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IRON AND ZINC GRAIN CONCENTRATIONS DIVERSITY AND AGRONOMIC PERFORMANCE OF COMMON BEAN GERMPLASM COLLECTED FROM EAST AFRICA

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ABSTRACT

Iron and zinc are essential micronutrients for normal human growth and development and are commonly deficient in diets of the most vulnerable. Common bean (*Phaseolus vulgaris* L.), one of the leading staple foods in East and Central Africa, is a valuable source of quality protein and micronutrients, specifically iron, zinc, and vitamins. Natural variation in micronutrient concentration exists among bean germplasm. Identification of varieties with high iron and zinc seed concentration (FESEED/ZNSEED) for promotion in food systems and utilisation in breeding programs is one strategy of addressing the problem of malnutrition in Africa. Three hundred and four lines sourced from the International Centre for Tropical Agriculture (CIAT) and its partners through the Pan Africa Bean Research Alliance (PABRA), were evaluated for agronomic traits, disease response, yield, FESEED, and ZNSEED. They were organized in four groups; PABRA fast track, Rwanda seeds of hope, HarvestPlus regional nutrition nursery and Uganda collection. Six checks were included; a universal high FESEED climbing bean (MIB465), low FESEED regional climbing bean (Decelaya), universal low FESEED bush bean (DOR500), regional high FESEED bush bean (RWR2154), and two yield checks (CAL96 and Vuninkingi for bush and climbing bean). The FESEED checks were selected based on their relative performance to other genotypes in several experiments by a community of bean breeders through the H+ program. Field trials were established at the National Agricultural Laboratories, Kawanda from 2011 to 2013. Days to maturity and flowering, vigor, yield, and reaction to diseases were evaluated. Micronutrient analysis was conducted using X-ray Fluorescence (XRF) and data confirmed using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Lines were significantly different ($P \leq 0.05$) in all the parameters assessed. FESEED and ZNSEED varied highly between seasons and among the entries ranging between 36-90 ppm and 24-47 ppm across the four nurseries. Twenty-six lines were selected as high iron beans (HIBs) based on XRF data; 12 of these were confirmed as HIB based on ICP data that is more accurate. Of these, two bush beans, Jesca (large purple speckled) and RW547 (medium grey) and two climbing beans, CAB2 (medium white seeded) and Ndimirakaguja (small cream) were the most superior in FESEED across seasons. With the exception of CAB2, these lines were relatively high yielding >2000 $kgha^{-1}$. There was no significant correlation of FESEED or ZNSEED to yield. Nonetheless, FESEED and ZNSEED positively (0.59) correlated indicating that selection for superiority in one variable would result in a high value in the other.

Key words: Common bean**,** Micronutrients, Anemia, Genetic variation, Yield, Diseases

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INTRODUCTION

Malnutrition is a major contributor to infant mortality in sub-Saharan Africa [1]. Deficiency of micronutrients such as vitamin A, zinc (Zn) and iron (Fe) affect at least half of the world's population [2]. In 2008, Horton [3] also reported that malnutrition contributes to over a third of child deaths in the world. Iron deficiency anemia is prevalent worldwide and occurs in both industrialized and developing countries [4]. According to the World Health Organization (WHO) (2008), the highest proportion of individuals affected by Anemia, which is evidently linked to poverty, is in Africa (48-68 %), followed by South-East Asia (46-66 %); but the greatest number affected is in the latter (315 million) and not the former (171 million) [5]. The use of diverse micro-nutrient rich diets has not been effective considering that the affected people are poor [6]. They may not afford a diverse nutritious diet, and many are also uneducated hence may be unaware of the food options for a balanced diet. Malnutrition is also a problem among the urban-rich, probably due to limited knowledge of nutrition. Utilization of biofortified crops, those improved for nutritional quality, in diets provides a more feasible and sustainable option among such groups of people [6]. The common bean (*Phaseolus vulgaris* L.) is a leading staple after maize in East and Central Africa [7]. It is a cheap source of quality protein (20-28 %), energy (32 %), fibre (56 %) and micronutrients, especially iron (70 mg/kg), zinc (33 mg/kg), and vitamin A, that enhance normal body and mental growth and development. The crop is the world's most important legume food crop [8]. It has the potential to alleviate malnutrition and hunger-related problems because it is affordable and rich in nutrients. The crop feeds over 100 million people in the poor communities in Africa thus plays a significant role in human nutrition and livelihood [9, 10]. Additional health benefits were reported by US Dry Bean Council in the reduction of the development of heart disease, breast, and colon cancers [11].

High genetic diversity for micronutrients has been reported to occur naturally among bean germplasm [12, 13]. A few African countries have released farmer-preferred bean varieties introduced from elsewhere or improved/ collected locally, with comparatively higher Fe and Zn concentration in the grain among their routinely consumed bean types and among newly developed varieties [9, 14]. However, even though some of the East African countries have released biofortified varieties, the FESEED levels for most of them are well below the target of 94 ppm. Iron beans with the full target level, 94 ppm of iron, provide 127 % and 80 % of daily estimated average requirements of children and women respectively [15]. This paper is an attempt to provide a baseline of the Fe and Zn levels in existing African bean germplasm to inform bean breeding programs that do biofortification. Past studies have shown the potential to exploit genetic variation in seed concentration of iron and other minerals without the general negative effect on yield [16, 17, 18]. This relationship is particularly positive in mineral-deficient soils. Seed Fe and Zn concentrations have been shown to be significantly influenced by the environment [19, 20]. Bean genotypes that maintain relatively high micronutrient levels in comparison to others in different environmental conditions are preferred. To date, released varieties have a range of 55-110 ppm Fe concentration with the majority having concentration below 70 ppm necessitating the need to continue developing high-Fe concentration varieties [21, 22]. This study sought to identify potential genotypes for possible

promotion as high iron and zinc beans, and utilization in hybridization programs targeting high Fe and Zn, productivity and market traits.

MATERIALS AND METHODS

Site characteristics

Field experiments were conducted at the CIAT Uganda station based at the National Agricultural Research Laboratories (NARL) located in Kawanda, Wakiso District at 32° 31'E, 0°25′N in 2011, 2012 and 2013. The site has two rainy seasons within a year which have been denoted as "a" and "b", where "a" is the first rainy season which starts in March and ends in June, and "b" is the second rainy season which starts in September and ends in December. The Institute's elevation is 1190 m above sea level and it receives mean annual precipitation of 1200 mm. Its temperature ranges from 15 ^{o}C to 30 ^{o}C [23]. Results from the Soil and Plant Analytical Laboratories at Kawanda show that the soil has very high Fe concentration and relatively high calcium (Ca) and magnesium (Mg), in comparison to the critical values, but it is limited in potassium (K), phosphorus (P) and zinc (Zn). The soil is slightly acidic (low pH) for beans (Table 1).

Genetic materials assessed

Four common bean nurseries grouped according to their source and time of compilation were evaluated. The nurseries include: i)14 lines from the fast track biofortification nursery of the Pan Africa Bean Research Alliance (PABRA), ii) 61 landraces from Rwanda collected under the seeds of hope (SOH) project, iii) 42 lines from the regional nutrition nursery complied under HarvestPlus (H+) program, and iv) 187 lines from Uganda.

The PABRA fast track biofortification nursery evolved from the first efforts of PABRA to identify micronutrient-rich bean germplasm in 1996, among regionally grown varieties in Africa. The 14 materials evaluated in this study were a subset of the complete nursery that included 38 lines (≥ 70 ppm FESEED and 25-30 ppm ZNSEED), that were massively distributed within the PABRA network and a number of them officially released, notably in, Tanzania, Burundi, Rwanda, and Kenya. The SOH nursery included lines collected from Rwanda after the 1994 genocide purposely to conserve the locally adapted bean germplasm. Previous evaluation of Fe and Zn concentration had been conducted on these materials and based on that data, 98 materials, which contained ≥70 ppm FESEED and 25-30 ppm ZNSEED, were selected as high iron bean (HIB). In 2009, the seed was regenerated but only 61 lines were able to germinate after being stored for more than 10 years at CIAT-Kawanda. On the other hand, the regional nutrition nursery was developed from the efforts of the HarvestPlus program working with CIAT, IFPRI, and Rwanda Agriculture and Animal Resources Development Board (RAB) and INERA in 2009. This nursery comprised both bush and climbing bean lines from Rwanda and DRC that had been identified through the HarvestPlus program in addition to a number of materials released as high Fe in the second generation PABRA nursery (materials developed from targeted crossing programs aimed at increasing Fe levels), local landraces from Rwanda and the local bean breeding programs of Rwanda and DRC. The Uganda germplasm collection comprised 153 bean landraces, 17 officially released varieties, 15 pre-released varieties and two universal yield checks sourced from the

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Uganda National bean breeding program at the National Agricultural Crops Research Resources Institute (NaCRRI).

Trial establishment

Trials were set up over a three-year period, 2011, 2012 and 2013. The SOH nursery was evaluated during three seasons including; the first and second seasons of 2011 (2011a and 2011b), and the first season of 2012 (2012a). Thereafter, 26 lines were selected and evaluated in 2012b and 2013a from which six lines were selected. The regional nutrition nursery with 42 lines was evaluated in two seasons (2012b and 2013a) from which eight lines were selected. The PABRA fast track nursery was evaluated as a 14-line set in 2011b and, thereafter, eight lines were selected and evaluated in 2012a from which six were selected. The Uganda germplasm collection was evaluated for one season (2011b) and ten lines were selected. In 2013b, all the 26 selected lines from the four nurseries were evaluated as a single nursery with common checks. For all the trials, the variety CAL96 was used as the main local yield and low Fe check, DOR500 as a universal low Fe check and MIB465 as the universal high Fe check.

Field experimental designs

Different field experimental designs were utilized for each of the nurseries which were conducted separately but contained the same checks: a universal high Fe climbing bean (MIB465), low Fe regional climbing bean (Decelaya), universal low Fe bush bean (DOR500), regional high Fe bush bean (RWR2154), and two yield checks (CAL96 for bush beans and Vuninkingi for climbing beans). The FESEED checks were selected based on their relative performance to other genotypes in several experiments. Climbing beans were also evaluated separately from the bush beans. The alpha lattice design was used for the SOH, regional nutrition nursery and the Uganda collection nursery in all seasons with three replications. The PABRA fast track nursery had few lines (15) which were planted in a Randomized Complete Block Design (RCBD) with three replications. For all trials, plot sizes were 3 rows of 3 meters in length per entry. A spacing of 60 cm between rows and 10 cm within rows was used for climbers and 50 cm x 10 cm for bush beans. Each trial was weeded twice and an insecticide, Dimethoate and two fungicides (Mancozeb and Ridomil) were applied weekly until flowering. The recommended manufacturer's rate was used for each pesticide. Granular N:P:K 17:17:17 fertilizer was hand applied just before planting at the rate of 125 kg ha^{-1}.

Data collection

Agronomic data

Data was collected on yield performance and growth parameters at specific intervals based on the trait dictionary [24]. Days to flowering and physiological maturity were recorded as the number of days from planting to the day when 50 % of plants had at least one flower and number of days from planting to the day when the first pod began to discolor in 50 % of the plants respectively [25]. Growth vigor was recorded on 1-9 scale where 1 = Excellent, 3 = Good, 5 = Intermediate, 7 = Poor, 9 = Very poor [25]. Seed collection for yield began when 90 % of the pods had changed colour [26]. The seeds were sun-dried before recording total and clean seed weight per plot (g).

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Field disease incidence and severity

Response to occurring field diseases, specifically angular leaf spot (ALS), common bacterial blight (CBB) and bean common mosaic virus (BCMV) was assessed using a CIAT disease evaluation scale of 1-9 [24, 25]. Ranking of disease reaction was done as follows; 1-3 = Resistant: No visible symptoms or very light symptoms, 4-6 = Intermediate: Visible and conspicuous symptoms resulting only in limited economic damage, 7-9 = Susceptible: Severe to very severe symptoms causing considerable yield losses or plant deaths. Plants with black rot (BCMNV-bean common mosaic necrotic virus) were also counted.

Fe and Zinc seed concentration determination

Bean lines in SOH nursery were evaluated for Fe and Zn concentration in the second (2011b) and first seasons (2012a) of 2011 and 2012, PABRA fast track and Ugandan collections were only evaluated in 2011b and regional nutrition nurseries in 2012b before the confirmatory trial in 2013b.

For each nursery, a sampling protocol, authored by Stangoulis and Sison in 2008 was followed [27]. Twenty-five well-filled pods hanging above the soil from each plot were randomly sampled, and placed in new clean envelops, before the main harvest. The pods were hand threshed, and a seed sample weighing about 200 g per plot was obtained [27]. They were cleaned with distilled water to remove any soil contamination, packed in new paper bags and sent to Rubona Agriculture Research Station (RARS), of the Rwanda Agriculture and Animal Resources Development Board for analysis of Fe and Zn concentrations using the Oxford instruments X-Supreme 8000 energy dispersive X-ray Fluorescence (XRF) model. The 200 g seed samples were each subdivided into 10 smaller samples of 15-20g, prepared and analyzed using the method described by Mukamuhirwa *et al*. [28]. The XRF technology is less sensitive although it is non-destructive, requires no dissolution and has good precision for major elements [29]. This makes it appropriate for the analysis of large samples.

Lines with high Fe and Zn concentration levels selected based on XRF data were replanted in replicated trials with the yield and Fe checks. Harvesting was conducted as previously described and 5-10 g samples obtained per plot. Samples were shipped to Waite Analytical Services in Adelaide, Australia for confirmatory tests using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) [30]. The samples were oven dried at 80ºC and milled using a Fritsch Rotor and digested using a nitric/perchloric acid mixture on a programmable digestion system in open glass tubes [31]. They were then analyzed using Method-3BR by Radial CIROS ICPAES. Duplicate analyses, which give an indication of the homogeneity of the sample, were also carried out. Inductively Coupled Plasma (ICP) determines many elements quickly and with great accuracy [32]. However, it is destructive, expensive and in most cases requires dissolution.

Data analysis

Data were analyzed separately to assess within-season variability before performing combined analyses. They were subjected to analysis of variance (ANOVA) using GenStat [33]. Where ANOVA revealed significant differences, Fisher's protected least

significant difference (LSD) was obtained to separate the treatment means. Simple correlation coefficients among some traits were determined using replication means in GenStat [33].

RESULTS AND DISCUSSION

This study highlights the wide variability of grain iron concentration among regionally grown bean germplasm in East Africa. The germplasm evaluated included landraces that are routinely grown by farmers, released varieties in a number of countries and breeding lines that are utilized by plant breeders to improve different traits. High differences were shown to exist among genotypes for measured variables.

Variation of Fe and Zn grain concentration among the evaluated lines

There were significant differences ($P \leq 0.001$) in FESEED and ZNSEED of the evaluated germplasm among the different nurseries, indicating diversity. Similarly, FESEED and ZNSEED varied among the germplasm across seasons. The coefficient of variation (% CV) ranged from 2.0 to 8.6 for FESEED and 3.0 to 7.6 for ZNSEED. Coefficients of variation were calculated as the ratio of the standard deviation to the mean measure the dispersion of data points in the data series. The low values reflect a low degree of dispersion in both FESEED and ZNSEED datasets suggesting a good model fit.

The SOH lines were collected from Rwanda after the 1994 genocide. Among them, 15 entries showed FESEED ≥ 75 ppm in 2011b while 28 had FESEED ≥ 75 ppm in 2012a (Table 2). Across season means ranged from 61 to 90 ppm with a grand mean of 73 ppm for Fe, and 30 to 34 ppm with a grand mean of 36 ppm for Zn; 13 entries including; RW439, RW500, RW547, RW580, RW582, RW721, RW806, RW839, RW849, RW880, RW1087a, RW1179 and RW1180 had FESEED ≥ 75 ppm in the both seasons. However, only six of these entries namely: RW547, RW839, RW849, RW580, RW582, RW1180 had FESEED greater than the concentration obtained in the high iron check (MIB 465) in both seasons. In addition, these lines also had high ZNSEED (Table 2). Rwanda released seven locally bred and three CIAT bred HIB varieties in 2010 and 2011 with 71-91 ppm of FESEED [34]. The target increment (47-94 %) for FESEED in each of the released varieties was not achieved [34]. This suggests that more efforts are still needed in breeding for micronutrient dense beans. Although the FESEED of the six identified lines are just within the range of the already released varieties, they could still be promoted as HIB, and utilized in varietal development especially if they possess other preferred market traits. The use of wild relatives of beans and several breeding methods have resulted in lines with 92 – 102 ppm [22, 35, 36]. These could be utilized to provide more genetic gains.

Among the 14 PABRA fast-track lines**,** two varieties, Nain De Kyondo (89 ppm FESEED) and Jesca (82 ppm FESEED) had the highest FESEED significantly different ($P \leq 0.05$) from 75 ppm obtained for the high FESEED check, MIB 465 (Fig. 1). The FESEED for three other lines including Roba1, LMB49, and NABE3 were close to and not significantly different from that of MIB465. The ZNSEED and FESEED ranged from 27.4 ppm to 39.9 ppm and 44 ppm to 89 ppm, respectively (Fig. 1). Of the superior lines, Nain De Kyondo was released in the Democratic Republic of Congo in 2013 as HIB [34]

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while Jesca and Robai.1 are popular varieties in Tanzania but are not being promoted as HIB. These data provide evidence that could be used to promote these varieties as biofortified beans in Tanzania. Roba1 has been promoted in Uganda for high iron grain concentration and tolerance to multiple pests [37]; NABE3 (MCM2001) was released in Uganda for high yield and tolerance to BCMNV and this additional property of high FESEED offers traction to its promotion.

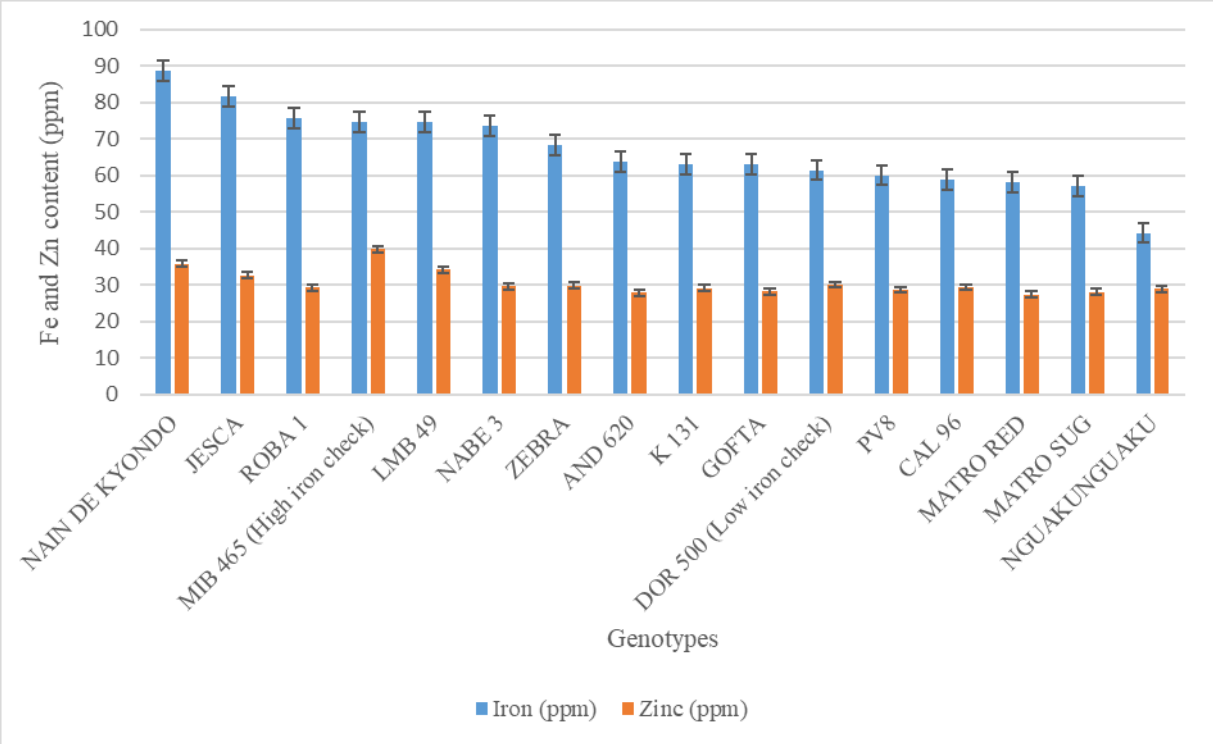

Figure 1: Variation of Fe and Zn grain concentration among PABRA fast-track lines (2012a)

Iron seed concentrations (FESEED) ranged from 56 ppm (SER30) to 76 ppm (MIB465), and ZNSEED from 24 ppm (RWR3042) to 34 ppm (DOR500) among the bush beans in the regional nutrition nursery (Table 3). Five entries had FESEED not significantly different from the 76 ppm that was obtained in the universal high FESEED check, MIB465. They included, ECAB0019 (75 ppm), KAB06F2-8-27 (74 ppm), RWR2076 (70 ppm) and KAB06F2-8-12 (69.9 ppm). For the climbing beans, FESEED ranged from 63 ppm (Icaya) to 89 ppm (Ndimirakaguja) while ZNSEED ranged from 26 ppm (MAC9) to 42 ppm (RWV3006). The ZNSEED obtained in RWV3006 was significantly higher than that obtained in CAB2 (37 ppm), the high FESEED check and in all other entries (Table 3). The entries Ndimirakaguja and RWV1129 (82 ppm) had significantly ($P \leq 0.05$) higher FESEED than all entries including the high FESEED check, CAB2 (76 ppm) whereas four other lines including; MBC32 (76 ppm), MAC74 (75 ppm), RWV3006 (74 ppm), RWV3316 (74 ppm) had relatively equal FESEED to CAB2 (Table 3). Several of the lines in this collection have been released as HIB in Africa [34]. However, their iron concentrations are lower than those in newly developed lines. Some of the lines have also been phenotyped for common biotic stresses, and broad and narrow resistance have been observed [38, 39]. There is potential to increase the FESEED by

utilizing wild relatives of beans or HIB that are continuously being developed [40], and improve tolerance to plant stresses. In addition, the means for bush (FESEED = 65 ppm, ZNSEED = 30 ppm) and the climbing bean (FESEED = 71 ppm, ZNSEED = 32 ppm) lines show that climbing beans recorded significantly higher FESEED compared to the bush beans. The use of climbing beans as a source of high Fe, biofortification studies seem to suggest they could have higher FESEED levels [41, 42]. Climbers are known to yield three times more than bush beans in land-constrained areas and are relatively more tolerant to biotic and abiotic stresses, making them an attractive option for small-scale farmers.

Among the lines collected from Uganda, FESEED ranged from 36 ppm to 89 ppm while ZNSEED ranged from 25 ppm to 40 ppm. Ten lines with FESEED > 74 ppm (Table 4) were selected for further testing. The ZNSEED in all the selected lines were > 30 ppm (Table 3). The overall performance revealed that 13 lines had FESEED ranging from 35 ppm to 50 ppm, 160 lines had FESEED ranging from 51 ppm to 71 ppm lines and 15 lines had 70-89 ppm. Forty-two lines had ZNSEED ranging from 20 ppm to 30 ppm, 104 lines from ranging from 31 ppm to 35 ppm, and 41 lines from 36 and 40 ppm. Results from this study show the existence of several HIB beans among farmers in Uganda but the majority of these lines succumb to prevailing pests, diseases, and several abiotic stresses. Considering their market preference, the identified superior lines could be considered for both biofortification and plant stress improvement. Six HIBs with FESEED of 64 to 78 ppm have been released in Uganda [34]. These have an added advantage of tolerance to common plant stresses but more HIB varieties are needed to capture the diverse market preference.

Confirmation of Fe and Zinc concentration using ICP-AES

Based on ICP-AES analysis, there was significant variation ($P \leq 0.05$) in seed concentration of iron (FESEED) and zinc (ZNSEED) among the 26 test genotypes (Table 5). The % variation (% rsd) between the two duplicates analyzed to indicate the homogeneity of the sample was < 10 %, which is usually obtained in case of soil contamination. The magnitude of error variance in comparison with entry variance was also small for both variables (Table 5), suggesting that the influence of extraneous factors was properly controlled. HarvestPlus emphasizes the importance of minimizing contamination right from the field to the laboratory in Fe and Zn experimentations [43].

Four entries namely, CAB2, RW547, Ndimirakaguja, and Jesca showed FESEED higher than 62.3 ppm obtained in the high FESEED check, MIB465. The FESEED of 12 entries including the four above and UGK72, RW846, UGK116, UGK85, RW1180, RWV3006, RWV1129 and UGK39 was not significantly different (P=0.05) from that of the high FESEED check MIB 465 (Table 6). The 12 lines had previously recorded distinctively high FESEED using XRF. With the exception of RWV1129, these materials also had ZNSEED greater than 30 ppm. The line CAB2 had the highest FESEED (69.8 ppm) and UGK116, the highest ZNSEED (37.6 ppm). The lines RW582, Nain De Kyondo, Roba1, LMB49, NABE3, UGK95, UGK4, and UGK149 had FESEED less than and significantly different ($P \leq 0.05$) from the value obtained in the high FESEED check although they had previously appeared among the best lines within nurseries. The study showed a range of FESEED of 36-90 ppm and 24-47 ppm for ZNSEED across the four nurseries using

XRF. Using ICP, the FESEED in the confirmation trial ranged from 48-70 ppm and ZNSEED from 27-38 ppm. These findings are comparable to values reported by other authors [35, 36, 44]. The FESEED obtained in this study appear relatively lower than the HarvestPlus threshold for high iron beans, that is, >90 ppm FESEED [45]. This indicates the absence of such materials in circulation currently. However, studies have reported FESEED of up to 105.5 ppm among African germplasm [12]. Due to such high variations, the actual FESEED obtained could be misleading if used as the only guide to identify high iron beans. Environmental factors, as well as sampling and laboratory analysis procedures, may affect the actual values obtained. Relatively high FESEED may result from dust or soil contamination [46]. These findings support the ongoing efforts to develop high iron beans by the International Centre for Tropical Agriculture and its partners. It also highlights the importance of utilizing threshold genotypes (high and low Fe checks) in selecting high iron germplasm away from focusing on a target figure for mineral content. Thus the relative performance of test lines to the FESEED levels obtained in the proven check varieties, in this case, the universal checks could help to select high iron beans. In this study, soil Fe concentration was sufficient although Zn was limited. The samples were also thoroughly cleaned thus, the difference observed among experiments could be attributed the method of Fe and Zn analysis and/or spatial variation within the experimental field. For comparison, both high and low Fe checks were included in each experiment. Repeated checks could also be adopted to adjust for spatial variability. It was also observed that some germplasm that had high FESEED and ZNSEED within nurseries did not express similar results in the confirmation trial suggesting the variability was both due to genetics and the fields in which the germplasm was grown. In this study, most genotypes performed differently in FESEED and ZNSEED between seasons, though a few showed stability. Planting site and season have been reported as a source of variability in FESEED [36, 47]. A study on a 10.24 ha agricultural field showed high spatial variability in Fe and Zn with a tendency of deficiencies occurring in patches [48]. More strongly reported are the high genotype and environment interaction for FESEED [44, 46]. However, this was not determined in this study. A 2002 study by Gregorio [16] found consistency in some genotypes across environments. A sustainable biofortification of beans requires consideration of the influence of climate, soil, agronomic practices [49, 50]. Varieties that consistently present FESEED and ZNSEED greater than the universal high FESEED check under different environmental conditions should be the target.

Agronomic and yield characterization, and reaction to field disease by selected high iron bean lines

Analysis of variance revealed that the 28 entries were significantly different ($P \leq 0.05$) in their response to field diseases; angular leaf spot (ALS), anthracnose (ANT), common bacterial blight (CBB), bean common mosaic virus disease (BCMV), bean common mosaic virus necrosis disease (BCMNV)/black rot (BR), bean rust, and yield. However, there were no significant differences ($P \leq 0.05$) among the test genotypes for days to 50 % flowering (DF) (Table 5).

Disease pressure was relatively adequate to cause infection during the evaluation season. All lines except UGK95 scored < 5 for ALS while BCMV scores ranged from 1 in UGK72 to 6 in LMB49 on a score scale of 1-9. LMB49 also scored a 6 for CBB on leaves

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whereas UGK72 was the only line that scored ≤ 3 for all the measured diseases. The line MIB456 had 21 plants with black root followed by NABE3 with seven plants. Others with the incidence of black root/BCMNV included, RW1180, UGK4, Zebra, UGK111, UGK149, UGK72, UGK116 showing the absence of the bc3 gene in these entries. Anthracnose and growth vigor ranged from 1-3 whereas rust scores ranged from 1-4 based on a scale of 1-9. A study conducted in the fields in Kenya showed that some lines used in this study possess moderate resistance to the common prevailing diseases; for example, Jesca was clean for CBB and showed minimal symptoms for BCMV, ANT, and root rot while NAINDEKYONDO showed minimal symptoms in CBB, Anthracnose and root rot [38]. These two varieties also showed minimal symptoms for most of the diseases observed in this study; however, Jesca had fairly higher rust symptoms and NAINDEKYONDO, BCMV symptoms in Kenya [38]. These variations could be caused by the existence of different pathogen races in the trial sites or by differences in disease pressure. The line, Ndimirakaguja had minimal scores for ALS, ANT, BCMV, and rust but a fairly high score for CBB in the field. This shows the potential to select or breed for HIB with broad resistance to common biotic and abiotic stresses. Significant yield variation was observed in the combined experiment. However, clean yield was not significantly different for SOH and regional nutrition in the previous trials because the genotypes had been selected for high yield within nurseries. All the lines except CAB2 yielded greater than 1000 kg ha^{-1} while Jesca, Ndimirakaguja, UGK39, and UGK85 yielded greater than 2000 kg ha^{-1} (Table 6). Disease scores ranged from 2-4 on Jesca, 1-5 on Ndimirakaguja, 2-6 on UGK39, 1-6 on UGK85 and 2-5 on CAB2. The yield loss attributed to field diseases was below economic damage but the genotypes responded differently to specific disease pathogens in the screen house. Selection for high yielding, disease resistant bean genotypes with market preference is expected to enhance adoption of micronutrient-rich beans by farmers [13].

Correlation of iron, zinc, response to field diseases, days to flowering and physiological maturity and yield of selected HIB

A simple correlation conducted using data sets from the confirmation trial revealed that a positive, moderate and strong significant ($P \leq 0.01$) correlation existed between FESEED and ZNSEED (0.59). Several other studies reported a similar positive correlation between FESEED and ZNSEED [12, 35, 44, 46]. This implies that some genetic factors for FESEED and ZNSEED are co-segregating and thus selection for superiority in one trait will most likely result in a high value in another element. A weak negative relationship was obtained between FESEED/ZNSEED and yield. A 2001 Moraghan and Grafton [46] study showed that FESEED and ZNSEED were not significantly correlated with grain weight. These two elements negatively correlated to most of the diseases but the relationships were not significant except ($P \leq 0.05$) for FESEED and ALSF (-0.44). This result suggests that high ALS disease pressure possibly has a negative effect in Fe pathway, but more interesting, it could mean that it is possible to breed for both ALS resistance and high FESEED. Several studies have revealed broad and narrow resistance in HIBs [38, 39, 51]. Common bean is an important source of Fe and Zn for human nutrition [52]. Therefore, understanding the correlation of these elements to the important farmer and consumer-preferred traits is an important consideration in a breeding program.

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CONCLUSION

There is variability in grain Fe and Zn concentration among genotypes collected from across East Africa. This variability could be exploited in breeding and potential genotypes such as CAB2, RW547, Ndimirakaguja, Jesca and others, that had consistent high FESEED/ZNSEED throughout the study could be promoted as potential HIB genotypes. However, it is necessary to test for bioavailability of these micronutrients in the selected high iron bean. Several genotypes that were superior in FESEED/ZNSEED were also high yielding, and some expressed broad resistance to common diseases. This is an important attribute to both the breeders and the farmers. It is key to note that the use of universal high and low FESEED/ZNSEED checks in selection cannot be underestimated in addition to using trial designs that consider spatial differences.

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Table 1: Soil analysis from soil and plant analytical laboratories at NARL-Kawanda

Season	pH	OM	N	P	Ca	Mg	K	Fe	Zn
		------%	------	----------	ppm	----------			
2011	5.3	9.8	0.4	5.9	1716.9	361.8	240.4	147.7	4.9
2012	5.1	5.3	0.3	4.8	1888.2	522	214.3	80.6	4
2013	5.2	3.6	0.2	9.6	1070.0	430.3	19.2		
2016	4.8	4.2	0.2	3.7	1054.0	374.7	77.5	241.6	2.8
Critical values	5.2	3.0	0.20	5.0	350.0	100.0	150.0		
Sufficient levels	5.2-7.0	6.0	0.30	20.0	2000.0	600.0	500	50	20

OM Organic matter, *N* Nitrogen, *P* Phosphorus, *Ca* Calcium, *Mg* Magnesium, *K* Potassium, *Fe* Iron, *Zn* Zinc

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Table 2: Variation of Fe and Zn concentrations in bean grains in SOH nursery in 2011b and 2012a

Entry	Iron (ppm)		Zinc (ppm)	
	2011b	2012a	2011b	2012a
RW 1180	89.4	90.3	38.1	47.0
RW 580	88.4	91.7	36.4	46.3
RW 547	88.2	92.5	36.3	46.3
RW 839	87.2	91.7	36.3	47.2
RW 582	86.8	92.5	36.0	44.8
RW 849	85.4	92.5	37.2	46.7
RW 1179	81.1	83.7	35.6	43.0
RW 806	79.9	76.7	34.9	39.7
RW 880	78.0	85.5	36.6	45.5
RW 1172a	77.4	74.5	33.6	36.7
RW 439	77.2	77.2	32.1	42.5
RW 1087a	77.1	78.3	34.2	39.8
RW 500	77.1	81.8	32.5	40.8
RW 721	76.6	83.5	32.6	37.5
RW 971	75.2	72.3	34.7	39.8
RW 683	74.9	70.3	29.9	36.7
RW 846	74.9	84.5	35.6	45.3
RW 438	74.7	70.8	32.4	41.0
RW 801	74.6	76.8	33.7	39.7
RW 648 (Sugar)	73.5	67.0	29.7	35.0
RW 1087b	73.1	72.7	34.2	45.3
RW 267	73.1	81.0	34.6	43.0
RW 600	72.8	74.2	32.2	41.3
RW 700	72.7	79.8	29.4	38.3
RW 298	72.7	80.0	31.0	37.5
RW 942	72.5	78.2	33.8	44.0
RW 601	72.1	73.3	31.1	39.0
RW 611	72.1	66.2	34.9	39.5
RW 1172b	72.0	79.3	31.6	43.0
RW 744	72.0	67.0	32.4	34.7
RW 447	71.9	70.5	32.9	39.2
RW 1326	71.7	72.8	32.5	38.5
RW 896	71.0	78.3	34.9	42.8

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RW 593	70.7	72.3	30.9	44.2
RW 833	70.2	76.2	31.7	36.2
RW 184	68.9	75.3	31.9	40.7
RW 613	68.4	67.8	32.9	40.0
RW 805	68.4	76.2	34.1	46.0
RW 583	68.3	68.3	35.9	39.0
RW 474	68.2	67.0	32.6	38.0
RW 667	67.2	66.8	32.3	35.7
RW 1234	66.2	78.3	33.5	41.3
RW 1046b	65.6	64.2	33.5	31.8
RW 684	65.3	73.7	31.9	39.3
RW 825	65.3	75.0	32.0	38.0
RW 986	65.1	66.0	29.4	40.3
RW 693	65.0	71.5	32.1	42.7
RW 820	64.4	70.5	31.4	36.3
RW 28	63.8	71.2	29.4	37.7
RW 745	63.3	69.0	26.6	45.0
RW 731	63.1	65.5	28.4	36.5
RW 216	62.7	70.7	29.5	37.2
RW 835	62.6	61.7	30.6	39.3
RW 221	62.0	67.3	30.5	34.0
RW 375	61.9	63.3	31.2	36.3
RW 1046a	60.8	77.5	27.6	41.3
RW 648 (zebra)	59.5	63.8	23.9	35.3
RW 655	58.2	68.5	32.9	38.3
RW 615	58.1	66.8	30.3	36.7
RW 324	56.9	66.0	28.6	35.0
RW 218	56.2	65.8	29.7	34.5
MIB 465 (High iron check)	74.4	88.0	44.3	39.3
DOR 500 (Low iron check)	63.0	58.8	33.6	28.3
Mean	71	74.6	32.6	39.9
CV (%)	6.1	2	5.3	3
LSD (5%)	7	2.4	2.8	1.9

CV (%) Coefficient of variation, *LSD (5%)* least significant difference

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Table 3: Iron and zinc concentration in bush and climbing bean genotypes in the regional nutrition nursery

Entry	Fe (ppm)	Zn (ppm)	Entry	Fe (ppm)	Zn (ppm)
ECAB0019	75.2	32.1	Ndimirakaguja	89.1	35.9
KAB06F2 -8 -27	74.1	32.5	RWV1129	81.9	30.6
RWR2076	70.4	30.6	MBC32	75.8	30.0
KAB06F2 -8 -12	69.9	28.0	MAC74	75.3	32.7
RWR2245	66.8	28.6	RWV3006	74.4	41.6
SEA16	62.1	31.6	RWV3316	74.0	33.8
ECAB0266	62.0	30.7	NGWIN X CAB 2 213111	72.0	28.5
RWR1668	61.2	31.2	MBC71	71.9	30.2
Piramide	59.0	31.3	RWV2359	70.4	31.7
RWR3042	59.0	23.6	CAB19	69.6	31.4
RWR1180	56.8	24.7	RWV2361	69.1	31.3
SER30	55.8	31.1	Igisubizo	68.8	30.8
RWR2154 (Regional high Fe check)	55.6	28.2	KIVU20 (KIVUZO)	68.3	31.3
DOR500 (Low Fe check)	63.5	33.5	MAC49	67.8	27.8
CAL96 (Local yield check)	65.8	27.8	Rugandura	67.7	33.3
MIB465 (Universal high Fe check)	76.1	39.1	Gasirida	67.3	32.1
			MBC23	67.2	30.1
			Nyiramagorori	67.1	32.7
			MAC44	66.7	31.1
			MAC9	66.7	25.9
			RWV2070	65.8	29.2
			RWV2887	64.7	31.4
			Icyana	60.3	27.4
			CAB2 (Regional high Fe check)	76.3	36.8
			MIB465 (Universal high Fe check)	76.1	39.1
			Vuninkingi (Local yield check)	67.2	28.6
Mean	64.9	30.4		70.6	31.4
CV (%)	5.9	6.0		4.90	4.60
LSD (5%)	6.4	3.0		5.7	2.4

CV (%) Coefficient of variation, *LSD (5%)* least significant difference

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Table 4: Grain concentration of Fe and Zn in genotypes collected from Uganda, which had ≥ 70 ppm Fe

Entry	Iron (ppm)	Zinc (ppm)
UGK116	88.8	36.2
UGK4	84.8	36.2
UGK103	78.7	36.7
UGK95	77.7	35.5
UGK149	76.7	35.8
UGK72	76.0	37.2
UGK111	75.8	38.5
UGK117	75.8	35.7
UGK39	74.7	34.7
UGK85	74.2	39.2
DOR500 (Low Fe check)	56.0	30.0
MIB465 (High Fe check)	75.0	43.0
Mean	61.3	32.9
CV (%)	8.6	7.6
LSD (5%)	10.3	4.9

CV (%) Coefficient of variation, *LSD (5%)* least significant difference

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Table 5: Variance of iron, zinc, yield, agronomic characterisation and field diseases of selected high iron beans in 2013b

Source of variation	Degree of freedom	Iron (ppm)	Zinc (ppm)	ALSF	ALSFP	ANTFP	BCMV	BR	CBBFL	CBBFP	RUSTFP	GV	DF	DPM	YDHA
Replication	1	0.3	23.4	0.54	1.60	0.59	0.80	2.2	1.45	0.31	1.34	1.54	6.0	10.5	277467
Replication/block	4	90.8***	43.8***	0.86*	1.36**	0.64*	2.25***	16.0***	4.83***	0.58	0.53	0.82	34.5	10.5***	333445***
Entry	27	36.0***	9.7***	0.64**	0.68*	0.40*	1.32***	15.2***	0.78**	1.03**	0.72**	0.62*	23.2	5.8***	561237***
Error	23	6.3	2	0.24	0.32	0.17	0.24	0.3	0.30	0.32	0.23	0.32	19.0	1.0	30084
Total	55	26.2	8.8	0.48	0.58	0.32	0.91	8.6	0.84	0.69	0.51	0.52	21.9	4.1	314633

ALSF Angular leaf spot in field, *ALSFP* angular leaf spot on pods in field, *ANTFL* anthracnose on pods in field, *BCMV* bean common mosaic virus, *BR* black root, *CBBFL* common bacterial blight on leaves in field, *CBBFP* common bacterial blight on pods in field, *RUSTFL* rust on pods in field, *GV* growth vigour, *DF* days to 50% flowering, *DPM* days to physiological maturity, *YDHA* clean yield estimated in Kg ha^{-1}, $^{*, **, ***}$significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

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Table 6: Iron, Zinc, yield, agronomic characterisation and response to field diseases of selected high iron beans in 2013b

ENTRY	IRON (ppm)	ZINC (ppm)	ALSF	ALSFP	ANTFP	BCMNV	BR	CBBFL	CBBFP	RUSTFP	GV	DF	DPM	YDHA	SW100 (g)
UGK4	52.3	31.2	3	4	2	5	1	4	5	3	2	44	57.5	2931.7	33.4
JESCA	62.7	34.3	3	4	2	2	0	4	4	2	2	46.6	58.5	2909.3	47.5
NAINDEKYONDO	54.6	29.1	3	2	1	3	0	4	2	2	1	46.4	56	2904.3	25.6
ZEBRA	54.7	28.9	3	2	1		3	3	4	2	2	41.6	55.5	2564.7	23.8
UGK111	55.7	30.6	3	3	2		2	4	3	2	1	49.6	55	2411.5	25.4
NDIMIRAKAGUJA	62.7	34.4	2	2	1	2	0	5	3	2	1	45.6	62	2295	28.9
UGK39	57.8	33.2	3	3	2	4	0	4	6	3	2	46.6	56	2254.8	40.2
UGK95	52.9	28.9	5	3	2	5	0	5	3	4	3	42	54.5	2213.2	62.2
UGK85	58.7	32.9	4	4	1	4	0	6	5	3	2	40	57	2090.3	
UGK149	49.6	30.5	3	4	2	5	1	6	4	3	3	46	56	2065.4	45.0
NABE3	56.6	30.3	3	3	1		7	4	3	1	3	46.6	55.5	2056.5	29.1
LMB49	48.4	30.9	3	3	2	6	0	6	3	2	2	40.5	56.5	2032.9	30.6
UGK72	61.1	33.1	2	3	2	1	1	3	3	1	3	47.6	57.5	1990.1	28.8
RW547	66.3	32.3	2	2	2	4	0	4	4	1	2	46.1	58	1946.4	27.5
ROBA1	56.3	32.9	3	3	1	4	0	6	3	2	2	46.5	58	1874.7	20.6
UGK116	60.8	37.6	4	4	2	4	1	3	5	2	2	45.6	57.5	1585.6	26.6
UGK117	55	31.9	3	3	2	2	0	3	4	2	2	47.4	56	1502.8	21.2
RWV1129	57.9	27.4	3	3	1	3	0	4	4	2	1	48.9	59	1495.3	49.9
RW846	61.1	37.3	2	4	2	4	0	6	4	3	3	45.6	57.5	1491.1	25.4

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RW1180	58.6	35.6	3	3	2	5	1	3	3	1	3	48.5	60.5	1453.7	17.9
RW184	54.5	32.6	3	4	2	5	0	5	4	2	2	44	56.5	1373.4	27.5
RW805	51.4	34	3	4	2	4	0	5	4	3	3	46	57	1373.3	24.3
RW582	51.4	28.3	4	4	2	4	0	6	5	4	2	55.5	54.5	1369.9	36.5
RWV3006	58	32.3	4	2	1	2	0	5	3	2	1	49.5	61	1202.6	41.4
GARUKURARE	53.1	30.8	3	2	1	4	0	5	3	1	2	47	61.5	1121.6	31.0
CAB2	69.8	35.3	2	2	2	5	0	4	3	2	3	47.6	60	378.7	38.0
DECELAYA1 (Low Fe check)	49.3	30	3	4	2	4	0	4	4	3	4	59.8	56.3	1557	40.0
MIB456 (High Fe check)	62.3	34.4	3	4	2		21	5	4	3	3	44.5	56	1068.7	24.0
Mean	56.9	32.2	3.1	3.1	1.6	3.8	1.4	4.5	3.8	2.1	2.1	46.6	57.4	1839.8	
CV (%)	4.4	3.5	15.7	18.1	24.8	13	38.1	12.2	15	22.7	26.9	9.4	1.8	9.4	
Se	1.8	0.8	0.34	0.4	0.29	0.34	0.37	0.38	0.4	0.34	0.4	3.08	0.72	122.64	
LSD (5%)	5.2	2.3	1	1.2	0.8	1	1.1	1.1	1.2	1	1.2	ns	2.1	355.9	

ALSF Angular leaf spot in field, *ALSFP* angular leaf spot on pods in field, *ANTFL* anthracnose on pods in field, *BCMV* bean common mosaic virus, *BR* black root, *CBBFL* common bacterial blight on leaves in field, *CBBFP* common bacterial blight on pods in field, *RUSTFL* rust on pods in field, *GV* growth vigor, *DF* days to 50% flowering, *DPM* days to physiological maturity, *YDHA* clean yield estimated in Kg ha^{-1}, *SW100* weight of 100 seeds in grams, *ns* not significantly different, *CV (%)* coefficient of variation, *Se* standard error of the mean, *LSD (5%)* least significant difference

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Table 7: Correlation coefficients for iron, zinc, field diseases, days to flowering and physiological maturity, and yield of selected HIB

	IRON	ZINC	YLDHA	SW100	DF	DPM	GV	ALSF	ALSFP	ANTFP	CBBFL	CBBFP	RUSTFP
IRON	-												
ZINC	0.59**	-											
YLDHA	-0.23	-0.30	-										
SW100	-0.11	0.39*	0.09	-									
DF	-0.10	-0.17	-0.29	0.11	-								
DPM	0.45*	0.41*	-0.31	-0.06	0.03	-							
GV	-0.07	0.23	-0.32	0.04	0.16	-0.24	-						
ALSF	-0.48*	-0.35	0.04	0.44*	0.03	-0.35	-0.07	-					
ALSFP	-0.31	0.19	-0.01	0.08	0.16	-0.43*	0.44*	0.21	-				
ANTFP	0.01	0.35	-0.12	0.07	0.06	-0.37	0.49**	0.00	0.61***	-			
CBBFL	-0.32	-0.08	-0.17	0.18	0.06	-0.04	0.02	0.11	0.22	-0.04	-		
CBBFP	-0.06	0.12	0.01	0.13	0.11	-0.28	0.10	0.13	0.55**	0.44*	-0.06	-	
RUSTFP	-0.38*	-0.17	0.03	0.45*	0.12	0.50**	0.21	0.47*	0.53**	0.37	0.46*	0.46*	-

Number of observations: 28, *YDHA* clean yield estimated in Kg ha^{-1}, *SW100* weight of 100 seeds in grams, *DF* days to 50% flowering, *DPM* days to physiological maturity, *GV* growth vigour, *ALSF* Angular leaf spot in field, *ALSFP* angular leaf spot on pods in field, *ANTFL* anthracnose on pods in field, *CBBFL* common bacterial blight on leaves in field, *CBBFP* common bacterial blight on pods in field, *RUSTFL* rust on pods in field, $^{*, **, ***}$significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

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