

# Effect of Soil Moisture Regimes on Seed Iron and Zinc Concentration of Biofortified Bean Genotypes against Malnutrition in Sud-Kivu Highlands

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Received: June 10, 2017

Accepted: October 22, 2017

Online Published: November 15, 2017

doi:10.5539/jas.v9n12p241

URL: <https://doi.org/10.5539/jas.v9n12p241>

## Abstract

This study investigated the influence of three soil moisture irrigation regimes on concentration of seed iron and zinc content of four biofortified bean varieties promoted for eradication of malnutrition in Sud-Kivu highlands. A field experiment was conducted in the Hogola marsh highlands during two cultural seasons B2013 and B2014. The experiment design was a RCBD with a split plot arrangement where the main plots were 110 m<sup>2</sup> and split plots 20 m<sup>2</sup>. A strategic application of homogenisation of the experimental site's soil fertility by chemical fertilizers of the type: CaCO<sub>3</sub>, KCl and DAP was conducted out. Four biofortified varieties (CODMLB001, RWR2245, HM21-7 and RWK10) constituted main factor, while water regimes respectively [bottom of the slope: R1 = 48% soil moisture, at the middle of the slope R2 = 37% soil moisture and at the top of the slope: R3 = 29% soil moisture according to the gradient of humidity] represented secondary factor and seasonality, tertiary factor. The study showed that the concentrations of iron and zinc were highly correlated with soil moisture regimes. The variety HM21-7 demonstrated better adaptability because it showed a low rate of reduction of iron and zinc concentration under the three soil moisture regimes and was therefore best suited to fight malnutrition in the Sud-Kivu province.

**Keywords:** micronutrients, malnutrition, Sud-Kivu highlands, CaCO<sub>3</sub>, KCl and DAP

## 1. Introduction

Bean (*Phaseolus vulgaris*) is one of the oldest world cultures preferentially consumed in the human diet due to its gastronomic (Broughton et al., 2002; Beebe, 2010; Silva et al., 2012; Casinga et al., 2016a, 2016b), culinary (Dinste, 2012; Garden-Robinson & McNeal, 2013) and socio-cultural (Dinste, 2012) specificities. Although faced with several environmental constraints throughout its vital life cycle, it is cultivated in more than 20 countries in Southern, Central and Eastern Africa owing to its adaptability to several ecological niches and so occupies more than four million hectares (Broughton et al., 2002; Hacisalihoglu et al., 2005; Yasar et al., 2008; Casinga et al., 2016a). Under swamped conditions, beans stomata remain closed for long periods, resulting in reduced respiration, transpiration and photosynthesis (Boru et al., 2003; Casinga et al., 2015a). Plants may be slow to recover when water recedes. Long-term impacts on the crop are often related to disease infection and retarded root development that limits access to available subsoil moisture later in the season (Naeve, 2002, Boru et al., 2003). Researchers noted that flooding during critical reproductive stages affect yield components including pod number and seed size (Casinga et al., 2015a, 2016a). Despite its agro-ecological potential,

Sud-Kivu is confronted with a strong population explosion, which, coupled with the absence of a coherent land policy (Casinga et al., 2015b, 2016a, 2016b, 2016c), leading to shortage of arable land and the consequent decline in the production of food crops, including beans due to the use of marginal land, including marshes constituting more than 40% of arable land and varieties in full degeneration (Mugangu, 2008; UtshudiOna, 2009; Casinga et al., 2016b, 2016c). This plunges its population into poverty and food insecurity following a rate of chronic protein-energy malnutrition of about 43.7% (Mastaki, 2006; PRONANUT, 2013; Casinga et al., 2016b). Owing to its nutritional richness due to the presence of several macro and micronutrients whose concentrations vary according to the genotype and within genotype (Hacisalihoglu et al., 2005). Besides, this culture has several medicinal properties such as lowering cholesterol (Rosa et al., 1998; Brown et al., 1999; Bourdon et al., 2001; Drewnowski, 2010; Casinga et al., 2016a), coronary heart disease and hypertension (Anderson et al., 2009; Bazzano et al., 2001; Papanikolaou & Fulgoni, 2008), decrease of diabetes, obesity and overweight (Geil & Anderson, 1994; Ledikwe, 2004), favorable effects against cancer (Hangen & Bennink, 2002), high capacity to act as oxidizer (Heimler et al., 2005), the ability to act against gene mutation (Azevedo et al., 2003), produces an antiproliferative effect (Aparicio-Fernández et al., 2006) and reduces digestive disorders (Williams et al., 2008 and stamps out anemia (Holland et al., 1991). Moreover, its cultivation cycle lasts only three months and thus can be grown throughout the year and used as a bridging food due to the agro-ecological diversity of the study area and its multiple products (Wortmann, 1998; Grubben et al., 2004). In addition, through biofortification which is a varietal improvement approach which consists in introgressing, by crossing, the genes that confer micronutrient content, which are the trace elements (mainly iron and zinc) and vitamins (Vitamin A) in plants containing them Less or not (Harvest Plus, 2010).

The objective of this study is to identify the best-performing biofortified bean varieties in Iron and Zinc concentration under periodically flooded swampy lowlands conditions in order to popularize them in households to eradicate malnutrition in this constrained area and to improve the livelihoods of the population.

## 2. Method

### 2.1 Location

The experiment was carried out in Hogola marsh, Karhongo-Nyangezi grouping, that is located in the Ngweshe chieftaincy of Walungu Territory, Sud-Kivu, Democratic Republic of Congo. The geographical coordinates of the station are 28°51' East longitude and 2°28' South latitude whereas the altitude is 1563 m.

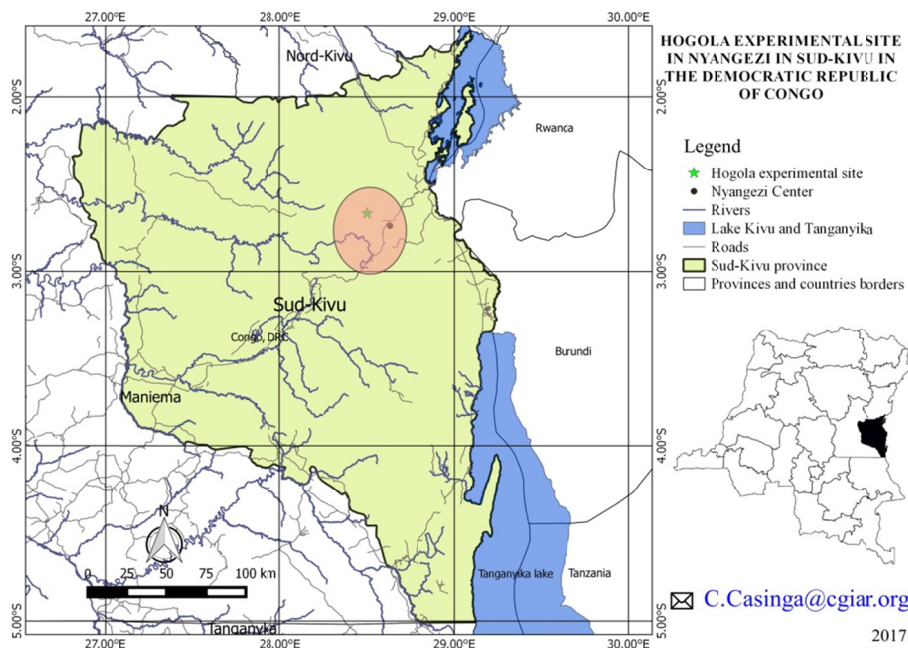


Figure 1. Hogola experimental site in Nyangezi in Sud-Kivu in the Democratic Republic of Congo

Hogola enjoys a  $AW_3$  climate type of Köppen's classification and its soil are classified as Ferralsol according to the FAO-UNESCO (Baert, 1995; Beernaert, 1999; Botula et al., 2012) while its texture is clay-silty (Casinga et al., 2015a, 2016b, 2016c). Subsistence agriculture remains the main activity in our experimentation area in spite of land degradation correlated to the exponential population increase.

## 2.2 Materials

The experimental material consisted of four varieties of biofortified bean: CODMLB001, HM21-7, RWR2245 and RWK10, and chemical fertilizers;  $CaCO_3$ , DAP (18-46-0) and KCl fertilizers with 60% concentration in K.

## 2.3 Implementation of Tests

After clearing the land of weeds using a machete, the experimental site (1280 m<sup>2</sup>) was plowed twice with a hoe to a depth of 30 cm in an interval of 10 days and then manually harrowed with a rake. The land was then subdivided into blocks, plots and sub-plots. Conducted under RCBD design with a split plot arrangement. The blocks were three and parallel on a slope. Their dimensions were 5.5 m × 62 m and separated from each other by a distance of 0.75 m. Three plots of 5.5 m × 20 m were delineated within each block at the bottom of the slope ( $R_3 = 48\%$  soil moisture), at the middle of the slope ( $R_2 = 37\%$  soil moisture) and at the top of the slope ( $R_1 = 29\%$  soil moisture) according to the gradient of humidity, so as to represent three water regimes within each block. Four sub-plots of 1 m × 20 m parallel to the slope were in turn demarcated within each plot.

At the physiological maturity stage, 60 well filled and non-soiled pods were collected on 30 plants located on three central lines of each experimental unit (excluding edging plants) and then shelled and sorted. The healthy seeds were then analyzed to determine the iron and zinc content by atomic absorption after extraction with Triamine Pentacetique acid according to Chauhan et al. (1981) and Okalebo et al. (2002).

Four soil samples were collected using a hole at 25 cm depth in each experiment plot. The latter were mixtured to form a composite sample on which the measurements were made before the sowing date.

Soil moisture was measured by the gravimetric method and in addition soil moisture characteristics  $\theta$  ( $\varphi_m$ ) were determined by the table methods of suction and pressure membrane, respectively for the range of matric potential superior to -0.01 MPa and lower to -0.01 MPa according to the procedure described by Okalebo et al. (2002). Two composite soil samples from each sub-plot taken 25 cm subterranean at the vegetative stage  $V_3$  and harvest stages were analyzed according to Kjeldahl (1883) method' for Nitrogen, while Olsen et al. (1954) method' was used for the estimation of available phosphorus in soils. In addition, potassium was analyzed by Dognin's (1981) method. DAP (18-46-0), KCl with 60% concentration in K and  $CaCO_3$  chemical fertilizers were applied strategically according to the level of the NPK elements in each experimental unit in order to standardize the fertility level of the experimental units. The development of the plots described above made it possible to conduct the experiment according to a factorial model in an experimental split-plot device.

In this study, biofortified bean varieties randomized and sowed in-line in sub-plots at 4 cm depth with two seeds per pooled at 20 × 20 cm were the main factor, while water regimes represented the secondary factor.

The experimentation was conducted in two seasons (February-June 2013 and February-June 2014). These constituted the tertiary factor.

## 2.4 The Observed Parameters

The concentration of iron and zinc content in dry plant biomass at the vegetative stage  $V_3$  and in seeds, micronutrient loss content, as well as water regimes were determined.

## 2.5 Statistical Analyses

The experimental results were evaluated by the analyses of variance, correlation and multiple regression whereas the means were separated by the  $LSD_{\alpha=0.05}$  test.

Excel 2013, R 3.3.0 and Assistat 9.5.1 software were used as a calculation tools.

## 3. Results

### 3.1 Iron and Zinc Content in Dry Biomass and Seeds

Statistical analyzes revealed significant differences both between water regimes and among varieties within different water regimes on micronutrient content ( $P = 0.02$ ;  $\alpha = 0.05$ ). Specifically, zinc showed significant difference between the two experimental seasons and a significant difference between different water regimes whereas for iron no significant difference was observed both seasonality and water regimes for all varieties.

Table 1. Micronutrient content in dry biomass and seed

Varieties	Micronutrient content in biomass												Micronutrient content in seeds											
	Iron (mg/g)						Zinc (mg/g)						Iron (mg/g)						Zinc (mg/g)					
	R1		R2		R3		R1		R2		R3		R1		R2		R3		R1		R2		R3	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
CODMLB001	42	30	54	59	75	76	15	18	25	28	31	34	26	29	54	59	75	76	19	22	25	28	31	34
RWR2245	28	32	28	26	46	49	17	14	25	25	28	32	24	28	28	26	46	49	22	23	25	25	28	32
HM21-7	36	39	45	42	58	60	18	15	26	28	32	33	29	29	45	42	58	60	22	20	26	28	32	33
RWK10	29	29	31	32	44	47	21	19	29	27	33	34	23	24	31	32	44	47	26	23	29	27	33	34
Seasons average	34	33	40	40	56	58	18	17	26	27	31	33	23	23	31	32	44	47	26	23	29	27	33	34
Content in water regimes average	33.1	39.5	56.9	17.1	26.7	32.1	23.4	31.4	45.5	24.5	28	33.7												
Micronutrient loss content average	HM21-7 > RWK10 > RWR2245 > CODMLB001																							

Note. R1 = The top of the landscape, R2 = The middle of the land scape, R3 = The bottom of the landscape, S1 = B2013 season and S2 = B2014 season.

Cumulative seasonal averages of Iron and Zn contents in the dry aerial biomass at the vegetative stage V3 drastically decrease from the top to the bottom of the hill, starting from the water regimes R3 and R1 according to the seasons and the biofortified bean varieties (Table 1). Thus, in season S1, the reductions in Fe content were 55.8 and 33.6 compared with 58 and 32.5 in season S2. Similarly, the reductions in Zinc content were 31 and 17.8 in the S1 season against 33.1 and 16.5 in the S2 season.

Iron and Zinc contents in seeds followed the same trends as observed in aerial parts of the crop. However, rates of reduction of nutrient contents in varieties varied in the order of HM21-7 > RWK10 > RWR2245 > CODMLB001.

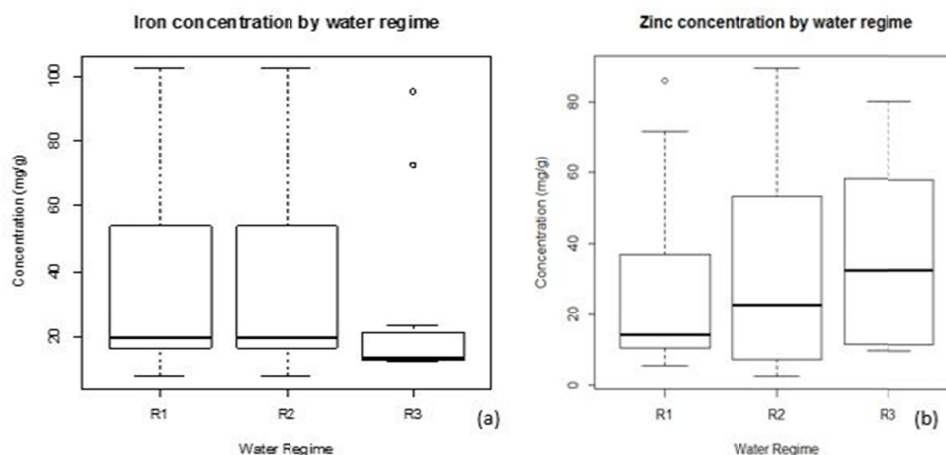


Figure 2. Correlation between micronutrient content and water regime in dry biomass and seed

Note. R1 = The top of the landscape, R2 = The middle of the land scape, R3 = The bottom of the landscape.

The Figure 2 above highlight correlation between the concentrations of iron and zinc content and the water regime, thus showing the influence of water nutrition on the nutrient concentration.

In the Results section, summarize the collected data and the analysis performed on those data relevant to the discourse that is to follow. Report the data in sufficient detail to justify your conclusions. Mention all relevant results, including those that run counter to expectation; be sure to include small effect sizes (or statistically nonsignificant findings) when theory predicts large (or statistically significant) ones. Do not hide uncomfortable results by omission. Do not include individual scores or raw data with the exception, for example, of single-case designs or illustrative examples. In the spirit of data sharing (encouraged by APA and other professional associations and sometimes required by funding agencies), raw data, including study characteristics and individual effect sizes used in a meta-analysis, can be made available on supplemental online archives.

#### 4. Discussion

Plants respond differently to environmental stress by adopting and developing adaptive mechanisms according to Chaves et al. (2003) and Casinga et al. (2015c, 2016b), confirming the results of the different varieties of biofortified bean obtained in the study site. HM21-7 developed good adaptation to by maintaining high Iron and Zinc concentration under this water excess stress. The major effect of excess of moisture on plants is the alteration of the hormonal balance and dry matter concentration and inhibition of photosynthesis and micronutrient uptake (Nunez-Elisea et al., 1999; Pezeshki, 2001; Dat et al., 2004; Kreuzwieser et al., 2004) confirming the decrease of our four genotypes of the micronutrient content of Iron upstream [CODMLB001 (81 > < 40.5 mg/g); RWR2245 (52 > < 42.5 mg/g); HM21-7 (62 > < 40.7 mg/g) and RWK10 (55 > < 37.9 mg/g)] and of Zinc content downstream [CODMLB001 (34 > < 13 mg/g); RWR2245 (34 > < 16 mg/g); HM21-7 (33 > < 18 mg/g) and RWK10 (35 > < 11 mg/g)] during the two experimental seasons. Besides, it confirms the results of Pfeiffer and McClafferty's (2007) work, as well as that of Silva et al. (2012) attesting that biofortified bean's contents in Iron and Zinc is not only genotypical, but like any quantitative character, it is also influenced by environmental factors (agronomic, climatic and edaphic) and G×E interactions such as differential genotype responses to agronomic, climatic and edaphic factors.

#### 5. Conclusion

The concentrations of Iron and Zinc contents are highly correlated with water regimes. It is up to the population living in the mountainous and marginal soils of mountainous Sud-Kivu to cultivate the variety HM21-7 under the three water regimes in case of inaccessibility to arable land. As to national and international organizations, they should popularize the variety HM 21-7 according to the agro-ecological zones because it is best suited to curb malnutrition in the province of Sud-Kivu.

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