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Response of Tissue Zinc to Zinc Fertilisation by Zinc Biofortifier Bush Bean Genotypes Targeted for Low Income Communities

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Authors' contributions

This work was carried out in collaboration between all authors. Author JST designed the study, wrote the protocol and wrote the first draft of the manuscript. Author EN implemented the experiment and analyzed data. Author SN supervised the implementation of the experiment and contributed to manuscript preparation. Author LNM was responsible for laboratory activities and overall logistical control. All authors read and approved the final form of the manuscript.

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ABSTRACT

Aim: This study investigated the influence of applied zinc on the richness and distribution of Zn in the leaves and grain of Zn dense bean genotypes.

Study Design: Treatments of this study were laid out in a completely randomised design (CRD), three replications, repeated three times.

Place and Duration: The study was conducted at National Crops Resources Research Institute (NaCRRI), in Uganda during 2011-2012.

Methodology: Two Zn biofortifier bush bean genotypes (KaboF6-2.8-27 and NUA69) and Zn rates of 0, 5, 7.5 and 12.5 mg pot^{-1} were considered in this study. Soil used was an Oxisol obtained from continuously cultivated bean producing soils. Data collected included leaf and grain Zn and estimations of the quantities of these bean consumable parts required to meet the thresh hold daily

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dietary requirements for pregnant and breast feeding mothers.

Results: There was a significant ($p<0.05$) interaction between Zn rates and genotypes in terms of tissue Zn. Zinc application caused a slight effect on leaf zinc in KaboF2.8-27, but had no significant effect ($p>0.05$) in NUA69 genotype. Both genotypes maintained a similar response pattern with respect to leaf Zn. Kabo6F2.8-27 was superior and had the highest leaf Zn at 44.3 mg pot^{-1}. As for the grain, KaboF2.8-27 and NUA69 peaked with 43 and 35.9 mg Zn kg^{-1} both within the application rate of 7.5 mg pot^{-1}.

Conclusion: The quantity of leaves required by pregnant mothers to meet their daily dietary Zn requirements is 248 g; while that of breast feeding mothers is 271 g of KaboF2.8-27 genotype. There is need for evaluation of the status of bioavailability of plant tissue Zn in order to ensure its effective and efficient utilisation by communities.

Keywords: Biofortifier; dietary requirements; Phaseolus vulgaris; Uganda.

1. INTRODUCTION

Beans (*Phaseolus vulgaris* L.), like several other plants, possess a renowned capacity for accumulating minerals in their tissues, especially in the consumable parts to the extent that they can act as sources of critically needed dietary micronutrients such as zinc. Zinc is vital in various aspects of human health such as infection, wound healing, health skin development, cell division, brain development and immunity improvement [1]. Zinc is also essential for the functioning of a large number of enzymes in the human body such as carboxypeptidase, liver alcohol dehydrogenase, and carbonic anhydrase. It is also essential in DNA transcription [2-4] and regulation and activation of other micronutrients, such as vitamin B-complex, which are better, absorbed and assimilated by the body when combined with Zn.

In Sub-Saharan Africa (SSA) and other developing regions, Zn is conventionally obtained through routine food staples, which are largely starchy and do not supply it in sufficient quantities. Thus, most communities in SSA develop Zn deficiency symptoms in the form of maladies such as delayed growth and development in children and adolescents, hair loss, diarrhoea, delayed wound healing, loss of appetite and weight loss and can also lead to under development of male sexual organs [5-7]. The economic implications of ameliorating Zn deficiency among communities using food fortification and supplementation, especially in SSA are enormous. Taking the example given by [8] Cakmak, a population of 50 million persons affected by Zn deficiency requires an estimated expenditure of 25 million US dollars; which is inconceivable in terms of burdened communities in SSA.

Efforts to leverage from Zn biofortification by beans to supply dietary Zn have been spearheaded by research institutions, particularly the International Centre for Tropical Agriculture (CIAT); and several genotypes with traits for remarkable Zn accumulation exist at research level. Zinc biofortifier beans rely on their biosynthetic or physiological capacity, as well as tolerance to accumulate Zn in the different plant parts to levels unattainable by other varieties or species [9].

Some areas on Zn biofortifier beans that still require research attention include information on the relative distribution of Zn within the edible plant parts, namely leaves and grain. What exists in literature is the relationship between bean leaf and root Zn, yet presently bean roots have no known dietary value [10]. In SSA, there is overwhelming consumption of bean grain compared to leaves. The objective of this study was to determine the influence of applied Zn on the level and distribution of Zn in the consumable parts of Zn dense bush bean genotypes.

2. MATERIALS AND METHODS

2.1 Site and Treatments

A screen-house was conducted at the National Crops Resources Research Institute (NaCRRI) in Uganda, during 2011-2012. Soil used in this study was an Oxisol collected in bulk (approx. 50 kg) from a continuously cultivated field at NaCRRI. It was air-dried on polythene sheets, manually homogenised and screened through a 2 mm sieve. Subsequently, 5 kg were filled in 5-litre capacity plastic pots.

Treatments included Zn applied at rates of 0, 5, 7.5 and 12.5 mg pot^{-1}, as $ZnSO_4$. In addition, two bush bean genotypes renowned for tissue Zn accumulation were used in this study. These included Kabo6F2.8-27 and NUA 69, selected from a set of other Zn biofortifying genotypes under the custody the International Centre for Tropical Agriculture germplasm [11]. KaBo6F2.8-27 and NUA 69 have capacities to accumulate Zn up to 43 and 40 mg kg^{-1}, respectively. Both NUA 69 and Kabo6F2.8-27 genotypes belong to the Andean genepool. They were generated from a series of crosses between CAL96 and mineral dense (Fe and Zn) parents, largely by collaborative work of the International Centre for Tropical Agriculture based at Kabete in Kenya [12]; Dr. Clare Mukankusi, 2015, Regional Breeder, East and Central African Bean Network, Kampala Uganda, personal communication).

2.2 Experimental Design and Management

Treatments were laid out in a completely randomised design and replicated three times. This setup was repeated three times. For each cycle, three bean seeds were planted per pot at about 3 cm depth. Two weeks after emergence, the seedlings were thinned to two plants per pot. At planting, the soil in the pots was watered with distilled water to field capacity (20%) determined. The pots were then weighed to obtain the reference weights for subsequent watering. At two-day intervals, each pot was reweighed before watering to restore the original weight to maintain field capacity. In order to prevent loss of nutrients through leaching, a plastic cover was placed beneath each pot, and any leachate trapped was returned into the respective pot.

At three weeks after planting, a set of trifoliate from the tip of one plant per pot was collected manually and packed in a paper bag. The leaf samples were oven dried at 60°C for 48 hr, before being subjected to analysis for tissue Zn concentration [13]. Seeds from the remaining plant in each pot were harvested at physiological maturity and further oven-dried at 105°C for 48 hr before analysis for Zn [13].

2.3 Estimation of Dietary Requirements Women

Considering women who are most vulnerable to Zinc deficiency, the amount of bean grain and leaf required to meet their minimum threshold Zn requirement was computed using the following equation below:

$$Quantity\ required = \left[\left({}^{1}/_{Tissue\ Zn}\right) \times Recommended\ intake\right] \times 1000$$

Where:

Quantity required = quantity of Zn required for daily dietary consumption by a given individual; tissue Zn = the quantity of Zn in grain or leaf; recommended in take = the amount of Zn required by an individual to meet the daily dietary Zn requirement by an individual.

2.4 Statistical Data Analysis

All data collected were analysed using Analysis of Variance in GenStat statistical package Version 13 [14]. Fisher's protected Least Significant Difference (LSD) at 5% probability level was used to determine differences among significant treatment means. The standard errors (SE) were calculated using the following formula:

$$SE = \frac{\sqrt{2\ \mathrm{Residual\ SS}}}{\sqrt{\mathrm{r}}}$$

Where: Residual SS = variance for each treatment, and r = number of replications, which in this case was 3.

3. RESULTS

3.1 Leaf Zinc Content

There was a significant interaction ($P<0.05$) between Zn rates and bean genotypes for foliar Zn accumulation (Fig. 1). Genotype Kabo6F2.8-27 consistently out-performed NUA 69 by 19 and 15.2% in Zn leaf content, at the Zn application rates of 0 and 5 mg pot^{-1}, respectively. However, there was no marked difference between application rates 5 and 7.5 kg Zn pot^{-1} (15.2 and 15% leaf Zn, respectively). The performance of both genotypes in terms of leaf Zn diminished sharply from the application rate of 7.5 up to 12.5 mg pot^{-1} (Fig. 1), leading to appearance of foliar Zn toxicity symptoms. Despite the appearance of toxicity symptoms, Kabo6F2.8-27 still emerged superior to NUA69 with 32.7% Zn accumulation.

3.2 Seed Zinc Content

There was a significant effect ($P<0.05$) of Zn rate on biofortifier bush bean genotypes (Fig. 2).The response pattern was typically hyperbolic, with peaks occurring at a Zn application rate of 7.5 kg

Zn ha^{-1}. However, a sharp decline of seed content in both genotypes was observed from the Zn rate of 7.5 mg kg^{-1}. For all Zn rates, Kabo6F2.8-27 genotype maintained superiority over NUA69.

3.3 Quantity of Bean Grain and Leaves Required to Meet the Recommended 11 mg Zn for Pregnant Women

Taking the example of pregnant women and breast feeding mothers, who are considered vulnerable to Zn deficiency, the quantities of beans required to meet their daily dietary needs are estimated in Tables 1 and 2. It was evident that the lower the Zn content of the genotype, the more quantities of bean grain and leaves required by an individual. Thus, considering the rate of 7.5 mg Zn pot^{-1} which resulted in the highest Zn accumulation in leaves and seed, Kabo6F2.8-27 seeds would be required in the smallest quantities to meet the dietary needs. On the other hand, RWR2154 (37) which accumulated the lowest quantities of Zn in the tissues, would be required in the greatest amounts.

3.4 Quantity of Bean Grain and Leaves Required to Meet 12 mg of Zn for a Breast Feeding Mother

Data for the estimated quantity of Zn in the leaves and seed of the bean genotypes equivalent to the recommended daily dietary needs for a breast feeding mother are presented in Table 2. Again, the pattern of the quantity of bean grain and leaves equivalent to the required daily dietary needs for this vulnerable group was similar to that observed for breast feeding mothers described above. Thus, considering the rate of 7.5 mg Zn pot^{-1} which resulted in the highest Zn accumulation in leaves and grain, Kabo6F2.8-27 grain would be required in the smallest quantities to meet the dietary needs. On the other hand, RWR2154 (37) which accumulated the lowest quantities of Zn in the tissues, would be required in the greatest amounts.

4. DISCUSSION

4.1 Leaf Zinc Content

The marked rise in leaf Zn concentration resulting from application of Zn (Fig. 1), irrespective of bean genotypes, implies that the physiological sinks of the genotype had not yet been saturated to capacity, by the inherent soil supply sources. Previous studies on these materials [14] indicated that Kabo6F2.8-27 genotype had average accumulation of 43 mg kg^{-1}, which was close 44.3 mg kg^{-1} achieved at 7.5 kg Zn pot^{-1}. On the other hand, NUA69 presented maximum Zn uptake of 37.6 mg kg^{-1} also at 7.5 kg Zn pot^{-1} application rate; which was just less than the value of 40 mg Zn kg^{-1} previously reported by [14]. It is, therefore, clear that to achieve maximum Zn biofortification of the study materials, additional Zn must be applied to this Oxisol. Similar observations were reported by [16,17] This may arguably attract extra NARL/8costs for procuring Zn fertilizers; however, use of minimal amounts of Zn fertilizers innovatively packaged to suit the farmers' circumstances, may be a better alternative to procuring Zn drugs which normally attract attention after the human health has already been burdened morbidity. This is supported by the conclusion that investment in breeding for biofortified Zn can save up to US 20 per year in Africa [18]. This is critical in the SSA where up to 40% of population mostly ranking below the abject poverty line.

Consumption of bean leaves is spread within Sub-Saharan Africa; however, the relative quantities, consumed compared to the grain, are still low though contestable. It is imperative that research is undertaken to generate technologies that will balance consumption of bean leaves to avoid disadvantaging bean grains production. Indeed, since the present study was based under screen-house conditions, efforts should be made to include farmer conditions to permit possible socio-economic feasibility integration. The other pending issue is the consideration of the bioavailability of leaf Zn, since there have been observations that although the plants are able to accumulate this micronutrient, its bioavailability as a supplement is still questionable due to coexistence of phytate in plants, which is an anti nutritional factor, that binds micronutrients [19].

4.2 Seed Zinc Content

The significant effect ($P<0.05$) of Zn rate on biofortified Zn among bush bean genotypes (Fig. 2) poses a challenge of sourcing Zn fertilizers among the otherwise resource poor farmers in SSA. Fortunately, in the case of Africa, some national governments are in the process of prioritising use of fertilizers to bolster land productivity through implementation of fertilizer policies and strategies [20]. Information emerging from this study is, therefore,

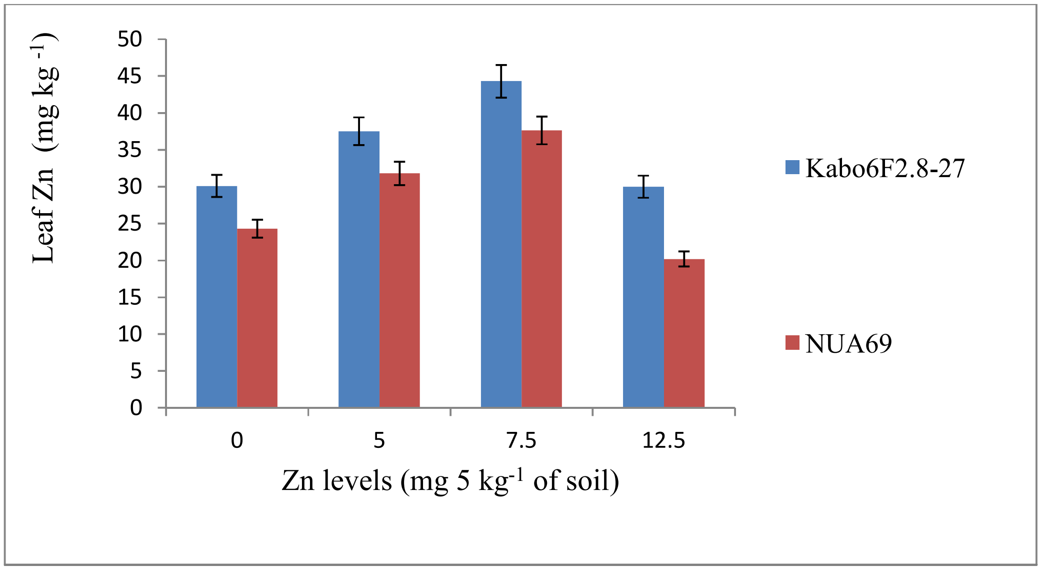

Fig. 1. Influence of Zn treatment on leaf Zn content of Zn biofortifier bush bean genotypes

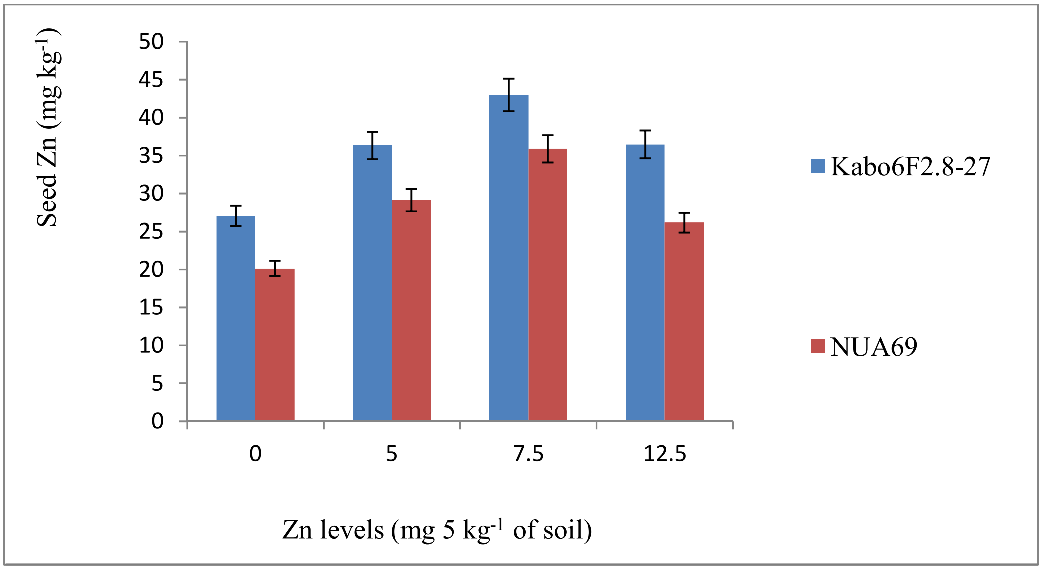

Fig. 2. Influence of applied Zn on Seed Zn content in biofortifier bush bean genotypes

of fundamental value to input into the relevant national fertilizer strategies. The reduction in seed Zn after the application rate of 7.5 mg Zn pot^{-1} (Fig. 1) is indeed important to note under the circumstances of this study. This could imply that Zn phytotoxicity was beginning to take a toll on Zn uptake and redistribution within the plant sinks, and subsequently disrupt some physiological processes in the plant. This inference is not conclusive since this issue was beyond the scope of this research.

Nevertheless, [21] noted that Zn phytotoxicity causes condensation of chromatin material resulting into disintegration of cell organelles and subsequently reduces rate of protein synthesis. [22] also attested to the fact that Zn when present in excess levels in the soil, may become phytotoxic to protein synthesis through partly binding the sulfhydryl group of protein causing deleterious effects in normal protein formation. Similarly, [23] revealed that that Zn toxicity inhibits ATP synthesis, and therefore, impairs generation of energy needed for metabolism including syntheses of proteins. Thus, it is important that Zn management is done carefully to avoid such occurrences that can otherwise diminish the nutritional value of staple crops such as beans.

4.3 Leaf versus Seed Zinc Content

Generally, leaves accumulated more Zn than seeds in the study genotypes (Figs. 1 and 2). The high accumulation of Zn in the leaves is a normal phenomenon since the contents of the seeds highly depend on the richness of the same resources in the vegetative parts of the plant. On the other hand, the low accumulation of Zn in the seed could have been due to poor translocation of Zn from the vegetative parts into the seed during the reproductive phase of the plants, as observed by [24,25]. In this respect, [25] estimated that up to 63% of Zn is retained in roots, during the advanced stages of plant growth. The consistent superiority of Kabo6F2.8-27 genotype in accumulation of Zn in both leaves and seeds, implies that this should be the genotype of choice for multiplication and distribution to farming communities, who are highly dependent on beans as a major dietary component. Despite the clear evidence emerging from this study that leaves would be superior sources of Zn to the grain in beans, bean leaves seem to be popular among the dishes that constitute diets of the less privileged communities in SSA. It is, therefore, imperative that protracted efforts are made to rollout campaigns for popularisation of consumption of the leaves. Different options could be sought for creating diversity of products from the leaves through value addition processes to enhance their readily acceptance.

4.4 Bean Grain and Leaves Equivalent to Daily Dietary Zn

Taking the example of pregnant women and breast feeding mothers, who are considered vulnerable to Zn deficiency, the quantities of beans required to meet their daily dietary needs are estimated in Tables 1 and 2. It was evident that the lower the Zn content of the genotype, the more quantities of bean grain and leaves required by an individual. Thus, considering the rate of 7.5 mg Zn pot^{-1} which resulted in the highest Zn accumulation in leaves and seed, Kabo6F2.8-27 seeds would be required in the smallest quantities to meet the dietary needs.

Data for the estimated quantity of Zn in the leaves and seeds of the bean genotypes equivalent to the recommended daily dietary needs for a breast feeding mother are presented in Table 2. Again, the pattern of the quantity of bean grain and leaves equivalent to the required daily dietary needs for this vulnerable group was similar to that observed for breast feeding mothers described above. Thus, considering the rate of 7.5 mg Zn pot^{-1} which resulted in the highest Zn accumulation in leaves and grain, Kabo6F2.8-27 grain would be required in the smallest quantities to meet the dietary needs.

Table 1. Estimated quantity of dry bean grain (g) and leaves (g) of zinc biofortifier genotypes required to supply the recommended daily dietary Zn (†11 mg) for a pregnant woman

Bean genotype	‡Seed biofortified Zn (mg kg^{-1})	Dietary grain equivalent (g)	‡Leaf biofortified Zn (mg kg^{-1})	Dietary leaf equivalent (g)
Kabo6F2.8-27	43.0	256	44.3	248
NUA69	35.9	306	37.6	292

†Average recommended Zn daily intake per pregnant woman per day [15]; ‡the data represent parameters of the genotypes grown with applied Zn at 7.5 mg per pot, which resulted in the highest Zn biofortification

Table 2. Estimated quantity of dry bean grain (g) and leaves (g) of zinc biofortifier genotypes required to supply the recommended daily dietary Zn (†12 mg) for a breast feeding mother

Bean genotype	‡Seed biofortified Zn (mg kg^{-1})	Dietary grain eqv. (g)	‡Leaf biofortified Zn (mg kg^{-1})	Dietary leaf eqv. (g)
Kabo6F2.8-27	43.0	27.9	44.3	271
NUA69	35.9	33.4	37.6	319

†Average recommended Zn daily intake per breast feeding mother per day [15]; ‡The data represent parameters of the genotypes grown with applied Zn at 7.5 mg per pot, which resulted in the highest Zn biofortification

5. CONCLUSION

The capacity of existing Zn biofortifier genotypes within SSA can be improved by supplementing the natural soil supply with externally sourced fertiliser Zn. Of the materials used in this study, KaboF2.8-27 emerged superior to NUA69 in terms of foliar and seed Zn accumulation, at application rates of 7.5 mg per pot. Overall, foliar Zn was greater that seed Zn for both genotypes. Application rates of beyond 7.5 mg Zn per pot ushered in phytotoxicity symptoms. Considering the daily dietary needs of pregnant mothers of 11 mg of Zn, a mother would have to consume up to 256 g of bean grain or 248 of leaves in order to satisfy her nutritional Zn needs. On the other hand, for a breast feeding mother, the consumption would be 27.9 g of grain and 27.1 g of leaves. However, noting that bean leaves are less popularly consumed in SSA than the grain, it is imperative that a proactive campaign is rolled out targeted at creating awareness of the value of bean leaves in sourcing nutrition of Zn in the region.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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