haricot beans constitute a major component of their diet.
Keywords: Haricot bean, leaf Zn concentration, seed Zn concentration, tissue Zn concentration, zinc fertilization

Introduction

Zinc is an essential trace element for plants, animals, and humans (Ai-qing et al., 2013). It is essential for activating plants' enzymatic systems, protein synthesis (Hafeez et al., 2013), photosynthesis, reproduction of genetic material (DNA) during cell division (Singh, 2004), and the synthesis of chlorophyll and carbohydrates (Kobraee et al., 2011). Zinc deficiency not only retards the growth and yield of plants, but also affects human beings. It is the most widespread micronutrient deficiency throughout the world particularly in developing countries (Cakmak, 2002). It is the fifth most important risk factor associated with illness and death in the developing world (Ai-qing et al., 2013). Zinc deficiency is a global nutritional problem affecting 50% of world's cultivated soils and about one-third of the world's population (Hacisalihoglu et al., 2004; Ai-qing et al., 2013; Fageria et al., 2014), particularly in areas where the population is heavily dependent on an unvaried diet of cereal-based foods (Hafeez et al., 2013). The authors also reported that the optimum dietary intake for adults is 15 mg Zn per day. Therefore, it is crucial to increase the Zn contents of food grains to raise food quality and thereby improve human health.

Cakmak (2002) also pointed out that increasing the concentrations of Zn in grains is a high priority research task, and would greatly contribute to the alleviation of Zn deficiencies in human populations worldwide. He also indicated that fertilization of plants via soils or foliar applications is one important strategy to increase micronutrient concentrations in grains.

Phaseolus vulgaris L. is an important leguminous crop of great nutritional status to poor communities in African countries. It is nicknamed "poor man's protein" due to its potential role in the daily diet of the poor who cannot afford expensive animal protein (Patrick et al., 2011). It can provide 15% of the Zn requirements (Beebe et al., 2000); however, it is recognized as being susceptible to zinc deficiency (Goh and Karamanos, 2003; Singh, 2004; Hossein et al., 2008; Imtiaz et al., 2010), which reduces its yield and nutritional value (Kobraee et al., 2011).

Zinc fertilization is one method to prevent zinc deficiency and increase its concentration in grains (Cabrera et al., 2003; Blair et al., 2009). As zinc is taken up by plants in small amounts, crop demands for zinc can be met through foliar sprays (Pivot, 2003). Ai-qing et al. (2013) reported that foliar Zn application increased grain Zn concentration in wheat more effectively than Zn application to soil. Foliar Zn application also reduced the grain [phytic acid]/[Zn] ratio and increased the estimated Zn absorption more than did soil Zn application. Foliar application is the most efficient method of supplying Zn, which is needed only in a small quantity and become available (FAO, 2000) as it does not directly contact the soil, avoiding losses through fixation (Nasri et al., 2011). Anu et al. (2014) also reported that foliar application of Zn resulted in better absorption than soil application. However, the narrow difference between its phytotoxicity and deficiency requires that appropriate application rates be determined. Further, crop responses to applied zinc vary depending on the soil type, crop, variety, available nutrient status, severity of deficiency, etc. Tryphone and Nchimbi-Msolla (2010) reported that haricot bean genotypes varied in response to the application of Zn in Tanzania. There is a lack of information in this regard in Halaba, Taba, and Butajira soils cropped with beans. Therefore, this study was conducted to evaluate the response of haricot bean varieties to different Zn levels applied via leaves in Halaba, Taba, and Butajira soils under greenhouse and field conditions.

Materials and Methods

The experiment was executed in a quadruple factorial design on farmers' fields and in a greenhouse. The two factors were the concentration of zinc fertilizer (0, 0.5, 1, or 1.5%) and the haricot bean variety (Nasir, Ibado, Hawassa Dume, or Sari-1). Zinc sulfate solutions of 0.5, 1, and 1.5% were prepared by adding 0.5, 1, and 1.5 kg, respectively, of zinc sulfate into 100 L of water (Pivot, 2003). The experimental sites were Halaba, Taba, and Butajira, and 20 sub-samples of soils (0–30 cm depth) per location were also collected from farmers' fields and composited for the greenhouse experiment. The types of soils include: Haplic Luvisols (Humic) in Butajira; Andic Lixisols (Humic) in Halaba; and Haplic Lixisols (Siltic) in Taba. The soil properties of each site are indicated in Table 1.

Table 1. Physico-chemical properties of the experimental soils

Soil properties	Butajira soil	Halaba soil	Taba soil
Textural class	Clay loam	Clay loam	Clay loam
pH (H_2O)	7.40	7.70	7.47
Organic Carbon (%)	2.05	2.35	2.35
DTPA Zn (mg kg^{-1})	0.49	1.33	1.80
DTPA Fe (mg kg^{-1})	1.26	1.60	1.50
Total N (%)	0.34	0.24	0.16
Available P (mg kg^{-1})	12.13	10.00	14.30

The source of zinc was heptahydrated zinc sulfate ($ZnSO_4 \cdot 7H_2O$), which contains 21% Zn (Tryphone and Nchimbi-Msolla, 2010). The zinc sulfate was sprayed at a volume of 100 L/ha on the plants three weeks and six weeks after the sowing date (Abdel-Mawgoud et al., 2011). Nitrogen (N) and phosphorus (P) were applied equally for all treatments. Phosphorus was applied just before planting as TSP at 50 mg P kg^{-1} soil (0.25 g P/pot) for the pot experiment and 20 kg P ha^{-1} for the field experiment. Nitrogen was also applied just before planting as urea at 0.045 g N/pot and at 18 kg N ha^{-1} for the field experiment. Four haricot bean seeds per pot were sown and two of the seedlings were thinned at 10 days after sowing.

For the field experiment, haricot beans were planted in rows with a spacing of 10 cm between plants and 40 cm between rows in 3 × 4 m plots during belg and meher (the small and large rainy seasons), while the greenhouse experiment was conducted in pots with 5 kg of soil per pot. All appropriate management practices were carried out equally for all treatments.

Plant height, number of pods per plant, number of seeds per pot, 1000 seed weight, and biomass and grain yields were recorded. Three fully developed leaves from the top of the plant during initial flowering and some seeds were collected from each treatment pot and plot, dried in an oven at 70 °C for 24 hours, and ground using a sample rotating mill. The ground plant materials were digested and analyzed for their zinc contents with the following procedures. A sample of 0.5 g was weighed on a sensitive balance to the nearest 0.00001g and put into a digestion tube. Six milliliters of HNO_3 was added to each tube and placed in a digestion block at 90 °C for 45 minutes. Then, 5 ml of H_2O_2 was added in two splits (3 ml and 2 ml) while the samples were in the digestion block and digestion continued for another 65 minutes. Finally, 3 ml of 6 M HCl was added and the samples were digested until the solution had turned completely clear (about 5 minutes). Then, the tubes were removed from the block and cooled for 20 minutes, shaken using a vortex, and the digests were transferred from digestion tubes to dram vials and stored after the solution was brought to a volume of 25 ml with deionized water. The concentrations of Zn were analyzed using a microwave plasma atomic emission spectrometer (MPAES) at 213.857 nm. Analysis of variance (ANOVA) was carried out using Proc GLM procedures in the SAS 9.3 program (SAS Institute Inc., Cary, NC USA) and Least Significant Difference (LSD) test was used for mean separation. All data from the two seasons and three locations were combined.

Results and Discussion

Pot experiment

Table 2. Haricot bean yield, yield components, and tissue Zn concentrations as influenced by soil

Soils	Plant height (cm)	No. of pods per plant	No. of seeds per pod	Grain yield (kg ha^{-1})	Leaf Zn (mg kg^{-1})	Seed Zn (mg kg^{-1})
Butajira soil	35.09c	6.00b	4.67a	17.62a	32.22b	15.74c
Taba soil	36.95b	5.88b	4.63a	14.51b	42.20a	20.94a
Halaba soil	44.34a	6.63a	4.31b	13.74b	33.13b	20.18b
CV (%)	9.38	18.64	14.41	21.31	18.76	6.22
LSD (5%)	1.47	0.47	0.26	1.32	2.73	0.48
P value						
Variety	<.0001	<.0001	<.0001	0.0002	0.0004	<.0001
$ZnSO_4{\cdot}7H_2O$	0.0024	0.0404	0.2880	0.0025	<.0001	<.0001
Variety × $ZnSO_4{\cdot}7H_2O$	0.0701	0.1867	0.0287	0.4966	0.0061	0.0051
Soil	<.0001	0.0040	0.0177	<.0001	<.0001	<.0001
Variety × soil	0.0648	0.0201	0.7370	0.8675	0.0012	<.0001
$ZnSO_4{\cdot}7H_2O$ × soil	0.1674	0.5157	0.7370	0.5847	0.0474	<.0001
Variety × $ZnSO_4{\cdot}7H_2O$ × soil	0.0164	0.8610	0.3612	0.1415	<.0001	<.0001

Means followed by the same letter(s) within a column are not significantly different at $P \leq 0.05$.

The pot experiment indicated that haricot bean production varied significantly among soils of the different locations (Table 2). Both yield parameters and tissue Zn concentrations were significantly influenced by location soils. The highest values of plant height (44.34 cm) and number of pods per plant (6.63) were observed in Halaba soil, while the lowest values of plant height and pods per plant, 35.09 cm and 5.88, were in Butajira and Taba soils, respectively. The highest values of seeds per pod (4.67) and grain yield (17.62 g/pot) were obtained in Butajira, but the lowest values were observed in Halaba soil. Though the highest number of pods per plant was observed in Halaba soils, grain yield was lowest in this location attributed to the lowest number of seeds per pod obtained. The highest leaf Zn (42.10 mg kg^{-1}) and seed Zn (20.94 mg kg^{-1}) were observed in Taba, while the lowest values, 32.22 and 15.74 mg kg^{-1}, respectively, were obtained in Butajira soil. This indicates that Butajira and Taba are better locations for haricot bean production in terms of quantity and quality, respectively.

The significantly higher seed Zn concentration observed in bean grown in Halaba soil than in Butajira could be attributed to the dilution effect by higher yield at Butajira and higher soil Zn content in Halaba soil. Nchimbi-Msolla and Tryphone (2010) reported that bean leaf and seed Zn concentrations were varied among locations due to the environments in which the crop was grown.

Haricot bean production varied significantly among varieties in terms of yield parameters and tissue Zn concentrations (Table 3). The tallest plants (44.07 cm) and fewest pods per plant (4.61) and seeds per pod (3.36) were observed with Ibado. The shortest plants (36.22 cm), lowest leaf Zn (32.39 mg kg^{-1}), and highest grain yield (16.78 g/pot) were recorded with Nasir. The most pods per plant (7.47) was obtained with Hawassa Dume. Sari-1 produced the most seeds per pod (5.33) and highest leaf Zn (39.40 mg kg^{-1}). Nasir and Sari-1 had the highest seed Zn, 20.13 and 20.14 mg kg^{-1}, respectively. Although the highest grain yield was observed with Nasir, it was not significantly different from the value for Hawassa Dume.

The application of Zn fertilizers significantly increased plant height and tissue Zn concentrations (Table 4). A similar finding was reported by Itamar et al. (2004), who found that foliar Zn fertilization significantly affected plant height. Kobraee et al. (2011) and Kumar and Babel (2011) also reported that the application of increasing levels of Zn fertilizer significantly increased both leaf and seed Zn concentrations. All levels of Zn fertilizers resulted in higher values of plant height than the value obtained with no Zn application. The highest leaf and seed Zn concentrations, 50.71 and 20.53 mg kg^{-1}, were observed at applications of 1.5 and 1% zinc sulfate, respectively, indicating that the levels of Zn fertilizer yielding the highest concentrations of Zn in leaves and seeds may differ. It also suggests that the amount of Zn fertilizer to be applied may depend on the purpose of production. The lowest concentrations of both leaf and seed Zn, 19.83 and 17.55 mg kg^{-1}, respectively, were observed with the control (no Zn fertilizer). Therefore, the application of 1.5 and 1% of Zn sulfate resulted in concentrations of Zn in leaves and seeds that were 155.72 and 16.98% higher, respectively, than the control (no Zn). The concentrations of Zn in leaves and seeds were different (higher in leaves), which are expected, suggesting that plant mineral concentrations may vary among plant tissues. Narwal et al. (2010) also reported similar findings on wheat in Australia. The number of pods per plant, number of seeds per pod, and grain yield were not significantly different among different levels of Zn fertilizer application and the control. Ai-qing et al.

(2013) also found that the application of Zn fertilizer usually had no significant effect on grain yield in wheat.

Significant differences in the concentrations of Zn in leaves ($p = 0.0061$) and seeds ($p = 0.0051$) were observed due to the interactions between varieties and levels of Zn fertilizer (Fig. 1). All varieties produced the highest leaf Zn concentrations with the highest level of Zn fertilizer (1.5% $ZnSO_4 \cdot 7H_2O$), but they produced the highest seed Zn concentrations at the application of 1% zinc sulfate except for Hawassa Dume, which produced highest seed Zn at 1.5%. However, the highest leaf and seed Zn concentrations differed among the varieties. The highest leaf Zn concentrations of Nasir, Ibado, Hawassa Dume, and Sari-1 were 43.65, 56.69, 49.62, and 52.90 mg kg^{-1}, respectively, whereas the lowest values, 20.24, 20.86, 18.58, and 19.64 mg kg^{-1}, respectively, were observed with no zinc fertilizer. On the other hand, the highest values of seed Zn in the above varieties were 22.47, 19.07, 18.90, and 22.00 mg kg^{-1}, respectively. These indicate that all varieties accumulated Zn in their tissues similarly except for Hawassa Dume, which differed in how much was accumulated in seeds. Therefore, the same amount of zinc fertilizer is recommended to be applied for all varieties.

Both leaf and seed Zn concentrations were significantly influenced by the variety × location interaction (Fig. 2) at $p = 0.0012$ and $p < 0.0001$, respectively. All varieties had their highest tissue Zn concentrations in Taba soil except for Ibado, which had its highest seed Zn in soil of Halaba. The highest leaf Zn concentrations in plants grown on Taba soil, with values of 41.38, 43.30, 38.61, and 45.51 mg kg^{-1} in Nasir, Ibado, Hawassa Dume, and Sari-1, respectively. Nasir, Hawassa Dume, and Sari-1 had their lowest leaf Zn values, 26.61, 32.93, and 34.77 mg kg^{-1}, respectively, in Halaba soils, but Ibado had its lowest leaf Zn (27.40 mg kg^{-1}) in soil from Butajira. The highest seed Zn values for Nasir (21.80 mg kg^{-1}), Hawassa Dume (20.43 mg kg^{-1}), and Sari-1 (23.08 mg kg^{-1}) were observed in Taba soil, but Ibado had its highest seed Zn (20.25 mg kg^{-1}) in soil from Halaba. All varieties had their lowest seed Zn concentrations in Butajira soil, with values of 17.37, 13.81, 16.06, and 15.89 mg kg^{-1} for Nasir, Ibado, Hawassa Dume, and Sari-1, respectively. Therefore, Taba is the best location for the production of all haricot bean varieties in terms of quality, whereas Halaba was the best location for seed Zn production for Ibado.

Tissue Zn concentrations were significantly influenced ($p < 0.0001$) by the zinc fertilizer level × location interaction (Fig. 3). The seed Zn concentration was significantly influenced by the variety × zinc fertilizer × soil interaction ($p < 0.0001$), but leaf Zn concentration was not. For all levels of zinc fertilizer, the highest tissue Zn concentrations were observed in Taba soil, but with no zinc fertilizer, the highest seed Zn was recorded in soil of Halaba. With no Zn fertilizer, Nasir, Ibado, and Sari-1 had their highest seed Zn in Halaba, and Hawassa Dume in Butajira soil. The higher seed Zn concentration of Nasir, Ibado, and Sari-1 obtained in Halaba soils with no Zn fertilizer could be attributed to the higher Zn absorption caused by favorable soil conditions like low soil P content. On the other hand, despite the lower soil Zn content, Hawassa Dume contained higher Zn content in Butajira soil with no Zn fertilizer, which could be attributed to the nature of its roots that might enable effective Zn uptake. At all levels of Zn fertilizer, the highest seed Zn concentrations for Nasir, Hawassa Dume, and Sari-1 were observed in Taba, but in Ibado the highest seed Zn concentration was observed in Halaba soil (Fig. 4).

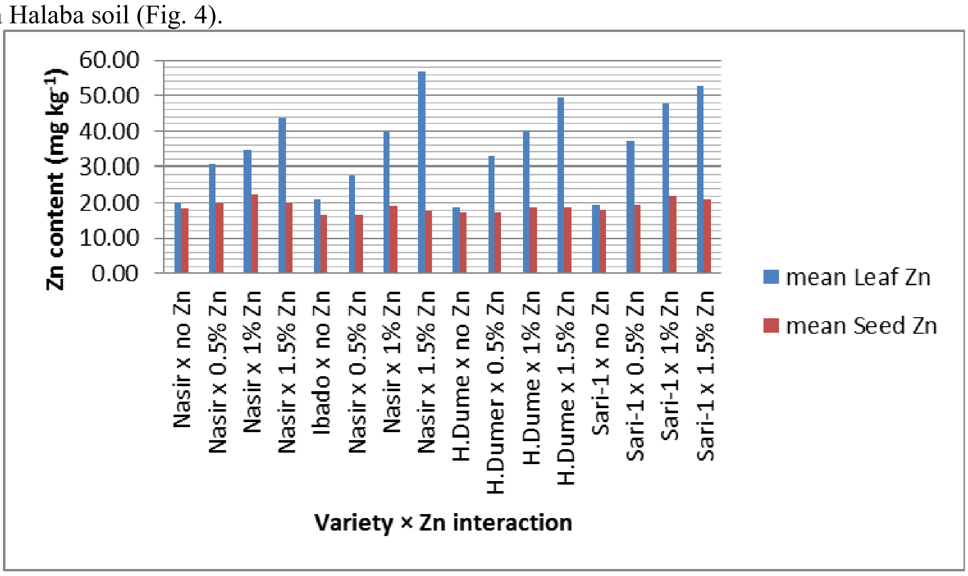

Fig. 1 Tissue Zn concentrations of bean varieties as influenced by the variety × Zn interaction. *H.Dume = Hawassa Dume.*

Table 3. Haricot beans yield, yield components, and tissue Zn concentrations as influenced by variety

Yield components and tissue Zn	variety				CV (%)	LSD (5%)
	Nasir	Ibado	Hawassa Dume	Sari-1		
Plant height (cm)	36.22b	44.07a	37.14b	37.75b	9.38	1.70
No. of pods per plant	6.72b	4.61d	7.47a	5.86c	18.64	0.54
No. of seeds per pod	5.00b	3.36d	4.44c	5.33a	14.41	0.31
Grain yield (kg ha^{-1})	16.78a	14.39b	16.27a	13.73b	21.31	1.53
Leaf Zn (mg kg^{-1})	32.39c	36.30ab	35.31bc	39.40a	18.76	3.15
Seed Zn (mg kg^{-1})	20.13a	17.51b	18.04b	20.14a	6.22	0.55

Means followed by the same letter(s) within a row are not significantly different at $P \leq 0.05$.

Table 4. Haricot beans yield, yield components, and tissue Zn concentrations as influenced by different levels of Zn fertilizer

Yield components and tissue Zn	$ZnSO_4{\cdot}7H_2O$ (%)				CV (%)	LSD (5%)
	0	0.5	1	1.5		
Plant height (cm)	36.76b	39.83a	39.33a	39.25a	9.38	1.70
No. of pods per plant	6.33a	6.42a	6.20ab	6.22ab	18.64	0.54
No. of seeds per pod	4.44a	4.42a	4.67a	4.61a	14.41	0.31
Grain yield (kg ha^{-1})	16.29a	15.18a	15.67a	16.1a	21.31	1.53
Leaf Zn (mg kg^{-1})	19.83d	32.19c	40.66b	50.71a	18.76	3.15
Seed Zn (mg kg^{-1})	17.55d	18.41c	20.53a	19.32b	6.22	0.55

Means followed by the same letter(s) within a row are not significantly different at $P \leq 0.05$.

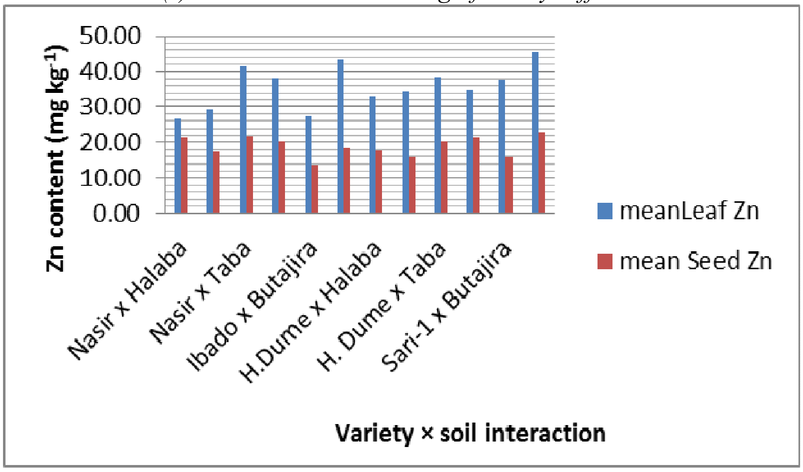

Fig. 2 Influence of the variety × soil interaction on tissue Zn concentrations of bean varieties.

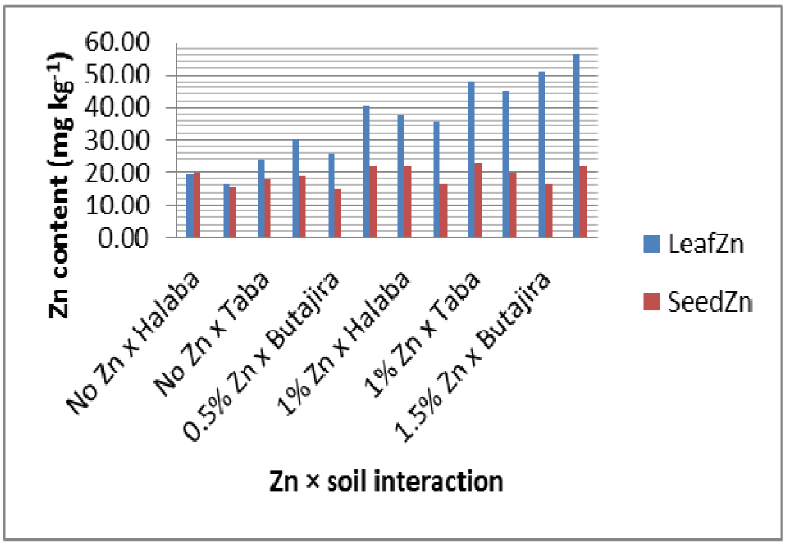

Fig. 3 Influence of the Zn × soil interaction on tissue Zn concentrations of bean varieties.

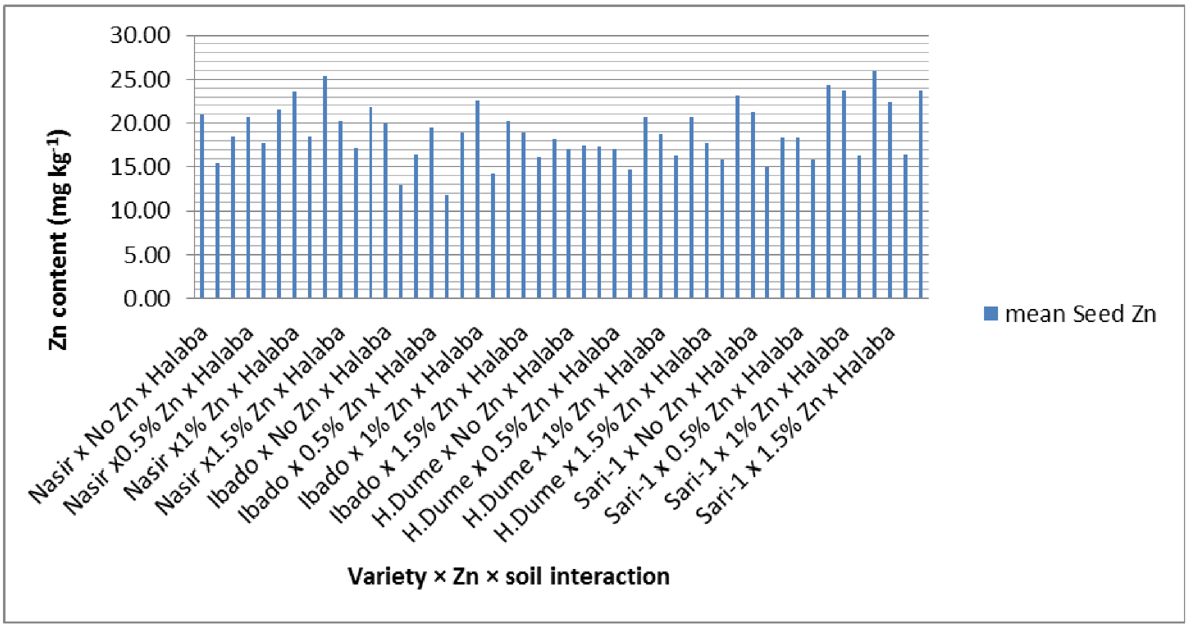

Fig. 4 Seed Zn concentration in haricot beans as influenced by the variety × zinc fertilizer × soil interaction.

Field experiments

Like the pot experiment, the field experiments showed that haricot bean production varied significantly among locations (Table 5). The highest values for yields and all yield parameters were observed at Butajira except for biomass yield, which was highest at Halaba. The highest grain yield was 3553.6 kg ha^{-1} while the lowest, from Taba, was 2250.4 kg ha^{-1}. The highest concentrations of both leaf and seed Zn, 26.93 and 29.52 mg kg^{-1}, respectively, were observed at Halaba, while the lowest values were obtained at Taba. This could be because Zn absorption in Taba fields might be more affected by environmental factors than in Halaba. However, under controlled conditions, where environmental factors have less effect, Zn absorption was higher in Taba than in Halaba that could be attributed to the higher soil Zn content of Taba soils (Table 1). Goh and Karamanos (2003) also found that yield and tissue Zn concentrations varied among locations. Growing season also significantly affected haricot bean production both in yield parameters and tissue Zn concentrations. Similar findings were reported by Ai-qing et al. (2013) on wheat in China. The highest (3559.1 kg ha^{-1}) and the lowest (1955.5 kg ha^{-1}) grain yields were observed during the belg and meher seasons, respectively. Plant height, the number of branches per plant, pods per plant, and seeds per plant; and biomass yield were all significantly higher during the belg season than the meher season. The highest concentrations of both leaf and seed Zn concentrations, 23.39 and 25.45 mg kg^{-1}, respectively, were observed in the meher season, but the lowest values, 21.59 and 23.62 mg kg^{-1}, respectively, were observed in the belg season. These differences in tissue Zn concentrations across seasons may be due to dilution effects associated with higher yields and yield parameters during belg. Similar findings were reported by Sebuwufu (2013) on haricot beans in Uganda. The poor bean production in meher might be attributable to the higher rainfall (Ogola, 1991) as it is a warm season crop (Directorate Plant Production, 2010).

Table 5. Haricot beans yield, yield components, and tissue Zn concentrations as influenced by location and growing season.

	Location				Season			
Parameters	Butajira	Taba	Halaba	LSD (5%)	Belg	Meher	CV (%)	LSD (5%)
Plant height (cm)	46.57a	39.25c	43.39b	1.46	45.72a	40.42b	13.73	1.19
No. of branches per plant	4.58a	4.23b	3.94c	0.19	4.39a	4.11b	17.95	0.15
No. of pods per plant	19.26a	14.30b	14.30b	0.79	16.94a	14.96b	20.06	0.65
No. of seeds per pod	5.37a	5.06b	4.92b	0.17	5.41a	4.83b	13.33	0.14
Grain yield (kg ha^{-1})	3553.6a	2250.4b	2467.8b	277.6	3559.1a	1955.5b	40.92	226.66
Biomass (kg ha^{-1})	6798.5b	4523.1c	7804.4a	328.66	8500.4a	4250.2b	20.95	268.35
1000 seed wt.	342.09a	293.37b	263.99b	30.63	307.32a	292.32a	41.52	25.00
Leaf Zn (mg kg^{-1})	21.45b	19.08c	26.93a	1.30	21.59b	23.39a	23.95	1.06
Seed Zn (mg kg^{-1})	22.06b	22.03b	29.52a	1.47	23.62b	25.45a	24.39	1.20

Means followed by the same letter(s) within a row are not significantly different at $P \leq 0.05$.

Similar to the pot experiment, the field experiments also indicated that haricot bean production, both in terms of yield parameters and tissue Zn concentrations, varied among varieties (Table 6). Similar findings were reported by McKenzie et al. (2001) on haricot bean in Canada. The highest values for plant height (45.71 cm), biomass (6838.1 kg ha^{-1}), and 1000 seed weight (470.56 g), and lowest grain yield (2503.4 kg ha^{-1}) were observed with Ibado. The combination of highest biomass and lowest grain yield indicates that Ibado produces the highest straw yield, suggesting that it is a good supplier of animal feed. Hawassa Dume had the highest grain yield (3149.3 kg ha^{-1}), and it was significantly higher than that of Ibado and Sari-1, but not significantly different from the yield of Nasir. Nasir produced the highest branches per plant (4.38), pods per plant (17.76), seeds per pod (5.67), leaf Zn (23.69 mg kg^{-1}), and seed Zn (25.79 mg kg^{-1}). Therefore, it was found to be the best variety both in terms of quantity and quality. Muhamba and Nchimbi-Msolla (2010) also reported that leaf and seed Zn concentrations varied among haricot bean varieties in Tanzania, ranging from 15.7 to 78.3 mg kg^{-1} and from 19.00 to 56.13 mg kg^{-1}, respectively. Ai-Qing et al. (2013) reported that the grain Zn concentration varied among wheat varieties in China.

The application of increasing levels of zinc fertilizer significantly increased tissue Zn concentrations (Table 7). Sharma and Bapat (2000) and Shaheen et al. (2007) reported that both the grain and straw Zn concentrations of wheat significantly increased with the application of Zn. Ai-Qing et al. (2011) and Ai-qing et al. (2013) also reported that the foliar application of Zn fertilizer significantly increased leaf and seed Zn concentrations, respectively, in wheat. Another report by Fageria et al. (2014) also indicated that Zinc concentration of tropical legume cover crops was increased with the addition of Zn to the soil. The highest concentrations of both leaf Zn (24.06 mg kg^{-1}) and seed Zn (25.97 mg kg^{-1}) were observed at the application of the highest level of zinc fertilizer (1.5%), whereas the lowest values of both leaf and seed Zn, 20.64 and 21.95 mg kg^{-1}, respectively, were recorded with no zinc fertilizer. These highest values of Zn in leaves and seeds were 16.57 and 18.31% higher, respectively, than the control. The value of seed Zn obtained with the application of 0.5% $ZnSO_4{\cdot}7H_2O$ was also 11.98% greater than the value obtained from the control. Although the highest seed Zn was observed at the application of 1.5% $ZnSO_4{\cdot}7H_2O$, it was not significantly higher than the values obtained at applications of 0.5 and 1% $ZnSO_4{\cdot}7H_2O$.

Table 6. Effect of varieties on the production and tissue Zn concentration of haricot beans

Parameters	Variety				CV (%)	LSD (5%)
	Nasir	Ibado	Hawassa Dume	Sari-1		
Plant height (cm)	42.38b	45.71a	41.74b	42.45b	13.73	1.68
No. of branches per plant	4.38a	3.93b	4.28a	4.41a	17.95	0.22
No. of pods per plant	17.76a	11.17b	17.22a	17.67a	20.06	0.91
No. of seeds per pod	5.67a	3.83c	5.52ab	5.45b	13.33	0.19
Grain yield (kg ha^{-1})	2870.4a	2503.4b	3149.3a	2506.1b	40.92	320.54
Biomass (kg ha^{-1})	6289.1b	6838.1a	6197.9b	6176.2b	20.95	379.50
1000 seed wt.	251.98b	470.56a	247.13b	229.61b	41.52	35.37
Leaf Zn (mg kg^{-1})	23.69a	22.75ab	21.97b	21.54b	23.95	1.50
Seed Zn (mg kg^{-1})	25.79a	23.87b	24.08b	24.40ab	24.39	1.70

Means followed by the same letter(s) within a row are not significantly different at $P \leq 0.05$.

Therefore, the application of 0.5% $ZnSO_4{\cdot}7H_2O$ is recommended for producing better quality haricot beans. The amount of seed Zn obtained from the 0.5% application was 24.58 mg kg^{-1}, which is enough for 1.64 days per person (Hafeez et al., 2013), i.e., 1 kg of haricot beans produced with the application of 0.5% $ZnSO_4{\cdot}7H_2O$ can supply a single person's Zn needs for 1.64 days. Fertilization with Zn did not significantly influence the yield and yield parameters of haricot beans (Table 7). Ai-qing et al. (2013) also reported that the foliar application of Zn fertilizer did not significantly increase the grain yield of wheat in China. Contrarily, Kobraee et al. (2011) reported that Zn fertilizer significantly increased the yield of soybeans. However, the highest pods per plant and biomass yield were observed with an application of 1.5% zinc sulfate. Mahbobeh et al. (2011) reported that the foliar application of zinc sulfate increased the number of pods per plant in haricot beans. The authors attributed the increase to the positive effect of zinc sulfate on the formation of stamens and pollen. Since the haricot bean is a self-pollinated plant, as the activity of stamens increases, the flowers are more fertile and more pods can be produced per plant. Other findings by Hossein et al. (2008) also showed that the application of increasing levels of Zn fertilizer did not significantly affect yield, but increased tissue Zn concentrations. Contrarily, Goh and Karamanos (2003) reported that foliar application of Zn significantly increased the yield of haricot beans compared to an unfertilized control.

Table 7. Effect of Zn fertilization on the production and tissue Zn concentration of haricot beans

$ZnSO_4{\cdot}7H_2O$ (%)	Plant height (cm)	No. of branches per plant	No. of pods per plant	No. of seeds per pod	Grain yield (kg ha^{-1})	Biomass (kg ha^{-1})	1000 seed wt.	Leaf Zn (mg kg^{-1})	Seed Zn (mg kg^{-1})
0	42.86a	4.15ab	16.00ab	5.04a	2741.3a	6056.9b	291.61a	20.64c	21.95b
0.5	42.82a	4.23ab	16.10ab	5.15a	2810.0a	6388.9ab	306.70a	22.54b	24.58a
1	43.40a	4.29ab	15.86ab	5.23a	2810.0a	6395.4ab	294.57a	22.72ab	25.64a
1.5	43.20a	4.38a	16.41a	5.05a	2748.4a	6660.2a	306.39a	24.06a	25.97a
CV (%)	13.73	17.95	20.06	13.33	40.92	20.95	41.52	23.95	24.39
LSD (5%)	1.68	0.22	0.91	0.19	320.54	37950	35.37	1.50	1.70
P value									
@V	<.0001	<.0001	<.0001	<.0001	<.0001	0.0015	<.0001	0.0278	0.1124
*Zn	0.8906	0.0753	0.2081	0.1891	0.9613	0.0213	0.7654	0.0002	<.0001
V × Zn	0.9537	0.6267	0.4146	0.1295	0.9937	0.6667	0.4314	0.7379	0.8878
#L	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
V × L	<.0001	0.0003	<.0001	0.0001	0.7260	<.0001	0.0008	0.0100	0.0010
Z× L	0.7644	0.5784	0.7161	0.5306	0.9905	0.1878	0.4164	<.0001	<.0001
V × Z× L	0.6092	0.3312	0.6925	0.0489	0.9846	0.5537	0.9135	0.9536	0.9558
$S	<.0001	0.0005	<.0001	<.0001	<.0001	<.0001	0.2387	0.0009	0.0031
V×S	0.0006	0.0429	0.0035	0.1179	0.0857	0.6264	1.0000	0.0825	0.0026
Z×S	0.5901	0.9074	0.8597	0.9266	0.9599	0.7784	1.0000	<.0001	0.0002
V×Z×S	0.9508	0.9972	0.8980	0.8922	0.9854	0.9998	1.0000	0.9198	0.8858
L×S	<.0001	<.0001	<.0001	0.0001	0.0073	<.0001	1.0000	<.0001	<.0001
V×L×S	0.0569	0.0119	0.0560	0.0795	0.0588	0.5084	1.0000	0.0002	0.0291
Z×L×S	0.6602	0.9409	0.6855	0.8924	0.8914	0.9861	1.0000	<.0001	<.0001
V×Z×L×S	0.9537	0.0727	0.7219	0.9793	0.9999	1.0000	1.0000	0.9974	0.9941

Means followed by the same letter(s) within a column are not significantly different at $P \leq 0.05$.
*Z = $ZnSO_4{\cdot}7H_2O$, @V = variety, #L= location, $S=season

Haricot bean production, yield parameters, and tissue Zn concentrations were significantly ($p < 0.0001$) influenced by the variety × location interaction (Fig.5) indicating that the diverse genotypes responded differently to local farm conditions. This could be attributed to the inherent genetic potential of these varieties and differences in local environmental conditions like soil fertility. Sebuwufu (2013) also reported similar findings on haricot beans in Uganda. Nasir produced its highest yield, all yield parameters and tissue Zn concentrations at Butajira. Ibado, Hawassa Dume, and Sari-1 produced their highest grain yield for most yield parameters at Butajira, but they produced the highest tissue Zn concentrations at Halaba.

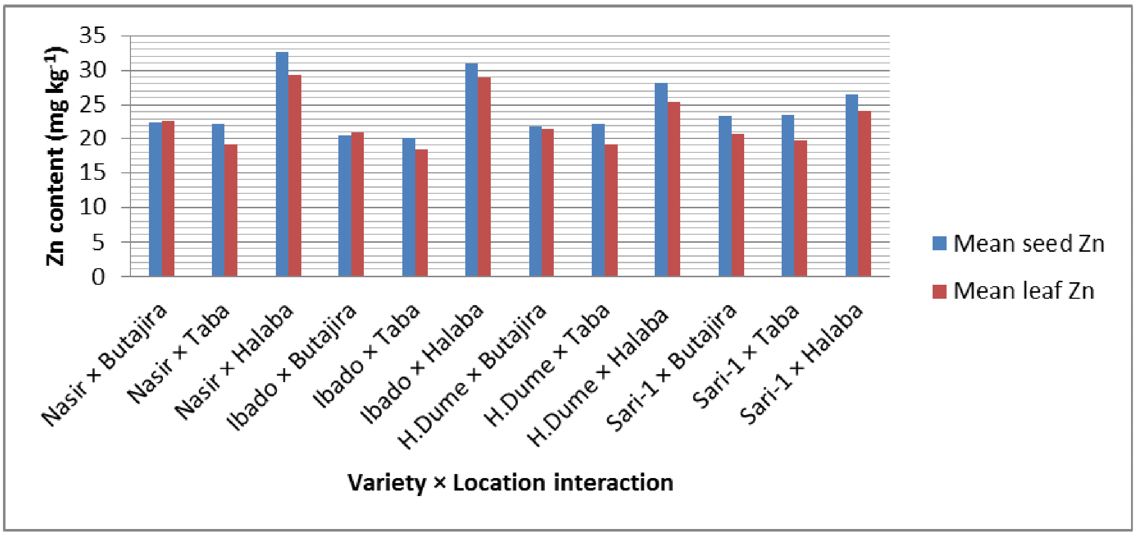

Fig 5. Influence of variety × location interaction on the tissue Zn concentration of haricot beans

Leaf and seed Zn concentrations were significantly ($p < 0.0001$) influenced by the interaction between the amount of zinc fertilizer and location (Fig. 6). At all levels of zinc fertilizer, the highest leaf and seed Zn concentrations were at Halaba, but, in the absence of zinc fertilizer, the highest leaf Zn was observed at Butajira.

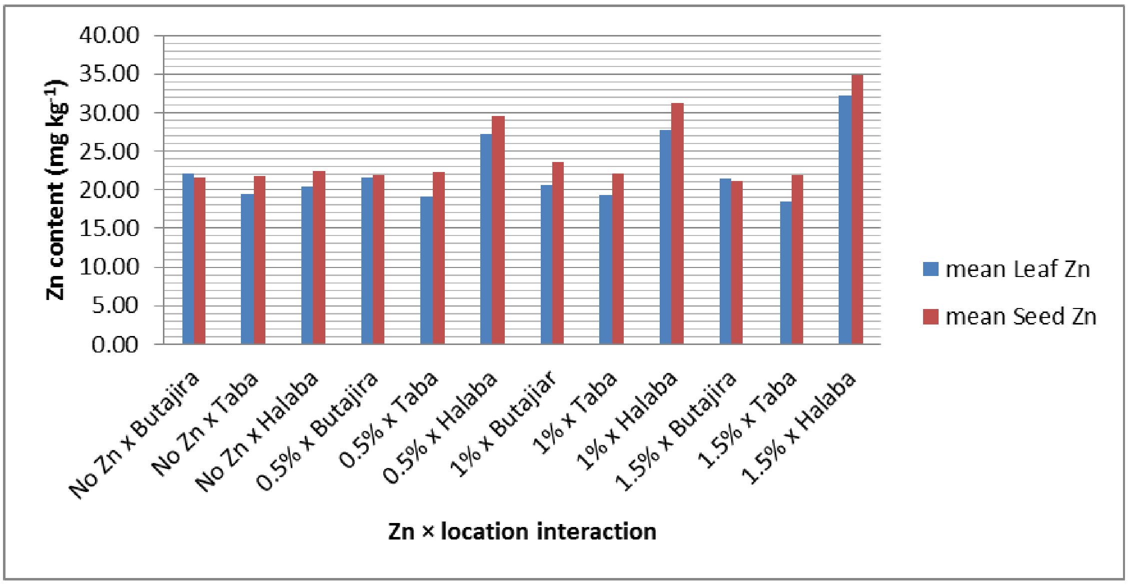

Fig. 6. Tissue Zn concentrations as influenced by the zinc × location interaction.

The interaction between the amount of zinc fertilizer and growing season significantly ($p < 0.0001$) influenced both leaf and seed Zn concentrations (Fig. 7). This indicates absorption of Zn by beans varies between growing seasons suggesting different amount of Zn should be applied during meher and belg for production of quality beans. With no Zn fertilizer, the belg season produced the highest leaf and seed Zn concentrations, but for all rates of applied Zn fertilizer, the highest leaf and seed Zn concentrations were in the meher season.

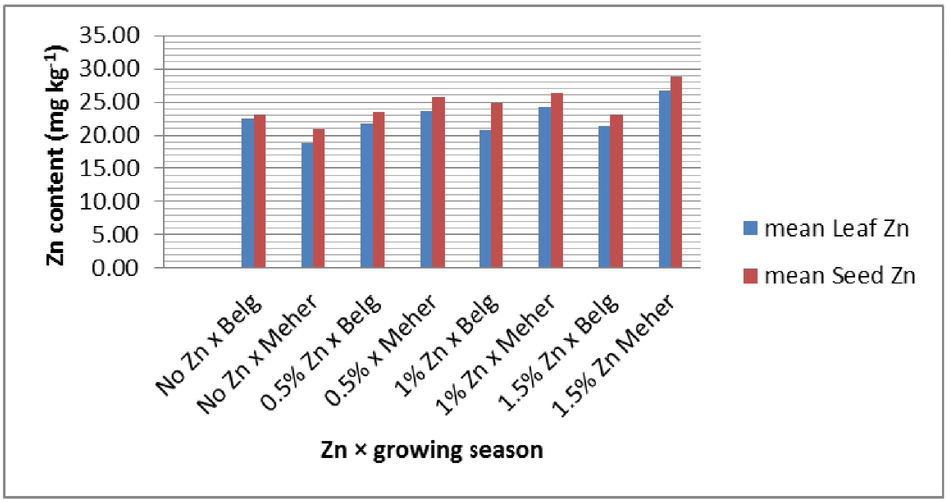

Fig.7. Leaf and seed Zn concentrations of haricot beans as influenced by the Zn fertilizer concentration × growing season interaction

Tissue Zn concentrations were also significantly ($p < 0.0001$) influenced by the amount of Zn fertilizer × location × growing season interaction (Fig. 8). With no Zn fertilizer, the highest leaf Zn concentration was observed at Butajira in the belg season. With all applications of Zn fertilizer, the highest tissue Zn concentrations were observed at Halaba in the meher season. The highest leaf and seed Zn concentrations, 44.10 and 46.41 mg kg^{-1}, respectively, were observed with the application of 1.5% $ZnSO_4 \cdot 7H_2O$ at Halaba in the meher season. In Butajira and Taba, for all levels of zinc fertilizer, the belg season produced better quality haricot beans. The values of tissue Zn concentrations in Butajira and Taba did not vary between the control and the beans treated with different levels of zinc fertilizer. Therefore, the production of haricot beans in Butajira and Taba would be best carried out in the belg season with no application of Zn fertilizer.

No parameters of haricot bean production were significantly influenced by the variety × zinc, variety × zinc × location, variety × zinc × season, and variety × zinc × location × season interactions. McKenzie et al. (2001) also reported that yield was not affected by the interaction between Zn fertilizer and haricot bean variety in Canada. The growth and production of the bean varieties were not affected by Zn supply indicating very high tolerance of these genotypes to Zn deficient soils. This finding is line with the finding reported by Hacisalihoglu et al. (2004) on haricot bean. The production of haricot bean varieties was significantly ($p < 0.05$) influenced by the variety × season interaction (Fig. 9). Nasir and Ibado had better yield parameters during the belg season but they had higher seed Zn concentrations during the meher season. Hawassa Dume and Sari-1 had better production in both yield parameters and seed Zn concentration during the meher season. The variety × season interaction points to genotypic differences in response to growing season, indicating possible differences in uptake and utilizations of supplied nutrients. Similar findings were reported by Sebuwufu (2013).

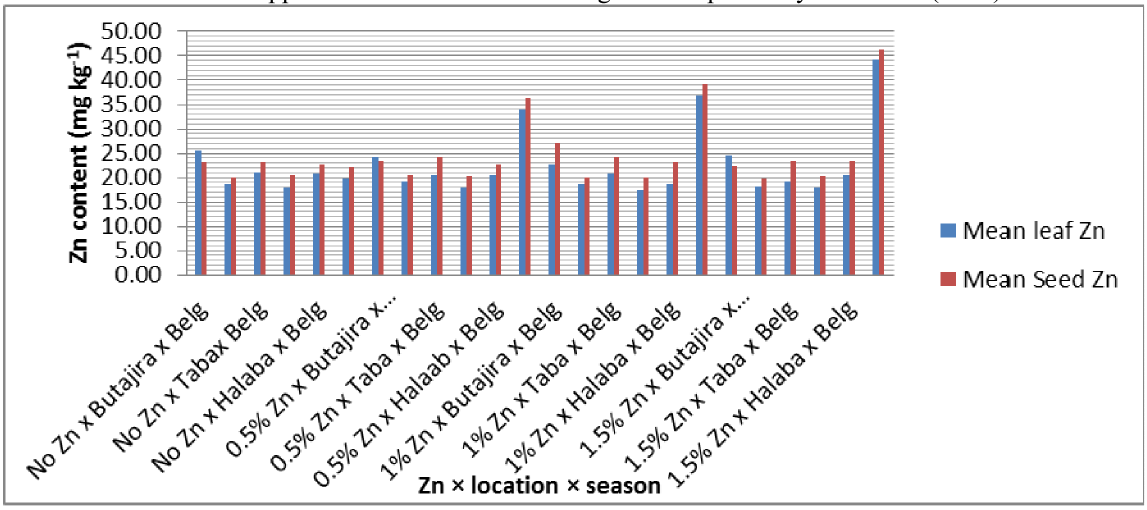

Fig. 8. Leaf and seed Zn concentrations as influenced by the Zn fertilizer concentration × location × growing season interaction.

Yield, yield components and seed Zn concentration were higher in the fields than pots with all varieties and Zn rates at all locations, which could be attributed to better root growth and distribution that could intern, enable the beans to absorb more Zn and other nutrients. This higher nutrient absorption resulted in more vigorous plant growth that could produce higher yield and yield components in the fields than pots. Leaf Zn concentration was lower in field experiments than the pots, which could be attributed to dilution effect caused by higher vegetative growth.

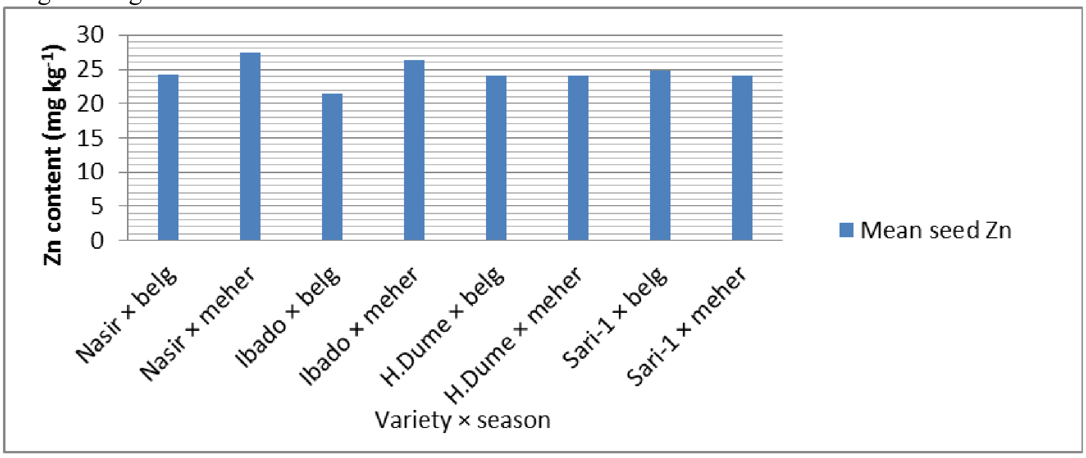

Fig.9. Effect of variety × growing season interaction on tissue Zn concentrations of haricot beans

Significant differences ($p < 0.0001$) in haricot bean production were observed due to the interaction of location and growing season (Fig. 10). In Butajira, except for the taller plant height observed during the meher season, all the yield parameters and tissue Zn concentrations were higher during the belg season. In Taba, better haricot bean production, both for yield parameters and tissue Zn concentrations, was observed during the belg season. In Halaba, all yield parameters performed better during the belg season, but both leaf and seed Zn concentrations were higher during the meher season. Therefore, the belg season is better for haricot bean production both in terms of quantity and quality in Butajira and Taba, but in Halaba, belg season is better for production in terms of yield parameters and the meher season is better in terms of quality production.

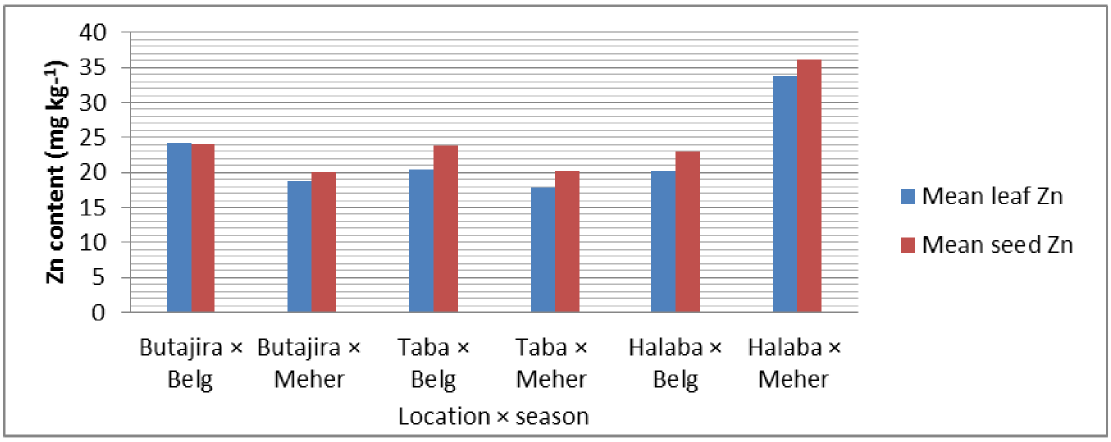

Fig.10. Influence of the location × growing season interaction on the tissue Zn concentration of haricot beans

Leaf and seed Zn concentrations were significantly ($p = 0.0002$ and 0.029, respectively) influenced by the variety × location × growing season interaction (Fig. 11). In Butajira and Taba, all varieties produced better tissue Zn concentrations (leaf and seed Zn) during the belg season, whereas in Halaba, all varieties showed better production during the meher season.

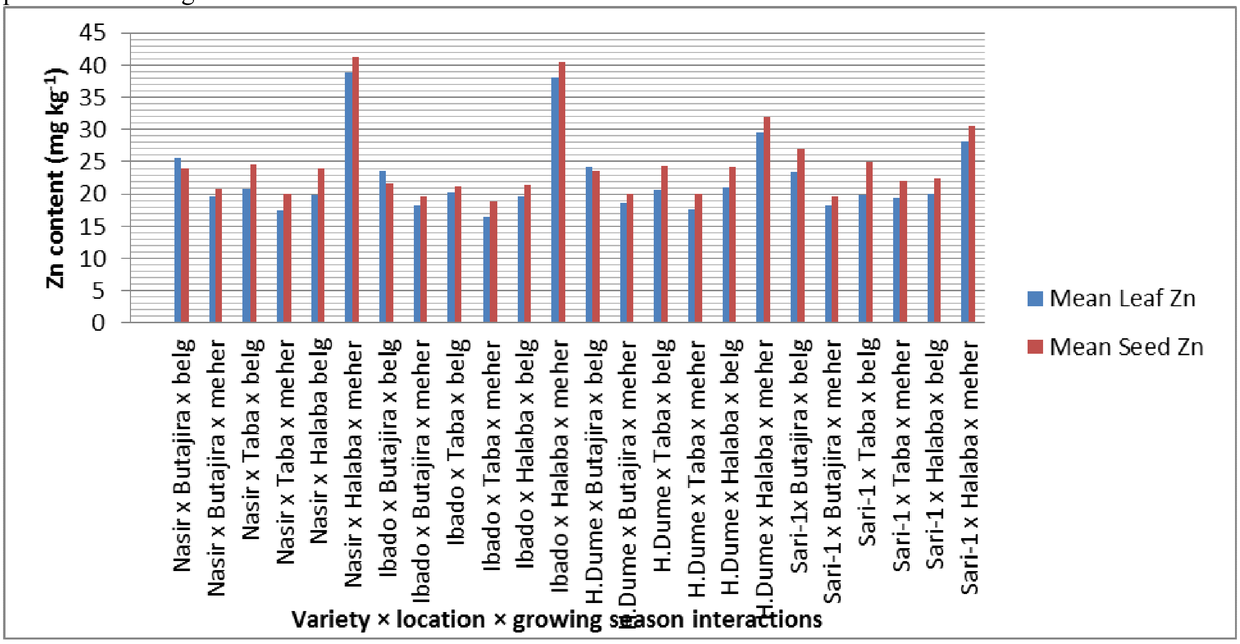

Fig. 11. Tissue zinc concentrations as influenced by the variety × location × growing season interaction.

Conclusion

Both pot and field experiments revealed that haricot bean production varied significantly among locations and bean varieties in terms of yield parameters and tissue Zn concentrations. The highest values for yields and yield parameters were observed in soils of Butajira. Nasir and Sari-1 had the highest seed Zn, 20.13 and 20.14 mg kg^{-1}, respectively, while Hawassa Dume gave the highest grain yield (3149.3 kg ha^{-1}) though not significantly different from Nasir did (2870.4 kg ha^{-1}). The application of increasing levels of zinc fertilizer significantly increased tissue Zn concentrations. The highest leaf and seed Zn concentrations, 50.71 and 20.53 mg kg^{-1}, respectively, were observed at applications of 1.5 and 1% zinc sulfate, respectively, indicating that the levels of Zn fertilizer yielding the highest concentrations of Zn in leaves and seeds may differ. Although the highest seed Zn was observed with the application of 1.5% $ZnSO_4·7H_2O$, it was not significantly higher than the values obtained at applications of 0.5 and 1% $ZnSO_4·7H_2O$. Thus, Nasir was found to be the best bean variety, and 0.5% $ZnSO_4·7H_2O$ was the best rate for the quality bean production.

The supply of Zn from haricot beans can be improved through foliar application of zinc sulfate and selection of bean varieties with high capacity to accumulate Zn. The consumption of such haricot beans with high Zn content can significantly improve the Zn status of people who consume the crop as a major component of their diet.

Acknowledgements
The research work was supported by research grant from the Government of Canada, Department of Foreign Affairs, Trade and Development/International Development Research Center through the Canadian International Research Fund Project.

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