Received: 27 November 2018 | Revised: 16 April 2019 | Accepted: 22 April 2019

DOI: 10.1002/fes3.173

ORIGINAL RESEARCH

Food and Energy Security | WILEY

Breeding second-generation biofortified bean varieties for Africa

Paul M. Kimani | **Ahmed Warsame**

Department of Plant Science and Crop Protection, College of Agriculture and Veterinary Sciences, University of Nairobi, Nairobi, Kenya

Correspondence
Paul M. Kimani, Department of Plant Science and Crop Protection, College of Agriculture and Veterinary Sciences, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya.
Email: pmkimani@uonbi.ac.ke

Funding information
Association of Strengthening Agricultural Research in East and Central Africa, Grant/Award Number: ECABREN 2004; Bioinnovate Network (SIDA), Grant/Award Number: 2011-2014; Government of Kenya, Grant/Award Number: 2004-2018; Syngenta Foundation for Sustainable Agriculture

Abstract

Micronutrient malnutrition is one of the most serious health challenges facing vast sectors of Africa's population, particularly resource-poor women and children. Development and utilization of drought-tolerant, biofortified varieties is probably the most effective, sustainable, and potentially long-lasting strategy for reducing micronutrient deficiencies and coping with frequent droughts. Our objective was to develop second-generation biofortified bean (*Phaseolus vulgaris* L.) varieties combining drought tolerance, multiple disease resistance, and higher concentrations of iron and zinc in grain than the first-generation varieties currently grown by farmers in east, central, and west Africa. Forty-seven F_2 populations segregating for mineral density, resistance to biotic and abiotic stress factors, marketable grain types, and yield potential were developed at Kabete Field Station, and advanced to F_4 as population bulks. During the 2010 long rain season, 6,612 F_4 single plants were selected and used to establish $F_{4.5}$ progeny rows during the 2011 short rain season at Kabete. These progenies were evaluated for resistance to angular leaf spot, anthracnose, root rots, and agronomic traits. In 2012, 102 $F_{4.6}$ lines were evaluated under drought stress and no-stress conditions at Kabete and Thika. During the 2012 short rain season, selected disease and drought-tolerant $F_{4.7}$ lines were evaluated for mineral density and for their agronomic potential at four locations representing major bean production environments. Results showed significant ($p < 0.01$) variation for mineral density, drought tolerance, disease resistance, growth habit, grain type, and maturity among the populations and their progenies. Iron concentration varied from 30 to 130 ppm. Zinc concentration varied from 10 to 60 ppm. Superior lines were selected from BF01, BF07, BF16, and BF36 populations. Eighty-four lines had 50% more yield under stress and no-stress conditions compared with the parental lines, suggesting transgressive segregation. Results indicate that varieties combining high micronutrient density, resistance to diseases and drought, and marketable grain types can be developed from these populations.

KEYWORDS
bean, biofortification, disease resistance, drought tolerance

1 | INTRODUCTION

Although iron and zinc micronutrient malnutrition is a global problem, it is most severe in developing regions of the world because of widespread food insecurity, poverty, and consumption of energy-rich diets, which are poor in minerals and proteins (Blair, Gonzalez, Kimani, & Butare, 2010; Development Initiatives, 2017; Kimani, Beebe, & Blair, 2003). The problem is further aggravated by low crop productivity, which results in inadequate food intake in rural farming communities, and limited knowledge of the nutritional value of local foods. Welch and Graham (2004) estimated that more than three billion people in the world are afflicted by micronutrient malnutrition. In Africa, the prevalence of iron deficiency anemia (IDA) varies from 8% in Ethiopia to 67% in Tanzania, and 69% in Burundi (Kimani, Mamiro, Ugen, & Musoni, 2016). Micronutrient deficiency is often referred to as "hidden hunger" because the problem does not show any easily recognizable symptoms in the early stages until considerable and often irreversible damage has occurred.

Micronutrient deficiencies have increased in recent decades due to a decrease in the quality of poor people's diets, both in developing and developed countries, even in areas where access to food is not limited (Blair et al., 2009). They are more widespread than deficiencies caused by inadequate consumption of protein and calories. For example, in Kenya, micronutrient deficiencies occur even among population groups that have sufficient food in terms of meeting energy requirements. Children under five years are particularly affected by deficiencies in vitamin A (84% of children), iron (73.4%), and zinc (51%) (GOK, 2012). Women, especially pregnant women, are among the most vulnerable to a high risk of iron deficiency (60%) and vitamin A deficiency (39%). An estimated 16% of adult males suffer from iron deficiency anemia (IDA).

Several strategies have been used to fight micronutrient deficiencies in Africa (Kimani et al., 2011) and worldwide (Pfeiffer & McClafferty, 2007). While supplementation is effective and relatively low cost for easy-to-reach groups, it requires an elaborate and costly distribution network which leaves out the hard-to-reach vulnerable groups. Food supplementation is effective for affluent urban communities able to purchase fortified foods regularly. However, it leaves out the majority of rural and urban poor and has had only modest success in a few African countries with appropriate legislation and processing capacity. Development and utilization of biofortified cultivars is probably the most effective, sustainable, and potentially long-lasting strategy for reducing micronutrient deficiencies in Africa because it ensures wide availability, regular access, and is low cost. The only major cost is the initial investment in variety development and dissemination of mineral-rich varieties.

Biofortification aims to increase dietary availability, regular access, and consumption of mineral- and vitamin-rich foods in at-risk and micronutrient-deficient groups of populations through the production of mineral-rich varieties on-farm and across agricultural regions. A global biofortification program was initiated in July 2003 through the Harvestplus Challenge Program to provide better iron, zinc, and pro-vitamin A carotenoid nutrition to poor at-risk populations in Africa, Asia, and Latin America, thereby improving food security and enhancing the quality of life (Bouis, Graham, & Welch, 2000; Pfeiffer & McClafferty, 2007; Stangoulis, 2010).

Following demonstration of adequate genetic variability which could be exploited to enhance micronutrient density, plant breeding programs focusing on biofortification of staple crops such as sweet potato (Hagenimana & Low, 2000), beans (Beebe, Gonzalez, & Rengifo, 2000; Kimani & Karuri, 2001), rice (Gregorio, Senadhira, Htut, & Graham, 2000), wheat (Monasterio & Graham, 2000), cassava (Chavez et al., 2000; Maziya-Dixon, Kling, Menkir, & Dixon, 2000), and maize (Banzinger & Long, 2000) were started. Development of mineral-rich bean (*Phaseolus vulgaris* L.) cultivars can contribute to the alleviation of micronutrient deficiency in Africa because bean is widely cultivated (>5.1 million ha annually in Africa), widely consumed, and is rich in protein (>20%), minerals, and calories. Common bean (*Phaseolus vulgaris* L) is relatively cheap compared to other sources of micronutrients and protein, and complements cereal and root crop-based diets. Bean also fits into many cropping systems. Rwanda and Democratic Republic of Congo have been the target countries for HarvestPlus bean biofortification activities (Pfeiffer & McClafferty, 2007). A regional breeding program led by the University of Nairobi was initiated in 2004 to develop and disseminate micronutrient-dense bean varieties in 10 member countries of the Association of Strengthening Agricultural Research in East and Central Africa (ASARECA). The early stages of this work involved the screening of available regional bean germplasm for variation in iron and zinc (Kimani & Karuri, 2001). Forty-five micronutrient-dense lines were identified from screening more than 2,800 germplasm accessions and distributed as a fast-track nursery to 25 African countries for local evaluation and release (Kimani, 2005; Kimani et al., 2005). Many countries have released at least one variety from this nursery (Kimani, Beebe, Blair, & Mamiro, 2008). Accessions with high grain iron and/or zinc but lacking in other desired agronomic traits were subsequently used to develop new populations combining the high mineral trait with resistance to biotic and abiotic stresses, agronomic potential, and consumer-preferred grain characteristics (Kimani et al., 2008). The objective of this study was to evaluate advanced lines selected from these populations in order to identify new lines combining high mineral trait with resistance to biotic stresses, high yield potential,

TABLE 1 Characteristics of high iron parental lines used to develop breeding populations

Genotype	Origin	Growth habit[a]	Seed color	Seed size[b]	Fe (ppm)	Zn (ppm)
Nakaja	DRC	IV	Brown	Small	77.6	43.0
AND 620	CIAT	I	Red mottled	Large	76.0	35.3
Simama	DRC	II	Red mottled	Large	82.7	34.5
HRS 545	Sudan	II	Navy	Small	89.7	45.6
Gofta	Ethiopia	II	Brown	Medium	74.4	40.1
MLB 49−89A	DRC	II	Black	Medium	95.6	30.1

Abbreviation(s): DRC, Democratic Republic of Congo.

[a]Growth habit: I, determinate bush habit; II, indeterminate bush habit, erect stems; III indeterminate bush habit with weak stems, prostrate, and tendency to climb; and IV, indeterminate climbing habit.

[b]Large (>40 g/100 seeds); medium (26–39 g/100 seeds); small (<25 g/100 seeds).

and commercial grain types for production by smallholder bean farmers in east, central, southern, and West Africa.

2 | MATERIALS AND METHODS

2.1 | Population development

Forty-seven multiparent populations were developed from crosses between six parental lines selected for high iron and zinc concentration, and diverse sources of resistance to diseases, and commercial varieties in the greenhouse at Kabete Field Station, University of Nairobi between 2005 and 2007 (Tables 1 and 2). The parental lines were selected from screening more than 2,800 bean accessions for iron and zinc concentration (CIAT, 2008). These accessions were collected from nine countries in East and Central Africa between 2001 and 2008. Seed iron concentration varied from 40 to 120 ppm and zinc concentration from 20 to 52 ppm. Although there was considerable variation for iron and zinc concentration, most of the accessions with high mineral density were deficient in important agronomic traits such as resistance to diseases, farmer preferred characteristics, and market demanded grain types. Six lines with high iron (>70 ppm) and/or zinc concentration (>30 ppm) were selected for a hybridization program aimed at transferring the high mineral trait to commercial varieties and landraces (Table 1). The objective of this program was to combine high mineral trait with resistance to major biotic stresses with emphasis on regionally important diseases such as angular leaf spot, anthracnose, root rots, and abiotic stresses, especially drought tolerance with other farmer preferred traits and commercial grain types following the gamete selection procedure (Singh, 1994). Origin and some characteristics of the six parental lines are shown in Table 1. Three of the parents (Nakaja, Simama, and MLB 49-89A) originated from the Great Lakes region which is known to have high genetic diversity for the high mineral trait (Blair et al., 2010). The other three parental lines originated from CIAT, Sudan, and Ethiopia. All the high iron parental lines, except Nakaja, have bush growth habit.

Multiparent male gametes were developed by combining 11 commercial varieties and/or sources of resistance to angular leaf spot, anthracnose, root rots, tolerance to low soil fertility into single, three-way, and double crosses (Table 2). In these crosses, Mex 54 and G5685 were used as sources of resistance to angular leaf spot (Mahuku, Iglesias, & Jara, 2009; Namayanja et al., 2006). Vunikingi (G685), a small-seeded climbing bean from Rwanda, was used as a source of resistance to fusarium wilt (*Fusarium oxsporum f.sp. phaseoli*) (Musoni, Kimani, Narla, Buruchara, & Kelly, 2010). RWR 719, SCAM 80CM/15, and AND 1,062 were sources of resistance to Pythium and fusarium root rots, and tolerance to low soil fertility (Buruchara & Camacho, 1999; Lunze et al., 2007; Mukankusi, Amongi, Sebuliba, Musoke, & Acam, 2018; Otsyula, Buruchara, Mahuku, & Rubaihayo, 2003). Umubano (G2333), a small red-seeded climbing is a well known and widely used source of resistance to anthracnose (Pastor-Corrales, Erazo, Estrada, & Singh, 1994). However, Umubano is highly susceptible to fusarium wilt (Musoni et al., 2010). Commercial varieties included GLP 2 (Rosecoco), a large-seeded red mottled variety popular in Kenya and Uganda. Lyamungu 85 and Selian 97 are large-seeded red mottled varieties popular in Tanzania. Canadian Wonder (GLP24) is a red kidney variety popular in Kenya and Tanzania. However, these varieties are susceptible to angular leaf spot, anthracnose, and root rots.

In the final cross of each population, the high mineral parent was used as the female parent. The multiparent male gametes contained combinations of resistance to angular leaf spot, anthracnose, root rots, tolerance to low soil fertility, bean common mosaic virus, climbing or bush growth, and major commercial grain types. The resultant F_1 progenies were advanced as population bulks to F_4 generation at Kabete Field Station.

TABLE 2 Pedigrees of the 47 populations developed for this study

Population	Pedigree
BF01	AND 620/// ((Lyamungu 85 / RWR 719)///(SCAM 80CM15/ Mex 54)
BF02	GOFTA /// (Selian 97/Mex 54)// Vuninkingi)
BF03	GOFTA ///(Selian 97/ Mex 54)// (RWR719/ Vunikingi)
BF04	AND 620 /// (Lyamungu 85/RWR 719)// (SCAM 80CM/15/Umubano)
BF05	Gofta ///(Lyamungu 85/Vuninkingi)// (Mex 54/AND 1062)
BFO6	AND620 /// (Canadian Wonder/Mex 54) // (AND 1062/Umubano)
BF07	NAKAJA ///(Canadian Wonder/Mex 54)// (AND 1062/Umubano)
BF08	GOFTA /// ((Lyamungu 85/Vuninkingi)//(Mex 54/ AND 1062)
BFO9	MLB 49-89A //// (Lyamungu 85/Vuninkingi)//(Mex 54/G5686) /// AND1062)
BF10	AND620 /// ((Lyamungu 85/ Umubano)//(Mex 54 / RWR 719)
BF11	GOFTA/// (Lyamungu 85/Umubano)// (Mex 54 / RWR 719)
BF12	MLB 49-89A//(Selian 97/ Mex 54)//Vuninkingi)
BF13	MLB 49-89A/// ((Lyamungu 85/RWR 719)// (SCAM 80 CM15/ Umubano)
BF14	SIMAMA /// (Lyamungu 85/RWR 719)// (SCAM 80CM15/ Umubano)
BF15	NAKAJA /// (Lyamungu 85/RWR 719)// (SCAM 80CM15/Mex 54)
BF16	NAKAJA /// (Lyamungu 85/Vuninkingi)// (AND 1062/Mex 54)
BF17	MLB 49-89A///(GLP 2/Vuninkingi)// (GLP 2/Mex 54)
BF18	AND 620 /// ((Selian 97/Mex 54)// (Vuninkingi/RWR 719))
BF19	NAKAJA ////((Lyamungu 85/Vuninkingi)// (Mex 54/ RWR 719)
BF20	SIMAMA ////((Selian 97/Mex 54)//(Vuninkingi/RWR 719)
BF21	MLB 49-89A /// (Lyamungu 85/Umubano)// (Mex 54/ RWR 719)
BF22	MLB 49-89A //// (Lyamungu 85/Vuninkingi)// (SCAM 80CM15/Mex 54)
BF23	AND620/// (Lyamungu 85/ Vuninkingi)// (Mex 54/ RWR 719)
BF24	AND 620/// (Lyamungu 85/RWR 719)//(SCAM 80CM15/Umubano)
BF25	SIMAMA /// (Canadian Wonder/Mex 54)// Vuninkingi)
BF26	GOFTA /// (Lyamungu 85/Vuninkingi)// (Mex 54/ AND1055)
BF27	AND 620 /// (Lyamungu 85/Umubano)//Mex 54)
BF28	MLB 49-89A /// (Lyamungu 85/Umubano) // Mex 54)
BF29	NAKAJA //// (Lyamungu 85/Vuninkingi) // (Mex 54/ G5686) ///AND 1062)
BF30	NAKAJA /// (Lyamungu 85/RWR 719) // SCAM 80CM15)
BF31	MLB 49-89 ////((Lyamungu 85/Vuninkingi)// (AND 1,062/Mex 54)
BF32	NAKAJA////((Seilan 97/Mex 54)//(Vuninkingi/ RWR 719))
BF33	GOFTA//// ((Lyamungu 85/Vuninkingi) // (AND 1,062/Mex 54) /// G5686))
BF34	MLB 49-89A/// (Lyamungu 85/Vuninkingi) // (Mex 54/RWR 719)
BF35	GOFTA //// (Lyamungu 85/Vuninkingi)//(RWR 719/ Mex 54)
BF36	AND 620 /// (Lyamungu 85/Vuninkingi) // (RWR 719/Mex 54)
BF37	NAKAJA ///((Lyamungu 85/ RWR 719) // (SCAM 80CM15/ Mex 54)
BF38	MLB 49-89A ///(Canadian Wonder/ Mex 54) // (AND 1,062/ Umubano)
BF39	SIMAMA /// (Lyamungu 85/ Vuninkingi) // (Mex 54/ RWR 719)
BF40	MLB 49-89A///((Selian 97/ Mex 54) // (Vuninkingi/ RWR 719))
BF41	NAKAJA/// (Canadian Wonder/Mex 54) // (AND 1,062/ Umubano)
BF42	NAKAJA///(Lyamungu 85/RWR 719) // SCAM 80CM15)
BF43	AND 620/// (Selian 97/Mex 54)// (Vuninkingi/ RWR 719)
BF44	HRS 545/// (Lyamungu 85/Umubano) // (Mex 54/ RWR 719)
BF45	NAKAJA/// ((Lyamungu 85/Vuninkingi) // (Mex 54/ RWR 719)
BF46	GOFTA/// (Canadian Wonder/Mex 54) // (AND 1,062/Umubano)
BF47	SIMAMA///(Canadian Wonder/Mex 54) // (AND 1,062/ Umubano)

2.2 | Micronutrient and protein analyses

The $F_{2.3}$ bulks and $F_{4.7}$ lines were evaluated for grain iron and zinc concentration to determine variability for mineral density using the perchloric-nitric wet acid digestion method of Zarcinas, Cartwright, and Spoucer (1987). Nitrogen was

determined by standard Kjeldahl digestion method. Elemental analysis was done by atomic absorption technique.

2.3 | Sample preparation

Seed samples for mineral analyses were taken at harvest. They were air-dried, washed in 0.2N HCl, and finally rinsed with distilled water to remove contaminants. The samples were then oven-dried at 70°C for 48 hr, after which they were ground using an iron-free mill (Retsch, type MM 200, Germany) with a Teflon grinding jar and zirconium grinding balls, at a frequency of 25/s for 10 min, and then passed through a 1-mm sieve. The samples were then packed in air-tight plastic bottles for mineral determination.

2.4 | Determination of iron and zinc concentration

Digestion of the bean samples was done using the perchloric–nitric acid mixture digestion method of Zarcinas et al. (1987). Ten milliliters of nitric and 1 ml of perchloric acid were added to duplicate 1.0 g bean sample in digestion tubes, and the mixture allowed to stand overnight at room temperature. Four ml of perchloric acid was added to each tube. The tubes were transferred to Gerhardt Kjeldatherm block digestion system (Gerhardt) and heated for 1 hr at 120°C (Zarcinas et al., 1987). During digestion, the temperature was raised to 175°C. Toward the end of the digestion, the temperature was further increased to 225°C for 30 min during which the digest cleared and complete digestion was achieved. The digest was cooled and diluted to 50 ml with 1% v/v nitric acid and transferred into screw-top polycarbonate vials. Mineral concentration was determined with atomic absorption spectrophotometer (Spectr AA-10, Varian Techtron Pty Ltd). The concentration of the standards was 2, 8, and 20 ppm for Fe, and 0.5, 1, and 3 ppm for Zn. The absorbance of iron and zinc was read at a wavelength of 248.33 nm for iron, and 213.86 nm for zinc.

2.5 | Determination of nitrogen

Nitrogen was determined by the semi-micro Kjeldahl method (AOAC, 2000). Five ml distilled water, 1 tablet of Kjeldahl catalyst, and 10 ml concentrated sulfuric acid were added to each tube containing 0.5 g of ground bean sample (Fritz & Schenk, 1971). The tubes were placed in a preheated (385–420°C) digestion rack and digested temperature until the mixture cleared. The digest was allowed to cool and then diluted with 75 ml of distilled water and transferred to a distillation unit (Tecator Kjeltec System, Model 1,002). The digest was steam distilled using 50 ml of 40% sodium hydroxide, and the liberated ammonia trapped in 25 ml of 0.1 N hydrochloric acid. When about 200 ml had distilled over, the distillation was stopped and the acid in excess back-titrated with standard 0.1 N sodium hydroxide with methyl orange indicator solution to an orange-yellow end point. Blank determinations without samples were made in a similar manner. The amount of ammonia liberated was the difference between sample and blank titrations. Nitrogen content of the sample was calculated using 14.01 as the equivalent weight. Total nitrogen was converted to percent protein by multiplying by a factor of 6.25 and expressed on dry weight basis.

2.6 | Experimental sites

Field experiments were conducted at Kabete Field Station, University of Nairobi, and at the Kenya Agricultural and Livestock Research Organization (KALRO) research stations at Thika and Tigoni, and in a farmer's field in Bahati, Nakuru County.

Kabete Field Station is located on latitude 1°15' S and longitude 36°41' E (Jaetzold, Schmidt, Hornetz, & Chisanya, 2006), at an altitude of 1,820 meters above sea level. It receives an average annual rainfall amount of 980 mm during the long rain (March to May) and short rain (October to December) seasons. The site has minimum and maximum mean temperatures of 13.7°C and 24.3°C, respectively. The soils are nitisols, which are a very deep, well-drained, dark reddish, deep friable clay type, and resistant to erosion (Wahome, Kimani, Muthomi, Narla, & Buruchara, 2011). KARI-Thika is located on coordinates 00 59′ South and 370 04′ East at an elevation of 1,548 m above sea level. It experiences a bimodal pattern of rainfall with an annual mean of 1,000 mm distributed over two seasons. Long rains occur between March and May, while short rains occur between October and December. The mean annual maximum and minimum temperatures are 25.1°C and 13.7°C, respectively (Ndegwa, Muchui, Wachuri, & Kimamira, 2009). Soils are well drained, extremely deep, dusky red to dark reddish brown, nitosols (Jaetzold et al2006).

KARI-Tigoni research station falls under the lower highland (LH1) agro-ecological zone (Jaetzold et al., 2006). It is located on altitude of 2,131 meters above sea level, and latitude of 1°15′ S and longitude 23° 46′ E (Jaetzold et al., 2006). The average annual rainfall is 1,400 mm. The soil type is humic nitosol. Soils are well drained, extremely deep, dusky red to dark reddish brown, friable clay, with an acid humic topsoil (Jaetzold et al., 2006).

In Nakuru County, the experiment was conducted in a farmer's field in Kabatini area of Bahati constituency, Nakuru North District. The area falls under lower highland (LH3) agro-ecological zone (Jaetzold et al., 2006). The trial site was located on latitude 0° 12′S and longitude 36° 10′E with altitude of 2070 masl (Jaetzold et al., 2006). The average annual rainfall is about 1,000–1200 mm. The mean annual maximum and minimum temperatures are 22.6 and 9.1°C,

respectively (Jaetzold et al., 2006). Soils at the site are vitric Andosols, which are well drained moderately deep to deep, brown to dark brown, very loam to sandy clay loam (Jaetzold et al., 2006).

2.7 | Advancement of early generations (F_1-F_4)

F_1 and F_2 generations were advanced as population bulks at Kabete Field Station during the 2008 and 2009 long rain seasons (April to July), to allow for recombination and segregation. The F_3 generation also was grown as bulks at Kabete during the 2009 short rain season (October to December). During the 2010 long rain season, the F_4 bulks of each of the 47 populations were space planted (15 × 50 cm instead of the normal spacing of 10 × 45 cm) at the same site. Single plants were selected based on plant vigor, reaction to prevalent diseases (angular leaf spot, anthracnose, and root rots), growth habit, and pod load. Six thousand six hundred and twelve single plants were harvested separately, and their seed used to establish $F_{4.5}$ progeny rows during the 2011 short rain season. The progeny rows were scored for plant vigor, growth habit, reaction to diseases, duration to maturity, pod load, and seed yield using the standard system for the evaluation of bean germplasm (van Schoonhoven & Pastor-Corrales, 1987).

2.8 | Selection in $F_{4.5}$ generation

One thousand six hundred and seventy-one $F_{4.5}$ progeny rows with resistance to two or more diseases, plant vigor scores of 1–6 (excellent to moderate), and good pod load were selected from the 6,612 progeny rows evaluated in single rows at Kabete Field Station during the 2011 short rain season (October 2011 to January 2012). Undesirable populations and progeny rows were discarded. A 1–9 scale was used for disease rating, where a score of 1–3 was resistant, 4–6 intermediate, and 7–9 susceptible (van Schoonhoven & Pastor-Corrales, 1987).

2.9 | Line development and evaluation for drought tolerance and other agronomic traits

One hundred and two $F_{4.6}$ lines from eight populations were evaluated for drought tolerance and reaction to diseases in moisture-stressed and nonstressed conditions at Kabete Field Station and KALRO-Thika during the 2012 long rain season. Parental lines and commercial varieties were included as checks. Field experiments at each test site were laid out in a split-plot design with irrigation regimes as the main plots and genotypes as subplots. The trial was replicated three times. A plot consisted of four 3 m rows. Spacing was 50 cm between rows and 10 cm within rows. A basal rate of 100 kg diammonium phosphate (18% N and 45% P_2O_5) was applied in furrows before planting. Plots were kept weed free by manual cultivation. Insect pests were controlled by spraying every 2 weeks with broad-spectrum insecticides, "Tata Alpha" (cypermethrin 100 g/L) and "Tata Mida" (Imidacloprid 200 g/L) which were applied at 0.5 L/ha. No fungicides were applied to facilitate disease scoring.

All plots were irrigated to 80% field capacity from planting to just before flowering to ensure good plant establishment. To impose drought stress, water was withheld 40 days after germination in stressed plots until crop maturity. Control plots (nonstressed) received three supplemental irrigations until physiological maturity. Soil moisture was monitored by weekly soil sampling from depths of 0–5, 5–10, 10–20, 20–40, 40–60, and 60–80 cm with a soil corer, followed by gravimetric determination of percent soil moisture. Supplementary irrigation was provided using overhead sprinklers at Kabete and Thika. Populations and lines showing susceptibility to diseases and poor agronomic potential were discarded. The inner two rows in each plot were used to determine grain yield adjusted to 14% moisture content. Grain yield under drought-stressed and nonstressed conditions was used as the primary selection criterion for drought tolerance and agronomic potential. Grain yield in stress and no-stress conditions was used to determine relative yield reduction, geometric mean, drought intensity index, and drought susceptibility index (Rao, Polania, Ricaurte, & Rangel, 2008; Teran & Singh, 2002). Drought intensity index (DII) for each location/environment was calculated as DII = 1-Xds/Xns, where Xds and Xns are the mean of all genotypes under drought stress (ds) and no-stress (ns) treatments, respectively. The relative yield reduction (RYR) for each genotype was estimated as: irrigated grain yield-rainfed grain yield/irrigated grain yield × 100. Geometric mean (GM) was determined as: $GM = (ns \times ds)^{1/2}$. Drought susceptibility index (DSI) for each genotype was calculated as: DSI = (1−Yds/Yns)/DII, where Yds and Yns are the mean yields of a given genotype in ds and ns environments (Fischer & Maurer, 1978).

2.10 | Multilocation testing

One hundred $F_{4.7}$ lines were selected based on their reaction to diseases, drought stress, and agronomic potential during the 2011 long rain season. They were evaluated at four locations (Kabete, Thika, Tigoni, and Bahati) during the 2011 short rain season under rainfed conditions. The experiments were laid out in 11 × 11 lattice design with three replicates. A plot consisted of two 3 m rows with 30 plants each making a total of 60 plants. Diammonium phosphate (18-46-0) fertilizer was applied at a rate of 150 kg/ha and thoroughly mixed with the soil. At seedling stage, plants were sprayed with dimethoate 40% EC at a rate of 30 ml per 20 liter to protect them from bean stem maggot. The fields were kept weed free

by manual cultivation. Data were collected on grain yield and reaction to diseases.

2.11 | Data analyses

Analysis of variance was performed to determine whether there were significant differences due to treatments (irrigation, locations, and their interactions) and among the test genotypes. In these analyses, replications and locations were considered as random effects, and method of analysis, irrigation, and genotypes were the fixed effects (McIntosh, 1983). Fisher's least significant difference at 5 and 1 probability levels was used for mean separation. Data were analyzed using Genstat statistical software (VSN International Ltd., UK, Version 13).

3 | RESULTS

3.1 | F_2 and F_3 populations

3.1.1 | Variation in Fe and Zn concentration

Results showed that there was considerable variation in grain iron, zinc, and protein concentration in the study populations (Table 3). Iron concentration varied from 30 to 130 ppm indicating that micronutrient-dense lines can be selected from these populations. Grain zinc concentration varied from 10 to 60 ppm, also suggesting adequate variation for this trait. The new populations also showed variation for protein concentration. Protein concentration varied from 17% to 28.5%. Grain iron concentration in eight NUA lines, which were obtained from CIAT, varied from 45 to 75 ppm (Table 3). Zinc concentration varied from 20 to 45 ppm. Protein concentration in NUA lines varied from 22.5% to 26.5%. Runner bean (*Phaseolus coccineus* L.) genotypes, which also were included for comparison, showed relatively higher levels of grain iron and zinc concentration (Table 3). Runner bean had a mean iron concentration of 90 ppm, compared with 71 ppm for NUA lines, and 57.7 to 77.5 ppm for the new populations. The runner bean genotypes also had higher grain zinc concentration (35.8 ppm) compared with NUA lines (24.3 ppm), and the bean populations, except BF08-01 families which had a mean zinc concentration of 40.6 ppm. However, the grain protein concentration in runner beans was comparable to that of common bean genotypes. Similar variation in micronutrients in African bean germplasm was reported by Blair et al. (2010). NUA lines were bred at CIAT, Colombia, for high micronutrient density (CIAT, 2008).

3.1.2 | Variation in $F_{4.5}$ generation

The $F_{4.5}$ progeny rows grown at Kabete Field Station during the 2011 short rain season showed considerable variation for growth habit, duration to maturity, reaction to diseases, and grain yield. Most of the families were segregating for determinate (type I) and indeterminate growth habits (types II, III, and IV). Some populations such as BF08-01, BF08-04, BF08-07, BF08-16, BF08-29, BF08-30, and BF08-37 showed strong tendency to indeterminate growth habit. Progenies from these populations had type III (semi-climbing) and type IV (strong climbing growth habit). In contrast, only BF08-25 had a strong tendency toward determinate growth habit. Progenies from this population had only type I and II bush growth habit. Duration to maturity varied from 86 to 105 days. Ten populations (BF08-02, BF08-11, BF08-12, BF08-13, BF08-22, BF08-28, BF08-31, BF08-34, BF08-38,

TABLE 3 Variation in iron, zinc, and protein concentration in $F_{2.3}$ populations [Formerly Table 1]

		Fe (ppm)		Zn (ppm)		Protein (%)	
Population	**No. of families**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**
BF01	67	30–105	60.6	10–40	40.6	18.8–26.5	24.3
BF03	16	55–125	73.2	10–35	28.2	19.87–23.6	18.5
BF07	70	30–130	72.9	10–55	21.4	17.4–23.7	21.0
BF13	47	30–115	69.0	10–45	20.4	19.1–28.3	23.6
BF16	30	40–115	77.5	10–40	23.1	20.0–28.5	21.5
BF26	25	35–100	73.4	10–40	20.8	19.8–24.4	23.4
BF36	26	50–115	57.7	10–60	25.6	21.3–28.5	22.2
NUA	8	45–75	71.0	25–50	24.3	22.9–26.5	24.0
Runner bean	11	45–110	90	20–45	35.8	15.5–21.5	24.2
Total	300						
Mean			72.3		23.6		22.6
LSD 0.05			16.5		12.7		0.45
CV (%)			11.6		27.3		1.0

and BF08-47) produced early maturing progenies, which matured in 85 days or less. In contrast, two populations (BF08-16 and BF08-29) produced late maturing progenies (>95 days). All other populations produced combinations of early, medium, and late maturing plants. The progenies showed variation in reactions to co-infection by root rot, angular leaf spot, and anthracnose pathogens, which were the most prevalent diseases at Kabete during the 2011 short rain season. Reactions to these diseases varied from resistant (disease scores of 1 to 3), intermediate (scores of 4 to 6), and susceptible (score of 7). Grain yield of the among progeny rows varied from 211 kg/ha (BF08-11) to 1,492 kg/ha for BF08-07, and 1,444 kg/ha for BF08-07 progenies. However, there was considerable variation in grain yield and plant vigor within populations. Populations B08-01, BF08-03, BF08-05, BF08-07, BF08-08, BF08-16, BF08-32, BF08-36, and BF08-44 produced the most vigorous progenies (vigor scores of 1–3). Based on plant vigor, growth habit, duration to maturity, reaction to diseases, and seed yield, 1671 progeny rows were selected from the 6,612 families evaluated. Number of lines selected varied from 8 for population BF08-11 to 68 for population BF08-10.

3.2 | Performance of populations

Of the 47 populations, eight showed outstanding performance at Kabete under severe mid-season and terminal droughts during the 2011 short rain season. The populations showed considerable variation in vigor, growth habit, resistance to diseases, and grain yield. The most outstanding populations were BF08-01, BF08-03, BF08-07, BF08-13, BF08-14, BF08-16, BF08-26, and BF08-36. The test lines used in this study were derived from these populations. Figures 1–5 show the performance of the best lines from five populations compared with the check varieties.

Mean yield of the 12 lines selected from population BF08-01 varied from 2,523 kg/ha to 3,562 kg/ha (Figure 1). Results showed that these lines had up to 100% higher yield compared with the best check variety, Gofta. The high grain yield was attributed to their indeterminate growth habit, plant vigor, and resistance to diseases. These lines had types II and III growth habit (semi-climbing) and were also rated resistant to angular leaf spot, anthracnose, and root rots (disease scores of 1 to 3).

The performance of the five top lines selected from population BF08-03 is shown in Figure 2. Yield of these lines varied from 2,468 kg/ha (BF08-03-01) to 3,076 kg/ha (BF08-03-44). These lines showed up to 80% yield advantage compared with the best check, Gofta. These lines showed types II and III growth habit and combined resistance to angular leaf spot, anthracnose, and root rots. They originated from the F_5 progeny rows selected at Kabete Field Station during the 2011 long rain season under severe terminal drought stress.

BF08-07 was one of the most superior populations selected at Kabete during the 2011 long rain season. It has shown consistently good performance across locations and seasons. The best performing F_7 lines from this population are shown in Figure 3. Mean yield of these lines in favorable and unfavorable environments varied from 2,635 kg/ha (BF08-07-80) to 4,577 kg/ha (BF08-07-21). These lines showed a yield advantage of up to 168% compared with the best check, Gofta. The high yield potential of these lines was attributed to vigorous type IV (climbing) growth habit and heavy pod loads. These lines also showed consistently high levels of resistance to angular leaf spot, anthracnose, root rots, common bacterial blight, and bean common mosaic virus. Progenies from this population also showed tolerance to drought and low soil fertility at Kabete during the 2011 short rain season.

Figure 4 shows the best performing lines selected from population BF08-13. Mean yield of these lines at Kabete and Thika varied from 2,499 kg/ha (BF08-13-92) to 3,162 kg/ha (BF08-13-181). These lines showed an advantage of up to 85% when compared with the best check variety, Gofta, which had a mean yield of 1707 kg/ha. These lines were rated resistant to angular leaf spot and anthracnose and moderately resistant to root rots (scores 2 to 5). They showed relatively

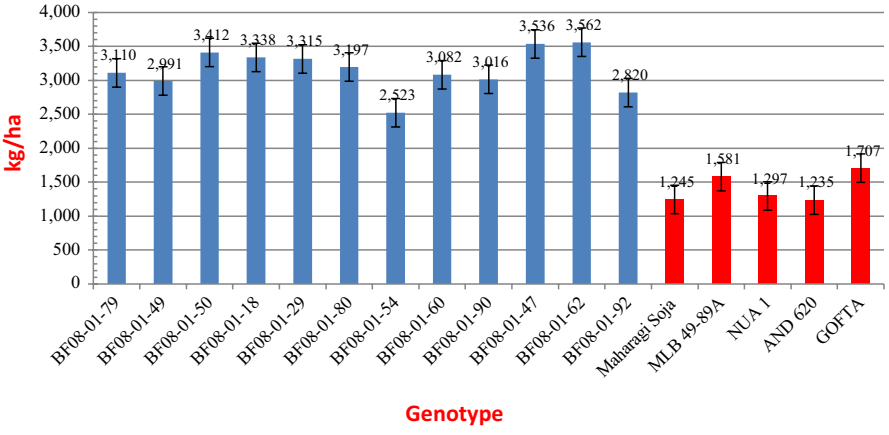


FIGURE 1 Mean grain yield of F6 lines from population BF08-01 grown at Thika and Kabete. I, standard error bars

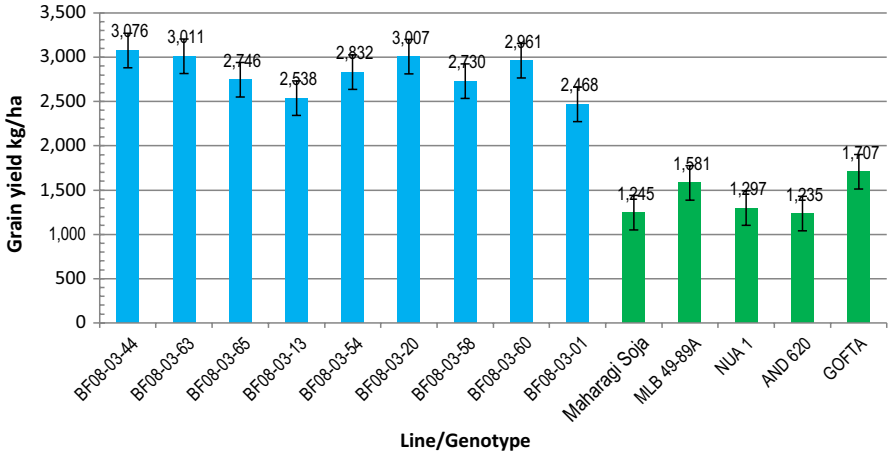
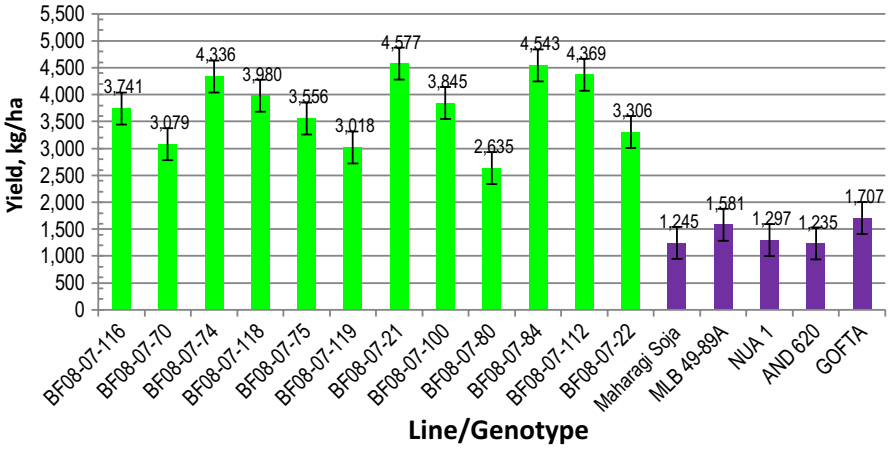


FIGURE 2 Mean grain yield of the top F6 lines from population BF08-03 grown at Thika and Kabete during the 2012 long rain season. I, standard error bars

FIGURE 3 Mean grain yield of the top F6 lines from population BF08-07 grown at Thika and Kabete. I, standard error bars



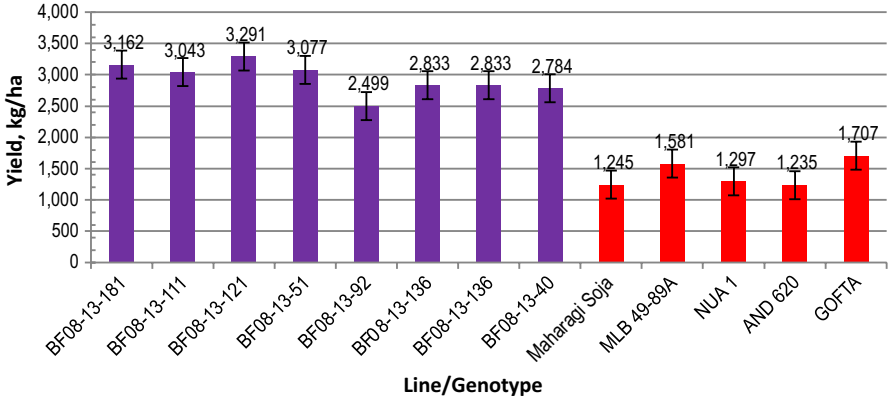


FIGURE 4 Mean grain yield of the top F6 lines from population BF08-13 grown at Thika and Kabete. I, standard error bars

early maturing compared to lines from populations BF08-01, BF08-03, and BF08-07. They have type II (upright, indeterminate bush) and type III (semi-climbing) growth habit but were moderate in vigor.

BF08-16 was one of the superior populations selected at Kabete during the 2011 long rain season. This population showed high levels of resistance to root rots, angular leaf spot, anthracnose, and tolerance to drought and low soil fertility.

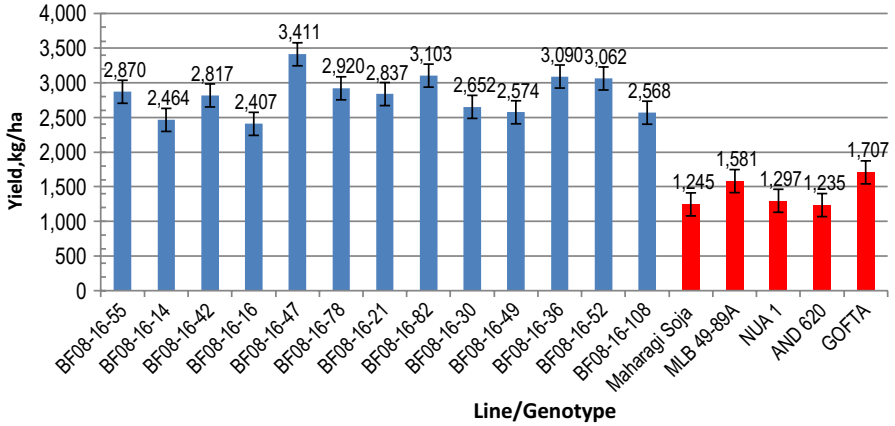


FIGURE 5 Mean grain yield of the top F6 lines from population BF08-16 grown at Thika and Kabete. I, standard errors bars

Source	*df*	Mean squares			
		Seed yield	RVR	GM	DSI
Replicates	1	129,400.0	49.3	61,585	0.0016
Locations	1	48,410,000**	616.4	17,785,288**	1.8668*
Treatments	1	469,400,000**			
Genotypes	106	6,306,000**	723.1	2,966,610**	0.3442
Location × treatment	1	46,130.0			
Locations × genotypes	106	1,587,000.0	877.5	1,305,704**	0.4128
Treatments × Genotypes	106	1,500,000.0			
Genotype × location × treatment	106	132,400,000.0			
Error	427	1,299,000.0	715	690,745	0.3281

*Significant at 0.05 probability level.
**Significant at the 0.01 probability.

TABLE 4 Mean squares of seed yield, relative yield reduction (RVR), geometric mean (GM), and drought susceptibility (DSI) indices of $F_{1.5}$ lines grown at Kabete and Thika during the 2012 long rain season

The best performing F_6 lines from this population are shown in Figure 5. Mean yield of these lines varied from 2,407 kg/ha (BF08-16-16) to 3,411 kg/ha (BF08-16-47). The new lines had a yield advantage of up to 99.8% compared with the best check variety. The high yield potential could be attributed to their climbing growth habit, heavy pod load, and resistance to major diseases.

3.3 | Performance of $F_{4.6}$ lines in drought-stressed and nonstressed conditions

Analysis of variance showed that there are significant differences in seed yield among the $F_{4.6}$ test lines at the two locations (Table 4). Results showed that location and irrigation treatments had highly significant ($p > 0.01$) effects on seed yield. Mean grain yield was higher at Kabete (2,636 kg/ha) compared with Thika (2,161 kg/ha). As expected, genotypes in irrigated plots had a higher yield compared to the moisture-stressed plots. Mean yield of the test lines was 3,138.9 kg/ha in irrigated plots compared with 1657.8 kg/ha for rainfed (stressed) plots at the two locations. Table 5 shows the mean performance of the best lines in moisture-stressed and nonstressed conditions. Mean yield of the 101 $F_{4.6}$ lines at the two locations varied from 658 kg/ha (BF08-36-140) to 4,577 kg/ha (BF08-07-21) with a trial mean of 2,398 kg/ha. In contrast, the yield of check varieties varied from 417 kg/ha for CAL96 to 1707 kg/ha for Gofta. CAL 96 is a red mottled commercial variety released in Uganda as K132. It is a widely used low iron check variety. Gofta is a commercial variety in Ethiopia and was selected as a high iron check variety. On average, drought reduced the yield of these lines by 47%, but the magnitude varied with genotypes. For example, some lines such as BF08-07-21, BF08-07-84, BF08-07-112, and BF08-07-74 showed almost 50% yield reduction due to drought stress (Table 5). However, location effects had no significant influence on relative yield reduction (RVR). The interaction between genotypes and locations was not significant for RVR. Drought intensity index (DII) was higher at Thika (DII = 0.516) than at Kabete (DII = 0.476) suggesting more severe moisture stress during the growing season.

TABLE 5 Grain yield, relative yield reduction (RVR), geometric mean (GM), and drought susceptibility index (DSI) of $F_{4.6}$ bean lines grown in moisture-stressed and nonstressed conditions at Kabete and Thika during the 2012 long rain season

Genotype	Irrigated (kg/ha)	Rainfed	Mean	RVR (%)	GM	DSI
BF08-07-21	6,148.8	3,005.4	4,577.1	48.3	4,262	1.00
BF08-07-84	6,079.2	3,007.7	4,543.4	47.6	4,194	1.02
BF08-07-112	5,870.4	2,867.5	4,368.9	48.4	3,985	1.03
BF08-07-74	5,806.8	2,864.3	4,335.6	48.1	4,025	1.03
BF08-07-118	4,528.0	3,431.9	3,979.9	24.1	3,889	0.52
BF08-16-47	5,008.8	1,812.5	3,410.7	51.9	2,790	1.07
BF08-01-18	4,089.1	2,587.5	3,338.3	45.9	3,083	0.94
BF08-01-29	4,350.8	2,278.7	3,314.8	63.6	2,903	1.36
BF08-07-22	4,463.2	2,148.3	3,305.8	51.9	3,059	1.08
BF08-13-121	4,606.3	1,975.6	3,291	53.2	2,306	1.14
BF08-01-80	3,911.9	2,481.4	3,196.7	50.7	2,942	1.06
BF08-13-181	3,564.1	2,760.0	3,162.1	24.6	3,116	0.51
BF08-01-79	4,536.2	1,684.0	3,110.1	77.5	1,640	1.68
BF08-16-82	4,180.1	2,025.9	3,103	49.5	2,686	1.01
BF08-16-36	4,105.8	2,073.6	3,089.7	62.2	2,622	1.28
BF08-01-90	4,569.3	1,462.8	3,016	66.5	2,557	1.41
BF08-03-1	2,952.7	1,983.2	2,467.9	55.9	2,274	1.18
BF08-14-83	4,003	668.5	2,335.7	86.2	1,298	1.85
BF08-26-163	2,758.4	1,873.7	2,316	29.0	2,214	0.62
BF08-14-82	2,495	2,131.7	2,313.4	18.4	2,285	0.38
BF08-13-102	3,027	1,144.1	2,085.6	63.1	1,622	1.35
BF08-36-127	2,131.9	1,643.5	1887.7	43.4	1,800	0.88
BF08-26-162	1,954.3	1,565.4	1,759.9	40.5	1,665	0.88
BF08-36-156	1,990.8	1,442.3	1,716.6	31.4	1,646	0.65
BF08-36-49	1,993.7	1,388.2	1,690.9	32.1	1,640	0.66
BF08-13-133	2,493.2	765.1	1,629.2	72.1	1,029	1.58
BF08-36-18	2,139.8	1,016.3	1,578	52.4	1,472	1.11
BF08-36-205	1,818.5	1,165.8	1,492.2	37.3	1,449	0.77
BF08-36-100	2,027.3	658.5	1,342.9	62.0	1,068	1.33
BF08-36-162	1,669.6	918.4	1,294	54.5	1,072	1.17
BF08-36-140	1,092.8	222.6	657.7	81.8	397	1.77
Checks						
GOFTA	2,363.4	1,050.7	1707.1	56.2	1,550	1.18
MLB49/89A	1,973.8	1,187.3	1580.5	38.5	1,521	0.82
NUA1	1,873.3	720.8	1,297.1	51.2	1,033	1.08
Maharagi Soja	1,726	763.3	1,244.7	50.2	922	1.11
AND620	1,587.4	882.3	1,234.8	39.9	1,136	0.84
CAL96	557.6	277.2	417.4	69.7	308	1.53
Mean	3,115.9	1,642.4	2,379.1	47.0	54.5	0.98
$LSD_{0.05}$: Locations = 154.9; Treatments = 154.9; genotypes = 1,138.5						

Thika received 476.7 mm of rainfall during the cropping season (248.5 mm in April, 182.6 mm in May, 38.1 mm in June, and only 7.5 mm in July), compared to 649.6 mm at Kabete (352.6 mm in April, 262 mm in May, 23 in June, and 12 mm in July). Therefore, plants at both sites experienced severe terminal drought.

Location and genotypic effects and their interaction were highly significant for the geometrical mean, GM (Table

TABLE 6 Grain iron and zinc concentration and disease scores of $F_{4.7}$ bean lines grown at Kabete Field Station [Formerly Table 3]

Genotype	Market class	Growth habit[§]	Iron (ppm)	Zinc (ppm)	Disease score[a]			
					Angular leaf spot	Anthracnose	Common bacterial blight	Root rots
BCB11-145	Red mottled	II	136.0	22.5	2	3	2	2
BF-08-13-181	Yellow	IV	105.5	39.5	3	2	3	3
BF-08-1-18	Yellow	IV	98.5	34.8	3	2	2	2
BF-08-7-74	Yellow	IV	96.5	41.2	4	2	2	2
BF-08-1-47	Yellow	IV	96.5	31.5	3	2	2	3
BF-08-16-36	Yellow	IV	92.5	39.2	5	2	3	3
BF-08-36-18	Red mottled	I	92.0	35.2	3	2	2	2
BF-08-7-84	Yellow	IV	91.0	36.5	3	2	2	3
BF-08-36-100	Red mottled	I	88.8	39.2	4	2	3	4
BF-08-1-80	Yellow	IV	88.0	38.8	3	2	2	3
BF-08-36-205	Red mottled	I	84.5	35.5	3	2	2	3
BF-08-36-49	Red mottled	II	82.2	38.8	4	2	3	4
BF-08-36-127	Red mottled	I	78.3	41.3	2	2	2	2
BF-08-13-102	Yellow	IV	78.0	38.5	5	2	3	3
BF-08-13-121	Yellow	IV	77.0	36.8	3	2	3	4
BF-08-3-1	Yellow	IV	76.8	38.8	4	2	2	3
BF-08-26-162	Small red	III	74.2	35.2	4	2	3	4
BF-08-1-90	Yellow	IV	72.5	34	3	2	2	2
BF-08-36-162	Red mottled	I	66.8	31.5	5	2	3	4
Checks								
Nain de Kyondo	Navy	III	105.2	25.8	3	2	2	3
MLB 49/89A	Black	II	83.5	24.2	4	2	3	4
Maharagi Soja	Brown	II	83.2	25.2	5	2	3	5
Mex142	Navy	II	78.5	24.5	6	5	5	2
NUA1	Red mottled	I	69.0	22.2	7	7	3	7
Mean			78.0	27.5	3.9	2.1	2.4	3.2
$LSD_{0.05}$			17.3	3.5	2.6	0.9	1.2	2.2
CV (%)			11.0	6.3				

[a]Based on 1–9 disease scale, where a score of 1–3 is resistant, 4–6 intermediate, and 7–9 is susceptible

[b]Growth habit I = determinate, erect stem; II = indeterminate bush, erect stem; III = Indeterminate with weak and prostrate stem and branches, and IV = indeterminate climbing habit with weak, long and twisted stem, and branches (van Schoonhoven & Pastor-Corrales, 1987).

4). Among the test lines, GM values varied from 397 for BF08-36-140 to 4,262 for BF08-07-21 (Table 5). In contrast, among the check varieties, GM values varied from 308 in CAL96 to 1550 for Gofta. GM values were higher at Kabete (2,306) compared with Thika (1898). GM has been suggested as a useful index for improving drought resistance in common bean (Ramirez-Vallejo & Kelly, 1998). Location effects were significant for drought susceptibility indices, DSI (Table 4). DSI values were higher at Kabete (DSI = 1.125) compared with Thika (DSI = 0.993). The higher DSI at Kabete could partly be due to confounding effects of high disease pressure which occurred during the vegetative phase. BF08-36-140 has the highest DSI value (DSI = 1.77) among the test lines. This line also had the highest RYR (81.8%) and the lowest yield (657 kg/ha), suggesting that it was highly susceptible to drought. BF08-14-82 had the lowest DSI (DSI = 0.38) but a moderate yield (2,313 kg/ha) in drought-stressed and nonstressed conditions.

3.4 | Fe and Zn concentration in $F_{4.7}$ lines

Results showed that there were significant differences ($p < 0.001$) among bean genotypes for grain iron and zinc concentration. Iron concentration varied from 66 to 136 ppm (Table 6). Zinc concentration varied from 10 to 60 ppm. The F_7 BF lines had higher iron and zinc compared to their parents and other check genotypes. The highest iron concentration was recorded on BCB11-145 (136 ppm), BF08-13-181(105.5 ppm), and BF08-1-18 (98.5 ppm). The lowest iron concentration among the F_7 lines was found in BF-08-36-162. Among the parental lines, Nain de Kyondo (105.2 ppm) had the highest grain iron concentration. This parental line originated from Great Lakes region which has been reported to have high diversity for grain mineral concentration (Blair et al., 2010). NUA 1 had the lowest grain iron concentration. NUA1 is a CIAT breeding line selected for high iron concentration and red mottled grain type. Except for NUA1, all the parental check varieties had grain iron levels above the target of 70 pm for the fast-track biofortified bean lines. Seven BF lines attained the higher target of 90 ppm iron set for the second-generation lines. Except for BF-08-36-162, all the new BF lines had higher grain iron concentration than the 70 ppm target for first-generation or fast-track lines, suggesting that selection for iron concentration was effective.

Results showed considerable variation for grain zinc concentration among the advanced lines. BF08-7-74 (41.2 ppm), BF08-36-127 (41.2 ppm), and BF08-13-181 (39.5 ppm) had the highest zinc concentration (Table 3). BCB11-145 had the lowest zinc concentration (22.5 ppm). This line was included among the test genotypes because of its outstanding drought tolerance and the popular red mottled grain type. However, it also had the highest iron concentration. The BF lines had significantly higher zinc concentration compared with the check varieties. However, only two lines attained the high zinc concentration target of 40 ppm. These were BF-08-7-74 and BF-08-36-127. Several BF combined high grain iron and zinc concentration. These included BF08-13-181, BF08-7-74, BF08-16-36, and BF08-36-18. Pearson's correlation analysis showed highly significant positive correlation ($r = 0.439^{***}$) between grain iron and zinc concentration.

3.5 | Reaction to diseases

The alternating wet and dry weather conditions at Kabete Field Station especially during the first half of 2012 long rain season (March–June) were very favorable for disease development. These conditions favored screening study lines for reaction to infection by angular leaf spot (*Pseudocercospora griseola*), anthracnose (*Colletotrichum lindemuthianum*), common bacterial blight caused by *Xanthomonas axonopodis* pv.*phaseoli*, and *Xanthomonas axonopodis pv phaseoli* var. *fuscans*, and bean root rots caused by *Pythium* and *Fusarium* spp. Results showed that there were highly significant differences for reaction to infection by the four diseases among the study lines. The disease score of selected F_7 lines is presented in Table 6. Fifteen of the 18 selected lines showed resistance to angular leaf spot under heavy disease pressure. NUA1, an advanced CIAT line selected for high mineral density in Colombia, was susceptible to angular leaf spot at Kabete Field Station. Among the check varieties, only Nain de Kyondo, which originated from DR Congo, showed a resistant reaction to angular leaf spot. Anthracnose was the most prevalent disease. More than 24 of the test lines succumbed to this disease (scores of 7–9). All the other lines were resistant to anthracnose (Table 6). Among the checks, Nain de Kyondo was susceptible to anthracnose. Mexico 142 showed an intermediate reaction to infection by anthracnose. All the selected lines showed resistant reactions to common bacterial blight and root rot. NUA1 was susceptible to root rot (score of 7) but resistant to common bacterial blight. Nine of the selected lines showed combined resistance to the four diseases under heavy disease pressure. All the other lines showed combined resistance to two or three diseases. In contrast, among the check varieties, only Nain de Kyondo

TABLE 7 Mean squares for grain yield of $F_{4\cdot7}$ bean lines grown at four locations during the 2012 short rain season

Source of variation	*df*	Mean squares
Replicates	2	4,275,000
Locations	3	63,370,000**
Genotypes	109	3,434,000**
Genotypes × locations	327	1,052,000*
Residual	878	851,600

*Significant at 0.05 probability level.
**Significant at 0.01 probability level.

TABLE 8 Grain yield of $F_{4.7}$ bean lines grown at four locations during the 2012 short rain season

Genotype	Kabete	Nakuru	Thika	Tigoni	Mean
	kg/ha				
BF08-7-75	4,791	3,018	3,068	2,417	3,323
BF08-16-82	4,316	2,162	2,872	3,167	3,129
BF08-1-79	4,304	2,448	2,767	2,682	3,050
BF08-13-102	3,979	2,299	2,285	3,477	3,010
BF08-1-90	3,363	2,235	2,698	3,643	2,985
BF08-1-18	4,097	2,916	1,917	2,752	2,920
BF08-1-80	3,600	2,531	2,098	2,973	2,801
BF08-16-36	2,867	2,084	2,295	3,710	2,739
BF08-3-1	3,883	1,541	2,793	2,317	2,633
BF08-13-181	3,645	2,096	2,000	2,017	2,439
BF08-1-47	2,985	2,081	1,997	2,393	2,364
BF08-7-84	2,709	2,468	1,148	2,497	2,206
BF08-13-121	2,027	1,769	2,222	2,697	2,178
BF08-7-74	2,534	2,666	1,393	2,028	2,155
BF08-26-162	2,503	1,598	2,063	2,247	2,103
BF08-36-18	1,820	2,094	1,440	3,023	2,094
BF08-36-162	1,793	2,060	2,443	1,945	2,061
BF08-36-205	1,909	1,990	1,605	2,455	1,990
BF08-36-49	2,254	1,541	1,707	2,032	1,883
BF08-36-100	1,897	1,774	1,858	1,568	1,775
BF08-14-135	1,816	1,110	163	1,532	1,155
BF08-36-127	1,357	1,125	1,140	877	1,125
BF08-36-153	1,697	93	697	1,047	883
BF08-13-216	798	805	508	1,100	803
Checks					
Nakaja	3,455	2,693	1,500	2,635	2,571
AND 620	1,831	1,985	2,103	2,020	1,985
Nain De Kyondo	1,609	2,023	1,480	1,733	1,712
Gofta	879	1,651	1,127	2,947	1,651
MLB49/89A	1,189	1,833	1,342	2,068	1,608
NUA1	1,609	1,590	1,833	1,328	1,590
CAL96	1,004	1,191	1,268	1,302	1,191
Maharagi Soja	772	926	772	1,673	1,036
Mean	2,867	2,076	1,843	2,248	2,258
$LSD_{0.05}$: Locations (L) = 141, Genotypes (G) = 739.4 and GXL = 1,478.8					

showed resistance to three diseases. All other check varieties showed intermediate reactions or susceptibility to two or three of the four diseases.

3.6 | Performance across agro-ecological zones

Analysis of variance indicated highly significant differences ($p > 0.01$) in grain yield due to locations and genotypic effects (Table 7). Grain yield of the genotypes was highest at Kabete (2,867 kg/ha) and lowest at Thika (1,843 kg/ha) (Table 8). The two high altitude test sites (Tigoni, 2131m and Nakuru, 2,070 masl) had higher yields than Thika. Mean grain yield was 2,248 kg/ha at Tigoni and 2,076 kg/ha at Nakuru. Among the test lines, grain yield varied from 803 kg/ha (BF08-13-216) to 3,323 kg/ha (BF08-7-75). Among the check varieties, mean grain yield across sites varied from 1,191 kg/ha (CAL 96) to 1,985 kg/ha (AND 620). This implied that BF08-7-7, the best yielding $F_{4.7}$ line, had a yield advantage of 67% over the best yielding check variety, AND

620. AND 620 is mottled bush variety which originated from CIAT. BF08-7-7 also had a 108.9% yield advantage compared with NUA 1, and 179% over CAL 96 (Table 8). These results suggest a significant yield improvement of the new bean lines compared with commercial varieties and their parents. Part of this superior performance could be attributed to the climbing growth habit of the new lines. AND 620, NUA 1, and CAL 96 have type I bush growth habit. Yield advantage of BF08-7-7 over the best climbing bean check variety, Nakaja, was 29.2%. The significant genotype × location interaction suggests differential response of the test genotypes to environmental conditions in study sites. Thus, BF08-16-42 was the best yielding line at Kabete (4,996 kg/ha), BF08-26-163 at Nakuru (3,654 kg/ha), BF08-01-08 at Thika (3,095 kg/ha), and BF08-16-36-36 at Tigoni (3,710 kg/ha).

4 | DISCUSSION

Breeding micronutrient-rich bean varieties in eastern Africa started in 2001 at the University of Nairobi as part of a regional effort supported by the Association of Strengthening Agricultural Research in East and Central Africa, ASARECA (Kimani & Karuri, 2001). More than 2,800 landraces were screened for grain iron and zinc concentration (Kimani et al., 2008). A few lines, which had over 70 ppm iron and more than 30 ppm, and met minimum agronomic criteria such as productivity and tolerance to diseases, were fast tracked and independently validated by variety release regulatory agencies. These fast-track lines were distributed to more than 25 collaborating countries in east, central, southern, and west Africa. Many countries have released these first-generation lines. For example, seven varieties were eventually formally released in 2012 in Kenya as the first biofortified bean varieties in this region (Kimani et al., 2016). However, several lines such as Gofta, Nakaja, MLB 49-89A, and AND 620, though high in micronutrients were deficient in important traits such as tolerance to drought, disease resistance, high yield potential, and commercial grain types desired by producers and consumers. These lines were therefore used as parents in this study to develop a second-generation biofortified bean varieties with better expression of key traits. Results of this study show that the new lines are not only more drought tolerant, but also have higher yield potential and multiple disease resistance. The superior performance of the new lines is probably due to transgressive segregation for agronomic traits and incorporation of new genes for disease resistance such as *co-4, co-4*^{2} and *co-5* genes found in G2333 (Pastor-Corrales et al., 1994), *phg* genes in Mex 54 (Namayanja et al., 2006), and root rot resistance and low soil fertility tolerance from RWR719 and AND 1,055 (CIAT, 2008), which were used in the development of male gametes in this study.

Results from this study compare well with the findings of other researchers. Among a core collection of over 1,000 bean genotypes, Beebe et al. (2000) found that grain iron concentration varied from 89 to 34 ppm, while zinc concentration varied from 54 to 21 ppm. Kimani et al. (2006) reported a range of between 59 and 131 ppm of iron, and 12 and 62 ppm of zinc among bean cultivars collected from eastern Africa. The significant correlation between the two elements found in this study ($r = 0.439^{**}$, $p < 0.001$) was also reported by previous studies. Tryphone and Nchimbi-Msolla (2010) found a correlation of $r = 0.416^{**}$ ($p < 0.001$). In another study, Zacharias et al. (2012) reported a correlation between iron and zinc concentration of $r = 0.87^{*}$ ($p < 0.05$). These positive and significant correlations suggest that genetic factors that increase Fe concentration cosegregate with genetic factors that increase Zn concentration. Blair et al. (2009) found that the two minerals were represented by a similar total number of quantitative trait locus (QTL) which colocalized together. They also found that inheritance of the two minerals was mainly controlled by additive genes. This may explain observed higher iron and zinc concentrations among BF lines which originated from crosses among parents with high and low grain iron and zinc concentration. Results also indicated that the higher targets for grain iron (90 ppm) and zinc concentration (40 ppm) appear achievable. They also indicate that for breeding programs where iron concentration is the primary selection criteria, an increase in zinc concentration can also be expected. Results of this study indicate that pyramiding genes of well-characterized parents from diverse gene pools and races of common bean can be a useful strategy for developing more productive varieties combining nutritional quality with multiple resistance to biotic and abiotic stresses and commercial grain types.

5 | CONCLUSION

Forty-seven new multiparent populations were created at the University of Nairobi by combining sources of high mineral trait, tolerance to drought, low soil fertility tolerance, resistance to major bean diseases, and commercial grain types. The populations showed wide variation for grain iron and zinc concentration, growth habit, plant vigor, drought tolerance, resistance to angular leaf spot, anthracnose, root rots and common bacterial blight, and other agronomic traits. Eighty-four lines were selected from eight outstanding populations. These lines were more drought tolerant, had consistent resistance to diseases across sites and seasons, and had more than 90% better yield compared to their parents. The 40 lines had 50% higher yield under controlled stress and no-stress conditions compared with the parental lines, suggesting transgressive segregation. Their high

yield potential was attributed to transgressive segregation, which was expressed as heavy pod load, plant vigor, indeterminate growth habit, and possession of genes conditioning resistance to diseases and drought. Mineral analyses of 46 promising lines showed that the new BF lines had higher grain iron and zinc concentration compared with their parents and the first-generation micronutrient-rich varieties. The second-generation lines can contribute not only to increased bean productivity, but also combating micronutrient deficiencies in eastern Africa and other parts of Africa.

ACKNOWLEDGMENTS

This research was supported by ASARECA, Bioinnovate Network, SIDA (Sweden), and the Government of Kenya. Their support is gratefully acknowledged. We sincerely thank Cathy Baker and Vivienne Anthony of the Syngenta Foundation for Sustainable Agriculture for their invaluable suggestions during the writing of this study.

CONFLICT OF INTEREST

None declared.

ORCID

Paul M. Kimani https://orcid.org/0000-0002-5770-256X

REFERENCES

AOAC. (2000). *Official methods of analysis of the Association of Official Analytical Chemists*, 17th ed. Gaithersburg, MD: AOAC.

Banzinger, M., & Long, J. (2000). The potential for increasing the iron and zinc density of maize through plant breeding. *Food and Nutrition Bulletin*, *21*, 397–400.

Beebe, S., Gonzalez, A. V., & Rengifo, J. (2000). Research on trace minerals in common bean. *Food and Nutrition Bulletin*, *21*, 387–391.

Blair, M., Astudillo, C., Beebe, S., Roa, I., Kimani, P. M., & Chirwa, R. (2009). Biofortification breeding of common bean (*Phaseolus vulgaris L.). Biozoom*, *1*, 25–28.

Blair, M., Gonzalez, L., Kimani, P. M., & Butare, L. (2010). Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (*Phaseolus vulgaris* L.) from Central Africa. *Theoretical and Applied Genetics*, *121*, 237–248. https://doi.org/10.1007/s00122-010-1305-x

Bouis, H. E., Graham, R. D., & Welch, R. M. (2000). The Consultative Group on International Agricultural Research (CGIAR) micronutrients project: Justification and objectives. *Food and Nutrition Bulletin*, *21*, 374–381.

Buruchara, R. A., & Camacho, L. (1999). Common bean reaction to *Fusarium oxysporum f.sp. phaseoli*, the cause of severe vascular wilt in Central Africa. *Phytopathology*, *148*, 39–45. https://doi.org/10.1046/j.1439-0434.2000.00457

Chavez, A. I., Bedoya, J. M., Sanchez, T., Iglesias, C., Ceballos, H., & Roca, W. (2000). Iron, carotene and ascorbic acid in cassava roots and leaves. *Food and Nutrition Bulletin*, *21*, 410–413.

CIAT (2008). *Breeding micronutrient dense bean varieties in eastern Africa*. Bean Improvement for the Tropics, Annual Report I-P1 Report, Cali, Colombia, pp. 15–25.

Development Initiatives (2017). *Global nutrition report 2017: Nourishing the SDGs*. Bristol, UK: Development Initiatives.

Fischer, R. A., & Maurer, R. (1978). Drought resistance in spring wheat cultivars I. Grain yield responses. *Australian Journal of Agricultural Research*, *29*, 897–912. https://doi.org/10.1071/AR9780897

Fritz, J. S., & Schenk, G. H. (1971). *Quantitative analytical chemistry*. (pp. 187–189 and 600–603). Boston, MA: Allyn and Bacon Inc.

GOK (2012). *Food and nutrition security policy*. Kilimo House, Republic of Kenya, Nairobi, Kenya: Agriculture Coordination Unit (ASCU).

Gregorio, B. N., Senadhira, D., Htut, H., & Graham, R. D. (2000). Breeding for trace mineral density in rice. *Food and Nutrition Bulletin*, *21*, 382–386.

Hagenimana, V., & Low, J. (2000). Potential of orange-fleshed sweet potatoes for raising vitamin A intake in Africa. *Food and Nutrition Bulletin*, *21*, 414–418. https://doi.org/10.1177/156482650002100414

Jaetzold, R., Schmidt, H., Hornetz, B., & Chisanya, C. (2006). *Farm Management Handbook of Kenya, 2 ed. Volume 2: Natural conditions and farm management information of Central Kenya, Ministry of Agriculture, Kenya*. Nairobi, Kenya: Cooperation with the German Agency for Technical Cooperation (GTZ).

Kimani, P. M. (2005). Fast tracking of nutritionally-rich bean varieties. Highlights No 24, CIAT in Africa. http://ciat-library.ciat.cgiar.org/Articulos_ciat/Highlight24.pdf [accessed March 2012].

Kimani, P. M., & Karuri, E. (2001). Potential of micronutrient dense bean cultivars in sustainable alleviation of Fe-Zn malnutrition in Africa. In: *Novel Plant breeding Approaches to fight micronutrient deficiencies*. International Center for Tropical Agriculture (CIAT), Bill and Melinda Gates Foundation and the Micronutrient Initiative. Cali, Colombia: CIAT.

Kimani, P. M., Karuri, E., & Mwaura, S. (2006). Iron, zinc and protein concentration in African bean cultivars. *Bean Improvement Cooperative (BIC)*, *49*, 155–156.

Kimani, P. M., Beebe, S., & Blair, M. (2003). *Iron and zinc variation in African bean cultivars and landraces*. Annual Report, CIAT, Cali, Colombia.

Kimani, P. M., Beebe, S., Blair, M., & Mamiro, P. (2008). *Breeding micronutrient dense bean eastern Africa. Bean Improvement for the Tropics*. Annual Report I-P1. CIAT, Cali, Colombia.

Kimani, P. M., Buruchara, R. A., Ampofo, K., Pyndji, M., Chirwa, R. & Kirkby, R. (2005). Breeding bean for smallholder farmers in Eastern, Central and Southern Africa: Constraints, achievements and potential. Proc. Pan-African Bean Research Alliance (PABRA) Millennium Workshop, 28 May – 2 June 2001, Arusha, Tanzania, pp. 11–28.

Kimani, P. M., Chirwa, R., Beebe, S., Buruchara, R., Pyndji, M., & Blair, M. (2011). *Breeding micronutrient dense varieties in eastern Africa*. Agro2011 Inaugural Biennial Conference, 26-28 Sepetember 2011 Kenya: University of Nairobi.

Kimani, P. M., Mamiro, P., Ugen, M., & Musoni, A. (2016). Development and release of new biofortified bean varieties in eastern Africa. *Bean Improvement Cooperative*, *59*, 147–148.

Lunze, L., Kimani, P. M., Ngatoluwa, R., Rabary, B., Rachier, G. O., Ugen, M. M., … Awad Elkarim, E. E. (2007). Bean improvement for low soil fertility adaptation in Eastern and Central Africa. In A. Bationo, B. Waswa, J. Kihara, & I. Kimetu (Eds.), *Advances*

in integrated soil fertility management in sub-Saharan Africa: Challenges and opportunities, tropical soil biology and fertility (TSBF) Institute of the International Centre for Tropical Agriculture (TSBF-CIAT) (pp. 325–332). AA Dordrecht, The Netherlands: Springer.

Mahuku, G. S., Iglesias, Á. M., & Jara, C. (2009). Genetics of angular leaf spot resistance in the Andean common bean accession G5686 and identification of markers linked to the resistance genes. *Euphytica*, *167*, 381–396. https://doi.org/10.1007/s10681-009-9897-4

Maziya-Dixon, B., Kling, J. G., Menkir, A., & Dixon, A. (2000). Genetic variation in total carotene, iron and zinc contents of maize and cassava genotypes. *Food and Nutrition Bulletin*, *21*, 419–422. https://doi.org/10.1177/156482650002100415

McIntosh, M. S. (1983). Analysis of combined experiments. *Agronomy Journal*, *75*, 153–155.

Monasterio, I., & Graham, R. D. (2000). Breeding for trace minerals in wheat. *Food and Nutrition Bulletin*, *21*, 392–396. https://doi.org/10.1177/156482650002100409

Mukankusi, C. M., Amongi, W., Sebuliba, S., Musoke, S., & Acam, C. (2018) Characterisation of *Phaseolus coccineus* interspecific germplasm accessions for disease resistance, grain market class and yield attributes. *African Crop Science Journal*, *26*, 117–135. https://doi.org/10.4314/acsj.v26i1.9

Musoni, A., Kimani, P., Narla, R. D., Buruchara, R., & Kelly, J. (2010). Inheritance of fusarium wilt (*Fusarium oxysporum*F. sp. *phaseoli*) resistance in climbing beans. *African Journal of Agricultural Research*, *5*, 399–404.

Namayanja, A., Buruchara, R., Kimani, P. M., Rubaihayo, P., Mahuku, G., Mayanja, S., & Eyedu, H. (2006). Inheritance of resistance to angular leaf spot in common bean and validation of resistance linked markers for marker assisted selection outside the mapping population. *Euphytica*, *151*, 361–369.

Ndegwa, A. M., Muchui, M. N., Wachuri, S. M., & Kimamira, J. N. (2009). *Evaluation of introduced snap bean (*Phaesolus vulgaris *L.) varieties for adaptability and pod quality*. KARI-CIAT report, 4p.

Otsyula, R. M., Buruchara, R. A., Mahuku, G., & Rubaihayo, P. (2003). Inheritance and transfer of root rot (*Pythium*) resistance to bean genotypes. *African Crop Science Society*, *6*, 295–298.

Pastor-Corrales, M. A., Erazo, O. A., Estrada, E. I., & Singh, S. P. (1994). Inheritance of anthracnose resistance in common bean accession G 2333. *Plant Disease*, *78*, 959–962. https://doi.org/10.1094/PD-78-0959

Pfeiffer, W. H., & McClafferty, B. (2007). Biofortification: Breeding micronutrient-dense crops. Chapter 3. In M. S. Kang, & P. M. Priyadarshan (Eds.), *Breeding major food staples for the 21st century* (pp. 61–91). Blackwell Scientific: Oxford, UK.

Ramirez-Vallejo, P., & Kelly, J. D. (1998). Traits related to drought resistance in common bean. *Euphytica*, *99*, 127–136.

Rao, I. M., Polania, J. A., Ricaurte, J., & Rangel, A. F. (2008). *Phenotyping protocol for evaluation of beans under drought stress* (14p). Cali, Colombia: CIAT.

Singh, S. P. (1994). Gamete selection for simultaneous improvement of multiple traits in common bean. *Crop Science*, *34*, 352–355. https://doi.org/10.2135/cropsci1994.0011183X003400020008x

Stangoulis, J. (2010). *Technical aspects of zinc and iron analysis in biofortification of the staple food crops, wheat and rice*. Proceedings of the 19th World Congress of Soil Science, Soil Solutions for a Changing World 1–6 August 2010, Brisbane, Australia, pp. 42–44.

Tryphone, G. M., & Nchimbi-Msolla, S. (2010). Diversity of common bean (Phaseolus vulgaris L.) iron and zinc contents under screenhouse conditions. *African Journal of Agricultural Research*, *5*, 738–747. https://doi.org/10.5897/AJAR 10.304

Teran, H., & Singh, S. P. (2002). Comparison of sources and lines selected for drought resistance in common bean. *Crop Science*, *42*, 64–70. https://doi.org/10.2135/cropsci2002.6400

van Schoonhoven, A., & Pastor-Corrales, M. A. (1987). *Standard system of the evaluation of bean germplasm* (54p). Cali, Colombia: CIAT.

Wahome, S. W., Kimani, P. M., Muthomi, J. W., Narla, R. D., & Buruchara, A. (2011). Multiple disease resistance in snap bean genotypes in Kenya. *African Crop Science J*, *19*, 289–302.

Welch, R. M., & Graham, R. D. (2004). Breeding for micronutrients in staple food crops from a humannutrition perspective. *Journal of Experimental Botany*, *55*, 353–364.

Zarcinas, B. A., Cartwright, B., & Spoucer, L. R. (1987). Nitric acid digestion and multi-elemental analysis of plant material by inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis*, *18*, 131–146.

Zacharias, J., Leilani, A., Jacob, D., Miklas, P. N., & Hossain, K. G. (2012). Genetic variability of mineral composition in common bean seed. *Annual Report of the Bean Improvement Cooperative*, *55*, 59–60.

How to cite this article: Kimani PM, Warsame A. Breeding second-generation biofortified bean varieties for Africa. *Food Energy Secur*. 2019;8:e00173. https://doi.org/10.1002/fes3.173