

*Full Length Research Paper*

# **Biofortified bean genotypes under integrated soil fertility management across sub-humid agro-ecological zones of The Democratic Republic of Congo**

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This study was implemented to evaluate the performance of biofortified bean under different integrated soil fertility management (ISFM) options and agro-ecological conditions in Democratic Republic of Congo (DRC). A Split-plot design with eight genotypes as main factors and four ISFM options as secondary factors was carried out in eight production zones distributed across South-Kivu, North-Kivu, and Katanga provinces. The application of lime + manure + NPK increased the bean yield by 173% in Lohutu. Compared to local variety in Lohutu, the CODMLB001 variety under the same option increased the yield by 252%. The same ISFM option allowed best response in terms of micronutrient content of bean in Rutshuru, inducing up to 80.3 mg.kg<sup>-1</sup> Fe, representing increase 41%. For Zn, the best response was obtained with lime + NPK applied in Kipopo that induced up to 32.2 mg.kg<sup>-1</sup> Zn. Lime + manure + NPK fertilizer option reduced root rot severity by 17.8% compared to the control. This study confirmed the potential of increasing bean productivity, micronutrient and reducing the severity of major diseases through application of soil fertility management options, which will vary with the bean genotype and the environment under which bean is cultivated.

**Key words:** Integrated soil fertility management, bean yield, micronutrient content, disease control, *Phaseolus vulgaris* L.

## **INTRODUCTION**

Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes for direct consumption in

the world (HarvestPlus Iron-bean, 2009; FAO, 1999; Juhi et al., 2010). The annual global bean production is

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approximately 12 million metric tons, with 2.5 million metric tons in Africa (Welch, 2001). Because of their high protein, mineral and fiber content, beans are consumed instead of meat in developing countries (Wortmann et al., 1998). In DRC, common bean is among the most widely consumed and cultivated food crops particularly in the provinces of North Kivu, South Kivu, and former Katanga; where bean consumption has been estimated up to 300 g per capita per day (HarvestPlus DRC, 2009).

Like in most of Sub-Saharan countries, bean yield in Democratic Republic of Congo (DRC) is generally low (Bationo, 2010; Lubanga et al., 2012) even if a large literature has reported the introduction of improved high yield varieties (Kanyenga et al., 2016). In bean-based agrosystems of DRC, bean yields range between 400 and 800 kg.ha<sup>-1</sup> (Bouwmeester et al., 2009) whereas, in research station, bush bean yield may reach 3,000 and up to 6,000 kg.ha<sup>-1</sup> for climbing beans (Kanyenga et al., 2012). Low yield in bean-based agrosystems is attributed to many biotic and abiotic factors; bio-aggressors, limited use of improved varieties, and low soil fertility – (Wortmann et al., 1998; Kimani et al., 2001). Bean agronomic performance and nutritional value (iron and zinc) – are function of several factors including genetic variability, environmental conditions and technical itineraries (Kanyenga et al., 2016). Therefore, in most sub Saharan countries like DRC, food is often sufficient in fat and carbohydrates but poor in protein, especially vitamins and minerals (Díaz de la Garza et al., 2007). Obviously, vitamin A, Fe and Zn deficiency likely cause malnutrition, irreversible blindness, anaemia, poor body and cognitive development, as well as increased vulnerability to life-threatening diseases (Gepts et al., 2008). Nowadays, extended literature is devoted to the research of sustainable practices and solutions for improving both bean grain yield and nutritional value (Welch and Graham, 2004; CIAT, 2008; Lunze et al., 2012; Marles, 2017). On one hand, to overcome low soil fertility challenge in bean production, previous studies suggested an integrated soil fertility management (ISFM) (Vanlauwe et al., 2015). This is a composite technology, including the use of improved germplasm, judicious mineral fertilizer application and improved organic matter management (Lambrecht, 2013). These strategies combine technologies that include crop and kitchen residues, manure, compost, biomass transfer, green manures, cover crops, liming, phosphate rock and mineral fertilizers in different combinations (Lunze et al., 2012). The ISFM techniques are recognized to not only improve soil fertility but also increase and stabilize bean yield in a sustainable way (CIALCA 2009). On the other hand, to overcome the deficiency in minerals, the International Centre for Tropical Agriculture (CIAT), through its HarvestPlus programme, developed different market class of dry beans. Generally, common beans contain 3.14-12.07 mg.kg<sup>-1</sup> Fe and <1.89-6.24 mg.kg<sup>-1</sup> Zn (Marles, 2017), whereas, biofortified genotypes contain 40-90 mg.kg<sup>-1</sup> Fe and 10-35 mg.kg<sup>-1</sup> Zn (CIAT,

2008). According to Welch and Graham (2004), biofortified crops are also likely to have a positive indirect impact on agriculture, with a higher mineral content that provides better protection against pests, diseases and environmental stresses besides increasing yields. Dissemination of such seeds to poor rural households is one of the best ways to improve their diet and to combat malnutrition.

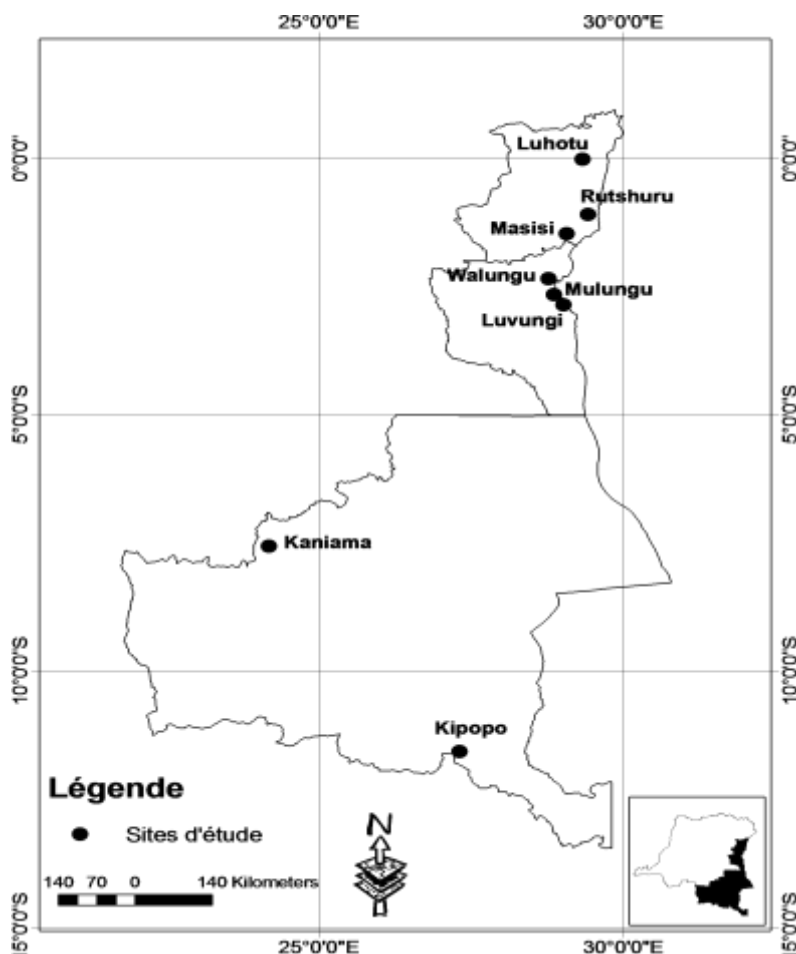
In Democratic Republic of Congo (DRC), the promotion of biofortified crops including bean by HarvestPlus programme and its partners (Institut National pour l'Etude et la Recherche Agronomique – INERA) is a great step in improving nutrition as well as food security. Most previous studies devoted to bean performance in contrasting soil and climatic conditions have admitted that micronutrient content in bean, in addition to its genetic component, may be influenced either directly by soil fertility status or indirectly by external inputs and the application of ISFM techniques (Beebe et al., 2008; Blair et al., 2008; Blair et al., 2009). In DRC, the knowledge on the agronomic and nutritional performance of biofortified bean genotypes across highly differing soil and climate types is still limited.

Therefore, soil and climate on the performance of biofortified bean are unknown. Actually, there is a need to improve the understanding on the coupling between ISFM and pedoclimatic conditions and their effects on Fe and Zn content in biofortified bean genotypes as well as their grain yield. This understanding of edaphic and climatic adaptation of biofortified bean in RDC will be a great step in attempt to disseminate high micronutrient source and contribute to nutritional security of the population. The purpose of this study is to evaluate the iron and zinc content, yield, and resistance or tolerance to disease of biofortified bean genotypes cultivated under ISFM and agroecological conditions of DRC.

## MATERIALS AND METHODS

### Characteristics and description of the study area

This study was conducted in eight bean production zones distributed across three provinces of DRC, namely South-Kivu, North-Kivu, and former province of Katanga. The South Kivu is characterized by a humid tropical climate influenced by the altitude, with two seasons: the dry season, from June to August, and the rainy season, September to May; with the average annual temperature of 19°C in the north and 10°C in the south, and the average annual precipitation ranging between 1200 and 1700 mm (Ellen, 2008; Pypers et al., 2010). During the rainy season, there is a short dry season from mid-January to mid-February dividing the rainy season in two: the long rainy season from September to February (known as season A) and the short rainy season (called season B) from February to June. The sites in Katanga belong to CW6 climate type according to the Köppen classification. This climate type is characterized by a well-marked dry season of six months (May-October), alternating with six months of rainy season (November-April). Their annual temperatures range between 11 and 28°C for an annual average of 20°C (Malaisse, 1983). According to Sys and Schmitz (1959), soils are heavily weathered and belong to the ferrallitic group with a red, ochre-red, and yellow-coloured foliar horizon categorized as zonal, intra-zonal, and azonal. In North Kivu, four seasons are observed: two wet



**Figure 1.** Localisation of study sites. Mulungu, Walungu and Luvungi are located in South-Kivu, Rutshuru, Masisi, and Lohutu are located in North-Kivu, whereas, Kipopo and Kaniama are located in the former province of Katanga.

seasons and two dry seasons. The first wet season is between mid-August and mid-January and the second is practically from mid-February to mid-July. As for the two dry seasons, they are very short. The soils of North Kivu could be divided into three great classes: recent volcanic soils (from lava flows from volcanoes), soils of alluvial plains (these soils are found in the plains of Semliki and come from lacustrine deposits, the Semliki River and its tributaries), and soils of ancient rocks: these soils are very deep and rich in humus. Three experiment sites are located in highland called Walungu, Mulungu, and Lohutu; two in midlands, Kipopo and Masisi, and three others in lowlands of Kaniama, Luvungi and Rutshuru (Figure 1). Soil characteristics (Table 1) as well as annual rainfall vary in these areas (Table 2).

## METHODOLOGY

### Set-up and implementation of experiments

Undisturbed soil samples were collected on each experimental site. In each study site, a Split-plot design with three replicates was installed. The treatments consisted of eight bush bean genotypes, CodMLB001, Hm1-7, RWK10, LSA144, Maharagi Soja, K132, NGWAKUNGWAKU, agronomically described by Kanyenga et al. (2016), local variety coupled to eight different options of ISFM with :10 t.ha<sup>-1</sup> farm manure, 2.5 t.ha<sup>-1</sup> lime, 0.2 t.ha<sup>-1</sup> NPK 10-20-10, manure + NPK, manure + lime, lime + NPK,

lime + manure + NPK, and a control. The different ISFM options were applied according to the doses, the timing and the method of application to each specific technique. Thus, lime was applied in depth of 10-15 cm in 2 weeks before planting. One-year-old cattle manure was applied at the same time and depth with lime and incorporated to the soil. NPK 10-20-10 fertilizers were applied on the day of planting. The planting density of 250.000 plants per hectare was performed and two to three manual weeding was done, and no treatment to pest or disease was applied.

The plant parameters were observed as, severity of the bean stem maggot (BSM) *Ophiomyia spp.*, bean root rot (BRR) of the genus *Fusarium* and *Pythium*, and the angular leaf spots (ALS) caused by *P. griseola* scored according to the CIAT rating scale (Van Scoonhoven and Voyset, 1991), as well as bean grain yield. At harvest, 15 pods were harvested from 15 plants on the two central rows removing the plants from the borders.

### Chemical analyses of soils and micronutrient analysis in bean grains

Soil samples from each experimental site were analyzed and following parameters were determined: pH, organic carbon, total nitrogen and phosphorus, total iron and zinc. Contents of Fe and Zn in bean grains were analyzed by spectrometry of X-ray fluorescence and determined as mg.kg<sup>-1</sup> as described in the protocol developed by Stangoulis et al. (2010).

**Table 1.** Some chemical proprieties of soils in different sites.

Sites	pH <sub>water</sub>	N (%)	OC (%)	P (mg.kg <sup>-1</sup> )	CEC (Cmol.kg <sup>-1</sup> )	Fe (mg.kg <sup>-1</sup> )	Zn (mg.kg <sup>-1</sup> )
Kipopo	5.5	0.8	19.82	32	10.2	84.74	1.98
Kaniama	5.4	0.1	22.26	202	11.15	270	4.88
Walungu	4.5	1.33	12.9	118	15.61	469.7	34.55
Mulungu	6.5	0.22	11.47	165	39.2	270.9	10.9
Luvungi	4.1	0.93	8.2	105	11.07	307.7	10.61
Rutshuru	5.8	0.3	3.13	73.6	42.3	124.6	3.56
Masisi	6.4	0.48	5.391	56.2	45.6	189	3.14
Lohotu	6.5	0.16	2.347	138.8	24.7	213.6	1.14

Source: University of Rwanda, Butare Campus, Soil laboratory.

**Table 2.** GPS coordinates, rainfall and landscape of experimental locations.

Sites	Altitude (mm)	Longitude (degree)	Latitude (minute)	Rainfall (mm)	Rainfall (days of rain)	Landscapes
Kipopo	1300	27°22' 32,7"	11°34'42,0"	1147	96	Midland
Kaniama	779	24°10' 30,9"	07°40' 18,0"	1425	131	Lowland
Walungu	2045	28°42' 39,1"	02°43' 03,4"	1779	165	Highland
Mulungu	1640	28°58' 04,5"	02°45' 32,1"	1803	187	Highland
Luvungi	907	28°01' 11,4"	02°51' 16,6"	899	128	Lowland
Rutshuru	1091	29°43' 58,7"	01.09.222	1009	142	Lowland
Masisi	2022	29°04' 04,8"	01.47.351	1648	190	Midland
Lohotu	2448	29°20' 29,3"	00.00.539	1850	204	Highland

### Statistical analysis

To detect the combined and individual effects of genotypes, ISFM techniques and environments one-way and multi-group analysis of variance (ANOVA) were applied. Correlation matrix (Pearson coefficient) was used to highlight the relationship between Fe and Zn contents and bean yield. All statistical analyses were performed using R 3.3.0 (The-R-Core-Team, 2016) and GenStat 17th considering  $P < 0.05$  as the level of significance.

## RESULTS

### Effects of ISFM options on bean grain yield of studied genotypes

Comparing ISFM techniques in North Kivu, (Table 3) the highest yield was obtained when lime is combined to mineral fertilizer NPK 10-20-10 in Lohotu, whereas, in Masisi and Rutshuru, the highest bean grain yield is obtained when lime is in combination with manure and mineral fertilizer NPK 10-20-10. Whatever the studied site, the lowest mean yield was obtained with the control, as well as, whatever the experimental site and the applied ISFM technique, the most productive bean genotype was CODMLB001 and the least productive genotype was the local variety. The best combination for North-Kivu of ISFM technique x bean genotype was lime + manure + NPK applied to CODMLB001 (Table 3). Based on bean yield disregarding ISFM and genotypic variability, the studied sites in North Kivu can be classified as follows: Masisi > Lohotu > Rutshuru. At

Lohotu application of lime + manure + NPK to CODMLB001 genotype compared to control, increased the yield by 173%, as well as, in comparing with local variety the biofortified CODMLB001 under the application of lime + manure + NPK, increased the yield by 252%.

Comparing ISFM techniques in South Kivu (Table 4), independent of the bean genotype, the highest bean yield was obtained when lime was combined to mineral fertilizer NPK 10-20-10 in Mulungu, whereas, in Luvungi and Walungu, the highest yield was obtained when lime was in combination with manure and mineral fertilizer NPK 10-20-10. Whatever the studied site, the lowest yield was obtained with the control, as well as, independent of experimental site and applied ISFM technique, the most productive bean genotype was CODMLB001, while the least productive was the local variety. The best combination ISFM technique x bean genotype in South Kivu was lime + manure + NPK applied to CODMLB001. Based on the obtained average bean yield disregarding applied ISFM and genotypic variability, the studied sites in North Kivu can be classified as follows: Mulungu > Walungu > Luvungi.

Results with and without liming from Mulungu, the Hm 21-7 genotype yield under liming improved to 65.5%. However, for local variety in Luvungi, lime induced a reduction of grain yield (Table 4). At Mulungu, application of lime + manure + NPK to CODMLB001 genotype compared with control increased the yield by 163% while, compared to local variety the biofortified

**Table 3.** Effects of ISFM options on the yield (kg.ha<sup>-1</sup>) of different bean genotypes in North Kivu. C: Control; L: Lime; N: NPK; M: Manure.

	Genotypes	ISFM options								Mean
		C	L	L+N	L+M	M	N	L+M+N	M+N	
Lohotu CVC0. 05 (88.5±45.0)	CODMLB001	953.9	1530.9	2404	1713.2	1838.3	2066.3	2604	2167	1909.7 <sup>a</sup>
	Hm21-7	788.3	1318.2	2197.2	1308.3	1708.3	1895.7	2292.1	1928.8	1679.6 <sup>b</sup>
	K132	676.6	1119.8	1645	1091.2	1148	1175.2	1766.8	1363.7	1248.3 <sup>d</sup>
	LSA144	669.6	822.6	1268.8	1112.3	986.8	896.4	1473.1	1502.4	1091.5 <sup>f</sup>
	MAHARAGISOJA	582.2	825.3	1601.8	1113	994.2	1018	1096.4	1179.8	1051.3 <sup>g</sup>
	NGWAKUNGWAKU	595.4	864.6	1867.1	990.1	1421	1223.8	1223.8	1208	1174.2 <sup>e</sup>
	RWR10	870.6	1513.7	2092.3	1245.2	1692.3	1353.9	2135.7	1724	1578.5 <sup>c</sup>
	Local variety	566	732	858.7	756.3	799.4	674.6	739.4	885.9	751.5 <sup>h</sup>
	Mean	712.8 <sup>h</sup>	1090.9 <sup>g</sup>	1741.9 <sup>a</sup>	1166.2 <sup>f</sup>	1323.6 <sup>d</sup>	1288.0 <sup>e</sup>	1666.4 <sup>b</sup>	1494.9 <sup>c</sup>	1310.6
Masisi CVC0. 05 (82.1±41.7)	CODMLB001	1239.9	2103.7	2208.1	2110.3	2462.1	2544.6	2677	2187.4	2191.6 <sup>a</sup>
	Hm21-7	1126.3	1904.1	2057.9	1924.8	2140	2185.8	2101.6	2018.7	1932.4 <sup>b</sup>
	K132	1042.8	1349.6	1436.8	1510.3	1592.7	1736.6	2122.3	1858.2	1581.2 <sup>c</sup>
	LSA144	915	1085.2	1386.7	1452.7	1262.6	1394.2	1821.1	1972.3	1411.2 <sup>d</sup>
	MAHARAGISOJA	704.2	995.6	1817.6	1227.6	1269	1330.8	1389.6	1526.7	1282.6 <sup>e</sup>
	NGWAKUNGWAKU	636	1100.4	1500.3	1190.9	1652	1253.4	1182.7	1449	1245.6 <sup>f</sup>
	RWR10	1168.2	1848.8	2079.2	1626.6	2188.4	1971.8	2548.8	2028.7	1932.6 <sup>b</sup>
	Local variety	823.7	871.1	1024.4	950.3	963.7	753.4	1141.7	1035.8	945.5 <sup>g</sup>
	Mean	957.0 <sup>g</sup>	1407.3 <sup>f</sup>	1688.9 <sup>c</sup>	1499.2 <sup>e</sup>	1691.3 <sup>c</sup>	1646.3 <sup>d</sup>	1873.1 <sup>a</sup>	1759.6 <sup>b</sup>	1565.3
Rutshuru CVC 0. 05 (114.5±59.2)	CODMLB001	1184.7	990.9	1506.4	1479.9	1390.4	1177	1963.2	1636.3	1416.1 <sup>a</sup>
	Hm21-7	949.4	878.7	1352.4	1323.6	1128	930.2	1798	1566.6	1240.9 <sup>b</sup>
	K132	743.1	769.7	1085.1	985.1	775.9	947.3	1232.2	1085.8	953.0 <sup>d</sup>
	LSA144	650.2	795.4	878.6	1046.6	642.7	831.4	987.4	929.7	845.3 <sup>e</sup>
	MAHARAGISOJA	621.7	632.7	893.2	817.6	627.1	740	1136.9	1043.6	814.1 <sup>e</sup>
	NGWAKUNGWAKU	713.9	479.2	894.6	855.8	553.3	638.4	1086.6	958.9	772.6 <sup>f</sup>
	RWR10	941.8	861.3	1220.2	1232.8	932.8	1023.7	1554.3	1302.7	1133.7 <sup>c</sup>
	Local variety	519.7	448.2	661.7	934.6	526.4	708.9	870.7	812.8	685.4 <sup>g</sup>
	Mean	790.6 <sup>e</sup>	732.0 <sup>f</sup>	1061.5 <sup>c</sup>	1084.5 <sup>c</sup>	822.1 <sup>e</sup>	874.6 <sup>d</sup>	1328.7 <sup>a</sup>	1167.0 <sup>b</sup>	982.6

Means with the same letter in line and column were not significantly different

**Table 4.** Effects of ISFM options on the yield (kg.ha<sup>-1</sup>) of different bean genotypes in South Kivu. C: Control; L: Lime; N: NPK; M: Manure.

Sites	Genotypes	ISFM options								Mean
		C	L	L+N	L+M	M	N	L+M+N	M+N	
Luvungi CVC <sub>0.05</sub> (77.8±39.5)	CODMLB001	1160.2	1003.9	1479.2	14799	1251	1320.2	1896.6	1447.4	1379.8 <sup>a</sup>
	Hm21-7	949.4	934.7	1266.3	1323.6	1087.1	1028	1486.9	1299.9	1172 <sup>b</sup>
	K132	743.1	769.7	1085.1	996.1	775.9	957.4	1183.9	1069.1	947.5 <sup>d</sup>
	LSA144	650.2	776.8	878.6	968.8	664.9	903.7	987.4	929.7	845 <sup>e</sup>
	MAHARAGI SOJA	621.7	743.8	893.2	850.9	738.1	797.8	988.8	1043.6	834.7 <sup>e</sup>
	NGWAKUNGWAKU	713.9	654.8	894.6	855.8	720	770.7	975.4	927.8	814.1 <sup>f</sup>
	RWR10	941.8	891.2	1197.1	1021.3	917.2	1025.9	1121	1141.2	1032.1 <sup>c</sup>
	Local variety	519.7	501.6	661.7	882.8	537.6	742.2	722.9	812.8	671.6 <sup>g</sup>
Mean	787.5 <sup>f</sup>	784.5 <sup>f</sup>	1044.5 <sup>c</sup>	1047.4 <sup>c</sup>	836.5 <sup>e</sup>	943.2 <sup>d</sup>	1170.4 <sup>a</sup>	1083.9 <sup>b</sup>	962.2	
Mulungu CVC <sub>0.05</sub> (79.9±40.6)	CODMLB001	1007.8	1561.3	2405.2	1714.4	1839.6	2096.8	2647.2	2204.3	1934.6 <sup>a</sup>
	Hm21-7	815.3	1348.7	2209.6	1309.6	1709.6	1926.1	2335.3	1166.1	1702.5 <sup>b</sup>
	K132	648.4	1150.2	1646.2	1081.3	1149.2	1205.7	1810	1401	1261.5 <sup>d</sup>
	LSA144	624.8	797.4	1270	1113.6	936.9	904.7	1516.3	1539.8	1091.3 <sup>f</sup>
	MAHARAGI SOJA	500.3	833.6	1603	1114.2	995.4	1048.4	1139.7	1217	1056.5 <sup>g</sup>
	NGWAKUNGWAKU	465.6	906.1	1868	991.3	1422.2	1254.2	1267	1245.3	1177.5 <sup>e</sup>
	RWR10	859.1	1544.1	2093.6	1246.4	1669.4	1383.3	2178.3	1761.3	1592.2 <sup>c</sup>
	Local variety	544.7	762.4	859.9	757.6	800.7	693.9	782.7	923.2	765.6 <sup>h</sup>
Mean	683.3 <sup>g</sup>	1113.0 <sup>f</sup>	1744.5 <sup>a</sup>	1166.1 <sup>e</sup>	1318.8 <sup>d</sup>	1314.3 <sup>d</sup>	1709.6 <sup>b</sup>	1532.3 <sup>c</sup>	1322.7	
Walungu CVC <sub>0.05</sub> (77.5±39.4)	CODMLB001	1308.3	1259.6	1608.2	1581.7	1624.2	1392.4	2017.2	1698.8	1568.6 <sup>a</sup>
	Hm21-7	1096.2	1147.3	1454.2	1425.3	1337.2	1145.7	2003.4	1629	1404.8 <sup>b</sup>
	K132	889.9	1038.3	1186.9	1086.9	985.1	1140.8	1374	1148.2	1105.3 <sup>d</sup>
	LSA144	797	1064.1	980.3	1148.3	851.9	1024.9	1109.2	992.1	996 <sup>e</sup>
	MAHARAGI SOJA	768.4	901.3	995	919.3	858.6	933.4	1229.3	1106	963.9 <sup>f</sup>
	NGWAKUNGWAKU	838.4	747.9	996.3	957.6	726.6	887.2	1113.7	1021.3	915.6 <sup>g</sup>
	RWR10	1088.6	1130	1322	1334.6	1142	1239.1	1709.8	1365.1	1291.4 <sup>c</sup>
	Local variety	666.4	667.7	763.4	1036.3	713.3	825.7	928.9	875.2	910.9 <sup>h</sup>
Mean	931.7 <sup>g</sup>	994.5 <sup>f</sup>	1163.3 <sup>c</sup>	1186.3 <sup>c</sup>	1034.4 <sup>e</sup>	1074.9 <sup>d</sup>	1442.9 <sup>a</sup>	1229.5 <sup>b</sup>	1132.2	

Means with the same letter down a column are not significantly different.

**Table 5.** Effects of ISFM options on the yield (kg.ha<sup>-1</sup>) of different bean genotypes in Katanga. C: Control; L: Lime; N: NPK; M: Manure.

Sites	Genotypes	ISFM options								Mean
		C	L	L+N	L+M	M	N	L+M+N	M+N	
Kaniama CVC <sub>0.05</sub> (77.8±39.6)	CODMLB001	1221.6	1130	1119.4	1096	1495	1263	1451.4	1142.1	1239.7 <sup>a</sup>
	Hm21-7	1007.3	1018	984.7	1136	1208	1016	1356.6	1277.2	1125.4 <sup>b</sup>
	K132	754.1	908.7	696.7	876.1	855.4	1011	1116.7	996.4	901.9 <sup>d</sup>
	LSA144	771.3	934.4	820.3	822.4	722.2	895.2	1030	1018.8	876.8 <sup>d</sup>
	MAHARAGI SOJA	611.8	771.7	751.7	889.8	706.7	803.8	1142.2	861.3	817.4 <sup>e</sup>
	NGWAKUNGWAKU	713.7	618.2	682.2	933.7	632.9	724.2	872.4	986.1	770.4 <sup>f</sup>
	RWR10	964.2	1000	800.1	909.8	1012	1109	1256.9	1063.2	1014.5 <sup>c</sup>
	Local variety	653.4	538	575.2	718.8	583.7	706	973.6	774.3	690.4 <sup>g</sup>
	Mean	837.2 <sup>f</sup>	864.9 <sup>e</sup>	803.8 <sup>g</sup>	922.8 <sup>cd</sup>	901.9 <sup>d</sup>	941.1 <sup>c</sup>	1150 <sup>a</sup>	1014.9 <sup>b</sup>	929.6
Kipopo CVC <sub>0.05</sub> (77.4±39.4)	CODMLB001	1239.9	2104	2797	2110	1824	2412	3132.6	2723	2292.8 <sup>a</sup>
	Hm21-7	1126.3	1904	2182.3	1925	1157	2119	2764.9	2458.4	1954.3 <sup>a</sup>
	K132	1042.8	1350	2403.4	1510	754.7	1684	2224.6	1858.2	1603.5 <sup>d</sup>
	LSA144	915	1085	1708.9	1453	732.4	1394	1921.1	1827.9	1379.7 <sup>e</sup>
	MAHARAGI SOJA	704.2	995.6	2049.7	1228	1095	1331	1389.6	1526.7	1289.9 <sup>f</sup>
	NGWAKUNGWAKU	636	1100	1987.8	1191	707.6	1231	1388.4	1449	1211.4 <sup>g</sup>
	RWR10	1168.2	1849	2472.6	1627	1221	1905	2671	2028.7	1867.7 <sup>c</sup>
	Local variety	535	662	982.2	950.3	465.1	711.4	936	913.6	769.5 <sup>h</sup>
	Mean	920.9 <sup>g</sup>	1381.2 <sup>e</sup>	2073 <sup>a</sup>	1499.2 <sup>d</sup>	994.3 <sup>f</sup>	1598.5 <sup>c</sup>	2053.5 <sup>a</sup>	1848.2 <sup>b</sup>	1546.1

Means with the same letter down a column are not significantly different.

CODMLB001 variety under the application of lime + manure + NPK, increased the yield by 238%.

In Katanga, comparing the ISFM techniques (Table 5) independently of bean genotype, the highest yield was obtained when lime was combined to manure and mineral fertilizer NPK 10-20-10 in both Kaniama and Kipopo. In Kaniama, the lowest yield was obtained when lime is combined with mineral fertilizer N PK 10-20-10, whereas, in Kipopo, the lowest yield was obtained with control. Whatever the experimental site and the applied ISFM technique, the most

productive bean genotype was CODMLB001, although in Kipopo, it did not differ from Hm21-7, while the least productive was the local variety. The best combination ISFM technique x bean genotype for Katanga was lime + manure + NPK applied to CODMLB001. Bean yield in Kipopo is higher compared to Kaniama. In Kipopo, application of lime + manure + NPK to CODMLB001 genotype increased the yield by 153%, while compared to local variety, CODMLB001 variety under the application of lime + manure + NPK, increased the yield by 235%.

#### Effects of ISFM options and genotype on micronutrient content in bean grains

The Fe content indicates that, in studied agroecological zones, the highest concentration was obtained in Rutshuru, whereas, the lowest is obtained in Kaniama. Considering ISFM, the highest Fe content was obtained with lime combined with manure and chemical fertilizer NPK 10-20-10 and the lowest was obtained with the untreated control. Based on applied ISFM techniques in relation to agroecological

**Table 6.** Effects of ISFM techniques on iron and zinc content ( $\text{mg.kg}^{-1}$ ) in bean grains in studied agro-ecological zones.

Micronutrients	ISFM options	Environments							Mean	
		Kaniama	Kipopo	Luhotu	Luvungi	Masisi	Mulungu	Rutshuru		Walungu
Fe	Control	52.2	52.3	53.6	56	55.3	60.1	57.1	55.6	55.3
	Lime	50.7	54.6	58.5	57.9	59.1	55.1	56.3	54.9	55.9
	Lime + NPK	58	64	63.4	67.6	59.6	62.3	68.7	67.3	63.9
	Lime + manure	58.3	57.5	59.8	62.6	57	63.5	63.1	62	60.5
	Manure	55	55.7	59.8	59.8	60	58.6	59.4	58	58.3
	NPK	55	56.7	61.4	60.7	61.2	59.5	60	58.9	59.2
	NPK + lime + manure	64.4	68.4	73.2	72.5	61.2	64.2	80.3	78.4	70.3
	NPK + manure	61.1	64.3	68.4	67.1	58.6	63.6	72	71.2	65.8
	Mean	56.8	59.2	62.3	63	59	60.9	64.6	63.3	61.1
	Zn	Control	26.3	25.2	26.8	25.7	28.4	28.4	27.6	28.4
Lime		27.9	27	26.8	27.4	27.7	27.7	28.3	27.7	27.6
Lime + NPK		28.2	32.2	29.5	29.2	31.8	31.8	28.9	31.8	30.4
Lime + manure		27	30.4	28.7	28.8	30.4	30.4	30.2	30.4	29.5
Manure		27.6	27.9	27.8	27.5	28	28	28.2	28	27.9
NPK		28.7	28.4	28.4	27.8	28.4	28.4	28.9	28.4	28.4
NPK + lime + manure		29.8	30	29.7	30.3	30.3	30.3	30.3	30.3	30.1
NPK + manure		30.1	29.8	20	29.1	30.9	30.9	30.9	30.9	30.3
Mean		28.2	28.9	28.5	28.2	29.5	29.5	29.2	29.5	28.9

zones, the best response was obtained with lime + manure + NPK applied in Rutshuru. Compared to control, the application of NPK + lime + manure increased Fe content in Rutshuru (Table 6).

Based on Zn content in bean grain, the highest mean content was obtained in Masisi, Mulungu, and Walungu. Considering ISFM options, the highest mean Zn content ( $> 30 \text{ mg.kg}^{-1}$ ) is obtained with lime + NPK, Manure + NPK, and lime + manure + NPK, whereas, the lowest Zn mean content is obtained with the control. Based on applied ISFM techniques in relation to agroecological zones, the best response was obtained with lime + NPK applied in Kipopo (Table 6). Contents of Fe and Zn in bean genotypes were influenced by ISFM techniques (Table 7). The highest Fe content was obtained with lime + manure + NPK, while the highest content among genotypes was obtained on CODMLB001 under lime + manure + NPK. The influence of ISFM on Zn content in grains was observed with the highest values obtained on application of three treatments, lime + NPK fertilizer, manure + NPK fertilizer and lime + manure + NPK fertilizer, while the best genotype was CODMLB001 under lime + manure + NPK supply (Table 7).

#### Effect of ISFM options and genotypes on the severity of diseases

The incidence of BRR among agroecological zones was lower in Luhotu and higher in Rutshuru (Table 8). The incidence of BRR in relation to applied ISFM techniques was higher with NPK alone and control. Comparing ISFM techniques in studied sites in relation to BRR, the highest incidence was observed in Rutshuru (with lime + NPK treatment) and Walungu (with lime + NPK and NPK

alone). This incidence is similar to what was observed on control. Comparing ISFM techniques, the highest incidence of ALS was observed with control and lime treatment, whereas, the lowest was observed with NPK + lime + manure. Comparing agroecological zones, the highest incidence of ALS was observed Walungu and the lowest in Luhotu. The comparison of ISFM in relation to BSM incidence indicates that the highest mean incidence was observed with the control, whereas, the lowest was observed with lime + NPK and NPK + lime + manure. Table 9 shows the incidences of BRR, ALS and BSM in relation to bean genotypes and agroecological zones. Among the evaluated bean genotypes, the lowest BRR incidence was observed with CODMLB001; the same observation was made for ALS and BSM. The lowest incidence of BRR and ALS was observed in Luhotu, and Masisi for BSM. The combined effects of ISFM and bean genotype are given in Table 10. The lowest incidence of BRR, BSM and ALS was observed when lime + manure + NPK was applied to CODMLB001. Local varieties were characterized by high incidence whatever the diseases and applied ISFM technique.

#### Correlations between yield, micronutrients and the severity of diseases

The effect of ISFM options revealed the existence of correlations either negatively or positively between the severity of all bio-aggressors and the yield; between bio-aggressors and micronutrient content; among the bio-aggressors and among the micronutrients (Table 11).

In respect to yield and bio-aggressors, there were negative and significant correlations with BRR, BSM



**Table 7.** Effects of ISFM techniques on iron and zinc content (mg.kg<sup>-1</sup>) in bean genotypes. C: Control; L: Lime; N: NPK; M: Manure.

Micronutrients	Genotypes	ISFM options							Mean	
		C	L	L+N	L+M	M+N	L+M+N	M+N		
Fe (mg.kg <sup>-1</sup> )	CODMLB001	66	68.5	84	75.3	68.4	71.8	91.5	79.5	75.6 <sup>a</sup>
	Hm21-7	62.3	66.2	76.7	68.7	65	66.5	79.4	77.4	70.3 <sup>b</sup>
	K132	52.2	54.2	55	56.8	61.2	55	64.8	59.7	57.4 <sup>ef</sup>
	LSA144	55.7	62.6	63.8	57.1	61.3	62.4	62.6	59.7	60.6 <sup>d</sup>
	MAHARAGI SOJA	51.8	47.4	62.3	52.9	54.6	52.1	61.4	58.2	55.1 <sup>fg</sup>
	NGWAKUNGWAKU	51.4	46.8	53.4	52.5	49	49.3	62.4	64.7	53.7 <sup>gh</sup>
	RWR10	55.6	67.2	68.1	72.2	64.5	68.8	74	71	67.7 <sup>c</sup>
	Local variety	49.8	46.1	50.1	44	49.5	51.2	59.1	58.7	51.1 <sup>h</sup>
	Mean	55.3 <sup>e</sup>	55.9 <sup>de</sup>	63.9 <sup>b</sup>	60.5 <sup>c</sup>	58.3 <sup>cd</sup>	59.2 <sup>c</sup>	70.3 <sup>a</sup>	65.8 <sup>b</sup>	61.1
	Zn (mg.kg <sup>-1</sup> )	CODMLB001	30.6	27.8	30.6	31.2	31.1	30	33.6	32.3
Hm21-7		29.1	27.2	30.9	32	28.5	28.6	29.7	31.6	29.7 <sup>ab</sup>
K132		24.6	27.6	27.8	29.1	26.1	28	30.3	27.4	27.6 <sup>d</sup>
LSA144		25.6	28.3	31.3	29.7	28.9	31	24.7	26.8	28.3 <sup>c</sup>
MAHARAGI SOJA		25.4	27.2	30.8	26.5	25.7	26.8	31.4	28.4	27.8 <sup>d</sup>
NGWAKUNGWAKU		25.3	24.6	29.2	27.9	28.1	27.7	29.6	29.8	27.7 <sup>d</sup>
RWR10		27.7	32.2	30	30.5	29.7	28.3	32.9	35.3	30.8 <sup>a</sup>
Local variety		28.4	25.8	32.6	29.4	24.9	26.9	28.8	31	28.5 <sup>c</sup>
Mean		27.1 <sup>e</sup>	27.6 <sup>de</sup>	30.4 <sup>a</sup>	29.5 <sup>b</sup>	27.9 <sup>cd</sup>	28.4 <sup>c</sup>	30.1 <sup>a</sup>	30.3 <sup>a</sup>	28.9

Means with the same letter down a column are not significantly different.

( $r = -31\%$ ) and ALS disease ( $r = -21\%$ ). The same trend was observed between iron and diseases, 36% with BRR, -24% with BSM and -19% with ALS. Positive and significant correlations were obtained between Fe content and yield ( $r = 72\%$ ), also bio-aggressors between them 20% between ALS disease and BRR, 38% between BSM and BRR and 35% between BSM and ALS disease.

## DISCUSSION

### Effects of ISFM options on bean grain yield of studied genotypes

Globally, the results of this study showed that, compared to control and local varieties, the application of lime + manure + NPK to CODMLB001 genotype

significantly increased the yield. The obtained yields vary with genotypes, environments, and applied ISFM. These observations indicated the crucial role of both bean genotype and ISFM in increasing bean yields. Obviously, liming is an effective and dominant practice to raise soil pH and reduce acidity-related constraints to improve crop yields (Fageria and Baligar, 2008). Benefits of liming include increased

**Table 8.** Effects of ISFM options on the incidence of Bean Root Rot (BRR), Angular Leaf Spot (ALS) and Bean Steam Maggot (BSM) in different study sites.

Diseases	ISFM	Environments						Mean	
		Kipopo	Luhotu	Luvungi	Masisi	Mulungu	Rutshuru		Walungu
BRR	Check	5.0	3.7	4.5	4.1	4.8	4.0	5.1	4.5
	Lime	3.8	3.4	4.2	4.1	4.5	5.1	4.6	4.2
<u>LSD</u>	Lime + NPK	3.3	3.4	4.1	4.1	4.8	5.1	5.1	4.3
E : 0.20	Lime + Manure	3.6	3.5	4.0	4.8	4.8	4.0	3.8	4.1
I x E : 0.33	Manure	3.6	3.7	4.6	3.8	3.0	4.6	3.6	3.9
	NPK	4.7	3.4	4.6	4.1	4.8	5.0	5.1	4.5
	NPK+ Lime + Manure	3.0	3.3	4.0	3.0	3.7	4.6	4.2	3.7
	NPK + Manure	3.6	3.9	4.4	4.3	3.0	4.6	4.6	4.1
	Mean	3.8	3.5	4.3	4.0	4.2	4.6	4.5	4.1
	LSD <sub>0.05</sub>	0.23	0.22	0.39	0.25	0.23	0.25	0.33	0.10
ALS	Check	4.9	4.9	4.0	4.8	4.9	4.8	4.0	4.6
	Lime	5.5	4.2	4.6	4.6	4.1	4.2	5.1	4.6
<u>LSD</u>	Lime + NPK	4.4	4.8	5.1	4.6	3.0	4.5	5.1	4.5
E : 0.15	Lime + Manure	4.6	4.5	4.0	4.6	3.0	3.6	4.0	4.1
I x E : 0.28	Manure	3.8	3.4	4.6	4.1	4.8	5.0	4.6	4.3
	NPK	4.3	3.0	4.5	4.8	4.5	4.5	5.0	4.4
	NPK + Lime + Manur	3.7	3.7	4.6	4.1	3.0	3.8	4.6	3.9
	NPK + Manure	3.7	3.0	4.6	4.1	4.5	5.1	4.6	4.2
	Mean	4.4	3.9	4.5	4.5	4.0	4.4	4.6	4.3
	LSD <sub>0.05</sub>	0.22	0.26	0.27	0.23	0.22	0.32	0.27	0.10
BSM	Check	5.0	4.1	5.0	3.5	4.9	4.9	4.8	4.6
	Lime	4.8	4.8	3.8	3.4	4.2	4.1	4.2	4.2
<u>LSD</u>	Lime + NPK	5.1	5.1	3.3	3.4	4.8	3.0	4.5	4.2
E : 0.15	Lime + Manure	4.0	4.0	3.6	3.5	4.5	3.0	3.6	3.8
I x E : 0.29	Manure	5.1	4.6	3.6	3.9	3.4	4.8	5.0	4.4
	NPK	4.2	4.6	4.7	3.5	3.0	4.5	4.5	4.1
	NPK + Lime+Manur	5.1	4.6	3.0	3.4	3.8	3.0	3.8	3.8
	NPK + Manure	5.1	4.6	3.6	3.9	3.0	4.5	5.1	4.3
	Mean	4.8	4.5	3.8	3.6	4.0	4.0	4.4	4.2
	LSD <sub>0.05</sub>	0.31	0.28	0.23	0.20	0.26	0.22	0.32	0.10

LSD: Least Significant Difference, E: Environment, I x E: Interaction between disease Incidence and Environment.

availability of essential nutrients (Ca, P, Mo) and the decreased solubility of toxic elements Al and Mn (Haynes and Ludekeet, 1981; Buni, 2014). In addition, liming also improves biological N<sub>2</sub> fixation in acid soils and enhances net mineralization of organic N (Edmeades and Ridley, 2003; Moreira and Fageria, 2010). However, liming experiments in plateau and hills of Rwanda showed that addition of lime alone is insufficient to rehabilitate poor or depleted soils; therefore, the best practice is one that combines lime, organic manure and inorganic fertilizers (Mukuralinda, 2007). Indeed, chemical fertilizer NPK 10-20-10 likely played an important role in rapidly releasing essential nutrient to plants, and manure application was essential in improving nutrient supply to plant. Manure is considered a source of major plant nutrients such as N, P and potassium (K) (Murmu et al., 2013). It can also provide many of the secondary nutrients that plants require. Benefits of manure amendments added to soil

include pH depressing and faster infiltration rate due to enhanced soil aggregation (Liang et al., 2011). Moreover, bean yield increase because of manure application has been observed by extended number of authors (e.g. Nabahungu, 2003; Alley and Vanlauwe, 2009; Bekunda et al., 2010; Vanlauwe et al., 2015; Musaninkindi, 2013; Khaim et al., 2013; Ndengu et al., 2017).

This study showed that independently to sites and applied ISFM technique, the most productive bean genotype is CODMLB001 and the least productive genotype was the local variety. Interesting results have been also obtained with RWK10 (2548.8 kg.ha<sup>-1</sup>) in Masisi and Hm21-7 in Mulungu (2335.4 kg.ha<sup>-1</sup>). Comparing the results obtained in Mulungu with and without liming, the Hm 21-7 genotype was able to improve its yield from 815 to 1348.7 kg.ha<sup>-1</sup> with lime application. This was an increase of 65.4% that is greater than that reported by Lunze et al. (2007) where

**Table 9.** Effects of genotypes on the incidence of Bean Root Rot (BRR), Angular Leaf Spot (ALS) and Bean Stem Maggot (BSM) in different study sites.

Diseases	Genotypes	Environments							Mean
		Kipopo	Luhotu	Luvungi	Masisi	Mulungu	Rutshuru	Walungu	
BRR	CODMLB001	1.8	3.0	3.6	2.0	3.0	4.1	2.8	2.9
	Hm21-7	2.8	3.6	4.3	3.3	3.7	4.5	4.7	3.8
<u>LSD</u>	K132	4.3	3.0	4.4	4.2	4.6	4.9	4.1	4.2
E : 0.20	LSA144	4.8	3.7	4.4	5.0	4.4	4.6	4.7	4.5
G x E : 0.32	MSOJA	4.6	3.8	4.4	5.2	4.2	4.4	5.0	4.5
	NGWAKU	4.0	3.7	4.7	4.5	4.2	4.5	4.0	4.2
	RWR10	3.3	3.6	3.9	4.2	4.0	4.7	4.5	4.0
	Local variety	5.0	3.9	4.7	3.8	5.5	5.4	6.1	4.9
	Mean	3.8	3.5	4.3	4.0	4.2	4.6	4.5	4.1
	LSD <sub>0.05</sub>	0.23	0.23	0.39	0.23	0.26	0.27	0.27	0.10
ALS	CODMLB001	2.3	3.0	3.6	2.4	2.5	2.8	4.1	3.0
	Hm21-7	3.4	3.3	4.3	3.7	3.3	4.3	4.5	3.8
<u>LSD</u>	K132	5.1	4.2	4.9	4.1	4.6	4.5	4.9	4.6
E : 0.15	LSA144	5.2	4.3	4.5	5.7	4.3	5.0	4.6	4.8
G x E : 0.29	MSOJA	4.7	4.2	4.4	5.3	4.3	4.9	4.4	4.6
	NGWAKU	4.5	4.1	4.5	4.8	3.9	3.8	4.5	4.3
	RWR10	4.1	3.5	4.7	4.4	3.8	4.2	4.7	4.2
	Local variety	5.6	5.0	5.3	5.2	5.3	5.8	5.4	5.4
	Mean	4.4	3.9	4.5	4.5	4.0	4.4	4.6	4.3
	LSD <sub>0.05</sub>	0.23	0.26	0.27	0.25	0.27	0.26	0.27	0.10
BSM	CODMLB001	2.8	3.8	1.8	2.7	3.0	2.5	2.8	2.8
	Hm21-7	4.0	4.4	2.8	4.0	3.3	3.3	4.3	3.7
<u>LSD</u>	K132	5.3	4.9	4.3	3.0	4.2	4.6	4.5	4.4
E : 0.15	LSA144	5.8	4.6	4.8	3.7	4.3	4.3	5.0	4.6
G x E : 0.28	MSOJA	4.8	4.4	4.6	3.8	4.2	4.3	4.9	4.4
	NGWAKUGWAKU	5.2	4.5	4.0	3.8	4.1	3.9	3.8	4.2
	RWR10	4.4	4.6	3.3	3.6	3.5	3.8	4.2	3.9
	Local variety	6.1	5.3	5.0	4.0	5.0	5.3	5.8	5.2
	Mean	4.8	4.5	3.8	3.6	4.0	4.0	4.4	4.2
	LSD <sub>0.05</sub>	0.21	0.28	0.23	0.24	0.27	0.27	0.26	0.10

LSD: Least Significant Difference, E: Environment, G x E: Interaction between Genotype and Environment.

Hm21-7 showed grain yields ranging from 1317 to 1455 kg.ha<sup>-1</sup>. To the local variety in Luvungi, lime induced a reduction in grain yield, from 519.7 kg.ha<sup>-1</sup> in control to 501.6 kg.ha<sup>-1</sup> with lime. This was similar to the results obtained by Ngongo et al. (2000), FAO (2006) and CIALCA (2009). The difference in bean yield under the same treatment of ISFM should be due to the inherent genetic potential of each variety to produce and respond to it, as well as to the both edaphic and climatic conditions (Ndengu et al., 2017). The low yield obtained with the control likely indicates the low natural ability of studied soils to induce bean yield. Within a same province, the difference in bean yield is likely a result of the combination between soil and climate, for example, in North Kivu soils in Rutshuru have been found moderately acid, poor in organic matter and with high CEC, contrary to Masisi where soils have been found slightly acid but rich in organic matter. In the same province, Rutshuru is the most enriched in phosphorus,

but with low CEC. Globally, results showed that biofortified varieties tended to yield better than local varieties. Furthermore, soil fertility improvement resulted in positive yield increases in most of the cases. This was similar to the observations of many other authors (Vanlauwe et al., 2015; Musaninkindi, 2013; Sebuwufu, 2013; Khaim et al., 2013; Ndengu et al., 2017).

#### Effects of ISFM options and genotype on micronutrient content in bean grains

The Fe content in different agroecological zones in relation with ISFM was higher in Rutshuru and lower in Kaniama. This study showed that the best genotypes under the best ISFM option were CodMLB001, Hm 21-7, and RWK 10. Notably, there is a wide genetic variability in seed mineral composition and their concentration is partly controlled by the environment in which beans are

**Table 10.** Effects of the interaction genotype x ISFM options on disease incidence of Bean Root Rot (BRR), Angular Leaf Spot (ALS), and Bean Steam Maggot (BSM). C: Control; L: Lime; N: NPK; M: Manure.

Diseases	Genotypes	ISFM options								Mean	
		C	L	L+N	L+M	M	N	L+M+N	M+N		
BRR	CODMLB001	3.0	2.5	3.1	3.3	2.9	3.7	2.2	2.5	2.9	
	Hm21-7	4.3	4.1	3.9	3.9	3.5	4.1	3.1	3.8	3.8	
	<u>LSD<sub>0.05</sub></u>	K132	4.4	4.2	4.4	4.0	4.3	4.0	3.9	4.3	4.2
	G : 0.10	LSA144	4.9	4.4	4.3	4.7	4.2	4.8	4.1	4.7	4.5
	I : 0.10	MAHARAGISOJA	4.6	4.9	4.8	3.9	4.4	5.1	4.1	4.5	4.5
	G x I : 0.29	NGWAKUNGWAKU	4.7	4.1	4.6	4.2	3.9	4.7	3.5	4.3	4.2
		RWR10	4.8	4.4	3.9	3.8	2.8	4.9	3.7	3.6	4.0
		Local variety	5.0	5.2	5.1	4.9	4.8	4.7	4.9	4.7	4.9
		Mean	4.5	4.2	4.3	4.1	3.9	4.5	3.7	4.1	4.1
	ALS	CODMLB001	3.3	2.9	3.2	2.8	3.6	3.0	2.4	2.5	3.0
Hm21-7		4.0	4.3	4.2	3.3	3.9	3.5	3.5	3.9	3.8	
<u>LSD<sub>0.05</sub></u>		K132	4.8	4.3	5.0	4.6	5.1	4.5	4.3	4.1	4.6
G : 0.10		LSA144	5.6	5.4	4.4	4.4	4.2	4.9	4.4	5.0	4.8
I : 0.10		MAHARAGISOJA	4.2	5.4	4.9	4.2	4.5	4.7	4.7	4.3	4.6
G x I : 0.28		NGWAKUNGWAKU	4.2	4.8	5.1	3.9	4.4	4.4	3.5	4.2	4.3
		RWR10	5.2	4.1	3.7	3.5	3.9	5.0	3.8	4.5	4.2
		Local variety	5.6	5.7	5.7	5.9	5.2	5.0	4.7	5.1	5.4
		Mean	4.6	4.6	4.5	4.1	4.3	4.4	3.9	4.2	4.3
BSM		CODMLB001	3.4	2.5	2.8	2.5	3.7	2.5	2.3	2.6	2.8
	<u>LSD<sub>0.05</sub></u>	Hm21-7	4.2	3.8	3.6	3.3	4.0	3.6	3.5	3.9	3.7
	G : 0.10	K132	4.6	4.1	4.7	4.3	4.8	4.2	4.1	4.5	4.4
	I : 0.10	LSA144	5.2	5.0	4.2	4.0	4.5	4.6	4.6	4.9	4.6
	G x I : 0.27	MAHARAGISOJA	4.4	4.8	4.5	3.9	4.7	4.7	4.1	4.4	4.4
		NGWAKUNGWAKU	4.5	4.5	4.5	3.7	4.2	4.3	3.5	4.3	4.2
		RWR10	5.0	3.5	3.6	3.5	3.7	4.4	3.3	4.3	3.9
		Local variety	5.3	5.4	5.5	4.9	5.3	4.9	5.1	5.3	5.2
		Mean	4.6	4.2	4.2	3.8	4.4	4.1	3.8	4.3	4.2

LSD: Least Significant Difference, E: Environment, I x E: Interaction between disease Incidence and Environment.

planted (Beebe et al., 2000; Gelin et al., 2007; Blair et al., 2009). Under these conditions, only genotypes selected for their physiological ability to adapt to acid soils such as Hm 21-7 can be considered as a prototype (Lunze et al., 2012; Beebe et al., 2008). The performance of Hm 21-7 was higher than reported by CIALCA (2009) in Walungu with 62 mg.kg<sup>-1</sup>, but corroborate with Lunze et al. (2012) for both Hm 21-7 and LSA144. Considering ISFM options, the highest Fe content was obtained with lime combined with manure and chemical fertilizer NPK 10-20-10, and the lowest was obtained with the control. Macronutrient fertilizers containing N, P, and K can have significant effects on the accumulation of nutrients in edible plant products (Allaway, 1986; Grunes and Allaway, 1985). However, providing more Fe to plants than required for maximum yield does little to further increase the Fe in edible seeds and grains (Bouis and Welch, 2010). Among factors pointed out to influence Fe uptake by plants, liming is cited due to its pH increasing effect, which limits the availability of Fe to plants. According to this statement, this study should have obtained lower Fe content with

the application of lime (Elgala et al., 1976; Bohn et al., 1979). This contrasting result should be due to many reasons including: the high potential of tested bean genotype to uptake Fe, initial high Fe content in soils, high buffering capacity of soils, etc. The Fe availability is dictated by the soil redox potential and pH, thus, in soils on aerobic conditions or high pH, Fe is readily oxidized, and is predominately in the form of insoluble ferric oxides. At lower pH, ferric-Fe is released from the oxide, and becomes more available for uptake by roots (Morrissey and Guerinot, 2009).

The result of this study showed that Zn content in bean grains in relation with ISFM was higher in Masisi, Mulungu, and Walungu compared to other sites. Considering ISFM options, the highest Zn content was obtained with lime + NPK, Manure + NPK, and lime + manure + NPK, whereas, the lowest Zn mean content is obtained with the control. Increasing Zn levels via Zn fertilization has been shown for navy beans (*Phaseolus vulgaris* L.), as well as other crops such as rice (Moraghan, 1980; Welch, 1986). It is stated that Zn content of grain was affected by its content in soils,

**Table 11.** Correlations between diseases of Bean Root Rot (BRR), Angular Leaf Spot (ALS) and Bean Steam Maggot (BSM), ISFM, grain yield, Fe, and Zn content.

Diseases	ISFM	Yield	Fe	Zn	BSM	BRR
ALS		-0.25	-0.29	-0.04ns	0.23	0.26
	Check	-0.17	-0.24	0.04ns	0.27	0.23
	Lime	-0.13	-0.32	-0.08ns	0.42	0.30
	Lime + NPK	-0.28	-0.40	-0.11ns	0.22	0.18
	Lime + Manure	-0.18	-0.34	-0.17	0.26	0.19
	Manure	-0.32	-0.31	-0.03ns	0.08ns	0.28
	NPK	-0.23	-0.23	0.07ns	0.14	0.23
	NPK + Lime + Manure	-0.37	-0.28	-0.04ns	0.17	0.33
BRR	NPK + Manure	-0.32	-0.15	0.03ns	0.22	0.27
		-0.24	-0.20	-0.03ns	0.23	
	Check	-0.16	-0.21	-0.07ns	0.26	
	Lime	-0.23	-0.19	0.01ns	0.25	
	Lime + NPK	-0.27	-0.23	-0.07ns	0.14	
	Lime + Manure	0.02ns	-0.05ns	-0.07ns	0.18	
	Manure	-0.27	-0.26	-0.02ns	0.28	
	NPK	-0.19	-0.09ns	0.17	0.13ns	
BSM	NPK + Lime + Manure	-0.49	-0.27	-0.01ns	0.13	
	NPK + Manure	-0.29	-0.16	-0.05ns	0.44	
		-0.19	-0.22	-0.04ns		
	Check	-0.33	-0.11	-0.07ns		
	Lime	-0.24	-0.36	-0.11ns		
	Lime + NPK	-0.25	-0.29	-0.09ns		
	Lime + Manure	-0.17	-0.24	-0.16		
	Manure	-0.14	-0.23	0.02ns		
NPK	-0.32	-0.27	0.00ns			
NPK + Lime + Manure	-0.14	-0.26	-0.01			
NPK + Manure	-0.20	-0.22	-0.03ns			

Legend: ALS = Angular Leaf Spots, BRR= Bean Root Rots, BSM= Bean Steam Maggots.

accordingly, when the amount of Zn increases in soil, the amount of Zn in grain also increases (Cakmak et al., 1989). The Zn absorption capacity is reduced by high P utilization and Zn in plant and soil has an antagonism state with P –negative interaction– (Mousavi, 2011). This statement indicated that the application of chemical fertilizers NPK or manure as a source of P could affect Zn uptake by plants and consequently reduce its content in grains. Furthermore, Zn availability decreases with increasing soil pH, because the minerals solubility reduced and Zn uptake increases with soil colloidal particles such as clay minerals, iron and aluminium oxides, organic matter and calcium carbonate (Irmak et al., 2008; Salimpour et al., 2010; Khorgamy and Farnis, 2009). The best genotypes under the best ISFM option for Zn are CodMLB001, RWK10, and Maharagi Soja. Nevertheless, some influence of the manure-NPK combination on Zn content with RWK10 was observed, likely meaning that this genotype has a better ability to accumulate Zn. Globally, manure-NPK combination allowed an increase of 27.4% of Zn. Lime alone has increased the Zn content for all sites, which was similar

to the results reported by other researchers (e.g. CIALCA, 2009; Sanginga et al., 2009). Based on applied ISFM techniques in relation to agroecological zones, the best response is obtained with lime + NPK applied in Kipopo which induced up to 32.2 mg.kg<sup>-1</sup> Zn.

Low Fe and Zn content observed in other locations such as Kaniama, should be a consequence of antinutrient substances. Antinutrients can depress Fe and Zn bioavailability (e.g. phytate and certain polyphenolics); there are current challenges in increasing micronutrient content in bean grains (Bouis and Welch, 2010). It has been shown that the Fe and Zn content; although dependent on genetic diversity with significant positive correlations (Tryphone and Nchimbi-Msolla 2010; Gepts et al., 2008; Blair et al., 2008), but environmental conditions (soils and climates) and farming practices could modify more or less their concentration in bean grains (Pereira et al., 2014; Beebbe et al., 2000). Obviously, physicochemical and biological properties of the soil as well as the rainfall determine the performance and stability of biofortified bean genotypes (Kanyenga et al., 2016).

### Effect of ISFM options and genotype on the severity of diseases

The incidence of BRR in agroecological zones was lower in Lohotu and higher in Rutshuru (Table 7). The incidence of BRR related to ISFM, was higher with NPK alone and control. Comparing ISFM in studied sites in relation to BRR, the highest incidence was observed in Rutshuru (with lime + NPK treatment) and Walungu (with lime + NPK and NPK alone). This incidence was similar to that observed on control. Comparing ISFM techniques, the highest incidence of ALS was observed with control and lime treatment, whereas, the lowest was observed with NPK + lime + manure. Comparing agroecological zones, the highest incidence of ALS was observed in Walungu and the lowest in Lohotu. The comparison of ISFM in relation to BSM incidence indicates that the highest mean incidence was observed with the control, whereas, the lowest was observed with lime + NPK and NPK + lime + manure. The lowest incidence of BRR, BSM and ALS was observed when lime + manure + NPK was applied to CODMLB001. Local varieties were characterized by high incidence whatever the diseases and applied ISFM technique. Lime-Manure-NPK Fertilizer option reduced root rot severity by 17.8% from 4.5 with control treatment to an incidence of 3.7 according to the CIAT rating scale (Van Schoonhoven, 1992). Furthermore, Lime + manure + NPK Fertilizer option induced the decrease of the severity of angular spots of beans by 15.3% from 4.9 to 3.9, and bean stem maggot by 17.4% from 4.6 to 3.8. This observation corroborates the statement according to which ISFM techniques enhance the natural resistance capacity of bean genotypes to diseases (Tolera et al., 2005). However, observation from Ochilo (2013) showed that, the addition of soil amendments had no influence on the levels of infestation of the bean stem maggot and the black bean aphid, and their associated plant mortality.

The best genotype allowing less severity for all bio-aggressors at all sites was CodMLB001 which, compared to the local variety, showed less severity with a reduction of 40.8% for root rot, 44.5% angular spot disease in Lohotu, 46.2% for the bean stem maggot in Masisi. This states that the use of more or less resistant genotypes as one of the least effective ways to use in the integrated fight against bio-aggressors by reducing the severity of diseases such as angular spots (Kanyenga et al., 2016), root rot (Otsyula et al., 2005), and bean stem maggot. Given that rainfall was generally abundant and well distributed in Masisi, this would contribute to the reduction of the severity of the bean maggot (Wortmann et al. 1998) while the drought would increase its severity in other sites.

### Correlations between yield, micronutrients and the severity of diseases

The effect of ISFM revealed the existence of correlations

negative and positive for the severity of all bio-aggressors and the yield; bio-aggressors and micronutrient content; among the bio aggressors and among the micronutrients.

Yield and bio-aggressors had negative and significant correlations with root rot ( $r = -33\%$ ), bean stem maggot ( $r = -31\%$ ) and angular spot disease ( $r = -21\%$ ). The same trend is observed between iron and diseases 36% with root rot, -24% with bean maggot and -19% with angular spots. This means that when the severity of bio-aggressor increases, the yield and Fe content decreases in the same proportions. This observation should be explained by plant resource allocation principle. Indeed, the allocation of energy for resisting vis-à-vis of diseases reduces the amount of energy that should be allocated to other plant functions and consequently affecting nutrient uptake from soil. Positive and significant correlations were obtained between Fe content and yield ( $r = 72\%$ ), also bio-aggressors between them. This states that the increase of one parameter leads directly to the increase of the other in the same proportions. These correlations between yield and bio-aggressors varied between -33% for BRR, -31% for BSM and -21% for ALS, and are lower than those reported by Jesus Junior et al. (2001). The correlations between different bio-aggressors are similar to the results cited by other researchers (Saettlers, 1991; PABRA, 2008; CIAT, 2010). The degree of severity of ALS disease is more often influenced by genetic, environment, production pathways (Kanyenga et al., 2012), specific strains and breeds of bio-aggressors (Van Schoonhoven and Voyset, 1991). CodMLB001, Hm 21-7 and RWK 10 genotypes remain genotypes with genetic resistance and are therefore a source of varietal resistance in integrated strategies to control diseases for farmers in developing countries (CIAT, 2003; Chataika et al., 2010). The beneficial effects of inputs of inorganic fertilizers and organic amendments have also been reported by several other authors (e.g. Davet, 1996; Isaac, 1992; Mulongoy et al., 1992) but whose effects on the physicochemical and biological properties of the soil and the plant, sometimes inhibit beneficial interactions between beneficial and pathogenic microorganisms. These beneficial effects also depend more on the degree of genotype improvement (Havlin et al., 2005), for example, hybrids respond better than local varieties and environmental conditions (Durrieu, 1993; Tolera et al., 2005; Rui Yu-kui et al., 2009; Lucas and Kunzovas, 2014).

The local variety is everywhere the most sensitive genotype, the least productive, the least performant with regard to Zn and Fe content (except at Kipopo), and the most sensitive to the angular spots disease as previously reported by Kanyenga et al. (2012). This justifies the relevance of ISFM in bean cropping system in particular and crop production in general, as confirmed by Vanlauwe et al. (2015). However, Kankwatsha et al. (2008) and Thung and Rao (1999) consider that the application of organic and humic amendments requires huge quantities that are not always

available and within reach of most producers who are essentially small farmers.

## Conclusion

The potential of increasing bean productivity, micronutrient content and reducing of the disease severities is possible through promotion of appropriate soil fertility management options and genotype of the biofortified bean according to the environment under cultivation. Use of high-performing bean genotypes under integrated soil fertility management, like the combination of lime, manure and mineral fertilizers (NPK 10-20-10); in almost all agro-ecological zones, has significantly increased yield, micronutrient content (iron and zinc) and reduced the severity of major diseases (root rot and angular spots) and pests (bean stem maggot) that are with low soil fertility and rainfall disturbances, factors limiting the production of beans in the DRC. However their technical efficiency (that is aspects related to the availability of large quantities of inputs to cover the needs of crops, and their incorporation in the soil), economic (that is cost of production compared to profitability and return on investment cost) and ecological (that is synergy and antagonism between decomposing microorganisms, pathogens and nitrogen-fixing agents), their application and adoption at the level of small farmers still pose some problems and require a lot of research for their adaptation, dissemination and adoption among producers.

To maximize the production of biofortified beans, and in addition to improve malnutrition due to iron and zinc deficiency; the selection and use of genotypes with good genetic potential (combining high yield with high micronutrient content) and the application of improved soil fertility management techniques should be recommended to farmers. However, this can only be possible in locations with high production potential showing a good response to integrated soil fertility management options and where rainfall variations, even if unpredictable, have only a limited influence. This makes it possible to contribute effectively and in a sustainable manner to the improvement of food security and the reduction of malnutrition due to Fe and Zn deficiency among vulnerable populations.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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