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Importance of common bean genetic zinc biofortification in alleviating human zinc deficiency in sub-Saharan Africa

Mashamba Philipo¹*, Patrick Alois Ndakidemi¹ and Ernest Rashid Mbega¹

Abstract: Zinc deficiency is among the leading risks to human health in sub-Saharan Africa, its adverse exposure leads to diarrhea, pneumonia, and malaria. Furthermore, it is the leading cause of stunting in children and negatively influences the human immune system, body iron, and vitamin A and D. High zinc deficiency in sub-Saharan Africa is due to the consumption of staple foods with low zinc contents. Genetic zinc biofortification of common bean among staple food crops is the best approach for alleviating zinc deficiency, as it is cost-effective and can easily reach low-income households. Genetic zinc biofortification by conventional breeding coupled with marker-assisted selection is the best strategy for sub-Saharan Africa, as the selection of crosses is precise and takes short time to develop high zinc-containing varieties. Zinc content increase in common bean seeds has a high impact on alleviating zinc deficiency as it is consumed whole compared to cereal grains which undergo milling, the process that removes zinc-rich parts before being consumed. This review explains the current status of zinc deficiency in sub-Saharan Africa, conventional methods for alleviating the problem, current and potential of modern genetic approaches for zinc biofortification of common bean in alleviating zinc deficiency in the region.

Subjects: Agriculture & Environmental Sciences; Agriculture; Agriculture and Food

ABOUT THE AUTHOR

Our research group deals with research on Sustainable Agriculture (plant breeding, pathology, biotechnology and soil fertility management). The first author is an Assistant Lecturer and Plant Breeder at the Nelson Mandela African Institution of Science and Technology (NM-AIST), Tanzania. This study is part of his Ph.D. thesis “Genetic Iron and Zinc Biofortification of Yellow Common bean (Phaseolus vulgaris L.) Varieties in Tanzania”. The overall objective of this study was to evaluate seed iron and zinc concentration of the common bean genotypes and increase their contents into seeds of the widely consumed yellow common bean varieties for improved nutrition security in Tanzania”. The second and third authors are supervisors (Professor and Senior Lecturer respectively) in the Department of Sustainable Agriculture, Biodiversity, and Ecosystem Management at the NM-AIST.

PUBLIC INTEREST STATEMENT

Genetic zinc biofortification of common bean is of much importance in sub-Saharan Africa, as zinc deficiency is still a public health problem regardless of some interventions going on to contain the problem. About 4–73% of the populations across different global regions are estimated to be affected by zinc deficiency, the effect is much more to the low-income households of which most reside in sub-Saharan Africa. Zinc deficiency is the leading cause of stunting in children under the age of 5 years and its effect leads to compromised human immune system, thus those with zinc deficiency are vulnerable to many diseases. This study aimed at understanding the current status of zinc deficiency in sub-Saharan Africa, intervention strategies, and potential of increasing seed zinc contents in common bean among other staple food crops, in reducing zinc deficiency in sub-Saharan Africa.
1. Introduction

Phaseolus vulgaris (common bean) is an important annual herbaceous grain legume mostly grown and consumed in sub-Saharan Africa (Philipo et al., 2020a). It belongs to fabaceae family, and grown mostly for its dry seeds (Mondo et al., 2019; Philipo et al., 2021). Common bean is an important source of nutritional zinc among other staple foods (Głowacka et al., 2015). It contains relatively high zinc concentration in dry seeds compared to most staple food crops (M. W. M. Blair et al., 2009). The crop is largely grown in eastern and Southern Africa (Petry et al., 2015). Even though there is high production and consumption of common beans in sub-Saharan Africa, most of the widely consumed varieties had low seed zinc contents compared to locally adapted varieties (Blair, 2013; Philipo et al., 2020b). Zinc is an important micronutrient for most living organisms, including human beings and plants (Sharma et al., 2013).

Zinc is essential for plants, as it is a constituent of enzymes involved in carbohydrate, proteins and lipid metabolism, the synthesis of auxin, formation of pollen and management of genes involved in environmental stress tolerance (Chattha et al., 2017; Sharma et al., 2013). Soil zinc deficiency causes plants spikelet sterility, chlorosis, reduced growth and tolerance to environmental stress (Broadley et al., 2007; Xue et al., 2016). Deficiency of zinc in soils also results in low nutritional quality edible parts in terms of zinc in edible parts, thus causing malnutrition in the human population (Bailey et al., 2015; Mulualem, 2015).

In humans, zinc makes up less than 0.005% of total body weight, and found in each and every type of cells (Bagherani & Smoller, 2016). It plays an important role in proper functioning of body defensive system, cell division and growth, brain function, wound healing, carbohydrate metabolism, reproduction and smell and taste senses(Ahmad et al., 2015; Liu et al., 2017). Zinc deficiency leads to reduced body immune response, slow wound healing, infertility and reduce growth and development (Bagherani & Smoller, 2016; Plum et al., 2010). Human body experiences zinc deficiency when food intake or supplements cannot meet body zinc demand, due to poor absorption, increased loss and high body system utilization (Lokuruka, 2012). Worldwide zinc deficiency affects 20% of the world’s population, with more effect to the resource poor population residing in developing countries (Darnton-Hill et al., 2005; Stein et al., 2007).

Over the decades, attempts to reduce zinc deficiency have been dominated mostly by supplementation and chemical fortification of staple foods (Goudia & Hash, 2015). Recently biofortification has been advocated as a compliment to supplementation and chemical fortification (Stein et al., 2007). Biofortification is the process of enriching staple food crops with vitamins and minerals to the edible parts, through plant breeding or agronomic practices, so that when consumed significantly improve nutritional status of the target population (Welch & Graham, 2004). Compared to supplementation and chemical food fortification, biofortification is a cost-effective intervention and has been conducted in several food crops. In grain legumes, zinc biofortification has been practiced in a number of crops including soybean, common bean, peas, cowpeas, chickpeas, and lentils (Jha & Warkentin, 2020; Kumar & Pandey, 2020). The impact of pharmaceutical supplementation and chemical food fortification have not yet reached many poor resource populations residing in rural areas and few cases in urban particularly in sub-Saharan Africa (García-Casal et al., 2017). In most cases the populations residing in rural areas of sub-Saharan Africa do not eat or eat less processed food, due to poor market and infrastructure, thus they often prepare their staple food by milling in locally available millers (Ferrão et al., 2017).

This review will discuss, the current zinc deficiency status in sub-Saharan Africa and cost-effective methods particularly the potential of genetic common bean zinc biofortification in reducing zinc deficiency among sub-Saharan African populations.
2. Zinc deficiency status in sub-Saharan Africa

Malnutrition due to micronutrient deficiencies is a global public health problem despite several ongoing interventions to combat the problem. Compared to other global regions, sub-Saharan Africa as home for most resource poor population is more affected by micronutrient deficiencies (Fanzo, 2012). Zinc deficiency is among major risks to human health, whose measured adverse outcome of exposure include: diarrhea, pneumonia and malaria (WHO, 2009). Zinc deficiency ranks number three, after iron and vitamin A among the micronutrient deficiencies (WHO, 2013). Globally zinc deficiency effects has an estimate range of 4–73% across subregions (WHO, 2002). In sub-Saharan Africa, zinc deficiency accounts for 18–22% attributable fractions for lower respiratory tract infections, 11–13% attributable fractions for diarrheal diseases and 10–22% attributable fractions for malaria (WHO, 2002, 2013). Lower respiratory infections, diarrheal diseases and malaria are among the leading cause of disability-adjusted life-year (DALY) in sub-Saharan (Figure 1).

Zinc deficiency increases the risk of incidence for these infectious diseases as it impairs multiple aspects of immune function, including barrier and non-specific immunity, specific immune components (lymphocytes, monocytes and macrophages, neutrophils, natural killer cells), and mediators of immune function such as glucocorticoid and thymulin activity, and cytokine function (Bagherani & Smoller, 2016). Zinc deficiency negatively influences human body iron and vitamin status. It triggers synthesis of hepcidin molecule in the human gut, which decreases iron absorption (Kondaiah et al., 2019). Vitamin A metabolism in humans depends on zinc-containing enzymes (Rahman et al., 2002). In most cases zinc deficiency is associated with insufficient intake or absorption of zinc from the diet, however to some extent excess losses of zinc during diarrhea may also contribute (Plum et al., 2010). People with gastrointestinal, chronic liver and renal, sickle cell and diabetes diseases are at high risk of suffering from zinc deficiency, due to reduction in zinc absorption and increased endogenous zinc losses (Bailey et al., 2015; Kondaiah et al., 2019). Pregnant and lactating women are also at risk of being zinc deficiency, due to high need of the mineral for the growth and development of the fetal, on the other hand lactation reduces

![Figure 1. Percentage of DALYs (disability-adjusted life years lost) attributed to 20 leading cause, in sub-Saharan Africa region by 2016. Data sourced from (WHO, 2018b) report on Global health estimates.](https://doi.org/10.1080/23311932.2021.1907954)
Figure 2. Estimates of DALYs attributable to 20 major health risks in sub-Saharan Africa region. Data sourced from global health risks report (WHO, 2009).

Figure 3. Estimates of mortality attributable to 20 major health risks in sub-Saharan Africa region. Data sourced from global health risks report (WHO, 2009).
maternal zinc store (Rahman et al., 2002; Ryz et al., 2009). Due to high zinc demand for growth and development, children are at high risk of becoming zinc deficiency, particularly when they consume food with low zinc contents, as the mineral is used for cell growth and growth hormone metabolism (Nishi, 1996).

The global health risks on mortality and burden of disease attributable to selected major risks report for sub-Saharan Africa ranked zinc deficiency number 7 among the 20 leading risk factor causes of DALYs (Figure 2). DALYs are calculated as the sum of the years of life lost due to premature mortality in the population and the years lost due to disability for incident cases of the disease or injury. Among the leading risk factor, zinc deficiency accounts for 2.4% of all DALYs cases, which translates to 8.96 million in sub-Saharan Africa (WHO, 2009).

Zinc deficiency was ranked number 9 among the 20 leading risk factor causes of deaths in sub-Saharan Africa (Figure 3). It was reported that 2.2% of all deaths which translates to 249 thousands deaths in Sub-Saharan Africa were caused by zinc deficiency (WHO, 2009).
In sub-Saharan Africa, zinc deficiency is mainly caused by utilization of food with low nutritional zinc (Kondaiah et al., 2019; World Health Organization, 2018b, 2018b). Low household income, poor availability of high zinc-containing animal and fish-source foods, negatively influence the availability, and affordability of these foods in most of the populations in sub-Saharan Africa, making most of them consume cereals, legumes and roots and tubers, which are low in zinc bioavailability (Rahman et al., 2002; Ryz et al., 2009). Additionally, there is low consumption of fruits and vegetables, foods rich in vitamin C, proven to increase absorption of zinc in the human gut (WHO, 2009). Even though bioavailability of zinc from plant foods in human gut is low, being negatively influenced by inhibitors that include phytic acid, tannins, dietary fibre and calcium (Hess & King, 2009; Liu et al., 2017). Bioavailability of zinc from plant-based foods ranges from 5.5% to 56.5% (Hemalatha et al., 2007).

Despite the measures being taken to alleviate zinc deficiency prevalence in sub-Saharan Africa, its effect among the populations showed no significant decrease (Figure 4). Thus there is a need to apply supplementation, food chemical fortification and currently biofortification so that there is a complementation of one another as there is no single existing method that can alleviate micronutrient deficiency in sub-Saharan Africa (Bouis & Saltzman, 2017).

3. Preventive interventions to reduce zinc prevalence in sub-Saharan Africa
There are several strategies that are used to reduce and control the effect of zinc deficiency in sub-Saharan Africa, these include supplementation, and food chemical fortification and recently biofortification (Goudia & Hash, 2015; Hemalatha et al., 2007; Vinoth & Ravindhran, 2017).

4. Zinc supplementation
Supplementation implies giving of minerals and vitamins in the form of low-cost pills, powder or syrups to the population groups exposed to micronutrients deficiencies. There are a number of zinc supplements present and used to improve human health status, these include zinc acetate, zinc gluconate, zinc picolinate, and zinc sulfate (Mayo-Wilson et al., 2014). Sufficient zinc intake is of much importance particularly to children and pregnant women, in most cases, the recommended dietary allowance (RDA) for zinc (NIH, 2019) as presented in (Table 1), should be achieved through food intake, when not met, zinc supplementation is used as an alternative.

In clinical management of diarrhea, particularly in developing countries like those found in sub-Saharan Africa, WHO recommends that children older than six months should be supplemented with zinc at a dose of 20 mg/day while infants under age of six months should be given zinc at a dose of 10 mg/day for 10 to 14 days (WHO, 2005). Zinc supplementation in a dose of 10 mg/day provided for 168 days has a significant increase in growth of children under age of 5 years (Imad A, 2011). According to American Society for Clinical Nutrition a zinc supplement at the dose of 400 μg/kg/day is recommended for the premature newborn (Bagherani & Smoller, 2016). Though zinc supplementation has been administered to children and other people in need for some decades, zinc deficiency is still a public health problem. Its coverage is influenced by health infrastructures, of which in most cases these are poor in developing world, the intervention needs always trained personnel and training programs for the populations, thus making it not cost-effective and difficult to reach poor resource population residing in rural areas (Mayo-Wilson et al., 2014; Stein et al., 2007). There is a need of adopting other interventions like development of staple food varieties rich in zinc contents, as it is cost-effective and sustainable solution to zinc deficiency.

5. Zinc fortification
Zinc fortification is the technique of adding zinc to food so as to improve zinc nutritional quality of the food for improvement of public health (Imad A, 2011; WHO, 2005). Chemical fortification of food can be done as mass fortification, where widely consumed foods are fortified; targeted fortification, where foods processed for certain population category, for instance, complementary foods for children and populations with HIV, diabetics etc.; market-driven fortification, this involves
food processors fortifying foods available in the market (Allen et al., 2006). The most commonly used fortificants of zinc food fortification are zinc oxide and zinc sulfate with zinc oxide being more preferred, as it is the most cheapest fortificants compared to others (Jha & Warkentin, 2020; WHO, 2005). In sub-Saharan Africa, zinc fortification is mostly applied to maize and wheat flour, whereas until 2017 a total of ten countries that include; Burundi, Kenya, Malawi, Mozambique, Nigeria, Rwanda, South Africa, Tanzania, Uganda, and Zimbabwe had mandatory zinc fortification of maize. While fourteen countries, which include: Burundi, Cameroon, Djibouti, Ghana, Kenya, Liberia,
Malawi, Mozambique, Nigeria, South Africa, Tanzania, Togo, Uganda, and Zimbabwe had mandatory zinc fortification of wheat flour (Global Fortification Data Exchange, 2020a). The levels of zinc added to wheat and maize flour varied from one country to another, with range of 15–95 and 15–50 mg/kg respectively (Figure 5).

Despite the facts that zinc fortification results into high and faster food zinc content increment to the satisfactory level, the intervention has not been successful in sub-Saharan Africa as it was expected. The technique requires infrastructure to develop fortificant, ability of consumers to buy or access to markets, and most grains are milled by small-scale millers in both urban and the villages (Ferrão et al., 2017). For instance, the proportion of industrially processed maize, which is a staple food in the region, is very low (Figure 6). In Tanzania only 2.5% and 33.1% of the population consume fortified maize and wheat flour respectively, whereas in Uganda 6.5% and 8.5% of the population consume fortified maize and wheat flour respectively (Global Fortification Data Exchange, 2020b). Based on the fact that, zinc deficiency prevalence is still high in the region, there is a need to include other sustainable, friendly and affordable interventions, like biofortification which can reach easily resource-poor populations for alleviation of this public health problem.

6. Zinc biofortification
The process of increasing zinc concentration to plant-edible parts can be done through agronomic practices and plant breeding (Jha & Warkentin, 2020; Menguer, 2014)

7. Agronomic practices
Agronomic biofortification is the practice of enriching mineral contents of the edible part of plants via soil, foliar fertilizers and inoculation with soil beneficial microorganisms (Global Fortification Data Exchange, 2020a; Mayo-Wilson et al., 2014). In most cases zinc mineral fertilizer is applied as zinc chelates (contain approximately 14% zinc), zinc sulphate (25–36% zinc) and zinc oxide (70–80% Zinc), where zinc sulphate is the widely used zinc mineral fertilizer (Chattha et al., 2017; Global Fortification Data Exchange, 2020b; Menguer, 2014).

Zinc mineral fertilizers are applied to soils when there is poor phytoavailability of zinc mineral (Ramzan et al., 2020). Application of zinc mineral fertilizer increases its availability, uptake by plants and contents in plant-edible parts (Aciksoz et al., 2011). A number of studies revealed increase in plants zinc content after zinc soil fertilization. An increase of up to 75.2% in wheat grain zinc content was reported after zinc soil fertilization in China (Wang et al., 2016). Rice grain zinc increase of up to 92.6% was reported in India, after basal soil zinc sulphate application at maximum tillering and flowering stage (Saha et al., 2017). In common bean 100% increase in seed zinc content was reported in Brazil when zinc sulphate was applied as a soil fertilizer (Cambraia et al., 2019). Application of zinc sulphate as foliar fertilizer increased wheat grain zinc content by 47.8–83.0% whereas an increase of upto 27% in rice grain zinc content was reported as a result of zinc sulphate foliar fertilizer application (Chattha et al., 2017; Saha et al., 2017). A non-significant to significant increase of up to 14.7% in grain zinc concentration was reported in common beans as a result of foliar zinc sulphate fertilizer application (Cambraia et al., 2019; Wang et al., 2016). Zinc increment in grains among other factors influenced by the variety type of the crop used and zinc soil status (Aciksoz et al., 2011; Saha et al., 2017). Zinc fertilization in crops apart from increasing grain zinc contents it reduces phytic acid an anti nutritional factor that negatively influences absorption of monovalent and divalent positively charged ions, thus increase bioavailability of zinc in human gut (Hoppler et al., 2014). (Aciksoz et al., 2011; Chattha et al., 2017) reported a decrease of about 30% in phytic acid contents in rice and wheat grains after application of zinc sulphate fertilizer. Some more examples are given in Table 2.

Although mineral zinc fertilization has quick advantage on increasing zinc content in edible parts and cause reduction in phytic acid, it was also reported to reduce grain iron content, thus leads to insufficiency grain iron contents (Saha et al., 2017). Zinc fertilization had challenges to resource poor farmers of sub-Saharan Africa as many of them cannot afford buying zinc fertilizers every
### Table 2. Some examples of food crops in which zinc grain have been increased through biofortification

| Crop      | Agronomic Biofortification | Genetic Biofortification | Genetic engineering                                                                 | References                                |
|-----------|---------------------------|--------------------------|--------------------------------------------------------------------------------------|-------------------------------------------|
| Cereals   |                           |                          |                                                                                      |                                            |
| Rice      | Application of Zn-fertilizer (ZnSO₄) Increased Zn grain content by 27% | Increased grain zinc contents by 66% | Over expression of NAS Increased grain zinc concentration by 3-folds | (Barrill et al., 2014a; HarvestPlus, 2014) |
| Maize     | Application of Zn-fertilizer (ZnSO₄) Increased Zn grain content by 9% | Increase of up to 52.0% in grain zinc content was reported | Over expression of ZmZIP5 led to increased Zn contents in vegetative tissues, but not in mature seeds | (Cakmak & Kutman, 2018; Li et al., 2019) |
| Wheat     | Application of Zn-fertilizer (ZnSO₄) Increased Zn grain content by 83% | Increased grain zinc contents by 66% | Over expression of TaYSL3-2A, TaYSL12-2A, TaYSL6, 9 are reported to increase zinc concentration in wheat | (HarvestPlus, 2014; Rashid et al., 2019; Kamaral et al., 2020) |
| Legumes   |                           |                          |                                                                                      |                                            |
| Common bean | Application of Zn-fertilizer (ZnSO₄) Increased Zn grain content by 35% | Increase of up to 89.0% in seed zinc content was reported | Limited information                                                                 | (Zemolin et al., 2016)                      |
| Soybean   | Application of Zn-fertilizer (ZnSO₄) Increased Zn grain content by 105% | Increase of up to 52.6% in seed zinc content was reported | Limited information                                                                 | (Ramamurthy et al., 2014; Oliveira et al., 2019) |
| Cowpea    | Application of Zn-fertilizer (ZnSO₄) Increased Zn grain content by 27% | Increase of up to 50.0% in seed zinc content was reported | Limited information                                                                 | (Umar, 2014; Bett et al., 2017)            |

season for increased grain zinc contents from their harvests. There is a need of adopting another cost-effective methods like genetic biofortification, which will easily reach resource-poor farmers through planting and consuming varieties that will have increased grain zinc contents as they grow.

### 8. Genetic zinc biofortification

Genetic zinc biofortification refers to the development of crop varieties which accumulate high zinc contents to their edible parts as they grow and has increased bioavailability of the mineral to consumers (Goudia & Hash, 2015; Ram et al., 2016). Zinc biofortification started with rice in 1995 in Asia (Gregorio et al., 2000) whereas in common bean it was reported in 1999 in South America (Welch & Graham, 2004). Compared to other interventions which require continual financial expenditure, genetic zinc biofortification is cost-effective and can easily reach rural and resource poor populations (Slamet-Loedin et al., 2015). Furthermore, farmers can plant and re-plant zinc biofortified varieties at zero cost and consume them for alleviating zinc deficiency among human populations (Swamy et al., 2016). The developed varieties can be evaluated for adaptation, stability and genotype by environment interaction into many other environments, thus expanding the benefits of initial investment (Philipo et al., 2020a; Ritchie, 2017).

Staple food crops, which include maize, rice, sorghum and legumes, have been the main focus for zinc biofortification in sub-Saharan Africa (Nestel et al., 2006). In contrast to other staple food crops, common bean has relatively higher seed zinc content, thus a good crop for genetic biofortification (Blair, 2013). In cereals including maize zinc is more localized in embryo and aleurone layer, whereas in common bean the mineral is highly concentrated in endosperm (Figure 7). In most cases cereal grains are consumed after milling, the process which removes
zinc highly concentrated parts (embryo and aleurone) leaving endosperm which has very low zinc concentration (Cakmak & Kutman, 2018). On the other hand, common bean grains are consumed as whole making the crop a good source of plant-based zinc and thus good for genetic biofortification (Jha & Warkentin, 2020).

In sub-Saharan Africa, common bean production ranks number one among grain legume crops cultivated (FAOSTAT, 2015), the production trend of the crop has been increasing with a sharp slope particularly in eastern Africa (Figure 8), showing the potential of the crop in genetic biofortification for the sub-Saharan African population.

Common bean is the most consumed grain legume in sub-Saharan Africa, with its consumption per capita per year increase year after year (Figure 9), therefore genetic zinc biofortification of...
common bean will be of high impact in reduction of zinc deficiency among population residing in the region.

Globally the target for genetic zinc biofortification in common bean has been to develop cultivars with 40% more seed zinc contents without compromising farmers and consumers preferred agronomic properties (Blair, 2013). In most cases common bean genetic zinc biofortification in sub-Saharan Africa has been treated as secondary mineral after iron regardless of the potential of zinc to human health (Ritchie, 2017; Yu et al., 2019). In this region, common bean zinc biofortification have been implemented in Democratic Republic of Congo (DRC), Ethiopia, Rwanda, Sudan and Uganda (FAOSTAT, 2018; Petry et al., 2015). The programme resulted into 50% increase in seed zinc contents, though the primary focus was breeding for high seed iron content (Ugen et al., 2009). Nine (6-bush and 3-climber type) high zinc-containing bean varieties have been released in these countries (FAOSTAT, 2018; Yu et al., 2019). Zinc biofortified common bean varieties have been reported to retain zinc concentration up to 99.4% after undergoing preparations for home recipes (Hummel et al., 2020). Thus there is a need of practicing genetic zinc biofortification by treating zinc as a primary mineral and not only focusing on collecting and assessing high iron-containing genotypes for zinc contents (Blair, 2013; FAOSTAT, 2018). Several studies have revealed that there is no significance correlation between iron and zinc mineral in grains (Liu et al., 2017; Philipo et al., 2020a; Ugen et al., 2009). Even though there are some zinc biofortification programmes going on in sub-Saharan Africa, there is limited information on the effect of zinc biofortified varieties on nutritional zinc status of the target populations. Thus there is a need for conducting genetic zinc biofortification in common bean in many countries that grow the crop, for domestic and export so that benefits of high zinc-containing bean varieties can reach many populations of sub-Saharan Africa the benefit of reducing nutritional zinc deficiency. Common bean genetic zinc biofortification can be done through several methods which include, conventional breeding, marker-assisted breeding and genetic engineering.
Table 3. Some of the identified QTLs and DNA markers associated with high seed zinc contents in some legumes

| Crop        | QTL associated with high seed zinc content | Marker (SSR/SNP) linked with the QTL | Source genotype | References                          |
|-------------|------------------------------------------|--------------------------------------|-----------------|-------------------------------------|
| Common bean | QznDaAA6.2                                | V1001B                               | G4825           | (Matthew W Blair et al., 2010)       |
|             | QznDaAA8.1                                | H1201A                               | G14519          |                                     |
|             | QznPaAA6.1                                | BM158                                | G14519          |                                     |
|             | QznPaAA8.2                                | H1201A                               | G4825           |                                     |
|             | QznPoAA2.1                                | PV15                                 | G4825           |                                     |
|             | QznPoAA3.1                                | BMd1                                 | G4825           |                                     |
|             | QznPoAA6.1                                | BM158                                | G14519          |                                     |
|             | Qzn_contDaAA1.1                           | W0901B                               | G14519          |                                     |
|             | Zn-AA52c                                  | PV11                                 | G21242          | (Matthew W Blair et al., 2011)       |
|             | Zn-AA57c                                  | BM239                                | G21242          |                                     |
|             | Zn-AA58c                                  | BM165                                | G21242          |                                     |
|             | Zn-ICPa3                                  | I161G                                | G19833          | (M. W. M. W. Blair et al., 2009)     |
|             | Zn-ICPa7                                  | M125D                                | G19833          |                                     |
|             | Zn-ICPa11                                 | BMd33                                | G19833          |                                     |
|             | Zn-IC Pa3                                 | L064D                                | G19833          |                                     |
|             | Zn-ICPb9                                  | AK067G                               | G19833          |                                     |
|             | Zn-ICPb11.1                               | BMd27                                | G19833          |                                     |
|             | Zn-ICPb11.2                               | K126G                                | G19833          |                                     |
|             | Zn-AA5b3                                  | F702G                                | G19833          |                                     |
|             | Zn-AA5b6.1                                | DA39                                 | DOR364          |                                     |
|             | Zn-AA5b6.2                                | AK061D                               | DOR364          |                                     |
|             | Zn-AA5b11.1                               | Bng91                                | G19833          |                                     |
|             | Zn-AA5b11.2                               | BMd27                                | G19833          |                                     |
|             | Zn-AA5b11.3                               | K126G                                | G19833          |                                     |
|             | Zn_cont2.1                                | PV109                                | Cerinza         | (Matthew W. Blair & Izquierdo, 2012) |
|             | Zn_cont5.1                                | BM155                                | Cerinza         |                                     |
|             | Zn_cont5.2                                | BMd28                                | Cerinza         |                                     |
|             | Zn_cont7.1                                | PV35                                 | G10022          |                                     |
| Chickpea    | CaqZn2.1                                  | SNP110                               | ICC 8261        | (Das et al., 2015)                  |
|             | CaqZn3.1                                  | SNP208                               | ICC 8261        |                                     |
|             | CaqFZ4.1                                  | SNP300                               | ICC 8261        |                                     |
|             | CaqFZ5.1                                  | SNP413                               | ICC 8261        |                                     |
|             | CaqFZ7.1                                  | SNP471 and SNP472                    | ICC 8261        |                                     |
| Pea         | Zn-Ps2.1                                  | TP31957                              | Aragorn         | (Ma et al., 2017)                   |
|             | Zn-Ps3.1                                  | TP2567                               | Kiflica         |                                     |
|             | Zn-Ps5.1                                  | TP61763                              | Kiflica         |                                     |
|             | Zn-Ps7.1                                  | TP44143                              | Kiflica         |                                     |
|             | Zn-Ps7.2                                  | TP60315                              | Kiflica         |                                     |

8.1. Conventional breeding
Conventional plant breeding is the process of generating cultivars with traits of interest through crossings of closely related individual plants followed by field or pot evaluation and empirical selection of the crosses (Hummel et al., 2020; De Valença et al., 2017). For genetic zinc improvement, cultivars with contrasting grain zinc contents are crossed, in order to transfer loci associated with high grain zinc
content from a high zinc-containing genotype (cultivated or wild related species) into a cultivar with low grain zinc content (M. W. Goudia & Hash, 2015; M. W. Blair et al., 2009). Conventional genetic zinc biofortification for crops is only possible when there is existence of genetic variation in zinc contents for the target crop gene pool (Acquaah, 2013; Blair, 2013). For easy adoption of the zinc biofortified varieties, one of the parents involved in developing crosses should have farmers and consumers preferred traits (Beintema et al., 2018).

Conventional breeding has been employed in developing most of the high zinc-containing bean varieties released to date in several sub-Saharan African countries (Mukamuhirwa et al., 2015; WHO, 2005). The technique has been successful in common bean due to a natural genetic variation in seed zinc content that ranges from 25 to 60 ppm (Blair, 2013). The release of high zinc-containing varieties developed via conventional breeding has taken quite a number of years due to large environment influence on the trait and recovering of the farmers and consumers preferred traits like seed color, size, growing type (bush or climber) and taste (Mukamuhirwa et al., 2015; Yu et al., 2019). Some more examples on zinc biofortification of the crops are presented in Table 2. Therefore there is a need for using advanced selection methods like molecular markers for accuracy, reduced time and number of field evaluation, thus shortening the period for release of high zinc-containing varieties for improved zinc nutritional status of bean consumers in sub-Saharan Africa.

8.2. Plant marker-assisted breeding

Molecular marker-assisted plant breeding is the process of developing plant varieties through the use of DNA marker(s) associated with traits of interest, together with linkage maps, genomics and bioinformatics (Jiang, 2013). The DNA markers associated with the trait(s) of interest are used for indirect selection of those trait(s) particularly quantitative traits e.g., pest resistance, drought and poor soil fertility tolerance, quality traits (micronutrients, aroma, taste), as these are difficult to select under conventional breeding (Bouis & Welch, 2010; Jiang, 2013). Selection of individual plants from a segregating and/or non-segregating population involves two main stages. First, design and validation of DNA markers associated with the trait(s) of interest in parents and second, use of the validated DNA markers to select individual plants from a target breeding population at early seedling stage, based on the presence of markers associated with trait(s) of interest (Diapari et al., 2015; Lim et al., 2014). The advantages of applying molecular markers-assisted breeding (MAB) over conventional breeding in improving plant traits, including grain zinc contents are, first decreased selection time, as plants with traits of interest are selected at early developmental stage and leaving away those without trait of interest (Jiang, 2013). Second, selection under MAB can be done in any environment, making easy for selection of traits with low heritability which require favorable conditions for selection e.g., drought tolerance, heat tolerance and disease resistance (Oblessuc et al., 2012). Third, traits controlled by recessive alleles can be easily selected by co-dominance DNA markers like SSR and SNP whereas in conventional breeding selection of these traits would require selfing of test crossing (Bernardo, 2008).

In plants, seed zinc content is controlled by many genes involved in uptake from soils, up the plant transport and distribution within the plant parts (Bernardo, 2008; Oblessuc et al., 2012). There several identified and validated DNA markers and quantitative trait loci (QTLs) that are associated with high seed zinc contents in common beans (Acquaah, 2013; Waters & Sankaran, 2011). Marker-assisted breeding for seed zinc improvement in common bean have been applied in South America, where several DNA markers and QTLs linked to high seed zinc contents were identified followed by selection of individual plants from breeding segregating populations (Hemalatha et al., 2007; Borrill et al., 2014a; Matthew Wohlgemuth Matthew Wohlgemuth Blair et al., 2016). In most cases, studies on common bean seed zinc content genetic bases have relied on linkage and quantitative trait locus (QTL) analysis using biparental populations, which has shallow resolution due to little number of recombination events and thus results into genetic markers which are cross specific and show only fractions of genetic variability underlying the common bean high seed zinc content (Hemalatha et al., 2007; M. W. M. W. Blair et al., 2009). Some of the QTLs associated with grain zinc contents and their linked markers found in some grain legumes are presented in Table 3. Due to advancement in plant molecular studies, it is of most important to apply other molecular techniques like Genome Wide Association Study (GWAS) in studying the genetic
differences underlying seed zinc contents in common bean. In GWAS, diverse germplasm of a crop is used to scan the whole genome and thus gives a clear picture of the candidate genes responsible for expression of the trait of interest (Cichy et al., 2009; White et al., 2009). In sub-Saharan Africa, there is limited information on application of marker-assisted breeding in improving seed zinc content in common bean, thus adoption of the technique is of much importance for reduced time in development of zinc biofortified varieties.

8.3. Genetic engineering

Plant genetic engineering is the practice of manipulating genetic makeup of the plant through genome editing and/or transfer of gene(s) from a closely related or distant organism aimed at developing superior plant varieties with traits of interest (Contreras-Soto et al., 2017; Upadhyaya et al., 2016). The advancement in DNA knowledge and biotechnology has enabled studies on plant genome, identification and validation of several genes controlling plant agronomic and biochemical traits including grain micronutrient contents (Masuda et al., 2013; Zang et al., 2017). In the process of transferring genes coding for traits of interest, the identified and validated gene(s) are isolated from the source organism and transferred into tissues of the target plant via DNA microparticle bombardment or Agrobacterium tumefaciens mediated transfer (Dias & Ortiz, 2012; C. C. Zhang et al., 2016). Transferring genes into unrelated species (e.g., from bacteria to plants) is called transgenesis, while transferring from similar species or sexually compatible species (e.g., from wild to cultivated varieties) is called cisgenesis (P. Byrne, 2014; Upadhyaya et al., 2016). The plant developed from transgenesis is known as transgenic while that from cisgenesis is called a cisgenic (Acquaah, 2013). The benefits of employing plant genetic engineering over conventional breeding in improving plant traits, including grain zinc contents are, first it is the fastest method of developing varieties, though it needs high initial financial investment (Keshavareddy et al., 2018). Second, genes from a distant species can be isolated and used to improve another plant species, thus can be applied even when there is no genetic variation in the trait of interest (Connorton et al., 2017). Third, only the genes of interest are transferred to the plant to be modified whereas in conventional breeding there is transfer of even the unwanted genes during artificial hybridization (Borrill et al., 2014b). Fourth, the technique can be used to develop plant varieties with reduced uptake of unwanted metals from the soils (Slamet-Loedin et al., 2015).

Gene transfer technique has been used in developing several plant varieties with increased grain zinc contents (Bernardo, 2008; WHO, 2005). Over expression of nicotianamine synthase (NAS) encoding genes, resulted into increase in grain zinc content of transgenic rice by 2–3 folds (Borrill et al., 2014b), whereas over expression of a metal transporter (HvMTP1) encoding genes in barley led to 25 % increase barley cis-genic grain (Menguer et al., 2018). To date, there is limited information on seed zinc content increase by gene transfer techniques in common bean, though the already identified and validated transporters and genes involved in grain zinc accumulations in other crops can be used to develop transgenic or bean plants with increased seed zinc contents (Connorton et al., 2017; Oblessuc et al., 2012). The limited information is reported to be due to very long common bean genetic transformation protocol, poor reproducibility and in vitro regeneration, though the transcriptional networks involved in Zn uptake Phvul.011G035700/bZIP23-like factors and those involved in root to shoot transportation Phvul.003G086500/OPT3-like factors have been identified (Connorton et al., 2017; Menguer et al., 2018).

Recently genome editing has been advocated as a precision breeding technique and a compliment to conventional genetic engineering gene transfer as it does not necessarily involve transformation (Castro Guerrero et al., 2016; Sperotto & Ricchenevsky, 2017). Genome editing involves several molecular biological methods, which include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) (Vinoth & Ravindran, 2017), and recently clustered regularly interspaced short palindromic repeats (CRISPR)/Cas systems (Contreras-Soto et al., 2017; Mao et al., 2019). These methods use sequence-specific engineered nucleases, which when induced results into identification of specific DNA sequences and give rise to double-stranded breaks (DSBs) (Y. Y. Zhang et al., 2018). The endogenous repair systems of plants correct the DSBs either by no homologous end joining (NHEJ), which can lead to the insertion or deletion of nucleotides causing gene knockouts, or by homologous recombination (HR), which can result into gene replacements and insertions (Mao et al., 2019). The DSBs repair outcomes are
predictable and thus selection of mutations with benefits to plant breeding can be done (Veillet et al., 2020). In most cases, genome editing techniques have been used in improving plants abiotic tolerance and biotic resistance traits with very few studies focusing on food nutritional quality (Ding et al., 2018; Veillet et al., 2020). For example, target genome editing of OsERF922 gene in rice using CRISPR/Cas9 technique resulted into development of rice with enhanced blast resistance (Y. Y. Zhang et al., 2018), whereas drought tolerance wheat was developed by editing TaDREB2 and TaERF3 genes (Ansari et al., 2020). Likewise, CRISPR/Cas9 genome editing was used in editing of soybean E1 gene and developed early flowering mutants (Han et al., 2019). There is limited information in common bean genome editing particularly for grain zinc improvement, though there are several genes identified to control different traits (agronomic, biotic and abiotic stress response and grain quality) (Ansari et al., 2020; Sperotto & Ricachenovsky, 2017; Veillet et al., 2020). The genome editing experiences acquired from many successful researches in improving a number of traits of interest in plants can be used in editing the genome of common bean and many other crops for enhancing grain zinc content.

9. Conclusion
Zinc is essential for normal functioning of human immune system and growth during childhood. Deficiency of this mineral is mostly caused by consumption of food with low zinc contents. Zinc deficiency is among the leading micronutrient deficiency in sub-Saharan Africa. Though zinc supplementation and fortification, have been in place for decades now, zinc deficiency is still a public health problem in sub-Saharan Africa thus a need for cost-effective intervention to compliment the already existing methods for alleviating zinc deficiency in the region, as there is no single method that has proved to control zinc deficiency.

Genetic zinc biofortification particularly of staple food is the most current cost-effective intervention in controlling zinc deficiency in sub-Saharan Africa. Development of high zinc containing common bean varieties, which is the mostly cultivated and consumed grain legume in sub-Saharan Africa, is the best approach. Unlike cereals which need to milled before being consumed and thus ending up losing high zinc containing parts (embryo and aleurone layer), common bean grain is consumed whole, providing sufficient zinc to consumers. Among the current strategies of genetic zinc biofortification, marker-assisted breeding is the best, as high zinc containing varieties can be developed precisely and within a very short time compared to conventional breeding. Development of high zinc-containing plant varieties by genetic engineering is the precise method, this method lacks acceptability in most of the sub-Saharan African countries due to fear of the unknown and thus no zinc biofortified variety developed by this technique have been released in the region. High zinc-containing bean varieties can easily reach resource-poor farmers and consumers particularly in remote areas of sub-Saharan Africa compared to supplementation and fortification which need advanced infrastructures to operate.

Cover Image
Source: Author.

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Competing Interest
The author(s) declare that they have no known competing interests.

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