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Full Length Research Paper

Interaction between potassium (K) and calcium (Ca) on the severity of Yellow Sigatoka in banana plants

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The main control measure of Yellow Sigatoka (*Pseudocercospora musae*) in banana plants (*Musa* spp.) has been the planting of resistant varieties, and fungicide application. However, the use of adequately nourished plants is also emphasized as a complementary control method. This study evaluated the influence of interaction between potassium (K) and calcium (Ca) in nutrient solution on the severity of Yellow Sigatoka in banana. Evaluation included severity of disease, chlorophyll *a* and *b* contents, nutrient contents, and total dry weight (TDW). There was no interaction between concentrations of K and Ca for area under the disease severity progress curve (AUDSPC), although the AUDSPC increased in leaves 1 and 2 with increasing concentrations of K from 1 to 6 mmol L^{-1}. Increasing K led to a reduction in chlorophyll *a* and *b* contents, and in nutrients N, P, Mg, B, Cu, Zn, and Mn. TDW increased with increasing K. Therefore, high concentration of K causes nutritional imbalance in banana plants, and favors the severity of Yellow Sigatoka.

Key words: Hydroponics, *Musa* spp., nutritional imbalance, *Pseudocercospora musae*.

INTRODUCTION

Banana (*Musa* spp.) is grown worldwide in tropical and subtropical countries. Yellow Sigatoka leaf spot disease, caused by the fungus *Pseudocercospora musae* Zimm (teleomorph *Mycosphaerella musicola* Leach), is a major factor affecting global production, particularly when susceptible varieties are grown in favorable microclimates (Aman and Rai, 2015; Rocha et al., 2012).

The disease reduces photosynthetic area and plant growth, thus affecting fruit quality with obvious consequences for productivity (Castelan et al., 2013). Disease management basically involves genetic and chemical control (Cordeiro and Matos, 2005; Ferreira et al., 2003; Patel, 2009).

In many cases, genetic control for resistance displeases the consumer with a supply of unpalatable varieties without commercial appeal. Also, misuse of chemical control agents results in risks to the applicator, environment, and selection of fungicide resistant

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populations. It also weighs the cost of production, thus reducing producer profits.

However, an alternative method using properly nourished plants can reduce severity of disease (Pozza and Pozza, 2012) without altering fruit taste. Among mineral nutrients, potassium (K) and calcium (Ca) participate in various plant defense responses to plant pathogens (Belan et al., 2014; Garcia Júnior et al., 2003; Pinheiro et al., 2011; Sugimoto et al., 2005).

In general, K-deficient plants are susceptible to infection (Balardin et al., 2006; Sharma et al., 2005; Marschner, 2012; Uchôa et al., 2011). This behavior is not a rule, however, as some studies report increased disease with increasing concentration of K, such as in the cases of *Cercospora coffeicola* in coffee and *Colletotrichum gloeosporioides* in strawberry, due to an imbalance of K and Ca (Garcia Júnior et al., 2003; Pozza et al., 2001; Nam et al., 2006).

Potassium participates in regulating various physiological pathways, such as enzyme activation, protein synthesis, photosynthesis, osmoregulation, transport, and stress resistance (Marschner, 2012). Potassium contributes to improving plant resistance to alterations in protein or amino acid availability, decreased cell permeability, and decreased susceptibility of tissue to maceration and penetration (Prabhu et al., 2007).

With respect to calcium, studies have shown reduction in intensity of diseases with increasing concentration of Ca up to balance point with other ions (Pozza and Pozza, 2012). Calcium plays a key role in recognizing invading pathogens in the plasma membrane, acting as a second messenger (Yang et al., 1997) to maintain biomembrane stability, thus avoiding the outflow of low molecular weight compounds from the cytoplasm to the apoplast (Marschner, 2012). Calcium also acts to build and strengthen the cell wall, thus hindering pathogen infection (Bateman and Lumsden, 1965).

In addition to the isolated effect, it is important to know how the interaction of these nutrients in plants influences the intensity of disease. According to Marschner (2012), cations K^+ and Ca^{2+} compete with each other, and with other nutrients for the same absorption sites, which results in unbalanced plant nutrition.

In other cultures, imbalance of K^+/Ca^{2+} ratio promoted changes in nutritional status, and favored pathogen infection (Carvalho et al., 2013; Garcia Júnior et al., 2003; Lima et al., 2010; Pinheiro et al., 2011; Pozza et al., 2001). In the case of banana plants, the intensity of Yellow Sigatoka was higher in plants grown in K-deficient nutrient solution (Freitas et al., 2015b), and lower in plants grown in soil with higher contents of Ca (Freitas et al., 2015a; Gerald et al., 2003).

Although there is no report emphasizing the effects of interaction of these nutrients in plants prone to *P. musae*, the culture was found to be sensitive to nutritional imbalance (Silva et al., 2008). Thus, knowledge of nutrient effects on the severity of Yellow Sigatoka can help develop management strategies, consequently reducing crop protection applications and increasing environmental and financial sustainability of banana crops. This study evaluated K and Ca interaction in nutrient solution in the severity of Yellow Sigatoka in banana plants.

MATERIALS AND METHODS

Plant material and growth conditions

The present study was conducted under greenhouse at the Plant Pathology Department, Federal University of Lavras (UFLA), Lavras. Lavras is situated in the Southeast region of Brazil at 21°14'S (latitude) and 45°00'W (longitude) at an altitude of 918 m above the mean sea level. The relative humidity and average temperature of greenhouse during the conduction of the experiment was 80% e 25°C, respectively.

Seedlings of micropropagated banana plants (*Musa acuminata* 'Grande Naine AAA Cavendish') with 49 days age obtained from tissue culture were adapted in trays containing 16 L nutrient solution (Hoagland and Arnon 1950) at 50% ionic strength, with continuous ventilation for 15 days.

Once adapted, plants were transferred to 6-liter pots containing nutrient solution with continuous aeration. Treatments consisted of five concentrations of K (1, 2, 4, 6, and 8 mmol L^{-1}) combined with five concentrations of Ca (1, 3, 5, 7, and 9 mmol L^{-1}) in a total of 25 treatments in factorial analysis of variance (5 x 5). The experiment was conducted in randomized block design with three replicated. The experimental unit consisted of one plant per pot, and the whole experiment was repetition once.

Concentrations of N (15 mmol L^{-1}), P (1 mmol L^{-1}) Mg (2 mmol L^{-1}), S (2 mmol L^{-1}) and micronutrients (1 mL of L^{-1} micronutrient stock solution) were the same in all treatments. Micronutrient stock solution was composed of 2.86 g L^{-1} boric acid, 1.81 g L^{-1} manganese chloride, 0.10 g L^{-1} zinc chloride, 0.04 g L^{-1} copper chloride, and 0.02 g L^{-1} molybdic acid. Iron was provided by adding 1 mL of iron stock solution (33.3 g Na_2 EDTA, 100.4 mL NaOH 1N, 24.9 g $FeSO_4.7H_2O$ and 4 mL HCl 1N) L^{-1} in the nutrient solution (Lima et al., 2010).

The pH of nutrient solution was monitored weekly, and kept at 5.5-6 with addition of HCl 0.1 mol L^{-1} or NaOH 0.1 mol L^{-1}. When necessary, volume of pots was supplemented with deionized water. Depletion of ions from the nutrient solution was checked weekly with a Compaction Meter device for K^+ (Horiba-CARDY®). Nutrient solution was changed in all treatments when depletion reached 30% of the initial value of K^+ (Braccini et al., 1999).

Inoculum preparation and inoculation

The isolate of *P. musae* came from diseased banana leaves (Cordeiro et al., 2011), and conidia was obtained using the method described by Freitas et al. (2015b) with modifications. Ten mycelial fragments (5 mm diameter) taken from the colonies after 26 days of growth in Petri dishes containing malt medium (20 g malt extract, 20 g agar and 1,000 mL distilled water) were macerated in mortar and pestle. Then, the mash was diluted in 15 mL tomato juice (200 mL tomato juice, 1g $CaCO_3$, and 900 mL distilled water) and transferred to a 9-cm Petri dish with solid tomato juice (200 mL tomato juice, 20g agar, 1g $CaCO_3$, and 900 mL distilled water).

To facilitate drying, dishes were left open in BOD incubator at 25°C with a 24 h photoperiod, and four 20-watt fluorescent bulbs. Once the culture medium was dry, about two days after incubation, 10 mL sterilized distilled water was added to each Petri dish, and

conidia were released from the dry mycelium with a toothbrush. This suspension was filtered on a double layer of cheesecloth, and concentration was adjusted to 4 x 10^4 conidia mL^{-1} using a hemocytometer, establishing an average of 4 readings.

Inoculation was carried out two months after transferring plants to the nutrient solution containing the treatments. Leaves 1, 2, 3 and 4 were inoculated on the abaxial surface by spraying 0.7 mL conidial suspension in a 36 cm^2 area delineated in the median region. After inoculation, plants were individually covered with transparent plastic bags for 60 h, remaining at 23°C, and relative humidity 92%.

Assessment of disease severity

Severity of disease was evaluated on the abaxial surface of each inoculated area after the first symptoms appeared. Five evaluations were performed at five-day intervals, based on the diagrammatic scale described by Stover (1971) and modified by Gauhl (1994). Scores ranged from 0 to 5, being: 0 - inoculated area without symptoms, 1- inoculated area with up to 10 stains, 2- inoculated area with 1 to 5% stains, 3 - inoculated area with 6 to 15% stains, 4 - inoculated area with 16 to 33% stains, and 5 - inoculated area with 34 to 50% stains. All five evaluations were integrated in the area under the disease severity progress curve (AUDSPC) according to the equation proposed by Shaner and Finney (1977):

$$\text{AUDSPC} = \sum_{i=1}^{n-1} \left(\frac{(y_i + y_{i+1})}{2} \right) (t_{i+1} - t_i)$$

In which:

yi proportion of disease in i-th observation
ti time, in days, in i-th observation
n total number of observations.

Chlorophyll assessment

Chlorophyll content was measured in samples of fresh leaf tissue of banana variety 'Grande Naine' with different shades of green, using a portable SPAD-502® for calibration. After reading, 0.2 g plant tissue was weighed, and macerated in liquid nitrogen, then transferred into tubes containing 10 mL of 80% acetone (v/v), remaining for 24 h in cold chamber protected from light.

After 24 h, extracts were filtered, and reading was performed with spectrophotometer at wavelengths 663 and 645 nm for chlorophyll *a* and *b*, respectively. Determination of chlorophyll (mg gmf^{-1}) was based on the following equations, according to Whitham et al. (1971):

$$\text{Chlorophyll } a = \frac{\left(12{,}7 \text{ x } A_{663} - 2{,}69 \text{ x } A_{645}\right) V}{1000 \text{ MMF}}$$

$$\text{Chlorophyll } b = \frac{\left(22{,}9 \text{ x } A_{645} - 4{,}68 \text{ x } A_{663}\right) V}{1000 \text{ MMF}}$$

In which:

A = absorbance at the indicated wavelength
V = final volume of chlorophyll-acetone extract
MMF= fresh weight in grams of plant material (mg $(g\ MF)^{-1}$).

Once calibration curve was built, readings were taken with SPAD-502® near the inoculated areas, 24 h before inoculation. With the data obtained, contents of chlorophyll a and b were determined using the following equations:

Ya = -0.0035 + 0.0007356X
Yb= -0.0001734 + 0.0002983X

In which:

X= SPAD reading
Ya = chlorophyll *a*
Yb = chlorophyll *b*.

Determination of leaf nutrient contents

Nutrient contents were determined at the end of the experiments, after evaluations were completed. Leaves 3, except the central ribs, were washed in distilled water, placed in paper bags and dried in oven at 60°C until constant weight, as established by Martinez et al. (1999). Then, samples were ground and analyzed according to the method proposed by Malavolta et al. (1997) to determine the contents of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, zinc and manganese.

Determination of plant dry weight

Total dry weight of plants was determined after evaluations were completed at the end of the experiments. Leaves, stems, and roots were washed, packed in paper bags, and placed on a greenhouse bench for preliminary drying, then dried in an oven at 60°C until constant weight.

Data analysis

The Shapiro-Wilk test (Shapiro and Wilk, 1965) was applied to the data of each repetition to evaluate normal distribution. As data were normally distributed, the variables required no transformation. Thus, data from both experiments were subjected to joint analysis over time to determine differences between them. Variables underwent analysis of variance (ANOVA) in a factorial 5 x 5, that is, 5 concentrations of potassium and 5 of calcium, in a total of 25 treatments. Linear regression models were fit to significant variables in F test ($p \leq 0.05$). Response surfaces were fit in case of significant interaction. Normal distribution of data was analyzed using R software, while the remaining analyses were performed using PROC GLM procedure in SAS software (v.9.2, SAS Institute Inc.).

RESULTS

Joint analysis of data

Joint analysis of variables over time showed no significant difference ($p \leq 0.05$) between experiments. Thus, results are the average of two repetitions.

Severity of Yellow Sigatoka

Early symptoms of Yellow Sigatoka in areas inoculated with *P. musae* were observed 26 days after inoculation.

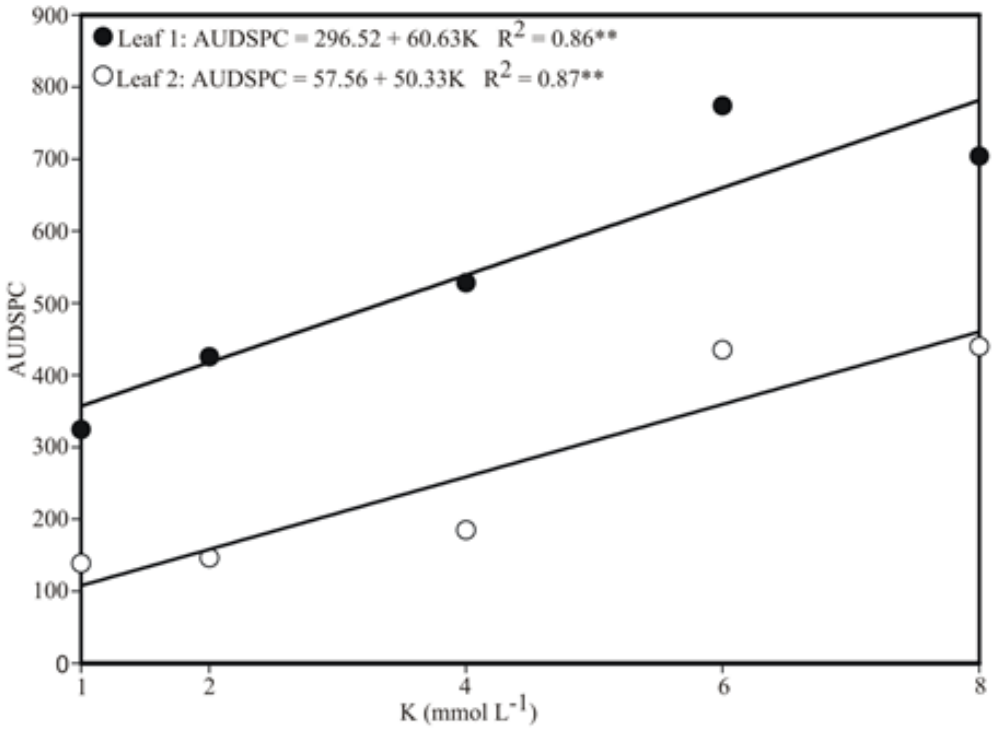

Figure 1. Area under the disease severity progress curve (AUDSPC) in leaves 1 and 2 of banana plants, depending on increase in potassium (K) concentrations in nutrient solution (**Significant (p≤0.01)).

There was no interaction between K and Ca concentrations for the area under the disease severity progress curve (AUDSPC). There was no effect of isolated concentrations of Ca on AUDSPC with overall average 551.17 and 268.96 in leaves 1 and 2, respectively.

However, increase in K concentrations from 1 to 6 mmol L^{-1} influenced (p≤0.01) increase in AUDSPC from 357.15 to 660.3 and 107.89 to 359.57 in leaves 1 and 2, respectively. AUDSPC in leaf 1 was on average 51.37% higher than in leaf 2 (Figure 1). AUDSPC in leaves 3 and 4 was not affected either by interaction of K and Ca concentrations or by isolated effect of these nutrients (Figure 1).

Content of chlorophyll *a* and *b*

There was no interaction between K and Ca concentrations for leaf contents of chlorophyll *a* and *b*. However, increase of these nutrients reduced the content of chlorophyll *a* and *b* in leaves in an independent way. With increasing concentrations of K from 2 to 8 mmol L^{-1}, leaf content of chlorophyll *a* and *b* decreased from 0.037 to 0.035 mg gmf^{-1} and 0.016 to 0.015 mg gmf^{-1}, respectively (Figure 2 a and b). Increase of Ca from 1 to 9 mmol L^{-1} decreased leaf contents of chlorophyll *a* and *b* from 0.038 to 0.035 mg gmf^{-1} mg, and 0.016 to 0.015 mg gmf^{-1}, respectively (Figure 2c and d).

Nutritional aspects of banana plants

There was an interaction between K and Ca concentrations for leaf contents of sulfur (S) and calcium (Ca). The highest Ca leaf content (25.77 g kg^{-1}) was found in concentrations 9 and 2 mmol L^{-1} of Ca and K, respectively (Figure 3 k).

Regarding S, the highest content (6.5 g kg^{-1}) was observed in the combination of concentrations 1 and 8 mmol L^{-1} of Ca and K, respectively (Figure 3 l). Separately, increasing K and Ca concentrations influenced nutrition of banana plants.

Regarding K, increase in concentrations from 1 to 8 mmol L^{-1} raised K leaf content from 28.80 to 40.14 g kg^{-1} respectively (Figure 3 a). However, increasing K (Figures 3 b to h) reduced leaf contents of N (46.34 to 37.45 g kg^{-1}), P (3.95 to 2.35 g kg^{-1}), Mg (5.31 to 2.41 g kg^{-1}), micronutrients B (46.44 to 32.62 mg kg^{-1}), Cu (7.73 to 3.4 mg kg^{-1}), Zn (15.16 to 12.1 mg kg^{-1}) and Mn (289.29 to 122.62 mg kg^{-1}).

Increased concentration of calcium from 1 to 9 mmol L^{-1} influenced reduction in N leaf content from 44.80 to 39.47g kg^{-1} (Figure 3 i). In addition, Mg leaf content decreased from 4.27 to 3.39 g kg^{-1} up to 4.70 mmol L^{-1},

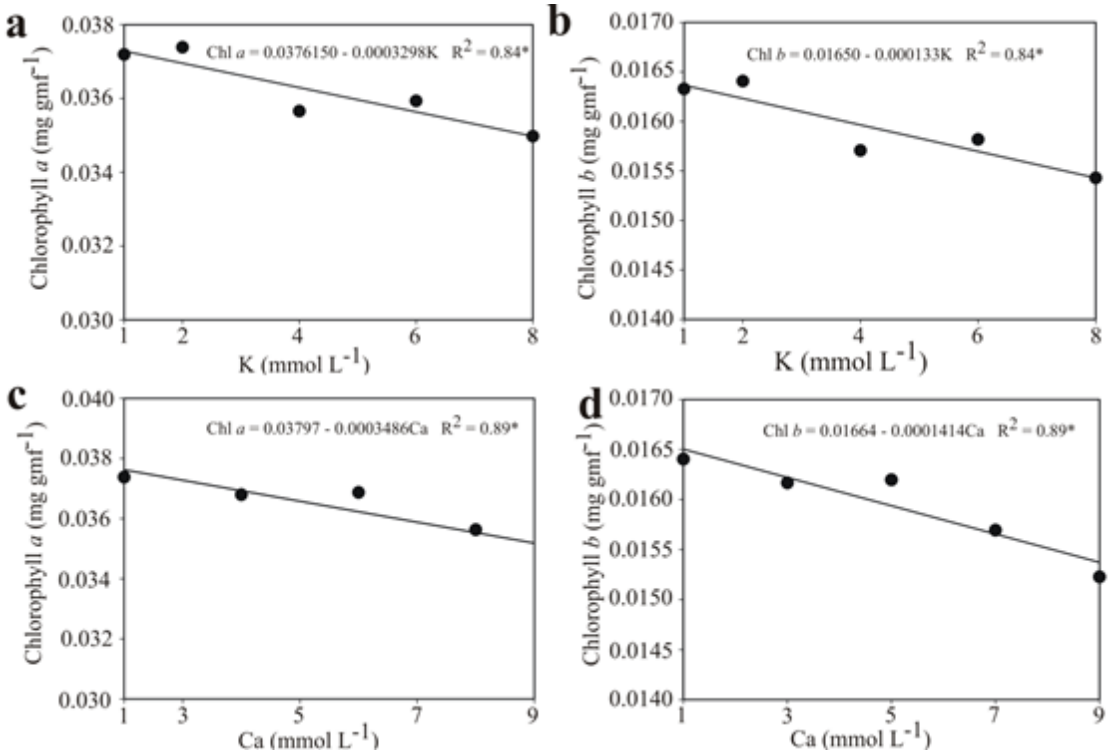

Figure 2. Leaf content of chlorophyll a (a and c) and b (b and d) in banana leaves, depending on increase in potassium (K) and calcium (Ca) concentrations in nutrient solution. gmf: gramm of fresh weight (*Significant (p≤0.05)).

and increased from this concentration up to 4.57g kg^{-1} at 9 mmol L^{-1} (Figure 3 j).

Plant dry weight

Total dry weight (TDW) of plants was not influenced either by interaction of K and Ca concentrations or by isolated effect of Ca concentration. However, increased K concentrations significantly influenced TDW increase, ranging from 59.43 to 170.52 g $plant^{-1}$ at K concentrations 1 and 8 mmol L^{-1} respectively, that is, TDW increased 15.87 g per 1 mmol L^{-1} of K supplemented in nutrient solution (Figure 4).

DISCUSSION

Addition of K to nutrient solution influenced increase of area under the disease severity progress curve (AUDSPC) for *P. musae* in banana plants. However, Uchôa et al. (2011) found reduction in severity of Black Sigatoka (*M. fijiensis*) from 260 to 117 intensity with increasing potassium concentration in soil of planting areas from 28 to 57.5 mg dm^{-3}, respectively.

Conversely, Freitas et al. (2015b) found that absence of K in nutrient solution resulted in higher severity of *P. musae* in banana plants. The findings of this study are different from those reported by Uchôa et al. (2011), obviously due to experiment conditions. Several soil factors can influence the nutritional status of plants. In nutrient solution, by contrast, it is possible to isolate the effect of each nutrient, enabling the study of relationship between nutrient effects and intensity of disease (Lima et al., 2010).

Experiments also conducted in nutrient solution for other pathosystems showed similar results to this study. The area under the progress curve of cercospora leaf spot (*C. coffeicola*) of coffee increased from 14.6 to 17.39 with increase of K from 4 to 7 mmol L^{-1}, respectively (Garcia Júnior et al., 2003).

Also in coffee, phoma leaf spot (*Phoma tarda*) increased the area under incidence progress curve (AUIPC), and area under severity progress curve (AUSPC) with K contents above 6.59 and 6.57 mmol L^{-1}, respectively (Lima et al., 2010). Excess of potassium, 30 mmol L^{-1}, also influenced increase of anthracnose (*Colletotrichum gloeosporioides*) in strawberries (Nam et al., 2006). Potassium applied appropriately in soil, 8% K_2O, reduced anthracnose (*Discula destructiva*) in *Cornus florida* L.

However, opposite results were found with double amount of K, 16% K_2O (Holzmueller et al., 2007). That is, as the effect of K on intensity of disease depends on

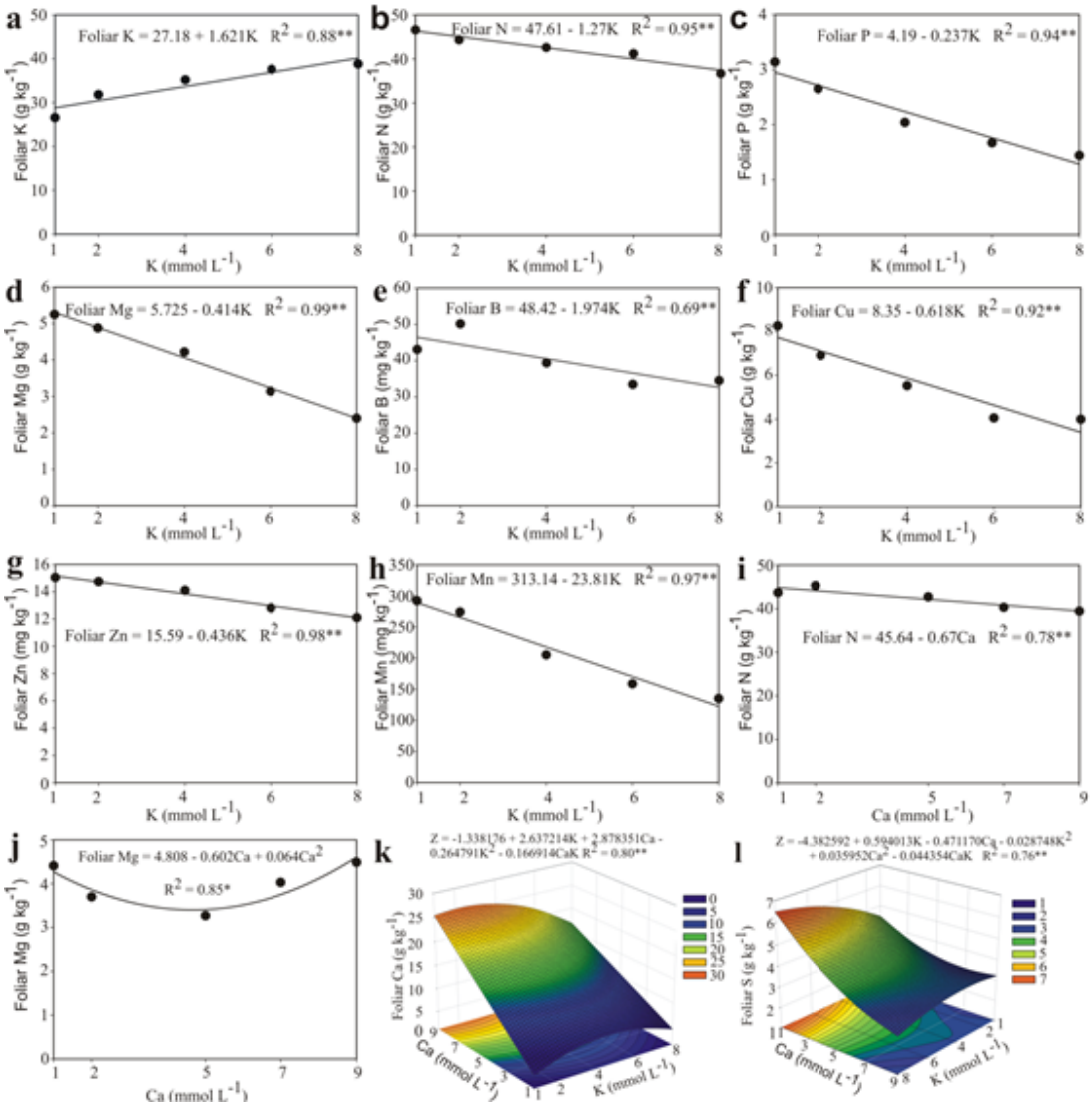

Figure 3. Leaf contents of potassium (a), nitrogen (b and i), phosphorus (c), magnesium (d and j), boron (e), copper (f), zinc (g), manganese (h), calcium (k) and sulfur (l) in banana leaves, depending on increase in potassium (K) and calcium (Ca) concentrations in nutrient solution. **Significant (p≤0.01) (*Significant (p≤0.05)).

the pathosystem, it is not possible to generalize the findings. Soil factors such as interaction between nutrients, organic matter and pH, and buffering capacity, texture, and structure should also be considered. Hosts may also influence this interaction depending on their nutritional requirements at each stage of life cycle. As it is known in the literature, banana plants require large amounts of K (Borges and Souza, 2004). Overall, leaf nutrient contents were outside both the range considered appropriate (Borges and Souza, 2004), and the reference values (Martinez et al., 1999) established for banana growing.

Thus, it can be concluded that increased severity of Yellow Sigatoka was due to plant nutrient imbalance. Freitas et al. (2015b) also found increasing severity of Yellow Sigatoka caused by nutrient imbalance in banana plants. Similarly, imbalance due to high contents of S and low contents of P, Ca, and Mg in the soil resulted in increased severity of Black Sigatoka from 117 to 340 intensity (Uchôa et al., 2011).

In coffee, increase of K contents up to 7 mmol L^{-1} reduced absorption of N and Ca, making plants more susceptible to cercospora leaf spot (Garcia Júnior et al., 2003; Pozza et al., 2001). In the same culture, imbalance in ratio N/K also promoted changes in the nutritional status of plants and favored infection by *P. tarda* (Lima et al., 2010).

According to Huber and Haneklaus (2007), nutrient

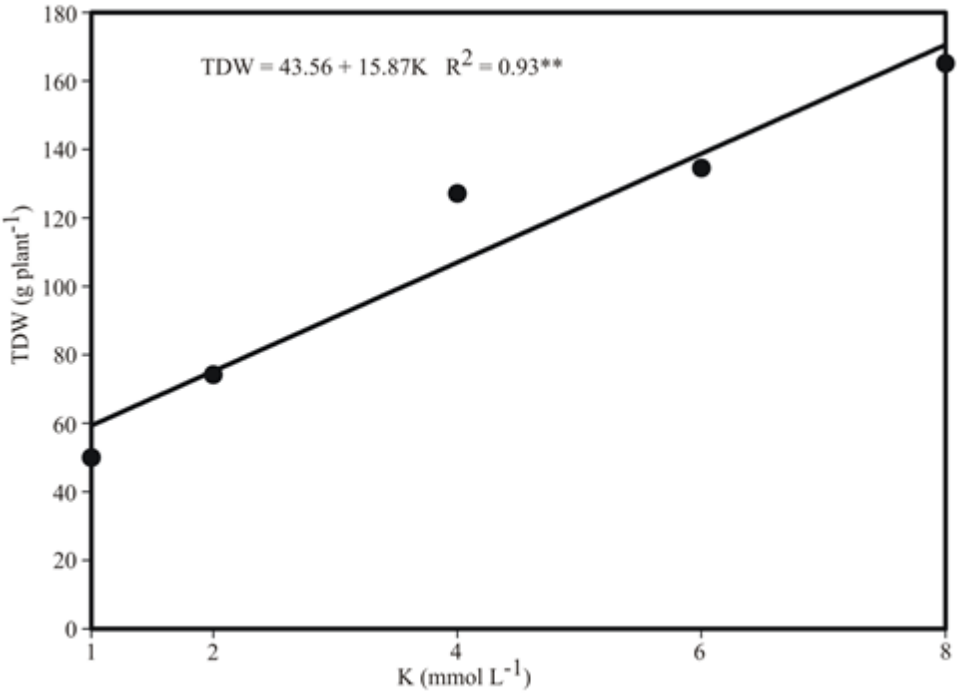

Figure 4. Total dry weight (TDW) of banana plants depending on increase in potassium (K) concentrations in nutrient solution (**Significant (p≤0.01)).

imbalance can be as harmful to plant resistance to disease as nutrient deficiency. According to Marschner (2012), high concentrations of K can cause nutrient imbalance by reducing the uptake of other nutrients. This result was confirmed by Pinheiro et al. (2011), who found a reduction of 26.4 % in Ca contents in soybean leaves with increasing K in nutrient solution.

Increasing K also reduced leaf contents of chlorophyll *a* and *b*. Chlorophylls are green pigments specialized in absorbing light and transferring energy and electrons during photosynthesis, and are thus related to this process efficiency (Taiz and Zeiger, 2013).

However, leaf chlorophyll content can be reduced under physiological stress (Larcher, 2004). Thus, reduction in contents of chlorophyll *a* and *b* can be attributed to nutritional imbalance, as, besides being part of plant molecular structure, mineral nutrients also participate in important reactions during chlorophyll synthesis (Neves et al., 2005).

As an example, Mg, N and Mn show contents outside the suitable range for banana. As magnesium is part of the structure of chlorophyll molecule (Marschner, 2012), Mg-deficient plants are more susceptible to disease (Huber and Jones, 2013).

In coffee, Alves et al. (2009) found higher intensity of rust (*Hemileia vastatrix*) in leaves and cercospora leaf spot in leaves and fruits in crops with Mg, S, N, and Cu deficiency. In rice, sheath blight (*Rhizoctonia solani*) was reduced with increasing Mg concentration from 0.062 to 0.5 mmol L^{-1} (Schurt et al., 2014).

Regarding N and Mn, the former is important as a chlorophyll constituent, and the latter for its role in photosynthetic reaction, in which oxygen is produced from water (Taiz and Zeiger, 2013). The positive effect of these nutrients in reduction of plant diseases has been demonstrated in other studies. Furtado et al. (2009) found higher incidence of Panama disease (*Fusarium oxysporum* f. sp. *cubense*) in banana plants with lower contents of nitrogen. Incidence of cercospora leaf spot of coffee was reduced by 20.7% with increase of N from 3 to 15 mmol L^{-1} (Pozza et al., 2001). In wheat, severity of spot blotch (*Bipolaris sorokiniana*) was reduced with increasing Mn in leaves (Zanão Júnior et al., 2009).

In addition to nutrient imbalance, plant growth stage may explain the difference in susceptibility to *P*. *musae*, as AUDSPC was higher in young leaves. Similar results were found in banana plants infected with *M. fijiensis* (Kablan et al., 2012; Romero, 1995). Kablan et al. (2012) attributed these results to the formation of physical or physiological barriers in old leaves, acting either during or after fungal invasion. In this study, as there was no significant difference for AUDSPC in leaves 3 and 4, the formation of physical barriers such as lignification and cuticle thickening helped reduce the intensity of disease.

Although addition of K had increased AUDSPC, and reduced leaf contents of N, P, Mg, B, Cu, Zn, Mn, and chlorophyll *a* and *b*, there was increase in total dry weight of plants. Likewise, Silva et al. (2008) found higher dry

weight of banana plants grown with higher doses of K in soil, up to 1.600 mg dm^{-3}. Increased dry weight in contrast to reduction in contents of most nutrients can be explained, since contents were still close to the suitable range for banana even after reduction (Borges and Souza, 2004; Martinez et al., 1999).

In addition, availability of water and nutrients to plants is constant during experiments in nutrient solution, as water level is kept constant and nutrients are changed when depletion reaches 30% of the initial value (Braccini et al., 1999). Under these conditions, plant growth is possible even with low contents of some nutrients.

According to Marschner (2012), although high concentrations of K decrease the contents of other nutrients, plant growth may increase. In banana culture, K and N are the most important nutrients for plant growth (Borges and Souza, 2004).

Thus, in this study, the favorable conditions of hydroponics and increase of K were responsible for increasing plant weight. Reduction in contents of chlorophyll *a* and *b* was insufficient to affect plant growth. Also, as disease manifested only in the inoculated area and not in the full leaf, there was no influence on total dry weight of plants.

Conclusions

High concentrations of K in nutrient solution promoted nutritional changes in banana plants, mainly by reducing leaf contents of N, P, Mg, B, Cu, Zn, and Mn. As a result of this imbalance, there was an increase in severity of Yellow Sigatoka. Therefore, proper and balanced fertilization can minimize nutritional changes in banana plants, and reduce the number of fungicide sprays for controlling *P. musae*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Crop yield potential as telltale indice of soil weathering extent and fertility status: The case of East African Highland Bananas

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In the African Great Lakes Region, bananas are grown on a diversity of soils with different weathering stages. However studies using the crop yield potential as a mean of soil weathering degree assessment are still scanty. Bananas were grown on five soils types to test if such a relationship could be ascertained. Mineralogical composition, elemental total analysis, routine chemical analysis, oxalates and dithionite-citrate-bicarbonate (DCB) extractions on the 0-20 and 20-40 cm soil layers were used as soil characteristics. Banana yield was higher in Cibitoke where the soil was characterized with relatively high values of total reserves in bases (TRB) and the weathering index of Parker (WIP). In contrast, no yield was recorded in Gitega where the soil had relatively lower values of TRB and WIP and high Fe_{DCB}/Fe_{total} ratio. Furthermore, banana yield was strongly and significantly ($p<0.05$) correlated with the TRB, the mineral reserves, $Fe_{oxalate}/Fe_{DCB}$ ratio, the silt content and poorly correlated with the soil pH, total carbon and nitrogen, available P, exchangeable bases and the CEC. It was concluded that banana yield potential reflected well the soil weathering extent and in complement to soil properties related the routine analysis, the total analysis provide even more precision to elucidate the snapshot of the soil properties in the light of the observed banana yield potential.

Key words: Banana, yield potential, correlation, routine analysis, soil weathering indices, total analysis.

INTRODUCTION

The scope of this study is restricted to Burundi and Democratic Republic of Congo (DR Congo). These two countries are located in African Great Lakes Region (AGRL). AGLR comprise a system of plateaus, mountains, valleys and lakes, located between 2°N and 10°S and extending over six countries namely Burundi, Kenya, Rwanda, Tanzania, Uganda and the eastern province of DR Congo (Davies, 1995). In the AGRL, bananas referred to East African Highland banana (EAHB-*Musa spp-AAA*) are largely represented (Karamura et al., 1998; Nyombi et al., 2009). EAHB group includes cooking bananas and beer bananas. With

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dessert bananas types and plantains, they compose the three banana categories encountered in East Africa (Karamura, 1998).

In that region, bananas are found on a diversity of soils, among which Ferralsols, Nitisols and Acrisols are most common (Davies, 1995; Rishirumuhirwa, 1997; Okalebo et al., 2006) and represent respectively 27, 18 and 8 % of the production area (Eledu et al., 2004). These soils are generally characterized by low levels of fertility (Davies, 1995; Rishirumuhirwa 1997; Eledu et al. ,2004) resulting from their high degree of weathering and leaching, which in turn are the cause of low crop productions in the majority of African countries (Okalebo et al., 2006). Thus for instance, by a simple observation, it is easy to recognize that plant growth and production is poor in one or another site and relatively better in others. As crop production depends on various environmental factors including climatic, edaphic, parasitic and human factors (Godefroy et al., 1991), it is not easy to assign the performance levels at one or another factor affecting the crop yield. Owing to the low inherent fertility of the AGLR dominant soils (Ferralsols, Acrisols and Nitisols) (Davies, 1995) and their more or less advanced weathering stage (Okalebo et al., 2006), the physico-chemical soils' characteristics, may constitute one of the major factors influencing yields variation. As plant growth, vigour and yield are dependent upon the availability of a number of essential element nutrients and other soils properties such as its texture or structure, salinity, acidity, waterlogging, or compaction, Plant growth and yield may be excellent indices of inherent soil fertility and weathering extent. The physico-chemical characteristics of soil can serve in the soil weathering status assessment (Souri et al., 2006)**.** In particular, weathering indices are commonly used to characterize in situ weathering soil profiles as well as for the assessment of soil fertility and soil development (Price and Velbel, 2002). Several types of indices have been used by different authors to characterize the weathering stage of tropical soils: the ratios between acid ammonium oxalate extractable iron and aluminum (Fe_o, Al_o) and Na dithionite-citrate-bicarbonate (DCB) extractable iron and aluminum (Fe_d, Al_o), the quantity of DCB extractable free iron and aluminum (Fe_d, Al_d) and total iron content (Fet) (Delvaux et al., 1989 ; Mahaney et al., 1994 ; Dalmolin et al., 2006; Zhang et al., 2007; Moody et al., 2008; Neto et al., 2010), the total reserve in bases (TRB) (Herbillon, 1986; Delvaux et al., 1989) and others indices types among which the weathering index of Parker (Delvaux et al., 1989; Price and Velbel, 2002; Patino et al., 2003; Haskins, 2006; Souri et al., 2006).

The ratio Fe_d/Fe_t, which expresses the transformation of iron-containing silicates into pedogenic iron oxides, is often employed as a weathering index (Kämpf and Curi, 2000). The sum of total alkaline and alkaline earth cations constitutes the TRB which estimates the content of weatherable minerals in mineral soil horizons (Herbillon, 1986).

TRB includes the bases occluded in primary minerals as well as those located on exchange sites and also possibly in the secondary clay minerals (Delvaux et al., 1989). Among numerous chemical indices used to characterize the profiles alteration degree, Price and Velbel (2002) found that the weathering index of Parker (WIP) had the merit to be valid as well as for profiles on homogeneous and heterogeneous parental material. The WIP has been suggested by Parker in 1970; it allows determining the alteration degree of different rocks. This index is very sensitive to chemical changes in the early stages of alteration (Palma et al., 2003).

There have been many studies on soils characteristics and crop performance relationship since Belgians period in the two aforementioned countries (Burundi and DR Congo) but these works have focused much more on relationship between industrial crops (cocoa, coffee, cotton, hevea, oil palm, tea, sugar cane) and classical agronomic indices of fertility (soil organic matter content, exchangeable bases, soil' nitrogen content, available P) throughout fertilization trials and management practices. Studies dealing with others chemical' soil quality indicators, that is, weathering indices for predicting yield especially on banana in the study area are very rare.

From recorded total banana yield, physico-chemical characteristics and some weathering indices of different soils types, this present study seeks to:

1. Assess the trends of the different physico-chemical soil characteristics and weathering extent of 5 soil types in the AGLR
2. Determine the relationship between the different parameters with focus on observed highland bananas yield levels and the soil chemical properties including the weathering indices; to find out what indicators correlate best with yield.

MATERIALS AND METHODS

Experimental sites

Five sites (three in Burundi and two DR Congo) located in different agro-ecological zones (AEZ) were selected as part of the CIALCA project in order to study mulch-based cropping practices (DMC) in banana-bean intercropping system. The sites were selected based on their representativeness of the AEZ regarded as principal banana production areas in those countries. Some characteristics of the experimental sites are summarized in the Table 1.

Agronomic potential evaluation according to Banana performance

A beer banana *(Musa acuminata* Colla *(AAA-EA)),* cultivar Igitsiri or Ndundu (respective local name in Burundi and DR Congo) which belongs to the group of EAHB was planted at the above mentioned sites to evaluate the agronomic potential of different soils types. Bananas were intercropped with common bean (*Phaseolus vulgaris* L.*)* (a legume). Various no-till treatments with mulching were

Table 1. Some general characteristics of the five experimental sites.

Country	Site	Soil type-FAO/ Geology[a]	GPS reading (longitude and latitude)[b]	Altitude[b] (m asl)	Annual mean rainfall[c] (mm)	Annual mean temperature (°C)[e]	AEZ	Previous crops
Burundi	Gitega	Ferralsol/Schisto-quartzites	29.90405°E 03.37791°S	1646	1101	20.0	Central Plateaus	Grassland with *Eragrostis* spp and *Hyparrhenia* spp
	Kirundo	Nitisol/shales	30.23874°E 02.54601°S	1604	1034	21.9	Northen depressions	Cassava
RDC	Cibitoke	Fluvisol/recent fluvial alluvium (from the cenozoic and the quaternary)	29.07282°E 02.84061°S	882	958***	24.1***	Ruzizi Plain	Sweet potato
	Mulungu	Nitisol/Basalts from volcanic ashes*	28.78826°E 02.33499°S	1699	1725	23.3	Kivu montagneux	Sweet potato
	Walungu	Ferralsol?/Basalts**	28.71889°E 02.68861°S	1638	1850	24.0	Kivu montagneux	Complex intercropping****

[a]Carte des sols du Burundi au 1/250000ème, Carte géologique du Burundi au 1/250000ème, *[B]Delvaux, personal communication (2009), **SOTERCAF; [b]Field records; [c]IGEBU, Web *LocClim* (FAO, 2006), *** Annual mean rainfall and temperature for the period 1985-1995 and 2007-2009 (IGEBU); ****Legumes, cereals and roots and tubers crops.

compared to a control treatment with tillage and no mulch in a randomized complete block experiment with 4 replicates. The plots without mulch were ploughed twice a year and superficial weeding with hand hoe was performed for the rest of the year to control weeds. No other inputs (fertilizers, manure, lime, mulch) were applied. In the present study, only the control plots were used because they were assumed to reflect as much as possible the natural soil fertility status and hence provide a proper estimation of the agronomic potential and a snapshot of the weathering stage. Banana performance (yield, growth rate) which partially reflects the agronomic potential of sites was evaluated on the basis of yield recorded 30 months after field establishment. Indeed, after this period, bananas were supposed to have completed the first cycle at all sites. This means that banana bunches were harvested and the corresponding weight recorded systematically, as each individual plant became physiologically mature within 30 months. Thus, the total yield per hectare was computed based on the total weight of bunches harvested in the 4 control plots of 100 m^2 each (Burundi) or 96 m^2 (DR Congo) in relation to the surface of 1 ha.

Soil sampling and analysis

In each site and just at the trial inception, soil samples were collected from two different soil layers (0-20 and 20-40 cm) with an auger. For each soil depth, a composite sample was made from 5 subsamples in each of the 4 control plots. For the 5 sites, a total of 10 composite samples (5 from the surface layer and 5 from underlying layer) were therefore subject to different physico-chemical analyses. Particle size analysis was performed on the fine fraction using the pipet method after ultrasonic dispersion in salt-free water with Na^+-resins without any pretreatment for organic matter destruction. Soil pH was measured potentiometrically in H_2O (pH_{water}) and 1 M KCl (pH_{KCl}) in 1: 2.5 soil-solvent suspension. The total N and carbon were determined by **g**as-liquid partition chromatography (GLPC) after a dry combustion using an auto Analyzer CN Flash EA1112. Available P was extracted using Mehlich-3 extraction solution (Mehlich, 1984) and was measured by Atomic Emission Spectrometry (ICP-AES). Cation exchange capacity (CEC) and the exchangeable cations content were determined according to Jackson (1965) (ammonium-acetate pH7 method). Effective cation exchange capacity (ECEC) was the total amount of exchangeable bases (Ca, Mg, K, Na) and the exchangeable acidity (Al and H). Exchangeable acidity was measured by titration with NaOH after H^+ and Al^{3+} ions displacement by a 1 N KCl solution. Al^{3+} ion was determined separately by fluoride complexation. Total carbon and total nitrogen content was determined by dry combustion. The clay fraction mineralogical composition was determined at 20°C by X-ray diffractometry after the clays saturation by K^+ and Mg^{2+} ions.

Total elemental analysis was carried out for the 10 soil samples according to Chao and Sanzolone (1992): Soil samples were crushed and powdered (particles diameter < 100 µm). A crushed and powdered sample of 0.1 g was mixed with 1.6 g of lithium metaborate and 0.4 g of lithium tetraborate poured into a graphite crucible. This mixture was then melted at 1000°C for 5 min in a kiln. After this time, the melt obtained was removed from kiln for cooling. The cooled melt was entirely dissolved in 100 ml of 2 M HNO_3 under magnetic agitation at 100°C. The analyses were then performed on the resulting solution. The total

Table 2. Selected chemical characteristics of 5 soils types of the RGL (Burundi and DR Congo).

Site	Depth (cm)	pH_{Water}	pH_{KCl}	Ca	Mg	K	Na	Al	H	S	ECEC	CEC	BS	N_{tot}	C_{tot}	P	C/N*
				cmolc kg^{-1} soil										%		(mg kg^{-1})	
Mulungu	0-20	6.0	6.0	21.71	4.89	2.10	0.06	0.02	0.02	28.77	28.84	46.9	61.3	0.42	4.86	115.2	10.5
	20-40	6.3	6.2	19.65	5.10	1.22	0.08	0.03	0.02	26.05	26.10	41.1	63.3	0.32	3.48	NM	9.8
Walungu	0-20	5.8	5.8	12.11	5.57	0.25	0.04	0.05	0.04	17.97	18.06	28.5	63.2	0.20	2.71	1.04	12.2
	20-40	5.7	5.6	9.34	4.76	0.14	0.03	0.04	0.00	14.27	14.31	24.3	58.6	0.16	1.85	NM	10.3
Gitega	0-20	4.2	4.1	0.45	0.32	0.08	0.01	0.48	0.66	0.87	2.01	10.1	8.6	0.13	2.04	5.23	14.0
	20-40	4.4	4.4	0.66	0.55	0.06	0.01	0.60	0.16	1.28	2.04	7.6	16.8	0.11	1.79	NM	14.4
Kirundo	0-20	5.6	5.5	13.60	4.55	0.11	0.02	0.03	0.01	18.28	18.35	29.3	62.5	0.27	4.20	5.40	14.1
	20-40	5.7	5.6	13.91	4.55	0.08	0.04	0.00	0.02	18.58	18.60	29.2	63.6	0.25	3.91	NM	14.1
Cibitoke	0-20	5.8	5.8	6.40	4.80	0.48	0.10	0.00	0.02	11.77	11.79	14.8	79.3	0.14	1.57	4.34	9.9
	20-40	6.6	6.5	5.90	6.16	0.33	0.15	0.00	0.00	12.53	12.53	13.4	93.4	0.08	0.82	NM	9.4

The C: N ratio is obtained from the organic carbon (C_{org}) estimation based on the relationship between C_{tot} and C_{org} established by Delstanche (2011) from soils with pH value < 7 as it is the case for soils under study; NM = analysis not performed.

contents of elements (Si, Al, Fe, Ca, Mg, K, Na, Ti, and Mn) were determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) spectrometry. As the soil of Mulungu was suspected to be an andisol; P retention was determined by ICP-AES spectrometry after the P extraction method described in Blackmore et al. (1981). For different elements, the analyses of various free (Al_d, Fe_d, Mg_d, Mn_d, Si_d) or amorphous (Al_o, Fe_o, Mg_o, Mn_o, Si_o) fractions were performed respectively on hot DCB and 0.2 M acid ammonium oxalate in the dark extractions. The Na-pyrophosphate extractions used for organically bound Fe and Al assessment were not performed. DCB is supposed to extract crystalline, amorphous and organically bound elements and acid ammonium oxalate extracts amorphous and organically bound elements (McKeague et al., 1971).

Weathering indicators calculation

In addition to indicators based on ratios and relationships resulting from different fractions (free, amorphous and total) of the elements (Al, Fe, Mg, Mn, Si), other types of indicators like the TRB, the mineral reserves (MR) and the WIP are also used. TRB was calculated as the sum of the total contents of alkaline and alkaline earth cations Ca+Mg+K+Na (cmolc kg^{-1}) (Herbillon, 1986; Delvaux et al., 1989). Mineral reserves in the soil (MR) referred to as "weatherable minerals" (Udo de Haes et al., 2012) were calculated as the sum of the differences obtained between the total quantity of each base and the equivalent exchangeable quantity (Titeux, personal communication, 2012) according to the following equation:

$$MR = \sum_{i=1}^{n=4}(X_{it} - X_{ie}) \quad (1)$$

Where i corresponds to each of the 4 cations (Ca, Mg, K and Na), X_{it} is the total quantity and X_{ie} is the exchangeable quantity of cation i. The WIP is calculated from the expression (Delvaux et al., 1989):

$$WIP = \left[(2x\frac{Na_2O}{0.35}) + (\frac{MgO}{0.9}) + (2x\frac{K_2O}{0.25}) + (\frac{CaO}{0.7})\right] x100 \quad (2)$$

The higher the value of this index, the lower the soil weathering degree. So it would be judicious to consider this index as a "non-weathering index".

RESULTS

Some soil chemical characteristics values

Different chemical characteristics such as pH (water and KCl), exchangeable cations content, the sum of bases (S), CEC pH7, ECEC, base saturation rate [(BS=S/CEC) x100], total carbon (C_{tot}), total nitrogen (N_{tot}), available P and C:N ratio are presented in Table 2. The analysis of this table shows that soil pH values of the 5 sites were quite different. Mulungu soil was slightly acidic in the two considered layers. In Walungu and Kirundo, soil pH was moderately acidic with values ranging from 5.6 to 5.8; Gitega soil was very strongly acidic with pH values of 4.2 in 0-20 cm layer and 4.4 in the underlying layer (20-40 cm). Cibitoke soil was moderately acidic in the surface (0-20 cm) and slightly acid in the 20-40 cm layer. In all soils and for both sampled depths, pH_{water} was equal or slightly higher than pH_{KCl} and ΔpH values (in modulus values) range from 0 to 0.1 units, which would mean that soils variables charges were dominant in those soils. Indeed, dominance of a soil by variable-charges colloids can generally be assumed when ΔpH is a small negative (less than -0.5), zero or positive value (Rayment and Lyons, 2011; Uehara and Gillman,

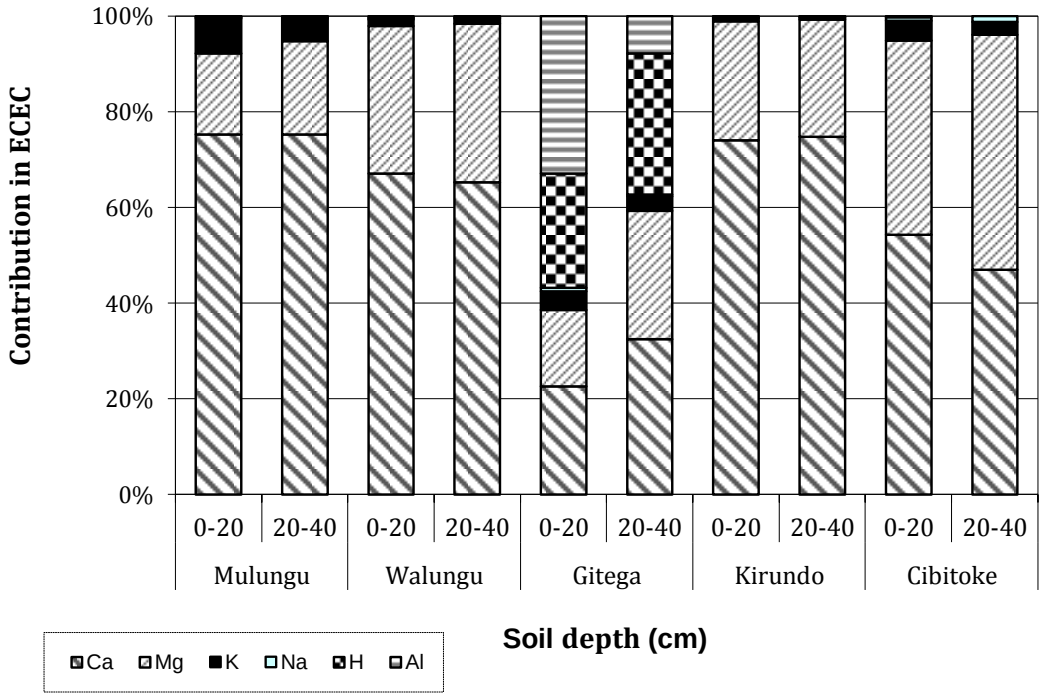

Figure 1. Different cations contribution to ECEC (sum of exchangeable cations and the exchangeable acidity).

1981). Globally, the average values of CEC were very high in soil of Mulungu while the soils of Kirundo and Walungu had a high CEC. CEC was low in Cibitoke and very low in Gitega (Tessens and Gourdin, 1993; Thiagalingam, 2000; Hazelton and Murphy, 2007). The trend was similar for ECEC. The sum of the bases (S) was close to the ECEC at all sites except Gitega, reflecting the high exchangeable acidity at this latter site (Table 2).

With the exception of Gitega where the base saturation rate was very low (< 20%), the average base saturation rate (BS) for both depths in the other 4 sites was greater than 60%, indicating a high (Mulungu, Walungu and Kirundo) to very high (Cibitoke) base saturation status (Table 2). The very low BS at Gitega site appears to be normal since the exchange complex is dominated by H and Al (Figure 1).

The cations contribution to charges (Figure 1) showed that Ca was the dominant cation in Mulungu, Walungu and Kirundo, followed by Mg and finally by K for both depths. In Mulungu the three cations formed respectively 75, 17 and 7% of exchange complex in the surface layer and 75, 20 and 4.7% in the underlying layer. In Kirundo, the respective contribution of the above mentioned cations was 74, 25 and 0.6% in the superficial layer (0-20 cm) and of 75, 24 and 0.5% in 20-40cm soil layer. In Walungu, the saturation percentage was of 67, 31 and 1.4% in surface and 65, 33 and 1% in the 20-40cm layer respectively for the Ca, Mg, and K. In Gitega, the trend was very different from what is observed in previous sites with 57% of exchange sites occupied by Al+H in the surface of which 33% with Al. The latter was taking up approximately 37% in 20-40 cm soil layer of which 8% by Al. In Cibitoke, Ca, Mg, and K respectively contributed to 54, 41 and 4 % in the superficial layer and to 47, 49 and 3% in 20-40 cm soil layer.

In the five soils, it was noticed that total carbon content was relatively higher in the surface layer (0-20 cm) than in the following layer (20-40 cm) (Table 2). Total carbon contents were the highest in Mulungu and Kirundo, and the lowest in Cibitoke. The same trend was observed with regard to total N content. Available P was very high in Mulungu and low in the four remaining sites. One should note the striking low P content in Walungu. C: N ratio was between 10 and 12 for all sites except in Gitega and Kirundo where it was fluctuating around 14. A ratio of 10 is an indicator of good biological activity. The highest ratio observed in Gitega and Kirundo indicates a relative slower degradation of organic matter and therefore a relative lower microbiological activity.

Particle size and mineralogical composition

Soils classification according to their particle size distribution is shown in Figure 2. The soil types fit in to three textural classes: Clay soils (Mulungu, Walungu and Kirundo), sandy clay loam soils (Gitega) and clay loam soil (Cibitoke). The mineralogical composition obtained from the X-ray diffraction (XRD) is presented in

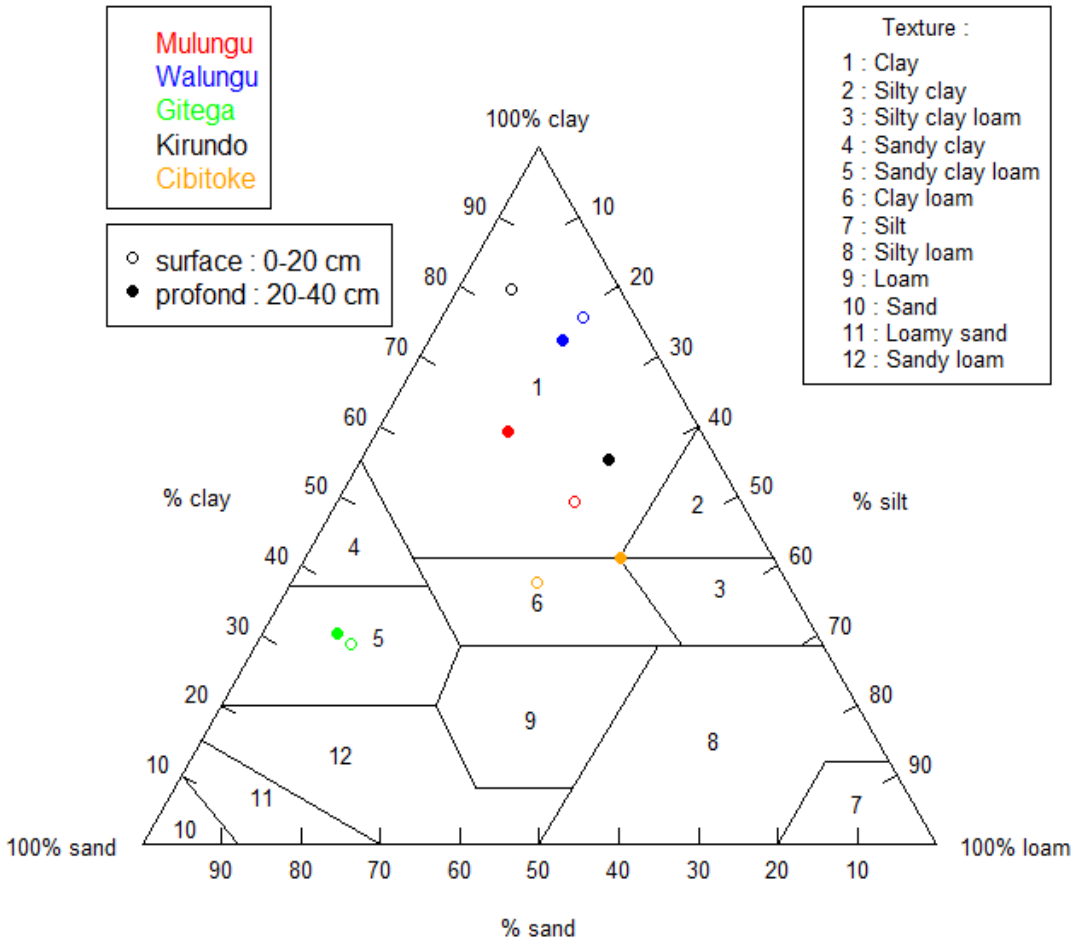

Figure 2. Soils textural classes of the soils under study.

Table 3. Mineralogical composition of 5 soil types.

Site	Depth (cm)	Clay minerals type in order of predominance
Mulungu	0-20	Halloysite, Kaolinite, Illite,
	20-40	Halloysite, Kaolinite, Illite
Walungu	0-20	Kaolinite, Illite, Gibbsite
	20-40	Kaolinite, Illite, Gibbsite
Gitega	0-20	Kaolinite, Gibbsite, Illite, Vermiculite
	20-40	Kaolinite, Gibbsite, Illite, Vermiculite
Kirundo	0-20	Kaolinite, Illite, Vermiculite
	20-40	Kaolinite, Illite, vermiculite
Cibitoke	0-20	Illite, Kaolinite, Interstratified-vermiculite, Smectite
	20-40	Illite, Kaolinite, Interstratified-vermiculite, Smectite

descending order of magnitude that is to say from the most abundant clay to the least abundant (Table 3). Mulungu soil was dominated by halloysite while Walungu, Gitega and Kirundo soils were dominated by kaolinite in the clay fraction. Cibitoke soil was dominated by illite.

Because of the confusion of kaolinite lines and halloysite lines in an XRD, CEC kg^{-1}clay values (CEC_{clay}) allowed realizing the dominance of halloysite on kaolinite especially in Mulungu soil. The CEC_{clay} value was determined taking into account the organic matter (OM)

Table 4. DCB and oxalate extractible elements contents (Al, Fe, Mg, Mn, Si), total iron content and different iron oxides ratios in the fine earth.

Site	Depth (cm)	Al_d	Al_o	Fe_t	Fe_d	Fe_o	Mg_d	Mg_o	Mn_d	Mn_o	Si_d	Si_o	Si_o/Si_d	$Al_o+1/2Fe_o$	Al_o/Al_d	Fe_o/Fe_d	Fe_d/Fe_t (en %)
		g kg^{-1}soil															
Mulungu	0-20	15.63	19.60	108.84	76.23	19.37	1.45	1.00	4.96	3.70	3.18	5.20	1.64	2.93	1.25	0.25	70.0
	20-40	13.67	19.80	112.33	73.02	20.01	1.51	1.24	4.39	3.49	3.49	5.78	1.66	2.98	1.45	0.27	65.0
Walungu	0-20	9.42	3.88	169.21	117.37	8.63	0.87	0.68	4.41	3.38	2.43	0.92	0.38	0.82	0.41	0.07	69.4
	20-40	9.49	3.47	162.04	113.81	8.42	0.72	0.59	3.80	2.83	2.48	0.88	0.35	0.77	0.37	0.07	70.2
Gitega	0-20	8.72	2.64	61.34	53.76	1.50	0.12	0.05	0.07	0.01	0.40	0.15	0.38	0.42	0.30	0.03	87.6
	20-40	8.99	2.91	65.42	54.66	1.68	0.09	0.05	0.06	0.01	0.41	0.15	0.37	0.38	0.32	0.03	83.6
Kirundo	0-20	7.70	4.98	89.04	66.75	3.78	0.71	0.59	0.82	0.69	0.95	0.65	0.68	0.69	0.65	0.06	75.0
	20-40	7.98	5.09	79.74	68.85	3.98	0.74	0.59	0.90	0.73	1.13	0.61	0.54	0.71	0.64	0.06	86.3
Cibitoke	0-20	1.51	1.20	35.01	17.45	3.21	0.70	0.61	0.52	0.45	0.87	0.27	0.31	0.28	0.79	0.18	49.9
	20-40	1.74	1.20	42.10	21.20	2.03	0.87	0.74	0.49	0.42	1.03	0.30	0.29	0.22	0.69	0.10	50.4

contribution to CEC. Landon (1991); Baize (2000) suggests to use a lump value of 200 cmolc kg^{-1}OM (i.e. 2 cmolc for each per cent of OM) for the CEC_{clay} estimation.

The study by Delstanche (2011) in AGLR showed that CEC_{OM} values for this region do not deviate so much from the 200 cmolc kg^{-1}OM average value. Indeed, this author found that CEC_{OM} values ranged between 180 and 236 cmolc kg^{-1}OM. Therefore, the CEC_{clay} was worked out by the following equation:

$$\text{CEC clay} = \frac{\text{CEC}-(\text{OM}\times 2)}{\text{A}} \times 100 \quad (3)$$

A being clay percentage and OM, organic matter rate determined by multiplying C_{org} by 1.728. On this starting point, CEC_{clay} values were of 67 and 47 cmolc kg^{-1} in Mulungu respectively in the topsoil (0-20 cm) and underlying soil layer (20-40 cm) and thus by far higher to the value of kaolinite. This seems to be a sign of halloysite dominance in comparison to the two other kinds of clay minerals namely kaolinite and illite (Table 3) given that the latter is not very represented.

Soils weathering indicators

Different DCB and oxalate extractible Fe, Al, Si, Mn and Mg contents as well as oxalate extractible Al and Fe ratios are shown in Table 3. Total analysis results, TRB and WIP of the different soil are shown in Table 5. From Table 4, one can note that in all the five sites, the different Fe extractible fractions occurred in the following trend: $Fe_o < Fe_d < Fe_t$. It was also noticeable that the total iron amount greatly varied according to sites in the following descending order: Walungu, Mulungu, Kirundo, Gitega and Cibitoke. The same trend as in the case of iron was observed in the case of Al with the trend $Al_o < Al_d < Al_t$ except at the Mulungu site. In the latter site, it was noted that Al_o and Si_o were superior to Al_d and Si_d respectively (Table 4) and this seems to indicate a particular characteristic of the soil type for DCB and oxalate extractible Al and Si. The Fe_o /Fe_d ratio which measures the reactivity of the sesquioxides was relatively higher in the Mulungu and Cibitoke and lower in the other three sites; the lowest values being observed in Gitega. Conversely Fe_d/Fe_t ratio that indicates the soil weathering extent was higher in Gitega site with an opposite trend to that observed in case of Fe_o/Fe_d ratio. This implies that the two parameters are negatively correlated. The relationship between the two ratios was relatively stronger in the top layer (0-20 cm) than for the 20-40 cm layer with R^2 values of 0.56 and 0.37, respectively (Figure 3). In other words, the higher the Fe_o/Fe_d ratio, the higher the amount of amorphous iron. Huang et al. (1977) reported that the amount of Fe_o as well as Al_o amount was related to OM content but not to soil acidity or clay content.

Feo was positively correlated with organic carbon rate. The correlation was relatively stronger in surface layer (0-20 cm) with R^2 = 0.51 than it was in 20-40 cm soil layer where R^2 = 0.26. The positive correlation between the total organic carbon content and Fe_o shows the role of iron oxides in the soil organic matter stabilization (Wagai and Mayer, 2007; Wissing et al., 2013).

From Table 5, it was noticeable that TRB, MR and WIP are the highest in Cibitoke and the lowest in Gitega. Walungu site was characterized

Table 5. Elemental total content, TRB and WIP of the five soil types in the fine earth (<2 mm).

Site	Prof.	Al	Si	Fe	Mn	Ti	P	Al_2O_3	SiO_2	Fe_2O_3	MnO_2	TiO_2	CaO	MgO	K_2O	Na_2O	P	Ca	Mg	K	Na	TRB	RM	WI
		g kg^{-1}						%									cmolc kg^{-1} soil							
Mulungu	0-20	114.46	169.27	108.84	4.90	14.12	9.95	21.63	36.21	15.56	0.77	2.35	1.56	1.01	0.13	0.09	2.28	55.78	50.87	2.72	3.06	112.43	83.66	4.92
	20-40	118.71	173.95	112.33	5.12	14.68	10.26	22.43	37.22	16.06	0.81	2.45	1.74	0.98	***0.06***	0.09	2.35	62.14	49.37	***1.28***	2.98	115.77	*89.72*	4.59
Walungu	0-20	111.04	156.62	169.21	4.90	26.29	1.08	20.98	33.51	24.19	0.78	4.39	0.57	0.59	***0.06***	0.04	0.25	20.36	29.89	***1.28***	1.17	52.70	34.73	2.16
	20-40	118.61	162.61	162.04	4.34	25.03	0.92	22.41	34.79	23.17	0.69	4.17	0.41	0.54	***0.06***	0.03	0.21	14.48	27.21	***1.28***	1.13	44.10	29.83	1.86
Gitega	0-20	89.53	292.14	61.34	0.16	7.86	0.39	16.92	62.50	8.77	0.02	1.31	0.07	0.11	***0.06***	0.02	0.09	2.50	5.31	***1.28***	0.78	10.87	9.00	0.83
	20-40	92.85	285.42	65.43	0.15	7.91	0.36	17.54	61.06	9.35	0.02	1.32	0.06	0.10	***0.06***	0.02	0.08	2.19	5.24	***1.28***	0.81	9.52	8.23	0.83
Kirundo	0-20	135.28	194.15	89.03	0.99	11.57	0.74	25.56	41.54	12.73	0.16	1.93	0.58	0.30	***0.06***	0.07	0.17	20.73	15.03	***1.28***	2.20	39.24	20.96	2.03
	20-40	141.64	196.43	79.74	1.03	11.96	0.71	26.76	42.02	11.40	0.16	1.99	0.61	0.31	***0.06***	0.08	0.16	21.66	15.42	***1.28***	2.49	40.85	22.27	2.13
Cibitoke	0-20	82.53	314.33	35.01	0.71	6.16	0.45	15.59	67.25	5.00	0.11	1.03	0.52	1.13	2.38	0.64	0.10	18.64	56.59	50.68	20.49	146.39	134.62	24.67
	20-40	95.49	297.57	42.10	0.74	6.32	0.38	18.04	63.66	6.02	0.12	1.05	0.52	1.40	2.65	0.61	0.09	18.74	70.52	56.37	19.70	165.33	152.79	26.98

In bold and italic, values at detection limit of the measuring device.

by TRB and MR values greater than those in Kirundo, but both soil types have identical WIP values of 2.0 if the average of the two depths was considered. However TRB, MR and WIP values related to the different soil layers, enable to separate clearly the two soil types with relatively higher values in Walungu compared with Kirundo in the 0-20 cm soil depth. In the 20-40 cm soil layer the same trend was maintained for both sites with respect to TRB and the MR but a reversed trend was noted for the WIP.

Cibitoke site markedly differs from the others by its higher total potassium, magnesium, silicon and sodium contents. Thus, it could be noted that in four out of five soil types namely Mulungu, Walungu, Gitega and Kirundo, total Ca and total Mg were by far two most dominant cations of TRB and MR. This was not the case in Cibitoke where total Mg and total K are rather the two most dominant cations. However the relative importance of the two dominant cations varies from one soil type to another. Thus in descending order, total Ca and total Mg were the most dominant in Mulungu and Kirundo soils. Total Mg and total Ca were the most represented cations of TRB and MR in Walungu and Gitega soils. In Cibitoke, total Mg followed total K and total Na were the cations taking over the TRB and MR. Thus, only based on this criterion, the five soil types boil down to three groups of soils (Mulungu and Kirundo, Walungu and Gitega, and Cibitoke) but the combination with other criteria such as the WIP indicated rather another trend and a clear difference between the soil types as it may be noticed in Figure 4.

TRB results comparison (Table 5) with those related to the sum of exchangeable bases (Table 2) indicated that the latter contribute to TRB respectively at 24, 33, 11, 46 and 8% in Mulungu, Walungu, Gitega, Kirundo and Cibitoke.

Soils agronomic potential

The agronomic potential of different soils was evaluated by comparing the performance level in terms of observed banana yield and different soil chemical characteristics including the computed weathering indices. Total banana yield according to different sites in the control plots is shown in Table 6.

Banana yield per cycle were different according to sites (Table 6) in the following descending performance sequence: Cibitoke ≥ Mulungu > Kirundo > Walungu > Gitega. The relationship between the different soil quality indicators above mentioned (soil textural composition, pH, total nitrogen, total carbon, available P, bases, sum of bases, CEC, Fe_o/Fe_d and Fe_d/Fe_t ratios, TRB, MR and WIP) and bananas yield potential in 5 soil types is highlighted in the correlations matrix of Table 7.

The analysis shows that except the pH, exchangeable K and silt content, other physico-chemical properties such total nitrogen and carbon, available P, exchangeable bases (Ca, Mg), S and CEC which are measures commonly used to account for the soils fertility status were poorly correlated to observed banana yield in the field compared with the correlation coefficients between total cations (total Ca, total Mg), MR and TRB. The best explanatory factors of the

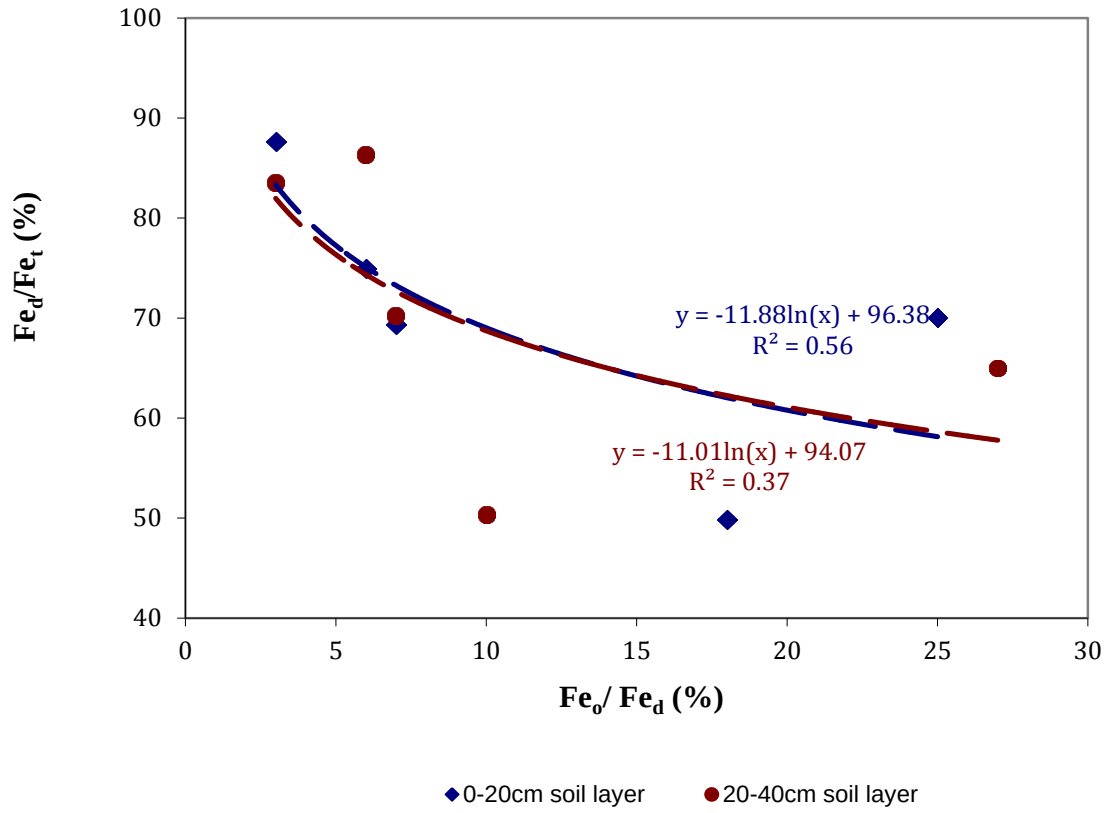

Figure 3. Relationship between reports Fe_o/Fe_d and Fe_d/Fe_t (established from 5 types soil).

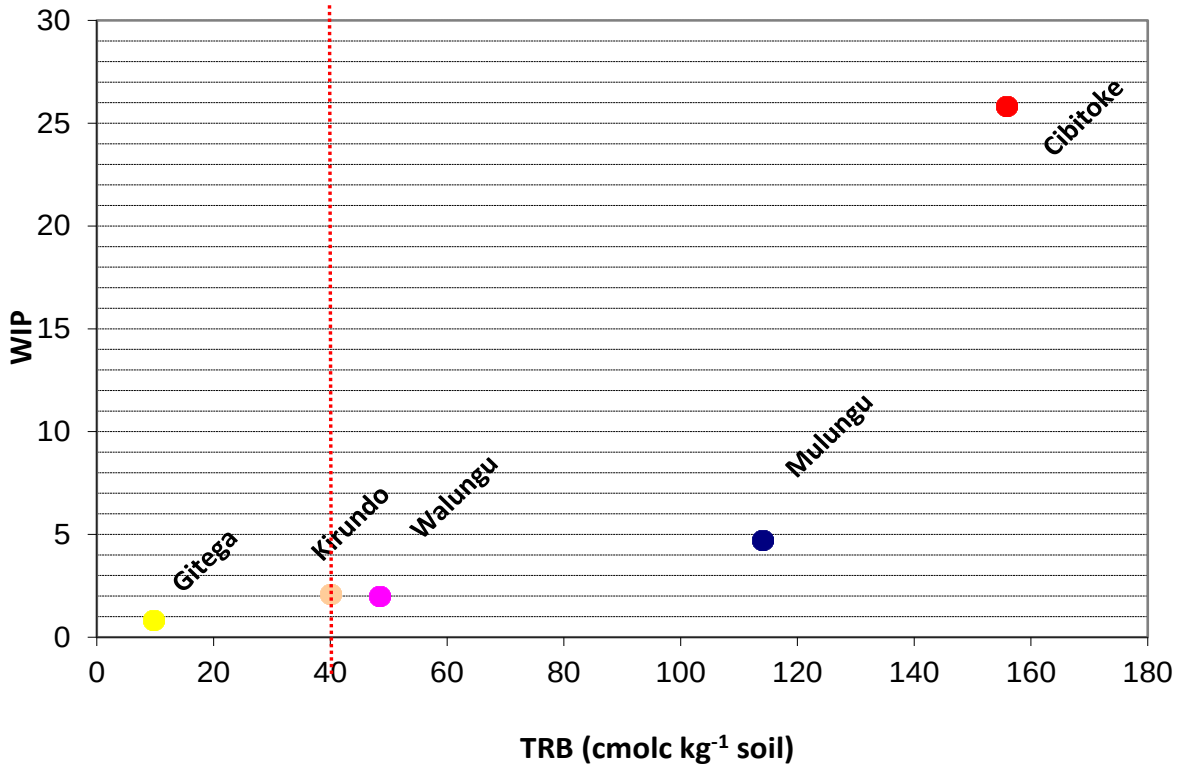

Figure 4. Sites relative position on the basis of TRB and WIP mean values. The vertical dashed line represents the upper limit of TRB = 40 cmolc kg^{-1} soil of highly weathered soils.

Table 6. Total banana yield observed in control plots.

Site	Total yield during 30 months **in t ha^{-1} (±SE)
Mulungu	30.8±5.1
Walungu	3.2±1.6
Gitega*	0.0±0.0
Kirundo	8.7±2.7
Cibitoke	31.3±6.8

SE, Standard error; *, no regime was observed during 30 months but rather banana mortality; **, Banana yield adjusted to that of the first cycle.

Table 7. Pearson correlation coefficients between observed banana yield and: (a) exchangeable cations, the sum of exchangeable bases (S), non-exchangeable bases (MR) and the sum of total cations (TRB); (b) other weathering soil indicators (data from 5).

Yield		(a)					(b)										
X →		Ca	Mg	K	Na	∑bases	% Clay	%Sand	% Silt	pH_{Water}	Ctot	Ntot	P	CEC	Fe_o/Fe_d	Fe_d/Fe_t	WIP
	Depth (cm)																
Exch	0-20	0.46	0.47	0.71	0.89*	0.52											
	20-40	0.47	0.71	0.74	0.89*	0.59											
No exch	0-20	0.77	0.93*	0.60	0.68	0.97**											
	20-40	0.76	0.92*	0.59	0.68	0.92*											
Total	0-20	0.67	0.92*	0.63	0.68	0.95*											
	20-40	0.70	0.92*	0.61	0.68	0.95*											
	0-20						-0.28	-0.15	0.87*	0.64	0.19	0.44	0.59	0.39	0.95*	-0.75	0.71
	20-40						0.00	-0.33	0.52	0.81	-0.03	0.24	-	0.38	0.73	-0.80	0.70

Exch: Exchangeable; No exch: No exchangeable; The arrow indicates that correlations are established between the yield and the features of part (a) or (b) of the table Note that ∑No exchangeable bases=MR and ∑Total bases=TRB The *, ** and *** indicate the level of significance corresponding respectively to 001<p-value<005; 0001 < p-value < 001 and p-value<0001 The absence of asterisk indicates that the correlation is not significant (p-value>005).

observed performance banana yield levels were the MR with r = 0.97 (p = 0.007), non-exchangeable Mg with r = 0.93 (p = 0.03), the total Mg with r = 0.92, the TRB with r = 0.95 (p = 0.01) and the silt content with r = 0.87 (p = 0.05). The observed yields were also correlated with Fe_o/Fe_d ratio in surface with r = -0.95 (p = 0.02).

In the underlying layer (20-40 cm), these were also the same parameters namely non-exchangeable Mg, total Mg, MR, TRB which were best correlated with banana yields compared with pH, total nitrogen and carbon, the bases, S as well as the CEC. It is also important to note that R values in the superficial layer (0-20 cm) are clearly greater than those of the underlying layer in the case of Fe_o/Fe_d ratio (Table 7).

Figure 5 illustrates the relationships between some of soil alteration indicators (silt content, TRB, RM, Fe_d/Fe_t and WIP) and observed banana yield in 5 soil types.

DISCUSSION

Soils physico-chemical properties and weathering extent trends according to sites

For the soils as a whole, CEC values are higher in

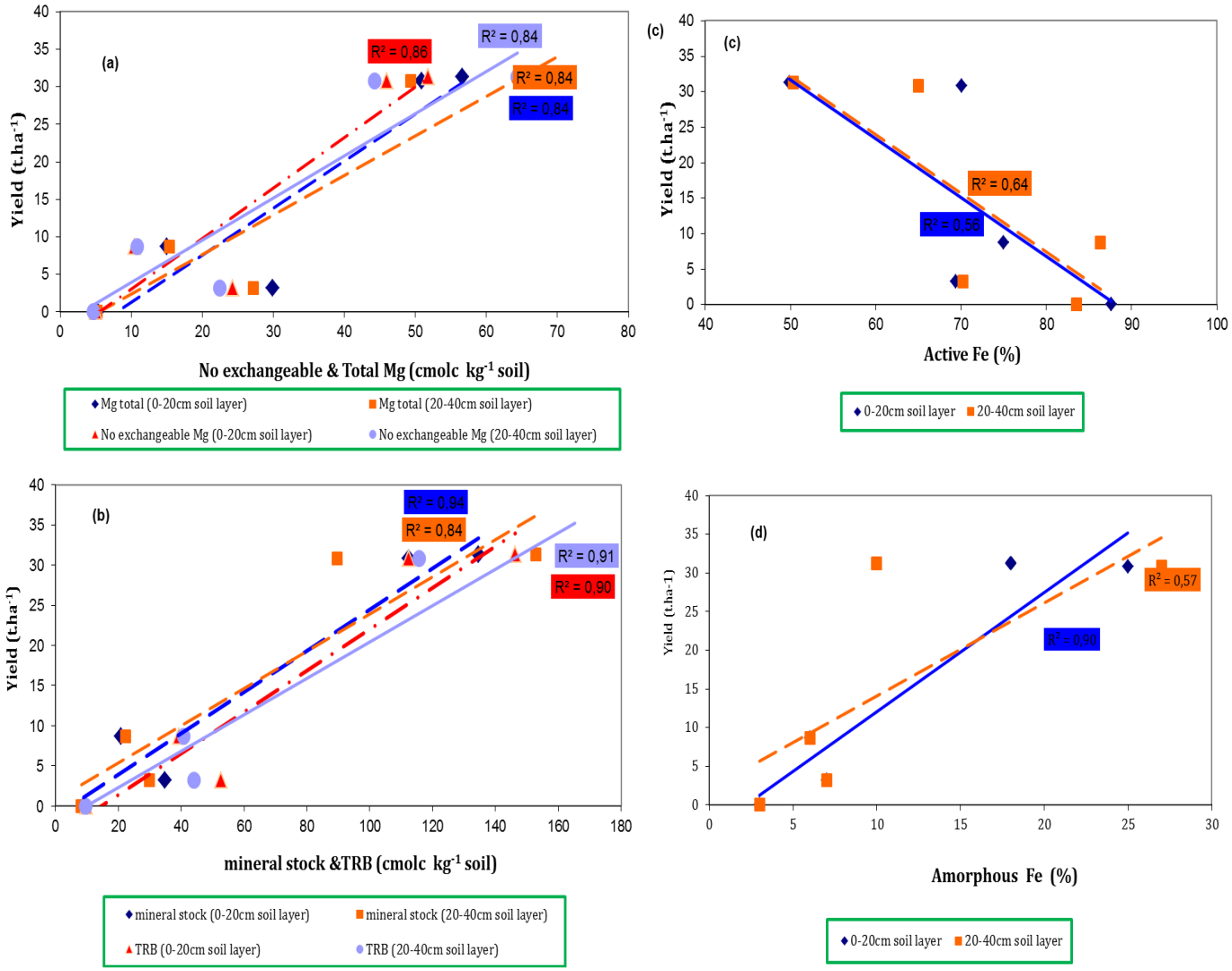

Figure 5. Relationship between observed banana yield and (a) non-exchangeable and total Mg (b) mineral reserve and TRB, (c) the quantity active iron in the soil, (d) the quantity amorphous iron in the soil.

0-20 cm soil layer than in 20-40 cm layer (Table 2), this may be related to the total carbon content and thus to organic carbon content which are similar given that soil pH values are < 7. Indeed, it is well known that soil CEC depends a lot on organic matter quantity as several studies on different soils types (Oxisols, Acrisols, ferric Lixisols) have pointed out (Oorts et al., 2003; Fritsch et al., 2007; Zhang et al., 2007; Mbonigaba et al., 2009; Delstanche, 2011) and to a less extent on soil clay content (Oorts et al., 2003; Delstanche, 2011) and the clay mineral nature. The influence of soil clay mineral nature is well illustrated when confronting Gitega and Cibitoke on the one hand, or Mulungu and Kirundo on the other hand.

It was noticeable that CEC values are higher in Mulungu and lower in Gitega. Soil in Mulungu contains halloysite, as a dominant clay mineral while kaolinite was clearly the dominant clay mineral in Kirundo with less illite, vermiculite being very little represented (Table 3). Therefore, high CEC values in Mulungu could be explained by many parameters among which the high OM content, the halloysite and illite presence as well as the presence of allophanic compounds. Boyer (1978) pointed out that halloysite, illite and allophanes are characterized by a high CEC; in the range of 20 to 30 cmolc kg^{-1}clay for illite and 20 cmolc kg^{-1}clay for halloysite. In Gitega, the low values of CEC can be explained not only by the low organic matter content, but especially by the texture and the mineralogical composition. Observed values in Gitega are consistent with those found in the soils where the clay fraction is dominated by kaolinite. Indeed, CEC in this site is about 11 and 5 cmolc kg^{-1}clay respectively in the topsoil (0-20 cm) and in the underlying layer (20-40 cm). This value of CEC (<16 cmolc kg^{-1}clay) is characteristic of kaolinite dominance in Ferralsols. Low values of CEC in Gitega

site can be also related to the soil texture. The soil is dominated by the sand fraction (Figure 2). Similarly the low CEC values in Cibitoke can be also partially explained by the soil texture. Indeed, the soil is characterized by relatively higher silt content. But then, CEC is inversely related to the particles sizes (Oorts et al., 2003) of which specific surface is decreasing as the particles size increases (Uehara and Gillman, 1981).

When considering the average values of different iron oxides fractions per soil layer as weathering indices, the following descending sequence was found: Mulungu > Cibitoke > Walungu > Kirundo > Gitega for Fe_o/ Fe_d. If the ratio Fe_d/Fe_t is considered, almost a reverse order in the sequence occurs as following: Gitega > Kirundo > Walungu > Mulungu > Cibitoke (Table 4). These values give an indication on the extent of the soil weathering. Low values of the Fe_o/Fe_d (Mahaney et al., 1994; Neto et al., 2010) and conversely higher values of the Fe_d/Fe_t ratio (> 70%) are indicators of strongly soil weathered soils as Ferralsols (Dalmolin et al., 2006; Zhang et al., 2007). Based on this criterion, Gitega has the most weathered soil in contrast to Cibitoke. This trend is confirmed by relative position of the sites when taking into account both the TRB values and the WIP (Figure 4). The values of these two indices show the decreasing sequence Cibitoke > Mulungu > Walungu > Kirundo > Gitega. The WIP values are very high at Cibitoke and low at Gitega. The more WIP values are smaller; the more is the weathering extent of the soils (Delaunay, 2005).

TRB values in Gitega are very low indicating that the soil is strongly weathered. Indeed, the average value (9.7 cmolc kg^{-1}soil) is by far below 40 cmolc kg^{-1}soil, the upper value limit suggested by Herbillon (1986) for strongly weathered ferrallitic soils. TRB values in Cibitoke soil were higher compared with other sites. For the sites classification according to TRB or MR values, the following sequence in descending order was noted: Cibitoke, Mulungu, Walungu, Kirundo and Gitega. Therefore, except the WIP which does not make a clear distinction between the soils in Kirundo and Walungu, or the CEC placing the soil in Cibitoke at the foot of the scale, a little before Gitega, other chemical indicators (Fe_d/Fe_t ratio, TRB, MR) show that the soil in Cibitoke was the least weathered, followed by Mulungu then Walungu, Kirundo and finally Gitega. This sequence was partially in agreement with results of the exchangeable bases contribution to TRB with an average of 7.8% for Cibitoke, 24% for Mulungu, 33% for Walungu, 46% for Kirundo and 11% for Gitega. Indeed, it was expected that exchangeable bases represent the soil significant portion of the TRB in Gitega, which was highly weathered and therefore with low quantities of mineral reserves. This was not the case because according to this criterion, Gitega lies between Cibitoke and Mulungu, sites characterized by a low contribution of exchangeable bases to TRB and thus less weathered soils. This trend of Gitega, that is, the low contribution of exchangeable bases to TRB may be explained by the high soil acidity which is evidenced by the fact that the bulk of the CEC is engaged in the exchangeable acidity (~57%, Figure 1) at the expense of the bases. There is also the fact that compared with other sites; the soil has very low clay content which further reduces the cations holding capacity. Therefore, the fact that CEC is low while WIP and TRB are still high like in Cibitoke may indicate that CEC use seems to be a good indicator of soil fertility when it is combined with the pH readings, but that others indicators like TRB could be better to judge unequivocally the soil fertility status especially in the sites that have not been affected by fertility management practices, because TRB is unlikely to be much affected by management.

The order of magnitude on the soils weathering stage (based on the TRB, MR and iron oxides ratios, CEC contribution to the TRB) seems to be also in agreement with the silt content values and the soils mineralogical composition (Table 3). The average silt content is higher in Cibitoke (35.2%) while soils silt content are 23.2, 18.9, 17.3 and 10.7% respectively in Mulungu, Kirundo, Walungu and Gitega. The silts mainly consist of primary minerals, they alter more quickly than the sands because of their high specific surface area due to their small size and more than clays which are often neoformed clay minerals and therefore more chemically stable. Therefore one understands why the TRB varies in the same direction than that of silts content given that the complex minerals that make up the silt and sand fractions are basically mineral reserves. As for the case of the silt content, mineralogical composition (Table 3) shows that in Cibitoke, the soil is the least weathered compared with others. Indeed, the mineral fraction is dominated by illite, kaolinite and vermiculite: illite and vermiculite being characteristic of young soils (Brady and Weil, 2002). According to Weaver (1989), illite, vermiculite and interstratified-smectite characterize soils at an intermediate weathering stage while the presence of kaolinite, gibbsite, goethite, hematite and anatase (TiO_2) depict a very advanced weathering stage. As such, Gitega and Walungu soils which contain in their mineralogical fraction, gibbsite and high TiO_2 levels (Walungu) in addition to kaolinite are more weathered than others. Gibbsite is appearing indeed in highly weathered soils (van Wambeke, 2002), in addition to iron and aluminum oxides as well as an increase in TiO^2 (Setterholm and Morey, 1995).

The assessment of the total or reserve base cations (Ca, Mg, K and Na) contribution to the TRB or MR indicates that Mg is the main contributor than Ca in Cibitoke, Gitega and Walungu soils. This case in point constitutes another indicator of soils weathering stage and the nature of the soils' parental material. Firstly, the fact that the total Mg is greater than total Ca in Walungu (Mg>Ca>K>Na) and Cibitoke with the sequence Mg>K>Na>Ca (Table 5) would confirm the basic rocks types from which these soils derived. Indeed, Godefroy

and Dormoy (1990) indicate that in most soils derived from basic rocks types, Mg is the dominant element of the reserves. According to Jaffré and Veillon (1991), in all soils on ultramafic rocks, calcium and potassium contents are at low levels while magnesium contents are relatively high in particular on alluvium soil, where a strong imbalance Ca/Mg in the exchange complex is found. On the other hand, Boyer (1978) points out that often, the soils having undergone a long pedological process have relatively low reserves more in Ca than in Mg. From this point of view, the soil in Gitega (Mg>Ca>K>Na) (Table 5) would hold the superiority in Mg reserves compared with Ca reserves on account of the highly advanced weathering degree. In the opinion of the same author, Mg can also be dominant in illite and montmorillonite rich soils, and this is the case in Cibitoke (Table 3).

With regard to the different soil properties including TRB, MR, the WI of Parker, or the amount of active iron (Fe_d/Fe_{total}), types of dominant cations of the TRB or the MR, particle size and mineralogical composition, all these parameters converge to indicate that the ascending order of soils alteration degree is Cibitoke (Fluvisol)- Mulungu (Nitisol)-Walungu (probably another type soil and not a Ferralsol)-Kirundo (rhodic Nitisol)-Gitega (typical Ferralsol). The Ferralsol of Gitega having reached an ultimate stage of weathering with regard to the TRB values.

EAHB yield potential as a snapshot of the soil inherent properties and weathering extent

The more TRB or Parker weathering index values were higher or even those of Fe_d/Fe_t ratio lower, better was banana yield. Hence Cibitoke site with higher values of these indices (TRB and Parker weathering index) is characterized by relatively good banana yield (in absolute terms) followed respectively by Mulungu, Kirundo, Walungu and Gitega (Table 6). The yields taken as a whole were strongly correlated with the MR, TRB, soil silt content, no exchangeable Mg, total Mg, Fe_o/Fe_d and Fe_d/Fe_t ratios, pH_{water} and WIP (Figure 5) and poorly correlated with the common routine analysis parameters such as C_{tot} (C_{org}), N_{tot}, P, CEC (Table 7) although the correlations coefficients are not always significant. The fact that banana yield is better correlated with the TRB or MR than the CEC or the sum of exchangeable bases (S) would be due to the fact that low values of CEC (or even those of S) are observed in less altered soils (with high primary minerals content) as well as in highly weathered soils. Conversely, TRB, MR, non-exchangeable cations and total fraction of cations, the silt content (Yakubu and Ojanuga, 2013) and banana yield values are higher in young soils than in very old soils and this explains the better correlation between those parameters. At the same time, this would indicate that the prediction of a soil potential production based only the routine analysis parameter, that is, the CEC or the sum of the bases is distorted and the distortion appears to be reduced when the results of total analysis are considered. Subsequently, one is tempted to say that the measurements in routine analysis (e.g. exchangeable cations, CEC) do not seem to be fair indicators to reflect the potential level of chemical soil fertility and therefore explain perceived banana yields levels in field. On the other hand, the fact that banana yield is much lower in Walungu than the yield observed in Kirundo (Table 6) while values of TRB or MR are relatively high in Walungu (Table 5) points out that the plants performance is a result of a lot of complex interactions of numerous factors rather than simple indicators.

For instance, the very low available P compared with the critical value for banana of 15 mg kg^{-1} (Okalebo et al., 1993) at Walungu combined with the high level of Mn in the topsoil (0-20 cm) or even the toxicity level observed in the 30-50 cm soil layer (Muliele, 2014) appears to be the most limiting factors hampering good banana yield in the site.

The relationship between active iron and banana performance is negative and more pronounced in the 20-40 cm layer. The negative influence of iron oxides, more marked in the underlying layer in comparison to the surface layer (Table 7) would be due to the influence of organic matter. The higher is the organic matter, the less is active iron (Fe_d) and more amorphous iron (Fe_o). As there is more organic matter in surface (0-20 cm) than in the 20-40 cm layer, there is theoretically more active iron in the underlying layer (20-40 cm) which has a negative influence on fertility because of the anionic exchangeable capacities (AEC) exhibition associated with the surface of kaolinite, iron and aluminum oxides as well as amorphous materials (Pansu and Gautheyrou, 2006; Brady and Weil, 2014). This means that iron oxides as well as aluminum oxides become positively charged and attract, retain, and supply negatively charged anions, such as sulfate, phosphate, nitrate, and chloride (Brady and Weil, 2014) but in reverse, soils with high AEC experience leaching of positively charged nutrients, such as calcium, magnesium and potassium. Moreover, phosphorus as an easily exchangeable anion may be limited, because it tends to form strong bonds with the oxides (Uehara and Gillman, 1981; Wandruszka, 2006; Brady and Weil, 2014) and therefore not readily available for the plant uptake (Brady and Weil, 2014). The presence of high quantity iron oxides being a sign of weathering, it is easy understand therefore that the relationship between the active iron and the yield is negative and more pronounced in the 20-40 cm layer where there is less organic matter than in the surface layer.

With respect to certain common routine analysis parameters (pH_{Water}, Ct_{ot}, N_{tot}, available P and exchangeable cations) and if all 5 sites are taken as a whole and correlation coefficients magnitude was

considered, the yield was respectively correlated with exchangeable K, pH, P, Mg, Ca and finally total nitrogen. The strong correlation between yield and exchangeable K seems to be consistent with common observations. Sure enough, many studies (Turner and Barkus, 1982; Smithson et al., 2004; Moreira et al., 2009; dos Santos et al., 2009; Nyombi et al., 2010; Galán Saúco et al., 2014), converge to indicate that with regard to cations, K is the most limiting factor and therefore the most important, followed by Mg. Even if the correlation coefficient are not very strong and significant, a positive correlation between available P and the yield was expected since, with the exception of Mulungu, the soil content in this variable is far away below the critical value for banana (Okalebo et al., 1993; Godefroy et al., 1991) and hence may overall constitute a limiting factor. Likewise, the absence of correlation between banana yield and C_{tot} (C_{org}) may be due to the fact this parameter is in sufficient concentration (Okalebo et al., 1993) in all sites but Gitega and therefore globally not a yield limiting factor. The strong correlation between yield and the soils pH while pH values in all sites except Gitega that fluctuate around 6.0 are much greater than the critical value of 5.2 suggested by Okalebo et al. (1993) may indicate that this parameter is still limiting and that the optimum pH value for EAHB banana production would be above the lower limit of the 5.8 to 6.5 optimum pH values range indicated by Galán Saúco et al. (2014).

Exchangeable Ca and Mg values (Table 2) illustrate that both cations are not limiting. Indeed, in all sites except in Gitega, Ca and Mg values seem high and therefore less limiting. In addition, the soil content in these two elements seems be in agreement with soil pH values that are all superior 5.5; Ca deficiencies occur in soils with low CEC and pH≤ to 5.5 (Landon, 1991). So, the soil in Gitega is the only one where Ca deficiencies are probable. In a study on banana systems in Kibungo (Rwanda) (dominance of Nitisols), Lassoudière (1989), Godefroy et al. (1991) indicate that critical values are respectively of 6, 2.5, 1.5 cmolc kg^{-1} soil respectively for exchangeable Ca, Mg K. According to Landon (1991), the deficiency threshold of exchangeable Mg in tropical regions is fixed at 0.5cmolc kg-1. On this basis, only the ferralsol (in Gitega) is deficient in exchangeable Mg (Lassoudière, 1989; Godefroy et al., 1991; Landon, 1991) and in exchangeable Ca (Lassoudière, 1989; Godefroy et al., 1991).

The nil banana yields in Gitega can be explained in part by the poor physico-chemical properties of the soil. Low CEC, lower rate of base saturation (BS), the low level of organic carbon, nitrogen and available P, highly acidic pH (Table 2) and higher aluminum saturation rate (33%, in 0-20cm layer) are soil fertility indicators far from conducive conditions for good banana production. A suitable land have a CEC > 16 cmolc kg^{-1}clay, a BS > 35% (Sys et al., 1993; Delvaux, 1995) and a pH_{Water} range of 5.8 to 6.5 or pH_{KCl} in the range of 5.0 to 5.8 for optimum banana production (Galán Saúco et al., 2014). In addition, with respect to the soil pH of the soil (around 4), textural composition (much more sand, Figure 2) and the low C org (SOM) content of the soil in Gitega, it seems coherent to have low levels of exchangeable Ca (Table 2) and in addition to aluminum toxicity expectation. Indeed, Fenton and Conyers (2002) reported that soil Ca content less than 0.5 cmolc kg^{-1}) was among others usually associated with sandy soils that are low in SOM and with a pH < 4 and that in addition, such soil properties will have a greater consequence on plants growth than the Ca deficiency, in most situations.

Many authors (Boyer, 1976; Delhaize and Ryan, 1995) indicated that exchangeable aluminum becomes toxic from pH_{water} < 5 or pH_{water}≤ to 5.5 (Meriño-Gergichevich et al., 2010), it is therefore easy to understand that this factor may constitute a yield limitation in Gitega because of its toxicity, but is not a problem in the other sites (Mulungu, Walungu, Kirundo and Cibitoke) whose pH values lie around 6 (Table 2). In fact, the content of exchangeable Al in this site of 33% is greater than the value of 30% reported by Gauggel et al. (2003). These authors reported that production experiences indicate that in soils with a pH range from strongly to extremely acid (which the case of Gitega), exchangeable aluminum concentrations are greater than 30%, and together with high soil manganese (Mn) concentrations, yields are reduced, usually to less than 2,000 boxes ha^{-1} $year^{-1}$ (~30 tha^{-1} $year^{-1}$). In optimal conditions, for a potential banana yield of 70 to 93 tha^{-1} $year^{-1}$ in the same area (Latin America) (López and Espinosa, 1998; Chia and Huggins, 2003), this corresponds to a yield loss of 50 to 63%. The fact that banana yield in Gitega is zero meaning a loss about a 100% while in Latin America yield loss range from 50 to 63% in similar conditions, would indicate a less tolerance to extremely acidic soils of EAHB than the varieties grown in Latin America. Although manganese toxicity was also expected in this site, this thesis does not seems be plausible. Indeed, there is naturally little manganese in the soil of which the total Mn content is approximately 160 ppm, a quantity which is much less (20-fold less) to 3275 ppm of total Mn indicated by Fouré and Marchal (1983) to observe a manganese toxicity in banana. These soil quality indicators (very low pH, exchangeable aluminum, very low base saturation, very low CEC and TRB or MR) in the site of Gitega could explain the poor banana performance subject to other many harmful phenomena to the plant at low soil pH such as the decrease in nitrification, the phosphorus deficiency and the availability of some heavy metals (Landon, 1991). The fact that exchangeable K is relatively well correlated with banana yield (Table 7) is possibly also an expression of a cationic imbalance. For all the sites, the values of K /(Ca + Mg) ratio are less than 10%, critical value proposed by some authors who worked in this zone (Lassoudière, 1989; Godefroy et al., 1991). Except the site at Gitega, the banana yield data analysis, site by site

indicates that very low Ca/K or Mg/K ratios or high K /(Ca+Mg) ratios correspond to better banana yields (case in Cibitoke and Mulungu), which reflect the prominent role of cation balance and K in banana nutrition and yield. However, in Gitega, Ca/K or Mg/K ratios are low and even the K/ (Ca + Mg) ratio is better than other sites but Gitega is the place where banana performance is the worst. This situation is to be linked to the very low soil fertility status in the site. According to Boyer (1978), balanced ratios of Ca, Mg and K are not of great significance for the plants nutrition in a soil with a BS < 10% as it is the case at Gitega; the very acidic pH and the exchangeable aluminum hamper the plants growth of in such cases. Soil acidity as the main handicap in Gitega seems more likely since this site presents good physical properties conducive to banana cultivation. Indeed, sandy clay loam soils, as it is the case of soil in Gitega (Figure 2), are the best for bananas cultivation (Robinson and Galán Saúco, 2010). Moreover, the average on the two depths for textural composition is 29.6% clay, 10.7% silt and 59.7% sand (Figure 2).These proportions do not differ from the optimum soil texture values reported by these authors (both for bananas and plantains) of 30% clay, 10% silt and 60% sand, because of a better aeration, a good infiltration coupled with a good soil drainage.

Conclusion

The production potential reflects the degree of soil alteration and generally speaking, the soil fertility status. In this study, it was found that banana yield levels were associated to the soil weathering stage and the other studied soil parameters. Banana yield levels were better and positively correlated with the total reserves in bases (TRB), the mineral reserves (MR), the Fe_o/Fe_d ratio and the silt content than the other routine soil analysis' parameters such as pH, total nitrogen, available P, exchangeable cations, the sum of the bases and the CEC. The more was the alteration degree of the soils, the less was the yield potential. Furthermore, soil weathering extent was better apprehended and associated to the level of production if the parameters from the soil routine analyses were completed by the total analysis parameters and iron oxides consideration. This was particularly patent for the most weathered soil of Gitega. Moreover, since the plants nutrition in low inputs agriculture system to is done at the expense of the primary minerals weathering which can be estimated by the TRB or mineral reserves, it appears appropriate to estimate the potential of soil chemical fertility and the type of interventions (fertilization and/or amendment) based on this parameter in addition to N and P levels assessment. Therefore, one might as well think about the plants nutrition balance by N, P, K; one might as well think about also, how to improve the soils mineral reserves including the reserves of Ca and Mg as they appear at low levels in weathered soils which make up the majority in the AGLR.

Conflicts of Interests

The authors have not declared any conflict of interests.

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LED in production systems of laying hens: An alternative to increase sustainability

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The Brazilian poultry production currently has a substantial impact on the national and international economy. Brazil ranks sixth in the world rankings of the largest egg producers. However, the activity still has barriers related to management and facilities, such as high waste of electrical energy due to the low energy efficiency of the lighting systems. The artificial lighting programs represent an important management tool to accelerate or delay sexual maturity of laying, stimulate the release of reproductive hormones, increase the egg production, besides promoting improvements in the shell quality and the size of the eggs. There is a large number of lamps that are available for use in artificial lighting systems, however, each light source has different characteristics (light intensity, wavelength, color, temperature and fluctuations) which may cause positive or negative effect on hens performance, egg quality and production costs. The LED technology is an innovative alternative that can improve the sustainability of the activity, since it has better energy efficiency, longer lifetime and emits light of different colors. This literature review aims to discuss the beneficial characteristics of the LED and its effects on the production of commercial layers.

Key words: Eggs, electric energy, layer production, layers, lighting program.

INTRODUCTION

The poultry production affects substantially the international economy. In 2015, the egg production in Brazil reached 39.5 billion units. This historical record surpassed 6.1 billion units as compared to the previous

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Table 1. Correlation between the wavelength (nm) of the light and the perceptions of the different colors.

Color sensation	Wavelength (nm)
violet	380 – 435
blue	435 – 500
green	500 – 565
yellow	565 – 600
orange	600 – 630
red	630 – 780

Lewis and Morris, 1998.

year (ABPA, 2016). Brazil is the sixth largest egg producer, after China, United States, India, Japan and Mexico. Brazil represents 3.34% of the world's total egg production (SEAB, 2012/2013).

Hens are photo responsive animals and the light stimulus acts positively on the physiology of the reproductive system triggering the release of hormones that are essential to maintain the egg production (Etches, 1994). The use of artificial lighting, for breeder hens and commercial layers, represents a low cost useful strategy (Mendes et al., 2010). The lighting programs encourage the growth and stimulate the reproductive system, resulting in an increased egg production (Freitas et al., 2010). The photoperiod manipulation can anticipate or delay the start of the eggs production, improve the quality of the shell and the size of the eggs, because of the feeding efficiency maximization by the light regime (Etches, 1994). Although, it seems to be a prominent picture, the poultry farming still has some barriers in terms of management and installations. One of them is the high electricity waste in its various stages of production. Therefore, the management of the electrical energy is important to reduce the final costs of the production (Jordan and Tavares, 2005; Jacomé, 2010).

Various light sources have already been tested in egg production sheds, however, studies are still performed to reach greater durability and lower cost (Nunes et al., 2013). Alternatively, to minimize energy costs, the LED has been used in aviculture due to its better energy efficiency and useful life. In addition, it is available in several colors (Molino et al., 2015).

Whereas, the LED technology is an innovative alternative and it can improve the sustainability of the activity, this literature review aims to present the beneficial characteristics of the LED and its effects on the production of commercial layers.

FEATURES OF THE VISUAL SYSTEM OF HENS

Light originates from a broad spectrum of electromagnetic waves, and the visible portion is relatively small and composed of wavelengths sized 350 to 800 nm (Nunes et al., 2013). The hens have two types of photoreceptor cells in the retina of the eyes, which are the rods and cones. The rods are numerous, with maximum sensitivity of 507 nm (blue light) and they allow the eyesight in low-light environments (<0.4 lux), but they are unable to distinguish colors. In contrast, the cones are sensitive to brighter levels of illuminance (from 0.4 to 44 lux) and they allow the color perception (Lighting, 1988; Lewis and Morris, 1998; Mendes et al., 2010).

There are three types of cones with sensibility peaks that are responsible for the primary colors perception, which are: 450 (blue), 550 (green) and 700 nm (red), which when stimulated together produce the white color sensation (Pritchard, 1995; Lewis and Morris, 1998). In humans, the cones are responsive to electromagnetic radiation between 400 and 730 nm, with a maximum response of 555 nm. However, the hens' eyes have an additional type of cone in the retina, and sensibility peak is close to 415 nm, but may transmit wavelengths shorter than 400 nm. It means that the hen can see a part of the ultraviolet range and see colors differently as compared to humans.

The maximum sensibility of hens occurs in the green-yellow band (545-575 nm) of the spectrum (Mobarkey et al., 2010), which is similar to the human beings. However, the spectral sensibility of the hens between 400 and 480 nm and between 580 and 700 nm is higher than the human's one. Due to this fact, birds can notice some brighter light sources as compared to the human perception, but the additional degree of brightness will vary with the source (Lewis and Morris, 1999). Table 1 presents the correlation between the wavelengths and the perceptions of the different colors.

Light is perceived by photoreceptors that transform the energy stored in the photons into biological signal. In the eye, the energy of the photons is transformed by the photosensitive pigments contained in the rods and cones, and transmitted by the neurons to the brain where the signal is integrated to an image (Etches, 1994). The perception of light through the eyes is strongly related to the behavior and the well-being of the hens. Prescott and Watches (1999) state that broiler may show behavior deviations when there is an excess or shortage of light, damaging their well-being and productivity.

Reproductive physiology and involved hormones

There are three theories to explain the effect of light on the reproductive activity of hens. One of them is through the eye, another is by the pineal gland and the third is the most accepted and is directly related to the hypothalamus (Etches, 1994). A study showed that

dimming the head of sparrows with black paint makes its sexual response to be blocked; however, there was no effect in blocking the light access to the eye (Sauveur, 1996).

Besides the retina, the hens can notice the light through the pineal gland located in the dorsal surface of the brain. The avian pineal gland is particularly involved in the control of circadian rhythms and sexual activity (Caneppele et al., 2013). The circadian rhythm coordinates a time schedule of biochemical, physiological, immunological and behavioral events, that will determine the productive performance (feed intake, motor activity, body temperature, among others) of health of lot (Abreu and Abreu, 2011).

In the dark period, there is melatonin release, produced by the pineal gland (Huang et al., 2013). Melatonin reduces the levels of LH and FSH, both bind to their receptors in teak and granular cells of the ovarian follicle, inhibiting the production of androgens and estrogens by small follicles and the production of progesterone by preovulatory larger follicles (Rocha, 2008).

The perception of light depends mainly on hypothalamic photoreceptors that are biological transformers that convert the energy of the photon into neural impulses. These neural impulses are then amplified by the endocrine system to control the ovarian and testicular function and consequently, the multiple reproductive functions, behavior and secondary sexual characteristics (Etches, 1994).

The hypothalamus is the main hormone control center, and it is located in the basis of the brain near the pituitary. Hypothalamus receives neural, environmental and hormonal signals, inside and outside the animal, and it uses this information to control the pituitary, gonads and other organs. The neurosecretory cells of the hypothalamus communicate directly with the pituitary through the bloodstream. The gonadotropin releaser hormone or GnRH is the primary hypothalamic hormone responsible for stimulating the anterior pituitary to release luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (Proudman, 1994).

Young birds have high levels of LH in the early development of the ovary. The small follicles produce androgens and estrogens that result in growth and pigmentation of the comb and the wattles and growth of the oviduct. As the puberty begins, the LH performs a negative feedback on the pituitary, which lowers its secretion. The estrogen levels declines while progesterone increases, as the follicles go through maturation until they reach the necessary size for ovulation. When the follicle is mature, the high secretion of progesterone culminates in releasing of LH and ovulation (Proudman, 1994).

One of the main functions of the ovaries is the production of steroid hormones which are essential for the growth and function of the reproductive system. Progesterone acts on the secretion of albumen (follicle maturation) and peak induction of LH (ovulation). The androgens act on secondary sexual characteristics (comb and wattle). The estrogens act on the egg yolk synthesis by the liver, the calcium transport from spinal bones from the bark to the gland (Bahr and Johnson, 1991; Rutz et al., 2007).

Following this concept, the short days are not able to stimulate the secretion of gonadotropins because the photosensitive phase is not entirely illuminated. In this situation, the production and release of LH are harmed and may interfere with reproductive functions, behavior and secondary sex characteristics of birds (Etches, 1994; Rocha, 2008).

Some works have been conducted in order to evaluate the effect of different light sources on the concentration of the reproductive hormones like estradiol in the bloodstream (Gonguttananun and Guntapa, 2012) and concluded that different types and colors of lamps can increase blood estradiol concentration.

Artificial lighting system for laying poultry

The lighting program that is used in laying poultry (laying hens and quail) aims to stimulate the reproductive tract of birds and increase the production of eggs (Etches, 1994). The artificial light is routinely used in lighting systems for commercial laying hens which may delay or accelerate sexual maturity, stimulate egg laying and improve weight gain in laying poultry and arrays (Freitas et al., 2005; Araujo et al., 2011). The delay in the beginning of the egg production through light control determines a better quality of shell, less eggs with two yolks or deformed eggs, and lower mortality due to prolapse (Araujo et al., 2011).

The light presence during the night improves the growth and adaptation to the environment in the first days of life and throughout the growing period (Nunes et al., 2014). Several light programs can be used in aviculture, but the ideal lighting program to be provided to laying poultry should be the one that provides the highest egg production with minimum feed intake and electric energy usage (Freitas et al., 2010). However, in order to achieve the required illuminance, the producers normally install artificial lighting systems composed of a large number of high-powered lamps, and low efficiency, which cause a substantial raise in the final costs (Nunes et al., 2013).

A new technology has been widespread in the poultry market as an alternative to increase the sustainability of the production system. They are the LEDs or LED. Some studies were developed in order to test LEDs use in poultry systems to replace traditional light sources (fluorescent and incandescent lights), and they have shown that the LED does not cause changes in the performance and egg quality of commercial laying hens

as compared to other light sources, therefore, it can be used to replace the traditional lighting systems (Borrile et al., 2013; Jacomé et al., 2012).

In Brazil, the use of open poultry houses allows the use of artificial light only to complement the natural daylight period, however it does not exclude the use of strategies to minimize wastes on electric energy, considering that recently the successive increases in the electricity cost have been a barrier to the sector. According to Gongruttananun and Guntapa (2012) and Santana et al. (2014), LED is an effective technological alternative to maximize the production and reduce expenses with electric energy.

CHARACTERISTICS OF LIGHT SOURCES

There are several types of available lamps, and each one offers a different light spectrum, which influences the production and quality of the eggs (Etches, 1994). Knowing the characteristics of different sources of light and the effects of these on the physiology and welfare of birds is essential for the implementation of an efficient light program.

The visual environment has some properties, such as: illuminance (luminous flux), spatial variation of lamps, color temperature (Kelvin) and the oscillations of the lamps. The perception of these properties depends on the spectral sensibility of birds which is different from humans (Prescott and Watches, 2000; Mendes et al., 2010). Thus, the hens and humans can perceive colors differently. For example, hens can perceive ultraviolet and infrared radiation (Menes et al., 2013). Several works have been conducted (Gougruttananun and Guntapa, 2012; Silva et al., 2012; Jacomé et al., 2012; Hassan et al., 2013; Borrile et al., 2013; Mendes et al., 2013) in order to analyze the effects of different light sources on the egg quality and the performance of commercial laying hens.

The luminous intensity provided to the birds can affect the productive performance, so it is necessary to adapt the type of the lamp, taking into account the chromatic intensity oscillation, the temperature, the illuminance distribution and the quantity of lux (lumen 1 perpendicular incidence on a surface of 1 square meter) (Mendes et al. , 2010). The luximeter should be used to determine precisely the luminous intensity in lighting systems (Borrile et al., 2014).

In addition to the wavelength and the light intensity that can vary according to each production phase as well as the type of production, other important principles involved in lighting are: light incidence duration and distribution of light sources (Mendes et al., 2013). These principles can influence positively the performance of the hens (Mendes et al., 2010; Gonguttananun and Guntapa, 2012).

In order to improve efficiency and minimize production costs, different light sources have been used. Compact fluorescent lamps and sodium vapor have been used in place of incandescent bulbs, however LEDs has been used in poultry houses demonstrating positive results superior to fluorescent. LEDs provide superior illuminance and life as compared to traditional light sources used in layer hen's production.

Types of lamps

Choosing the type of lamp for a lighting program depends on a few factors such as: cost, durability, maintenance and efficiency. Using a proper light program and a correct number of lamps according to the environment, there will be no differences in the performance. However, the energy consumption can be a limiting factor to the use of certain light sources (Araújo et al., 2011).

Incandescent bulbs are commonly used to provide uniform illumination, however its conversion rate from electrical energy to luminous energy is low, which generates a large amount of heat (Jordan and Tavares, 2005; Borrile et al., 2013; Mendes et al., 2013) and provides a low durability, increasing the production costs (Jordan and Tavares, 2005).

Fluorescent lamps may be an alternative to incandescent lamps, considering that they produce greater brightness per Watt. However, its luminous intensity decreases with time, which means that luminous flux depreciation occurs. Besides, they have a higher initial cost (Mendes et al., 2013), and they are susceptible to power fluctuations. These problems make it difficult to maintain uniform light intensity in the whole livestock production cycle (Long et al., 2015). Another drawback is that the fluorescent lamp maximum efficiency occurs when the air temperature is between 21 and 27°C. If the temperature is out of these limits, the efficiency is reduced (Araújo et al., 2011). The sodium vapor lamp has also been used as a useful and saving light source (Mendes et al., 2010).

According to Etches (1994), the type of lamp that is used (incandescent, fluorescent, sodium vapor) does not matter, however it is known that each lamp offers a different light spectrum and this factor can influence the production and quality of the eggs.

Environmental lighting technology has greatly advanced in the recent years, and traditional light bulbs are being gradually replaced by light emitting diode (LEDs) lamps (Gongruttananun and Guntapa, 2012; Santana et al., 2014). The main advantage of the LED is the energy saving (80% less energy waste as compared to incandescent bulbs and 50% compared to fluorescent lamps), longer shelf life and color diversity (Molino et al., 2015).

Light emitting diode (LED)

The LED emerged in the 60s and nowadays it is

known worldwide due to its high luminous efficiency and long life (Liu et al., 2010). In addition, the cost of the technology has decreased significantly since its first development and it has become more accessible to the poultry industry (Long et al., 2015).

The light emitted by the LEDs is monochrome and the color depends on the crystal and impurity of the material that is used in the production. The light frequency emitted by the electron also determines its color (Moreira, 2009; Valentine et al., 2010). Its colors include red, orange, yellow, green, blue, violet, purple, and also ultraviolet and infrared, and it can provide a more natural environment for the hens, ensuring better expression of their behavior (Araújo et al., 2013).

Studies have been conducted in order to test different LED colors on performance and egg quality of commercial laying hens. Borrile et al. (2015) worked with laying poultry in the second production cycle, in which the effect of different LED colors was not noticed in the performance characteristics and quality of eggs. In contrast, Borrile et al. (2013) observed a better performance in laying hens exposed to red, white LED and incandescent bulbs as compared to the blue, green and yellow LED.

Evaluating three light sources, Valentine et al. (2010) observed that the LED bulbs required 12 times lower electric consumption as compared to 60 W incandescent light bulbs that have the same luminosity. Besides, the LED bulbs are 5 times smaller than a 15 W fluorescent lamp. The lifetime of a LED bulb is approximately 50,000 h while the compact fluorescent and incandescent last for 8000 and 1000 h, respectively. Therefore, the LED bulb has an 8 times longer lifespan than a fluorescent lamp and 50 times longer than the incandescent (Liu et al., 2010).

Using LEDs lamps, Gongruttananun and Guntapa (2012) showed savings of 84.6% in the electric energy waste, as compared to red and white fluorescent lamps. Additionally, the birds exposed to the red LED have produced more eggs during the first week. Testing the same sources of light, Rozenboim et al. (1998) observed a 17% reduction in electricity using the LED.

Despite the considerable savings of the energy cost, there are still some limitations before introducing the use of LEDs. During an experiment with Dekalb white lineage, it was shown that hens exposed to the fluorescent lamps presented better feed conversion, greater egg size per housed bird and better uniformity. LED lamps caused less uniformity and its intensity decreases by 27% after 3360 h of use. Facing these results, it is necessary to deal with parameters like humidity and light spectrum. The sensibility of hens is different from humans, so it is necessary to evaluate the levels of light intensity in the different stages of production, and also the phases of the day (Long et al., 2015).

Molino et al. (2015) evaluated the effect of different sources and intensities of light in the production and the egg quality. These researchers observed a higher production, and better quality of eggs by the effect of LED and compact fluorescent lamps as compared to incandescents lamps. The best light intensity was 5 lux as compared to 10, 15 and 22 lux per m^2.

Nunes et al. (2014) evaluated performance, characteristics, reproductive system morphology (ovary and oviduct) and egg quality of Japanese quail exposed to green, blue, red LED and fluorescent lamps. The results showed that there was no significant difference between the treatments for performance and morphology. However, greater egg weights from the hens exposed to fluorescent lamp was observed as compared to green and blue LED. On the other hand, Jacome et al. (2012) evaluated the effect of orange, blue and white LED lamps in the production and quality of quail eggs and did not find any difference between the treatments.

Hassan et al. (2013) also evaluated the effect of LED on performance characteristics, ovarian morphology and reproductive hormones of laying hens exposed to red, green, blue LED and combinations: red x green, red x green x blue and fluorescent lamp. The results showed a greater egg production in treatments with red LED and combination of red x green, and heavier eggs were observed in treatments with blue and green LED. Estradiol and FSH levels, in the blood, were higher for LED red treatments and combination red x green. Hens treated with blue LED delayed the production with 15 days as compared to those treated with red LED. Ovarian weight and number of follicles were higher for the treatment with red LED.

CONCLUSION

According to the results presented in this analysis, it can be concluded that the LED lamps represent an efficient technology to reduce the energy costs without damaging the performance and egg quality of the commercial layers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Biological nitrogen fixation and yield of pigeonpea and groundnut: Quantifying response on smallholder farms in northern Malawi

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The global nitrogen (N) cycle is markedly, and increasingly, influenced by anthropogenic inputs. A large unknown remains the quantity of biological N fixation (BNF) inputs derived from agriculture. This leads to major uncertainties in modeling reactive N interactions with climate change, and understanding N biogeochemical processes. Understanding N dynamics is central to enhancing productivity in cropping systems. To fill this gap, we used ^{15}N natural abundance to quantify BNF and yield of groundnut and pigeonpea – on 18 on-farm sites in Ekwendeni, Northern Malawi. The study was conducted over the 2007/08 (2008) and 2008/09 (2009) cropping seasons under farmer management, for a range of edaphic environments. Overall, the soils are largely sandy with low to moderate organic carbon (0.12-1.56%), pH (5.5-6.5), and very low to moderately high inorganic P (3 to 85 mg kg^{-1}). Intercropping was efficient at utilization of growth resources than sole cropping as evidences by land equivalent ration (LER) >1. The main drivers of BNF were plant density, inorganic P and interspecific competition. The proportion of N derived from the atmosphere (22-99%) was influenced by soil P status across seasons and crop species, but not by cropping system. The mean proportion of BNF was high in both groundnut (75%) and pigeonpea (76%). Total N fixed, on the other hand, differed with cropping system in the dry year, where intercropping was associated with low levels of N fixed by pigeonpea (15 kg N ha^{-1}) compared to sole pigeonpea (32 kg N ha^{-1}). A short rainfall season could not support biomass production of pigeon pea, and this has negative implications for relying on BNF to drive productivity on smallholder farms.

Key words: Intercropping, Groundnut, Pigeonpea, Nitrogen fixation, ^{15}N natural abundance.

INTRODUCTION

Nitrogen (N) deficiency is a major factor limiting productivity of maize based systems on smallholder

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farms in Southern Africa. The major sources of N in agro-ecosystems are inorganic fertilizers, livestock manures, compost manures, and legumes. Legumes fix atmospheric N biologically into inorganic forms that can be used by plants (Giller, 2001).

Biological N fixation is important for small holder farmers as it is relatively cheaper source of N compared to inorganic fertilizers, less prone to losses through leaching and denitrification. In Malawi, farmers grow a diversity of legumes including groundnut, common beans, soybean, pigeonpea, cowpea and green manures such as velvet bean, fish bean and a number of agroforestry species. These legumes are planted in sole stands or intercropped with cereals or other legumes, and rotated with cereals. Intercropping is the growing of two or more crops simultaneously on the same piece of land (Hauggaard-Nielsen et al., 2008).

This helps to optimize the use of resources such as land, nutrients, labor and water. The traditional intercrop system consists of maize-legume intercrops at low density. The relative proportion of each component species depends on the main crop of interest to the farmers, complimentarity in growth habits and use of resources. Productivity of intercrops can be maximized with careful selection of component crops and appropriate agronomic practices (Szumigalski and Van Acker, 2008).

Legumes improve soil quality through BNF and crop residue incorporation, and increase productivity of cereal based systems (Kumar Rao et al, 1983, Sakala et al 2000). The amount of N fixed by legumes and residual benefits varies with plant species, agronomic practices and environmental factors. Groundnut can fix 32 to 206 kg N ha^{-1} (Giller et al., 1987; Unkovich and Pate, 2000)), and a net N contribution of 13 to 100 kg N ha^{-1} if crop residues are incorporated in the soil (Toomsan et al., 1995). Pigeonpea can fix 69 to 100 kg N ha^{-1} (Kumar Rao et al., 1987), and a net N contribution of 2 to 60 kg ha^{-1} depending on the genotype and environmental factors (Myaka et al., 2006; Egbe et al., 2007).

An intercrop of a legume and a legume has rarely been considered in research, in contrast to an intercrop of a legume and a cereal which is widely grown and has a nutritional complementarity. This includes the nitrogen fixation capacity of the legume and the high nitrogen requirement of the cereal. Complementary growth forms can also be the basis for a successful intercrop, such as a slow growing, deep rooted perennial grown in mixtures with a fast growing and shallow rooted annual. In India, some farmers intercrop pigeon pea with groundnut at low density (Willey at al., 1981), and has been tested in Zimbabwe (Natarajan and Mafongoya, 1992) which is an example of a doubled up legume system.

In this study, BNF and yield of intercropped groundnut and pigeonpea legumes was investigated. We hypothesized that N fixation rate per area basis will be higher under groundnut – pigeonpea intercrop (GNPP) than if either is sole cropped.

MATERIALS AND METHODS

Site description

Participatory on-farm researcher designed farmer managed trials were conducted in Ekwendeni area of Mzimba District, northern Malawi (33°53'E and 11°20'S; altitude 1200 m) in 2008 and 2009 growing seasons. Note that the growing season usually starts around November of the previous year but we refer to the season in terms of the year in which the majority of the season occurs. The annual precipitation is 800 to 1200 mm, with a unimodal distribution from November/December to March/April. Ekwendeni soils are classified as ferruginous latosols (Young and Brown, 1962).

In the 2008 crop season, rainfall was considerably below average at 669 mm. This was much less than 800 to 1200 mm, the expected range for this agro-ecological zone. Precipitation was high (45-50% of total) between last half of December 2007 to end of January 2008 which caused sheet erosion on some farm sites depending on the slope of the field, and could have resulted in leaching of some nutrients (personal observation).

Another concern was poor seedling development at some sites possibly due to saturated soil. From the second half of February 2008, the area experienced a dry spell and this coincided with grain and pod filling growth stages in maize and groundnut respectively while pigeonpea was still at vegetative stage. Scattered rain showers fell in March 2008 but this was not adequate to support optimum growth of the crops. In the 2009 season (November 2008 to April 2009), the area received 826mm of rainfall, and this was adequate for growth of legumes and maize.

Experiment description and data collection

The treatments for the cropping seasons 2008 and 2009 consisted of five cropping systems: three sole crops, maize (*Zea mays)*, groundnut (*Arachis hypogaea)* and pigeonpea (*Cajanus cajan*); and two intercrops, maize-pigeonpea and groundnut-pigeonpea. The varieties grown were CG7 groundnut, ICEAP00040 pigeonpea and ZM621 maize. CG7 groundnut is characterized by bunch growth habit and high oil content averaging 48% and a yield potential of 2500 kg ha^{-1} (Malawi Government Ministry of Agriculture, Irrigation, and Food Security, MoAIFS, 2005). Pigeonpea is a semi perennial legume that grows up to 2-4 m high (Werner, 2005). It has a deep root system and initial growth rates are slower compared to groundnut or maize.

The experiment was laid out as a randomized complete block design with six treatments. Each treatment was replicated 18 times on farm, one replicate per farm. Treatment plot size was 10m by 10m, and consisted of 11 rows (aligned on a ridge following farmer practice in Malawi), each 10m long and spaced at 0.90m. The net plot used for measurements of grain and biomass consisted of the interior 8m of 7 centrally located ridges, to reduce border effects by not monitoring the external 1m of row .

The goal for the planting pattern for intercrops was based on maximizing the plant population of the main crop for all cropping systems. The legumes were seeded at a 0.20m and 0.90m within row spacing for groundnut and pigeonpea respectively to achieve 43,210 plants ha^{-1} (0.90x0.2x1) and 55555 plants ha^{-1} (0.90x0.20x1) for intercropped and sole groundnut respectively, with an additional 37000 plants ha^{-1} of maize or pigeonpea in the intercrops. Maize and pigeonpea were seeded alternately in the intercrop, along rows in stations of three plants each, spaced at 0.45m intervals.

The planting pattern for maize and pigeonpea was an additive

design, sole crop and intercrop all planted at 37000 plants ha^{-1} density for both crops. Planting was done in December 2007 and 2008. All plots received a uniform basal application of 10 kg N ha^{-1} at one week after planting based on observations that soils were highly N deficient, to improve uniformity of plant stands and early vigor. All field management practices were conducted by participating farmers. In the 2009 crop season, year one treatments were replicated in time by planting adjacent plots. The varieties and planting pattern were the same as described for year 1.

Soil sampling and analysis

At planting in December of 2007, composite soil samples (8 to 10 subsamples) were collected from each farm using a Z-scheme to ensure random collection. Two depths were sampled, 0 to 15cm and 15 to 30cm, for site characterization.

These were air dried and sieved through a 2mm sieve. Soil texture was determined using the hydrometer method (Anderson and Ingram, 1989). Particulate organic matter (POM) were analyzed on ungrounded soil samples using a modification of the light-large particulate organic matter (POM) fractionation method described by Cambardella and Elliott (1993) and Cambardella and Elliot (1992).

Sodium polytungstate was recycled according to Six et al. (1999). After POM extraction and weighing, the sample was ground into powder with a clean mortar and pestle. POMC and POMN were determined using a dry combustion C and N Analyzer (Costech ECS 4010, Costech Analytical Technologies, and Valencia, CA). The remaining soil samples were ground and sent to A and L Great lakes Lab in Fort Wayne, Indiana, United States of America, for analysis of the following variables: pH in a 1:1 ratio in H_2O, inorganic P (Bray P), and Mehlich 3 extraction of Ca, K, Mg (Mehlich, 1984).

Plant sampling and analysis

Assessment of nitrogen fixation by legumes

Plant sampling for N fixation measurements: Two 1m x1m quadrants were demarcated in each plot for BNF measurements. At harvest, these plants were harvested and separated into grain and leafy biomass. Dried grain samples were ground into fine powder using a Wiley mill to pass a sieve size of 1mm, then carefully sub sampled and weighed into capsules before ^{15}N and ^{14}N mass spectrophotometer analysis conducted at University of California Davis, USA. The proportion of N fixed through BNF was determined using ^{15}N natural abundance method. The proportion of N derived from atmosphere (%Ndfa) was calculated according to Shearer and Khol (1986) and Peoples et al., (1989) as follows:

$$\%Ndfa = 100\frac{\left(\delta^{15}Nref - \delta^{15}Nlegume\right)}{\delta^{15}Nref - B} \quad (1)$$

Where $\delta^{15}N_{ref}$ is the ^{15}N natural abundance of grain of the reference plant (maize) grown on same soil as the legume; $\delta^{15}N_{legume}$ is the ^{15}N natural abundance of the grain of the legume crop; B is the $\delta^{15}N$ of the test legume where the only N source is atmospheric N. The lowest $\delta^{15}N$ for each legume was used as B value (Hansen and Vinther, 2001).

Plant biomass

Plant biomass at early vegetative stage and at harvest was determined from the net plot of 57.6m2 (8 middle ridges x 8m x 0.90m). Groundnut and maize were harvested in June whilst pigeonpea was harvested in September after full physiological maturity. As described above, quadrant samples were removed from the net plot to measure N fixation, and grain yields were adjusted for this removal. Grain moisture was determined by wet weight basis of oven dried sub sample of grain. Grain yields were reported on an adjusted basis at 8% and 15% moisture content for groundnut and pigeonpea, respectively.

Statistical analysis

Soil nutrient and physical properties were analyzed using a one way ANOVA for location. Whereas plant analysis, biomass yield were analyzed as a RCBD using SAS proc mixed procedure for a two-way model, cropping system by year as factors (SAS Institute, 2001). Where variances were not homogenous, data were analyzed with unequal variances assumption. All data were analyzed in SAS proc mixed procedure. Significant differences were determined at p=0.05.

RESULTS

Soil characterization

Table 1 shows results on soil chemical properties and texture. Soil pH ranged from 5.5 to 6.5 at 0 to 15cm depth with a mean of 5.8±0.3. The soils are largely light textured with 18±7.7% and 74±9.8% clay and sand respectively. Soil organic carbon (OC) was low in the range of 2.0 to 16 g kg^{-1}, with a mean of 6.4± 3.1g kg^{-1}; and total N (0.7±0.05 g kg^{-1}). Inorganic P was highly variable (3 to 85 mg kg^{-1} at 0 to 15cm depth). The mean CEC was 5.8±1.6 and 4.9±1.4 cmol kg^{-1} at 0 to 15cm and 15 to 30cm soil depths respectively. Exchangeable cations (calcium, potassium and magnesium) were adequate for growth of the maize, groundnut and pigeonpea (Table 1).

Plant growth in 2008 and 2009

Biomass of pigeonpea and groundnut at early vegetative stage (eight and half weeks after planting) averaged 8.9±1.7 g $plant^{-1}$ and 17±3.4 g $plant^{-1}$, respectively, with no effect observed of cropping system. The mean biomass/plant of sole and intercropped groundnut at harvest were 63±20 and 53± 20g respectively. However, in pigeonpea, intercropping reduced pigeonpea biomass by 30 to 60%, p=0.0023 (Figure 1). In 2008/09 season, similar observations were made on late season growth of pigeonpea.

Grain yield of groundnut and pigeonpea

Table 2 shows results on leafy biomass and grain yield of

Table 1. Soil chemical properties and texture of on-farm experimentation fields (Baseline analysis sampled early December, 2008. N=18).

Variable	0-15cm		15-30cm	
	Mean	Range	Mean	Range
pH (in H_2O)	5.8 ±0.3	5.5-6.5	5.9±0.4	5.1-6.9
OC (g kg^{-1})	6.5±2.1	3-11	5.0±2.2	2-11
Total N (g kg^{-1})	0.5 ±0.1	0.4-0.8	0.5± 0.2	0.3-1.1
POMC (g kg^{-1})	0.4 ±0.01	0.20-0.94	0.2± 0.12	0.03-0.45
POMN (g kg^{-1})	0.02±0.01	0.008-0.04	0.01±0.002	0.003-0.04
Bray P (mg kg^{-1})	10 ±8.4	3-85	3±1.9	1-66
Sand (%)	74 ± 9.8	-	72±10.7	-
Clay (%)	18 ± 8.2	-	21±8.8	-

Key: POMC=particulate organic matter carbon; POMN=particulate organic matter nitrogen, units for POMC and POMN are g per kg POM.

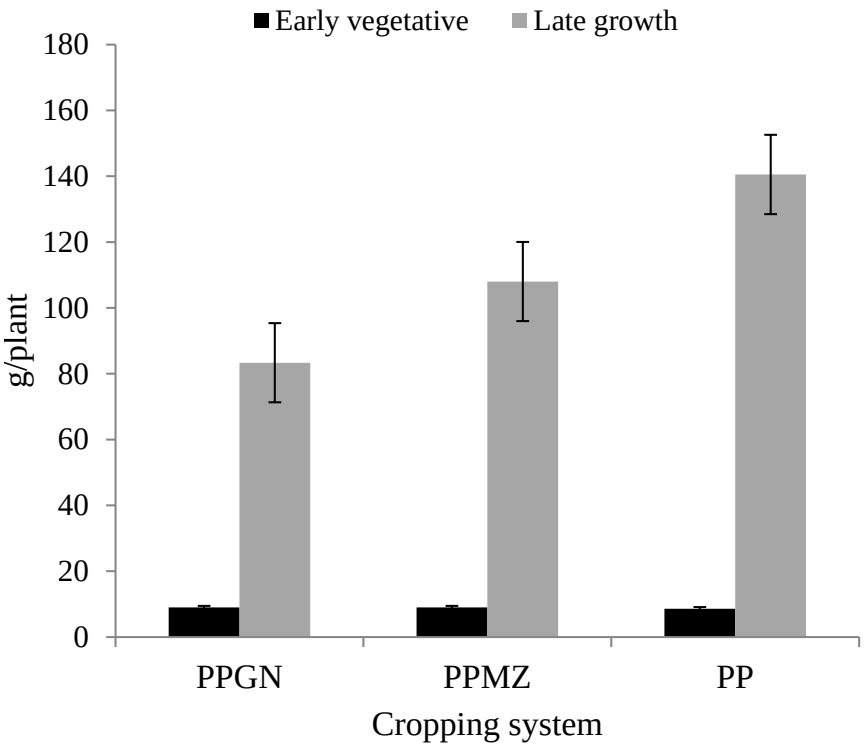

Figure 1. Pigeonpea growth at vegetative stage (8.5 weeks after planting) and at harvest in 2007/08 season (Key: PP=sole pigeonpea; PPGN=pigeonpea intercropped with groundnut; PPMZ=pigeonpea intercropped with maize. Standard error presented as error bar).

groundnut and pigeonpea. In groundnut, cropping system and season interactions were significant, p=0.0026. Sole cropped groundnut yielded 84% higher in 2008/09 season than 2007/08. The yield of groundnut haulms varied with season and cropping system (p<0001). Sole groundnut produced more haulms (2.5 t ha^{-1}) compared to intercropped groundnut (1.7 ton ha^{-1}).

Across seasons, the quantity of haulms was 89% higher in a 2009 than in 2008. In pigeonpea, grain yield averaged 284 kg ha^{-1} and was not affected by cropping system or season. However, leafy biomass of pigeonpea was significantly affected by cropping system (p=<0.0001) and season (p=0.03). Sole cropped pigeonpea produced 100% more leafy biomass than intercrop. In a wetter season, pigeonpea produced higher biomass than in 2008 season. The land equivalent ratio was equal to 1.52 indicating total productivity was 52% higher under groundnut-pigeonpea intercropping than

Table 2. Effect of cropping system on leafy biomass and grain yield of groundnut and pigeonpea, 2007/08 and 2008/09 cropping seasons.

Crop	Cropping system	Grain yield (kg/ha)		Leafy Biomassϕ		
		2008	2009	2008	2009	Mean
Groundnut	Sole	598^{aA}	1101^{bB}	1895	3156	2569^{B}
	Intercropped	435^{aA}	650^{aB}	1275	2042	1659^{A}
	Mean	516	876	1585^{A}	2599^{B}	-
Pr>F	Season (A)	<0.0001	-	<0.0001	-	-
	Cropping system (B)	<0.0001	-	<0.0001	-	-
	A × B	0.0026	-	0.247	-	-
Pigeonpea	Sole	310	302	3410	4341	3876^{B}
	Intercropped	238	284	1443	2320	1881^{A}
	Mean	274	293	2416^{A}	3333B	-
Pr>F	Season (A)	0.738	-	0.03	-	-
	Cropping system (B)	0.425	-	<0.0001	-	-
	A × B	0.816	-	0.947	-	-

Means in a row or column per variable category followed by same upper lower or upper case letter are not statistically significant at p<0.05; ϕIn pigeonpea, the biomass includes the leafy biomass and stems (2008= 2007/08 cropping season; 2009=2008/09 cropping season).

sole cropping, that is, 52% more land would be required in sole stands to produce same yields as in intercropping (Table 2).

Biological nitrogen fixation of groundnut and pigeonpea

Nodule number and weight

Cropping system had no effect on nodule numbers per plant and nodule weight of both legumes at eight weeks after planting (WAP). The mean nodule number per plant were 91±8; and 10±1.4 for groundnut and pigeonpea respectively. However, in groundnut, the nodules were bigger (p=0.004) in a wetter season (2009) than 2008 (2.7 vs 1.7mg); and no season effects observed in pigeonpea. The average nodule weight in pigeonpea was 9.6mg.

Proportion of nitrogen (N) derived from the atmosphere and N fixation by groundnut and pigeonpea

The proportion of N derived from the atmosphere (%Ndfa), total N fixed by legumes and correlation matrix between N fixed and selected variables are shown in Tables 3 and 4. In groundnut, the %Ndfa ranged from 29 to 99% with a mean of 78% with no effect of cropping system. For sole groundnut, the %Ndfa was positively correlated to crop N (r=0.98), POM (r=0.68), inorganic P (r=0.59) and plant density (r=0.65) (Figure 2). The relationship between total N fixed (kg ha^{-1}) and plant density was described by following fitted regression model:

Total N fixed = -93.404 + 0.003 plant density

However, no linear relationship was observed between the total N fixed per unit area and inorganic P or density when groundnut was intercropped with pigeonpea.

In pigeonpea, the %Ndfa ranged from 41 to 99%, mean =76±20. This did not differ between sole and intercropped pigeonpea. In 2008, the %Ndfa was positively correlated with total N fixed, r=0.73 and 0.68 for sole pigeonpea and pigeonpea intercropped with maize (PPMZ) respectively. Similar findings for %Ndfa were observed under sole pigeonpea and pigeonpea intercropped with groundnut (PPGN) in 2009. Inorganic P was positively correlated with total N fixed, r=0.86 for PPMZ in 2008, r=0.59 for PPGN (Table 4), r=0.78 and 0.65 for sole pigeonpea in 2008 and 2009 respectively (Table 3 and Figure 3).

On area basis, total N fixed ranged from 21 to 86, mean= 53 kg ha^{-1} under sole pigeonpea; 34 to 148, mean = 72 kg ha^{-1} for GNPP (Table 3); and 4 to 26 kg ha^{-1}, mean=12 kg ha^{-1} for PPMZ. However, there were no differences in total N fixed by sole groundnut and GNPP. A trend of higher total N fixed under GNPP than PP was observed (p=0.094). Total N fixed in aboveground leafy biomass of pigeonpea was twice as much in 2009 season than 2008 probably due to adequate soil moisture with

Table 3. Biological nitrogen fixed (proportion and total) in grain and leafy biomass by sole and intercropped groundnut and pigeonpea.

Season	Cropping system	% Ndfa	N fixed in grain (kg/ha)	N fixed in leafy biomass (kg/ha)	Total N Fixed (kg/ha)	Range, total N fixed (kg/ha)	Estimated N fixed in defoliated PP leaves* (kg/ha)
2008	Groundnut (GN)	78	17b	21c	50c	21-102	-
	Pigeonpea (PP)	76	2a	15b	31b	11-64	8.6
	GNPP	-	16b	19bc	42bc	23-69	6.2
	Pr>F	-	<0.0001	0.0005	<0.0002	-	-
2009	Groundnut (GN)	73	28b	33	62	21-96	-
	Pigeonpea (PP)	75	8 a	34	53	21-86	13.4
	GNPP	-	23b	39	72	34-148	6.5
	Pr>F	-	<0.001	0.726	0.238	-	-

2008 = 2007/08 season; 2009= 2008/09 season; GNPP= groundnut intercropped with pigeonpea; B values obtained from lowest 15N of legume (Hansen and Vinther, 2001). B values in 2008 are -0.45, -0.38 and -0.80 for sole GN, GNPP and sole PP. B values in 2009 are -0.26 and -0.21 for sole GN and GNPP; and -0.83, -0.74 for sole PP and PP intercropped with GN; Means in a column by year category followed by same letter are not statistically significant at p=0.05; *The estimated N fixed in defoliated leaves calculated based on determined proportion of defoliation in pigeonpea at harvest, 41% and 57% for intercropped and sole cropped ICEAP00040 pigeonpea.

Table 4. Correlation matrix of nitrogen fixation with Ndfa, Bray P and crop N of sole and intercropped pigeonpea and groundnut, 2007/08 and 2008/09 seasons.

Season	Variable	N fixed by pigeonpea			N fixed by groundnut	
		Sole PP	PPGN	PPMZ	Sole GN	GNPP
2008	N fixed	1.000	1.000	1.000	1.000	1.000
	Bray P	0.780**	0.175	0.857***	0.587*	0.352
	Ndfa	0.731*	0.488	0.676*	0.428	0.296
	Crop N	0.911***	0.92***	0.599*	0.98***	0.93***
2009	N fixed	1.000	1.000	nd	1.000	1.000
	Bray P	0.646*	0.590*	nd	0.263	0.170
	Ndfa	0.598	0.566*	nd	0.337	0.868***
	Crop N	0.894***	0.904***	nd	0.614*	0.981***

2008 = 2007/08 season; 2009= 2008/09 season; Values in bold are significant. Level of significance *p=0.05; ** p=0.01; *** p=0.0001; GN= groundnut; PP = pigeonpea; PPGN= pigeonpea intercropped with groundnut; GNPP= groundnut intercropped with pigeonpea; PPMZ=pigeonpea intercropped with maize; nd=no data for PPMZ in 2009.

the high precipitation. A short rainfall season could not support biomass production of pigeon pea and this has negative implications for relying on BNF to drive productivity on smallholder farms. Defoliation in senesced leaves was estimated at 41% and 57% for PPGN and sole PP. The

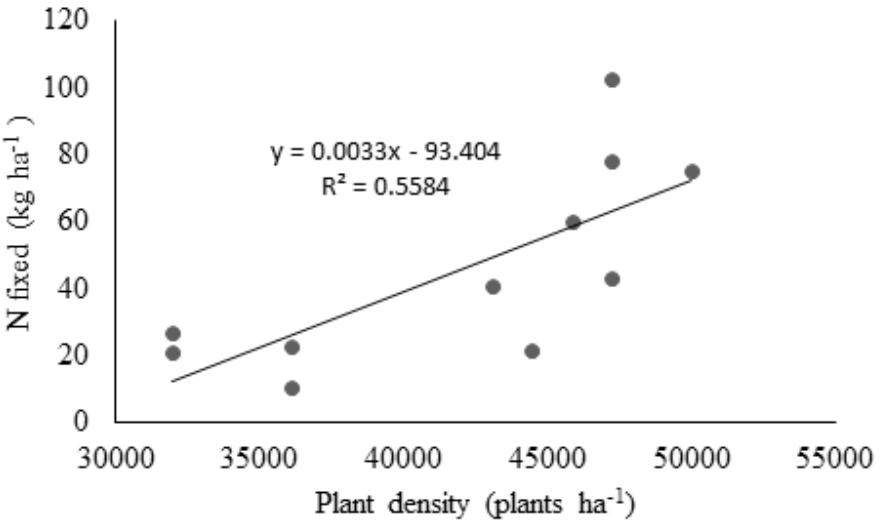

Figure 2. Relationship between nitrogen (N) fixed by sole cropped groundnut and plant density, 2007/08 season.

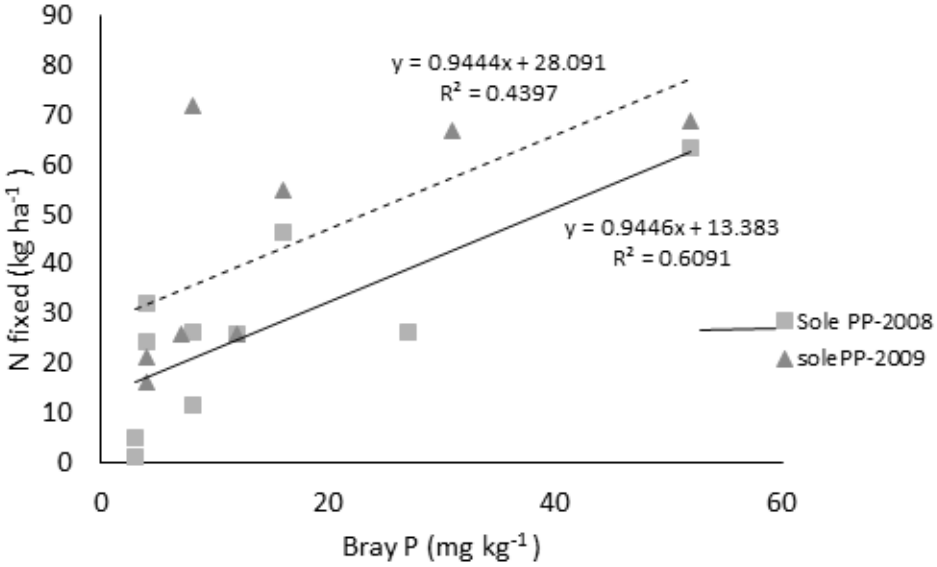

Figure 3. Relationship between nitrogen fixed by sole cropped pigeonpea and inorganic soil phosphorus, 2007/08 and 2008/09 seasons
Key: PP-2008=pigeonpea, 2007/08 season; PP-2009=pigeonpea, 2008/09 season.

estimates of N fixed in defoliated leaves are included in Table 2.

DISCUSSION

Soil fertility

Soil fertility was low and variable among smallholder farms and this is consistent with findings from earlier studies (Snapp 1998, Mhango et al., 2013). Inorganic P was variable and low, and yet this is important for BNF (Jemo et al, 2006). The high correlation of inorganic P and total N fixed by pigeonpea in maize-pigeonpea intercrops (Table 3) suggests interspecific competition and that the two crops were accessing P from same pools during part of their growth cycles. In contrast, groundnut and pigeonpea may access P from different pools because of the differences in growth habits. Phosphorus is important for root development and growth

of legume species. Positive correlations observed between inorganic P and N fixed by pigeonpea could be related effects of P on nodulation, biomass production and N fixation process.

Biomass production of sole and intercropped legumes

Early growth was not altered by crop system, indicating that competition was minimal early in the growing season. This is not surprising as pigeonpea has a slow growth pattern, and had 50% of groundnut dry matter accumulation during early vegetative growth.

This may have also been due to relatively low plant population densities, which follows farmer practice. Surprisingly, groundnut biomass was not affected in late growth stages and this could be due to differences in growth rates relative to semi-perennial pigeonpea. A follow up study in 2012 showed that different densities of pigeonpea, from 12350 to 37000 plants per hectare did not alter the growth and grain yield of CG7 groundnut. In contrast, the late season growth competition demonstrated by low pigeonpea biomass could be related to inadequate soil moisture due to early cessation of rain in 2008 which inhibited vegetative biomass production.

The grain yield of groundnut reported in this study is lower than the potential yield but is within the average yields (500 kg ha^{-1}) obtained on smallholder farms in Malawi (Kamanga 2002; Malawi Government, MoAIFS, 2005). Pigeonpea grain yield on smallholder farms are generally low and one of the major constraints are pests such as blister beetles (*Mylabris* species) that feed on flowers (Boehringer and Caldwell, 1989). Other on-farm studies in central Malawi have reported pigeonpea grain yield of 155-348 kg ha^{-1} (Twomlow et al., 2004; Chamango, 2004). The findings from this study also illustrate that there was efficient utilization of growth resources under groundnut-pigeonpea intercropping than sole stands, LER=1.52.

Biological nitrogen fixation of sole and intercropped groundnut and pigeonpea, and sustainability of cropping systems

Effective nodulation is important for maximizing N fixation by legumes. In this study, the reduction in nodule weight in a drier year can be attributed to drought. The number of nodules per plant in both legumes is comparable to previous work by Giri and De (1980) and Kumar Rao et al. (1996).

Interspecific competition is one of the determinants of intercrop productivity. It is an important finding that the proportion of Ndfa was not affected by cropping system. This is consistent with Katayama et al. (1995) but differ in that they reported higher %Ndfa in pigeonpea intercropped with cereals (84%) than sole or doubled up legumes (52 to 70%).

Groundnut met 78% of its N requirement from BNF. This was higher than 22 to 67% as reported by Katayama et al. (1995) and Phoomthaisong et al. (2003), and this could be due to low soil N (Table 1). The total amount of N fixed per area were lower than those reported by Ojiem et al (2007) for CG7 groundnut probably due to low inorganic soil P (Table 3), plant density and biomass production.

Ojiem et al. (2007) reported 115 to 124 kg ha^{-1} as N fixed with application of inorganic P fertilizer and higher plant densities of approximately 1.5 times than the density in this study. Since crop N was positively correlated with amount of N fixed by legume, inadequate soil moisture during reproductive growth stage of groundnut may have limited pod formation and grain filling consequently reducing total plant biomass.

The positive correlation between inorganic P and total N fixed by intercropped pigeonpea may suggest that the two crops were accessing the same P pools (Makumba et al., 2009). However, in a short rainfall season (2007/08), the lack of a correlation between inorganic P and N fixed by PPGN is probably due to poor growth of pigeonpea with inadequate soil moisture and hence less competition for nutrients.

Long duration legumes such as pigeonpea are expected to fix more N, and produce higher biomass than the early maturing varieties. In this study, pigeonpea fixed less N than groundnut in a short rainfall season probably because of inadequate soil moisture. The average total N fixed by pigeonpea is lower compared to 46-118 kg ha^{-1} as reported in earlier studies for ICEAP00040 variety in Malawi (Adu-Gyamfi et al., 2007). Sole pigeonpea fixed 30 and 53 kg ha^{-1} in a dry and wet season respectively and this is within 20-60 kg N ha^{-1}, values reported for the same variety on selected sites in Tanzania. These findings can be attributed to low inorganic P (Table 1) and inadequate soil moisture to support biomass production following a dry spell that occurred when pigeonpea was still at early vegetative stage in 2007/08 season.

Legumes have been promoted in farming systems as an alternative strategy to improving soil N and productivity of cereals. In Malawi, the recommended N rate for maize on most smallholder farms is 92 kg N ha^{-1}. The proportion of N requirement met by sole and intercropped legume systems is 12-50%. This implies that on low fertility soils (<15 g kg^{-1} OM), legume based cropping systems alone cannot sustain maize productivity and hence the need for integrated soil fertility management (ISFM) approaches.

CONCLUSION

This study evaluated biological nitrogen fixation and yield of sole, and intercropped groundnut and pigeonpea on smallholder farms. Soil P availability was not related to

general soil properties such as soil organic carbon and texture, yet it was an important determinant of nitrogen fixation in these legume diversified cropping systems. This indicates that it may be possible to support greater legume growth without building soil organic carbon to higher levels, rather the emphasis should be on judicious use of P-fertilizer and other P amendments such as compost.

Intercropping pigeonpea with groundnut or maize can help to improve crop productivity, maximize use of limited land and labor. The results have demonstrated that the drivers of biological N fixation are inorganic P, plant density and interspecific competition.

In a short rainfall season, interspecific competition may limit vegetative growth of semi-perennial pigeonpea, and this has negative implications for BNF. The findings from this study also suggest that different legume cropping systems should be recommended for farmers, sole cropping for grain maximization; and intercropping for smallholder farmers interested in multiple benefits.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of plant population and nitrogen rates on growth and yield of okra [*Abelmoscus esculentus* (L). Moench] in Gambella region, Western Ethiopia

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Okra is one of the most important crops next to maize and sorghum production in Gambella Regional States. Okra production and yield maximization has not been attained due to lack of appropriate production practices such as optimum plant spacing and fertilizer use. Therefore, the research was conducted to assess the effect of plant population, and nitrogen rate on growth and yield components of Okra (*Abelmoscus esculentus* (L). Moench). The treatments were factorial combinations of five spacings (45 cm × 20 cm, 45 cm × 30 cm, 60 cm × 20 cm, 60 cm × 30 cm and 60 cm × 40 cm, and four nitrogen rates (0, 23, 46 and 69 kg N ha^{-1}). The experiment was laid out in randomized block design factorial arrangement with three replications. Farmers' local variety of okra, 'Amula', was obtained from the same institute was used as a test crop. Results indicated that, plant population and nitrogen rate had significance influence on growth and yield components of okra. Maximum number of branches (2.93), number of leaves (15.95) and pod length (29.01 cm) was obtained from the interaction of 60 cm × 30 cm spacing, 60 cm × 40 cm and at 46 kg N ha^{-1}. The highest fresh pod yield (46.14 t ha^{-1}) and above ground biomass yield (119.34 t ha^{-1}) was obtained from 45 cm × 30 cm spacing and at rate of 46 kg N ha^{-1}. Similarly, maximum dry pod yield (16.65 and 16.31 t ha^{-1}) was obtained at 45 cm × 30 cm and at 46 and 69 kg N ha^{-1} respectively. Spacing of 45 cm × 30 cm and nitrogen rate of 46 kg N ha^{-1} appears to be optimum practice for higher yield of the local cultivar okra in Gambella area.

Key words: Growth, nitrogen, okra, plant population, yield.

INTRODUCTION

Okra (*Abelmoscus esculentus* (L). Moench) is one of the most well-known and utilized species of the family Malvaceae. It is also a chief vegetable crop grown for its immature pods that can be consumed as a fried or boiled vegetable or may be added to salads, soups and stews (Kashif et al., 2008).

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There is no available record on production area and productivity of the crop under Ethiopian condition; however, it has high diversity in some parts of the country particularly in the South Western low lands (550 to 650 m above sea level) of the country. The production and productivity okra is seriously affected due to the use of low yielder local varieties, suboptimal plant density, inappropriate planting date, and decline in soil fertility and a decreased use of organic amendments, heavy attack of various insect pests and weeds (Akanbi et al., 2010).

Maintaining optimum spacing or plant population and nitrogen fertilization dose are most important elements in improving productivity of okra. Optimum plant density is the key element for higher yield of okra, as plant growth, yield and quality are affected by inter and intra-row spacing (Amjad et al., 2002; Paththinige et al., 2008). With increasing plant population, yield per unit area increases until a certain limit, beyond which yield decreases due to limitation of environmental resources required for plant growth (Amjad et al., 2002).

The lack of nitrogen in soil may lead to poor plant growth due to a decline in soil productive potential and fertility status. Nitrogen is the most essential element of plant nutrition as plants take it up in significant amounts. Sufficient nitrogen supply improves cell division, foliage production, and photosynthetic activity of the plant, thus producing higher numbers of flowers and fruits (Sharma and Yadav, 1996). Optimal use of nitrogen improves dry matter, especially the economic parts of the plant (that is, flowers and fruits). However, nitrogen availability to plants depends on the source, soil type, and environmental conditions, which may affect crop performance (Salazar et al., 2011).

Many farmers are currently cultivating okra in Gambella Region at various spacings which are inappropriate for obtaining maximum yields. These inappropriate plant spacings often lead to poor plant growth, fruit quality, and low yields which are insufficient to offset production costs which results in substantial losses of yield. The farmer's low yield problem is further compounded by the utilization of low or no nitrogen fertilizer use.

The agro ecological condition of Ethiopia is favourable for home garden and commercial production of okra, however, the overall national production and consumption is neglected and only known and grown in a few part of the country like Gambella, Humera and Benshangul Gumuz. People grow okra conventionally although no any clear record on production area and productivity of the crop under Ethiopian condition. No attention has so far been given for the development of improved agronomic management practices like spacing and nitrogen fertilizer application to increase the productivity of the crop in the region. The hypothesis was to find out optimum plant population and nitrogen rate that could give maximum pod yield of okra.

Understanding the economic importance of okra in Gambella Region, research was carried out to evaluate the effect of plant population and nitrogen rates on growth and yield components of okra.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted at Gambella Research Institute (GARI), Pignkew station, which is located 18 km from Gambella town, during the period from July to November 2013. The experimental site is located at an altitude of 445 m above sea level. Gambella is situated in the sub-humid hot to warm agro ecological zone that is well known for okra cultivation by small holder farmers. The region's mean annual rainfall is 1245.8 mm with a uni-modal rainfall pattern which occurs from early April and extends to the end of November. The experiment was conducted on vertisol having a pH of 5.5. The average annual maximum and minimum temperatures are 36.7 and 22.3°C, respectively (Gambella Meteorological Agency, 2014, unpublished data).

Experimental material, treatments and experimental design

Local cultivar f okra ('Amula') was used as a planting material. The cultivar has been used by farmers in the region and it takes 55 to 75 days to mature under Gambella condition. It is short/ dwarf type of okra which is known to be high-yielding as compared to the other available cultivars.

The treatments consisted of five inter and intra row spacing (45 cm × 20 cm, 45 cm × 30 cm, 60 cm × 20 cm, 60 cm ×30 cm, and 60 cm × 40 cm) and 4 nitrogen rates (0, 23, 46 and 69 kg N ha^{-1}) having a total of 20 treatments. The experiment was laid out as a Randomized Block Design (RBD) in factorial arrangement of 5 × 4 with three replications. The gross plot size was 3.6 m × 3.0 m (10.8 m^2). The experimental blocks and plots were spaced 1.5 m and 1 m apart, respectively. Each plot consisted of 6 and 8 rows for 60 and 45 cm inter row spacing, respectively, the number of plants per row was 15, 10 and 7 for the intra-row spacings, 20, 30 and 40 cm respectively. The net plot consisted of the central 4 and 6 rows for 60 cm and 45 cm row spacing, respectively. Plants in a 0.6 m length at both ends of the row were considered as border plants, and were not harvested to avoid border effects. Thus, the harvestable plot area was 5.28 m^2 (2.4 m × 2.2 m).

Experimental procedures and cultural practices

Okra seeds were sown on 24 August 2013 by dibbling 2-3 seeds per hole on the prepared plots according to the treatment combination. Triple super phosphate was applied at sowing time to the control plots. Nitrogen at treatment levels were applied in the form of urea in three splits: emergency of plants, at active growth stage and the remaining at flowering. Nitrogen in the form of urea (4.86 kg) was applied to the plots accordingly. Nitrogen at the rate of 23 kg ha^{-1} (0.27 kg urea), 46 kg ha^{-1} (0.54 kg urea) and 69 kg ha^{-1} (0.81 kg urea) was applied to the experimental plots within blocks after crop emergency at 15 days interval. No potassium fertilizer applied.

Thinning operation was done after emergence of seedlings: one vigorous plant was maintained per hole. Weeding and other management practices like hoeing, pest and disease control were applied uniformly as required.

Soil sampling and analysis

Soil samples were collected from the entire experimental site in

zigzag pattern from 0 to 30 cm depth of soil using an auger. Then all samples were mixed together in order to get one composite sample weighing 1 kg for determination of selected soil properties. The composite soil sample was dried and crushed to pass through a 2 mm size sieve for the analysis of pH, texture. For the determination of total nitrogen and organic carbon, the soil sample was made to pass through 1 mm pore size sieve. The soil samples were analyzed for some parameters at Melkassa Research Center Soil Laboratory. The soil was analyzed for texture, pH, organic carbon, total N and available P. Soil pH was measured from a suspension 1:2.5 soil-water ratio using an electrodes pH meter (Motsara and Roy, 2008). The organic carbon content of the soil was determined by the volumetric method (Walkley and Black, 1934). Total nitrogen was estimated by the Micro-Kjeldahl method with sulphuri acid (Jackson, 1962). Available phosphorous was estimated by the Olsen method (Olsen et al., 1982).

The soil analysis results indicated that the experimental site had soil PH of 5.5 with 0.13% total nitrogen, 1.92 and 2.64% organic carbon, phosphorous and a texture of vertisol, respectively. The total nitrogen was low as it was below 0.15% as described by Bruce and Rayment (1982).

Data collection and measurement

Data were collected on crop phenology, growth, yield and yield related traits.

Crop phenology and growth parameters

Number of days to 50% flowering: The number of days to 50% flowering was determined from each plot by counting the number of days required for 50% of plants in a plot to reach flowering.

Days to pod set: The number of days taken for 50% of plants in each experimental plot set at least one pod per plant.

Plant height (cm): Plant height was measured at the last picking of the fruit from ten randomly selected plants in each plot from the central rows using a measuring tape from the soil surface to the tip of plant.

Length of pod bearing zone (cm): This refers to the length of stem from the point of first pod set to the point where pod formation ends.

Number of branches per plant: Total number of branches was counted from 10 randomly selected plants from the central rows and then mean was calculated at the final harvest.

Number of leaves per plant: The total number of leaves was recorded by counting the number of leaves per plant until the final harvest. Leaves were counted from the same ten randomly selected plants, which were selected for the measurement of plant height.

Yield and yield components of okra

Number of green pods per plant: The number of green pods per plant was counted at every picking day from ten randomly selected and tagged plants in each plot. The total number of pods obtained from the selected plants was divided to get the average number of pods per plant.

Length of green pod (cm): The length of 10 green marketable pods collected from sample plants was measured and averaged.

Pod diameter (cm): Pod diameter was measured using a vernier calliper and averaged.

Total green pod yield (g/plant and t ha^{-1}): Yield obtained at each harvest from the net plot area was summed up as marketable and unmarketable yield and converted to a hectare basis.

Pod fresh weight (g plant^{-1} and t ha^{-1}): Average fresh pod weight from 10 randomly taken pods from each net plot area was measured by using a digital balance.

Pod dry weight (g plant^{-1} and t ha$^{-1)}$: This refers to the dry weight from 10 pods selected randomly from the net plot area, and dried in an oven to a constant weight.

Above ground dry biomass yield (g plant^{-1} and t ha^{-1}): The sum of above ground parts of 10 selected plants along the pods was weighed and averaged after oven-drying to a constant weight.

Crop stand: The number of plant in the net area of each plot was counted fifteen days after emergence and at the time of the last harvest.

Data analysis

The data were subjected to analysis of variance (ANOVA) appropriate to randomized complete block design technique using the SAS computer software programme, version 9.2 (SAS, 2008). Least significant difference (LSD) at 5% probability level was carried out for means separation.

RESULTS AND DISCUSSION

Phenology and growth parameters

The results of analysis of variance indicated that main effect nitrogen showed significant ($P < 0.001$) difference on 50% flowering; pod setting, plant height and length of pod bearing zone (Table 1). However, the main effect of plant population and nitrogen fertilizer application rate significantly ($P < 0.05$) influenced the number of leaves produced per plant. The interaction effect of plant population and nitrogen fertilizer application significantly resulted in increased number of branches produced per plant (Table 1).

Application of nitrogen at the rate of 46 and 69 kg N ha^{-1} led to the longest days (51.40) to 50% flowering as compared to the control treatment, which gave least days to flowering (46.33). The application of the 69 kg N ha^{-1} increased flowering date by 10.94% as compared to the control (Table 3).

Nitrogen application from nil to the highest level (69 kg N ha^{-1}) prolonged the number of days to pod setting in a similar way that it prolonged the number of days required for 50% flowering. Increasing, the application rate of nitrogen from 0 to 46 and 0 to 69 kg N ha^{-1} prolonged the duration of 50% pod set by 7.6 and 8.8%, respectively (Table 3). At the control level (0 kg N ha^{-1}), days to pod setting was 49.93 while at 69 kg N ha^{-1} it was 54.33 days. The delay in number of days to flowering and fruit

Table 1. Mean squares values of crop phenology and yield components of okra.

Source of variation	DF	Days to flowering	Days to pod set	PH	LPBZ	NBP	NL	NPP	PL	PD
Replication	2	2.07^{NS}	1.61^{NS}	107.09 ^{NS}	100.58^{NS}	0.43*	9.02*	6.43**	0.68^{NS}	0.05N
Nitrogen	3	72.02***	56.93***	164.71***	204.21**	0.86**	16.38*	9.36***	134.93***	0.34***
plant spacing	4	0.31^{NS}	0.83^{NS}	7.43^{NS}	6.42^{NS}	0.45**	6.46*	18.32***	14.86***	0.17***
NXPS	12	1.05^{NS}	1.04^{NS}	33.46^{NS}	25.72^{NS}	0.06*	1.31^{NS}	0.32^{NS}	0.78*	0.03*
Error	38	1.89	1.66	26.48	44.81	0.03	2.01	0.66	0.29	0.006
CV		2.78	2.45	8.76	13.67	7.58	9.53	5.98	2.19	2.79

* Significant at P≤ 0.05; ** significant P≤ 0.01; *** significant at P≤ 0.001; NS = non significance at P≥0.05.N x PS, Nitrogen x plant spacing; DF, Degree of freedom; PH, Plant height; LPBZ, length of pod bearing zone; NBP, Number of branches per plant; NL= Number of leaves per plant; NPP, Number of pods per plant; PL, Pod length; PD, Pod diameter

formation in response to increasing the rate of N may be attributed to the prominent role nitrogen plays in promoting cell division and vegetative growth, which delays flowering and fruiting in crop plants.

Maximum plant height was recorded from the application of nitrogen fertilizer level of 23 kg N ha^{-1}, whereas the minimum plant height was recorded from the rest of nitrogen fertilizer levels. Application of 23 kg N ha^{-1} significantly increased plant height by 13.56% as compared to the control plots which received no nitrogen (Table 4). Results are in line with the finding of Bin-Ishaq (2009) who reported that increasing the rate of N up to 45 kg N ha^{-1} was associated with significant progressive increases in plant height of okra. The increase in plant height in response to increased application of nitrogen could be attributed to enhanced synthesis of protein in the plant, which is, fundamental building material of cells and a constituent of all enzymes.

The application of nitrogen rates at 23 kg N ha^{-1} rate resulted in maximum length of pod bearing zone. Application of 23 kg N ha^{-1} significantly increased length of pod bearing zone by 18.22% compared to the control plots. However, increase in the rate of nitrogen beyond 23 to 46 and 69 kg N ha^{-1} resulted in a significant reduction of length of pod bearing zone by 8.9 and 13.28%, respectively, compared to 23 kg N ha^{-1} application (Table 4).

The results of this study in consistent with Firoz (2009) who reported that inter-node length of okra plant was significantly influenced by the application of nitrogen fertilizer. Consistent with this result, Malik and Mondal (1996) revealed that okra pods are continually produced on new nodes of developing stems, which results in several weeks of harvesting.

The plant spacing of 60 cm × 40 cm and 60 cm × 30 cm, resulted in maximum number of leaves per plant (Table 4). This result is supported by that of Bin-Ishaq (2009), who reported that number of leaves per plant was significantly increased as the plant density decreased and number of leaves decreased as plant density increased. This is because at wider spacing, there are favourable growth conditions which enhance vegetative growths such as increase in number of branches which results in number of leaves per plant.

The application of nitrogen fertilizer rate of 46 kg N ha^{-1} significantly increased the number of leaves per plant by 16.81% compared to the number of leaves per plant recorded to control plots (Table 4). Increased application of nitrogen rate was associated with increase in vegetative growth components of plants and resulted in new leaf formation. This result is in line with the finding of Bin-Ishaq (2009) who reported that increasing N applied rate up to 45 kg N ha^{-1} was associated with significant progressive increases in number of leaves per plant. Similar results were reported by Ghoneim (2000) who reported that application of 60 kg N ha^{-1} to okra plants increased leaves per plant.

The highest number of branches was recorded from plant populations grown at the spacing of 45 cm × 30 cm and 60 cm × 30 cm plant and application of 46 kg N ha^{-1}, which was in statistical parity with the number of branches obtained from the spacing of 60 cm × 40 cm spacing and N application of 46 kg N ha^{-1} and 60 cm × 30 cm combined with N rate of 69 kg ha^{-1}. The results of the study is in line with the finding of Bin-Ishaq (2009) who reported that increasing N application rate up to 45 kg N ha^{-1} was associated with significant progressive increases in number of branches per plant. Application of more nitrogen beyond 46 kg ha^{-1} did not favour the production of more number of branches (Table 5). Consistent with the results of this study, Ekwu et al. (2010) reported that the application of nitrogen at the rate of 140 kg ha^{-1} produced the highest number of branches and fruits as compared to 0 and 70 kg ha^{-1}. Khan et al. (2010) also reported that the highest number of fruits per plant and of number of branches per plant of sweet pepper in response to increased application of nitrogen fertilizer up to 150 kg N ha^{-1}.

Yield and yield components of okra

The interaction effect of plant population and nitrogen

Table 2. Mean squares values of yield components of okra.

Source of variation	DF	PFW (g)	PDW (g)	ABMY(g)	GPY(kg)	PFW(t/ha)	PDW(t/ha)	ABMY(t/ha)
Replication	2	23332.80^{NS}	108.665**	17441.31 ^{NS}	20.03**	6.31 ^{NS}	0.41 ^{NS}	54.38 ^{NS}
N	3	27001.91***	1016.73***	105952.72***	41.30***	136.94***	7.13***	607.88***
PS	4	10620.77***	501.60**	71945.73***	4.32 ^{NS}	1471.63***	250.84***	8692.60***
NXPS	12	1124.84 ^{NS}	25.08 ^{NS}	10304.98 ^{NS}	3.03 ^{NS}	11.68*	0.61*	112.81***
Error		1108.02	30.93	6065.93	1.94	5.50	0.27	29.65
CV		11.06	4.69	10.76	10.17	10.13	5.64	9.80

*Significant at P≤ 0.05; ** significant P≤ 0.01; *** significant at P≤ 0.001; NS = non significance at P≥0.05; N x PS, Nitrogen x plant spacing; DF = degree of freedom; SV = Source of variance; PFW, Pod fresh weight per plant; PDW, Pod dry weight per plant; ABMY, Above ground biomass yield per plot; GPY, Green pod yield per plot; PFWH, Pod fresh weight per hectare; PDWH, Pod dry weight per hectare; ABMYH, Above ground biomass yield per hectare.

Table 3. Main effect of nitrogen rate on crop number of days to 50% flowering and number of days to 50% pod set of okra.

Plant spacing (cm)	Days to flowering	Days to pod set
N kg/ha		
0	46.33^{c}	49.93^{c}
23	49.33^{b}	52.67^{b}
46	50.40^{a}	53.73^{a}
69	51.40^{a}	54.33^{a}
SE±	0.29	0.26
CV (%)	2.78	2.45
LSD (5%)	1.01	0.95

Means followed by or sharing the same letters within a column are not significantly different at 5% level of significance; CV = Coefficient of variation; LSD = Least significant difference at 5%; SE = Standard error of the means.

fertilizer rate showed significant differences on length of green pods(cm), pod diameter(cm), pod fresh and dry weight yield(t/ha) and above ground biomass yield(t/ha). The main effect of nitrogen rate significantly affected green pod yield of okra per plot (kg) (P<0.001) (Tables 1 and 2).

Highest number of green pods per plant was recorded from plant population (45 cm × 30 cm and 60 cm × 30 cm) which was not significantly different from numbers of green pods produced in response to the spacing of 60 cm × 40 cm. Plant population spaced at 45 × 30 and 60 × 30 cm significantly increased number of pods by 19.78% and 19.71%, respectively as compared to the highest plant population spaced at 45 cm × 20 cm (Table 4). This result is supported by the finding of Amjad et al. (2001) who reported that the lowest planting density (37,000 plants ha^{-1}) resulted in maximum number of matured pods per plant in okra. Similarly, Ekwu and Nwokwu (2012) reported that the number of fruit of okra significantly increased with decrease in population density. The maximization of green pods in response to lowering plant population could be due to the fact that plants grown under low population density have good growth performance since competition for available resources are limited as compared to plants grown under high plant population density.

Maximum length of green pods was recorded from the interaction of low plant population (60 cm × 40 cm) and nitrogen application rate of 46 kg N ha^{-1} (Table 6). However, minimum length of green pods was recorded from high plant population spaced at 45 × 20 cm and from the control plots. Increased in length of green pods in relation to the interaction of the two factors could be due to the fact that low plant population responded well to the applied nitrogen rate which favours metabolic changes in plant resulting in the production of lengthy pods. It is obvious that wider spacing results in better growth performances of plant as a result of low competition for resources which can significantly result in increased pod length per plant.

Pod diameter was higher from the low plant population spaced at 60 × 30 cm and from the nitrogen application rate of 46 kg N ha^{-1} (Table 7). However, the minimum pod diameter was recorded from the control plots and from the high plant population spaced at 45 cm × 20 cm and 60 cm × 20 cm, respectively.

Table 4. Main effects of plant population and nitrogen rate on some growth parameters and number of pods per plant in okra.

Plant spacing (cm)	Plant height(cm)	LPBZ*	NLP**	NPP***
45×20	60.05	49.65	14.03^{b}	11.07^{b}
45×30	58.52	49.83	15.04^{ab}	14.46^{a}
60×20	58.27	48.48	15.15^{ab}	12.23^{b}
60×30	58.06	48.56	15.65^{a}	14.45^{a}
60×40	58.48	48.24	15.95^{a}	14.3^{a}
LSD (at 5%)	NS	NS	1.19	0.66
Nitrogen (kg/ha)				
0	55.84^{b}	45.70^{b}	13.62^{b}	12.42^{c}
23	63.41^{a}	54.03^{a}	15.62^{a}	13.44^{b}
46	58.33^{b}	49.23^{ab}	15.91^{a}	14.24^{a}
69	57.13^{b}	46.84^{b}	15.50^{a}	13.91^{ab}
SE±	1.49	1.38	0.24	0.20
CV (%)	8.76	13.67	9.53	5.98
LSD (5%)	3.88	4.94	1.19	0.59

*Length of pod bearing zone; **Number of leaves per plant; *** Number of pods per plant.

Table 5. Interaction effect of plant population and N-rates on number of branches of okra.

Nitrogen (kg/ha)	Plant spacing (cm)				
	45 × 20	45 × 30	60 × 20	60 × 30	60 × 40
0	1.97^{gh}	2.33^{c-h}	1.90^{h}	2.27^{c-h}	2.03^{f-h}
23	2.00^{f-h}	2.33^{c-h}	2.00^{f-h}	2.33^{c-h}	2.33^{c-h}
46	2.13^{e-h}	2.93^{a}	2.17^{d-h}	2.93^{a}	2.70^{ab}
69	2.37^{c-f}	2.47^{b-e}	2.30^{d-g}	2.60^{a-c}	2.53^{b-d}
CV (%)			9.75		
LSD (5%)			0.37		
SE±			0.05		

Means followed by or sharing the same letters are not significantly different at 5% level of significance; CV = Coefficient of variation; LSD = Least significant difference at 5%; SE = Standard error of the means.

Table 6. Interaction effect of plant population and nitrogen rate on length of green pods of okra.

Nitrogen kg/ha	Plant spacing (cm)				
	45 × 20	45 × 30	60 × 20	60 × 30	60 × 40
0	20^{j}	20.61^{ij}	20.32^{j}	21.50^{i}	21.47^{i}
23	23.47^{h}	24.44^{fg}	23.58^{gh}	24.93^{ef}	26.28^{d}
46	25.50^{de}	25.84^{de}	25.83^{de}	28.40^{ab}	29.01^{a}
69	26.33^{d}	27.46^{c}	26.07^{d}	28.00^{bc}	28.69^{ab}
SE±	0.37				
CV (%)	2.26				
LSD (5%)	0.92				

Means followed by or sharing the same letters are not significantly different at 5% level of significance; CV = Coefficient of variation; LSD = Least significant difference at 5%; SE = Standard error of the means.

The maximum pod fresh weight was recorded from low plant population from 60 cm × 30 cm plant spacing and was not significantly different from the pod fresh weight yield which was obtained from spacing of 60 cm × 40 cm (Table 8). However, minimum pod fresh weight was recorded from higher plant population of 45 cm × 20 cm

Table 7. Interaction effect of plant population and nitrogen rate on pod diameter of okra.

Nitrogen (kg)	Plant spacing (cm)				
	45 × 20	45 × 30	60 × 20	60 × 30	60 × 40
0	2.44^{f}	2.67^{e}	2.47^{f}	2.84^{d}	2.93^{b-d}
23	2.88^{cd}	2.97^{b-d}	2.65^{e}	2.97^{b-d}	3.00^{b-d}
46	2.91^{b-d}	3.07^{bc}	2.92^{b-d}	3.27^{a}	3.07^{b}
69	2.88^{cd}	2.91^{cd}	2.88^{cd}	2.93^{b-d}	2.93^{b-d}
CV (%)	3.25				
LSD (5%)	0.15				
SE±	0.02				

Means followed by or sharing the same letters are not significantly different at 5% level of significance; CV = Coefficient of variation; LSD = Least significant difference at 5%; SE = Standard error of the means.

Table 8. Main effects of plant population and nitrogen rate on yield attributes of okra.

Plant spacing (cm)	PFWP (g)	PDWP(g)	ABMP (g)	GPY (kg)
45x20	271.92^{c}	113.83^{c}	635.25^{b}	14^{ba}
45x30	299.67^{b}	118.93^{b}	733.17^{a}	14.62^{a}
60x20	271.17^{c}	110.40^{c}	652.33^{b}	13.58^{ab}
60x30	335.50^{a}	124.60^{a}	793.50^{a}	13.25^{b}
60x40	325.75^{ab}	125.01^{a}	801.83^{a}	13.17^{b}
SE±	7.20	1.38	16.76	0.28
LSD (5%)	27.51	4.59	64.36	1.15
N kg ha^{-1})				
0	261.73^{b}	108.90^{c}	637.07^{c}	11.37^{c}
23	266.47^{b}	114.52^{b}	688.67^{bc}	13.77^{b}
46	338.40^{a}	125.61^{a}	835^{a}	14.97^{a}
69	336.60^{a}	125.20^{a}	732.13^{b}	14.80^{ab}
CV (%)	11.06	4.69	10.76	10.17
LSD (5%)	24.60	4.11	57.57	1.03

Means followed by or sharing the same letters within a column are not significantly different at 5% level of significant; CV= Coefficient of variation; LSD = Least significant difference at5%; SE = Standard error of the means; PFWP = Pod fresh weight per plant; PDWP = pod dry weight per plant; ABMP = Above ground biomass per plant; GPY= Green pod yield per plot.

and 60 cm × 20 cm plant spacing, respectively. The result is in line with findings of Ali (1999) who reported that wider spacing leads to heavier individual pod weight in okra. Similarly, Amjad et al. (2001) reported that the weight of pods was highest at the closest plant spacing (50 cm × 25 cm). The maximum pod fresh weight per plant at low plant population might have been resulted from efficient utilization of growth nutrients favouring optimum vegetative growth of okra resulting in increased pod fresh weight as a result of limited or very less competition for resources.

Maximum green pod yield per plant was recorded from the application of nitrogen fertilizer rate of 46 and 69 kg N ha^{-1} while, minimum green pod yield was recorded from the control plots (Table 8). This result is in agreement with the finding of Yih-Chi Tan et al. (2009) who reported that nitrogen had highly significant effect on the yield of okra. Likewise, Akanbi et al. (2010) reported that application of N led to significant influence on fresh fruit yield of okra. The author stated that fruit yield increased with increases in N level reaching peak with the highest N level.

The main effect of nitrogen fertilizer application showed significance increase on pod fresh weight per plant (Table 8). The highest pod fresh weight per plant was recorded from the application of nitrogen fertilizer rate of 46 and 69 kg N ha^{-1} respectively. However, minimum pod fresh weight was recorded from the control plots and low N rate (23 kg ha^{-1}). The result is in line with the finding of Firoz (2009) who reported that increasing the rates of N from 0 to 40 or 40 to 80 kg N ha^{-1}, significantly increased total fresh pod yield and the mean fresh pod yield of okra. Pod fresh weight yield showed significant differences by the interaction effects of plant population and nitrogen rates (Table 9). The maximum fresh pod weight yield was recorded from the application of nitrogen fertilizer rate of

Table 9. Interaction effect of plant population and nitrogen rate on pod fresh weight of okra (t/ha).

Nitrogen (kg)	Spacing (cm)				
	45 × 20	45 × 30	60 × 20	60 × 30	60 × 40
0	25.54^{fg}	34.52^{c}	18.17^{hi}	14.82^{i-k}	8.45^{l}
23	26.14^{ef}	35.56^{bc}	18.59^{hi}	15.03^{ij}	8.47^{l}
46	29.65^{de}	46.14^{a}	22^{gh}	20.20^{h}	11.53^{j-l}
69	33.31^{cd}	39.14^{b}	24.67^{fg}	19.82^{h}	11.02^{kl}
CV (%)	10.17				
LSD (5%)	3.88				
SE±	1.37				

Means followed by or sharing the same letters are not significantly different at 5% level of significance; CV = Coefficient of variation; LSD = Least significant difference at 5%; SE = Standard error of the means.

Table 10. Interaction effects of plant population and nitrogen rate on pod dry weight of okra (t ha^{-1}).]

Nitrogen (kg)	Plant spacing (cm)				
	45 × 20	45 × 30	60 × 20	60 × 30	60 × 40
0	10.99^{e}	14.00^{b}	7.59^{gh}	6.04^{i}	3.55^{j}
23	11.63^{de}	14.69^{b}	8.00^{g}	6.40^{i}	3.69^{j}
46	12.47^{cd}	16.31^{a}	9.06^{f}	6.90^{hi}	4.04^{j}
69	12.89^{c}	16.65^{a}	9.30^{f}	6.59^{i}	3.86^{j}
CV (%)	5.72				
LSD (5%)	0.87				
SE±	0.54				

Means followed by or sharing the same letters are not significantly different at 5% level of significance; CV = coefficient of variation; LSD = least significant difference at 5% and SE = standard error of the means.

46 kg N ha^{-1} and from plant population spaced at 45 cm × 30 cm plant spacing respectively. Increase in pod fresh yield at this spacing level and N-rate could have been resulted from efficient utilization of resources leading to optimum morphological growth characters which favours pod fresh yield increase of okra (Onyegbule et al., 2012)..

Table 10 showed that the maximum pod dry weight yield was recorded from the application of nitrogen rate of 69 and 46 kg ha^{-1} and plant spacing (45 cm × 30 cm). However, the minimum dry pod yield was recorded from plant spacing (60 cm × 40 cm) and from all the applied nitrogen fertilizer rates This result is in consistent with the finding of Frezgi (2007) who reported that haulm dry matter yield significantly increased with increased nitrogen and high planting density; the highest yield of haulm dry matter was recorded for 75 cm inter row spacing, 20 cm intra row spacing and 150 kg N ha^{-1}treatment combination.

Significantly maximum above ground biomass yield per plant was recorded from low plant population spaced at 60 cm × 40 cm (Table 8). The maximum above ground biomass yield per plant was recorded from the application of nitrogen fertilizer of 46 kg N ha^{-1}. Application of nitrogen rate of 46 kg N ha^{-1} significantly increased yield by 31.06% over the control plots. Increase in nitrogen fertilizer rate beyond 46 kg N ha^{-1} resulted in yield reduction by about 23.70%.

The interaction effects of plant population and nitrogen fertilizer rate resulted in significant yield increase of the above ground biomass yield. Maximum above ground biomass yield was recorded from plant spacing (45 cm × 30 cm) and from the application of nitrogen at rate of 46 kg ha^{-1}. However, the minimum above ground biomass yield was recorded from low plant population (Table 11). The above ground biomass yield significantly increased relatively at narrow spacing because of favourable conditions for morphological growth characters which resulted from less competition between plants, that is, number of pods, number of leaves, number of branches, pod diameter and pod length which contributes for above ground biomass yield increase. Nitrogen at 46 kg ha^{-1} gave the highest yield due to efficient utilization of the resources and beyond that biomass yield showed a reduction trend.

Conclusion

This investigation conducted on growth and yield components of okra indicated that plant population and

Table 11. Interaction effects of plant population and nitrogen rate on above ground biomass yield of okra (t ha^{-1}).

Nitrogen (kg)	Spacing (cm)				
	45 × 20	45 × 30	60 × 20	60 × 30	60 × 40
0	63.84^{cd}	83.91^{b}	46.89^{efg}	32.93^{hi}	20.91^{j}
23	66.02^{c}	90.52^{b}	48.87^{ef}	39.21^{gh}	22.13^{j}
46	70.90^{c}	119.34^{a}	55.59^{de}	49.63^{ef}	27.45^{ij}
69	67.07^{c}	86.37^{b}	49.35^{ef}	43.52^{fg}	26.68^{ij}
CV (%)	10.01				
LSD (5%)	9.17				
SE±	3.32				

Means followed by or the same letters are not significantly different at 5% level of significance; CV = Coefficient of variation; LSD = Least significant difference at 5%; SE = Standard error of the means.

nitrogen rate played a significant role in increasing the yield of the crop. Growing okra at the spacing 45 cm between rows and 30 cm between plants with plant population of 74,047 plants ha^{-1} and at 46 kg N ha^{-1} resulted in the optimum growth and highest fruit yield of okra in Gambella Region.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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On-farm evaluation of integrated weed management in no-till rainfed crops in semi-arid Morocco

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Field studies were conducted from 2012-13 to 2014-15 to investigate the dynamics of germinable soil seedbank, density and community composition of weeds in crop rotations of barley (*Hordeum vulgare* L.) + pea (*Pisum sativum* L.) and bread wheat (*Triticum aestivum* L.) in Oued Zem, semi-arid Morocco. In September 2012, the initial seedbank in 6 fields was 2354 seeds m^{-2}. When herbicide-free barley + pea forage mixture (cut for hay) was followed by bread wheat, seedbank reductions were 35% after the two years. When bread wheat was followed by herbicide-free barley + pea forage mixture, seedbank reductions were only 5% in two years. Prior to wheat harvest, weed densities were 82, 8, and 14 plants m^{-2} in April 2013, 2014 and 2015, respectively. Prior to haying herbicide-free barley + pea, weed densities were 76, 109 and 34 plants m^{-2} in April 2013, 2014 and 2015, respectively. Weeds identified in bread wheat fields were 49, 36 and 40 species in 2012-13, 2013-14 and 2014-15, respectively. Weeds associated with herbicide-free barley + pea mixture were 68, 51 and 36 species in 2012-13, 2013-14 and 2014-15, respectively. The seedbank prior to planting and weed density prior to harvesting were strongly influenced by the most recent crop. Integrated weed management combining glyphosate before no-till planting, post-emergence herbicide use in bread wheat, haying barley + pea mixture, within the crop rotation (barley + pea/bread wheat) reduced weed seedbank by up to 35%, species richness by up to 47%, and weed density prior to wheat harvest or forage haying by up to 83%. Such changes suggest that integrated weed management practices in no-till system must be continued for more than 3 growing seasons to drastically reduce weed seedbanks and weed densities.

Key words: Weed, seedbank, wheat, barley + pea, no-till, Morocco.

INTRODUCTION

A no-till system has economic, ecological, environmental and social benefits. These include soil conservation, water use efficiency, nutrient cycling, time and fossil fuel saving, and less wear and tear on machinery (Kassam,

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2014). Such a system offers Moroccan farmers, who depend primarily on rainfed cereal-livestock production systems, the opportunity to reduce soil degradation and production costs (Boughlala et al., 2011). The estimated area under no-tillage in 2015-16 was only 7000 ha despite the research on tillage reduction in semi-arid Morocco that has been conducted since 1980 (Mrabet, 1993, 2000, 2008; El Gharras and Idrissi, 2006; El Gharras et al., 2010; Mrabet et al., 2012; Abail et al., 2013; Belmekki et al., 2014; Schwilch et al., 2015).

In Morocco, no-till practices are proposed as a substitute to conventional cropping systems based on tillage using discs and tine tools. However, in crop/livestock integrated production systems, weed weeds are an important challenge since most weeds are considered as free forage for livestock and the weedy fallow is widely practiced (El Brahli and Mrabet, 2000). Abandoning the plow induces a qualitative and quantitative change in the flora which is why no-tillage crop production relies primarily on herbicides, particularly glyphosate, for weed management (Aibar, 2006; Blackshaw et al., 2015; Loss et al., 2015).

Weed seedbank abundance and composition can be used as indicators of the success or failure of cropping systems and management practices (Buhler et al., 2001; Cardina et al., 2002; Rahali et al., 2010; Kleemann et al., 2016). Higher weed seedbank diversity has been reported in more diverse crop rotations (Sosnoskie et al., 2006). Murphy et al. (2006) observed that tillage and crop rotations impact weed seedbank diversity and density, with the highest diversity found in no-tillage systems with a three-year crop rotation and diversity decreasing as tillage intensity increases.

Integrated weed management systems have the potential to provide long-term management of weeds (Blackshaw et al., 2005; Holm et al., 2006; Harker and O'Donovan, 2013; Blackshaw et al., 2015). Agronomic factors such as crop diversification and rotations, combined with herbicides, need to be evaluated for their potential to manage weeds in no-till system in semi-arid Morocco. Therefore, field studies were initiated to determine the combined effects of pre-plant glyphosate, herbicide use in bread wheat, and herbicide-free barley + pea mixture for hay on the weed seedbank, and weed density within a bread wheat/barley + pea mixture rotation under a no-till production system in a semi-arid rain-fed environment.

MATERIALS AND METHODS

Study location and agronomic practices

A field study was conducted in Oued Zem (semi-arid region of central Moroccco). Soils were classified as sandy-loam. Annual precipitation varied from 290 mm in 2013-14 to 400 and 480 mm in 2012-13 and 2014-15, with more than 60% occurring from November to April (Table 1). Minimum temperatures were 5-10°C in winter (December to February) and maximum temperatures 25-35°C in summer (June to August).

Table 1. Monthly precipitation at Oued Zem, Morocco, from 2012-13 to 2014-15.

Month	2012-13	2013-14	2014-15
	mm		
September	32.0	32.4	7.0
October	130.5	0.2	4.2
November	91.4	32.6	195.4
December	11.6	20.0	64.4
January	43.5	81.0	52.0
February	10.6	19.6	14.5
March	107.8	50.8	61.8
April	41.9	44.0	0
May	0	12.0	7.2
June	10.6	0	6.8
July	0	0	0
August	0	0	0
Total	479.9	290.6	406.5

Six farms were selected in September 2012 and 4 ha on each farm were used to conduct no-till trials. 2 ha were no-till planted to bread wheat (*Triticum aestivum* L.) and two ha were no-till planted to a forage mixture of barley (*Hordeum vulgare* L.) + field pea (*Pisum sativum* L.). The wheat/forage mixture rotation was managed using conservation management practices. Glyphosate at 720 g ha^{-1} was applied prior to no-till planting in November 2012 and 2014. Before 2012-13, all fields had been planted with cereals (wheat and barley) using a conventional tillage system in rotation with the traditional grazed weedy fallow for several years, and no herbicide applications was practiced. During the rainy seasons, the weedy fallow was cut, faded and hayed by the end of March or mid April as forage.

Bread wheat (cv. "Arrehane") and local barley and pea varieties were planted in November 2012, 2013 and 2014. At each farm, two hectares of land were planted with bread wheat and two hectares for barley + field pea, each growing season. The wheat crop was fertilized at 100 kg ha^{-1} of diammonium phosphate (18-46-0) and no-till planted at 120 kg ha^{-1} of seed. The forage mixture was fertilized the same way as the wheat and no-till planted at 25 + 75 kg ha^{-1} of barley + pea seed, respectively. Nitrogen fertilizer was applied at the rate of 33 kg ha^{-1} to wheat at tillering. The experiment was done using a tine seed drill of 18 cm row spacing with little chains behind to improve seed covering and no press wheels.

The broadleaf herbicide mixture of florasulam + 2,4-D (3.75 + 180 g ha^{-1}) was used at tillering for post-emergent weed control in the wheat. A foliar fungicide (epoxyconazole at 125 g ha^{-1}) was sprayed on wheat at the booting stage. The herbicide and fungicide were sprayed with a tractor-mounted sprayer, and volumes of spray were 120 L ha^{-1} for herbicides and 200 L ha^{-1} for the fungicide. In November 2012 and 2014, glyphosate (720 g ha^{-1}) was applied in all fields prior to no-till planting wheat and barley + pea mixture. In November 2013, no vegetation was present at planting and there was no need to use glyphosate prior to planting. In all cropping seasons, no post-emergent herbicide was used in the barley + pea forage treatments.

Weed seedbank assessment

Soil seedbank was determined in September 2012, 2013, and

2014. Soil sampling was done before the first autumn rain. Each year, ten soil cores (7.2 cm diameter by 10 cm deep) were taken every 10 m along diagonal transects of each field of 2 ha, bulked, air dried, placed in polyethylene bags, and stored in the greenhouse at room temperature (25/10°C) until November. Sampling in the fall has been shown to provide reliable estimates of the viable weed seedbank because it allows natural dormancy-breaking mechanisms to operate over the summer (Baskin and Baskin, 2014). Germinable weed seedbank determinations were conducted using the greenhouse emergence method (Smith and Gross, 2006; Gulden et al., 2011; Leon et al., 2015; Kleemann et al., 2016). Soil samples from each field were used to determine weed seedbank by placing soil samples on plastic trays that had been partially filled with potting mix. They were placed in trays in a 2-cm-thick layer, placed in a greenhouse with a day/night temperature of 25/10°C, and watered as necessary to keep moist. Weed emergence counts were made twice a month from November to April. Seedlings that emerged were identified and removed. Those that could not be identified were counted, removed, and indicated as "others". Total seedling counts in each soil sample, from November to April, were considered the germinable fraction of the weed seedbank, and this was converted to seeds per square meter for each field.

Weed density assessment

Weed densities in bread wheat strips were estimated in 10 randomly chosen (0.5 m x 0.5 m = 0.25 m^2) quadrats at each site 3 times during the year:

1. In early November prior to application of glyphosate (720 g ha^{-1}) at planting time,
2. In mid-February prior to application of florasulam + 2,4-D and
3. In mid-April prior to wheat harvest in May.

In barley + pea mixture fields, weed densities were estimated in 10 randomly chosen (0.5 x 0.5 m = 0.25 m^2) quadrats at each site at the same times as reported above.

Weeds were identified using various floras. Scientific names used in this paper are those recommended for the North Africa plants by Dobignard and Chatelain (2010-13).

Data analysis

Since this study was conducted in six farmers' fields in the same region, each field was considered as one replicate. Mean seedbank density values per field were calculated as an average of the six sampled fields. After crop emergence, mean weed densities per field and the standard error of the mean were calculated as an average of the six sampled fields (John and Quenouille, 1977).

RESULTS AND DISCUSSION

Weed seedbank dynamics

In September 2012, the average germinable soil weed seedbank from six farmers' fields was 2354 seeds m^{-2} in the surface layer of 0 to 10 cm (Table 2). Such high seed densities may be due to the lack of weed management in the traditional cereals/weedy fallow rotation. In fact, most farmers were not familiar with herbicide use. Traditionally, weeds in cereal fields are hand pulled for animal feed. In September 2013, the crop rotation that started with bread wheat which was planted in November 2012 and harvested in May 2013 had 1720 seeds m^{-2}, while crop rotation starting with the forage mixture barley + pea that was planted in November 2012 and hayed in April 2013 had 2013 seeds/m^2 (Table 2). In comparison with the initial seedbank which was estimated in September 2012 to be 2354 seeds m^{-2}, the reductions in the seedbank in the first growing season (2012-13) were 27% after bread wheat, and 14% after barley + pea, respectively.

In September 2014, the weed seedbank in bread wheat planted in November 2013 and harvested in May 2014 after the barley + pea mixture (2012-13) was 1520 seeds m^{-2}, while the forage mixture barley + pea, drilled in November 2013 and hayed in April 2014 after bread wheat (2012-13) was 2238 seeds m^{-2}. As compared to the initial seedbank (2354 seeds m^{-2}) estimated in September 2012, seedbank reductions in two years (from September 2012 to September 2014) were 35% in the rotation of barley + pea (2012-13) with bread wheat (2013-14) and only 5% in the rotation bread wheat (2012-13)/hayed barley + pea (2013-14). Higher seedbank reductions in crop sequences were essentially due to glyphosate pre-plant and florasulam + 2,4-D (3.75 + 180 g ha^{-1}) post-emergence in bread wheat. In a 3-year crop rotation study, Kleemann et al. (2016) found that the use of oaten hay in year 1, followed by effective weed control in field pea and wheat crops, depleted the high initial seedbank (4820 seeds m^{-2}) of rigid ryegrass (*Lolium rigidum*) to moderate levels ($\leq$200 seeds m^{-2}) within 3 years. Smith and Gross (2006) found that seedbanks were strongly influenced by the most recent crop.

More than 30 weed species were present in the soil samples (Table 2). Eleven of these species accounted for 45 to 80% of the seedbank. From the total seedbank, 8 to 43% were from seeds of the endemic annual broadleaf, *Diplotaxis assurgens*. In two years, seed declined by 76% in the bread wheat/barley + pea rotation and 84% in the barley + pea/bread wheat rotation. In a 4-year cropping rotation of oaten hay/field pea/wheat/barley, rigid ryegrass (*Lolium rigidum*) seedbank declined by 86% after oaten hay in year 1 (Kleemann et al., 2016). In a seed persistence study, Lutman et al. (2002) found that seed loss rates of 16 weed species over 6 years varied from 8 to 58%.

Weed density

Before applying glyphosate and planting wheat, weed densities were 442, 68 and 261 plants m^{-2} in November 2012, 2013 and 2014, respectively (Table 3). The weed density observed in the fields in November 2013 (68 seedlings m^{-2}) was due to low rainfall (32.6 mm in the whole month, Table 1) and low weed emergence, therefore no glyphosate was applied at planting. Glyphosate was sprayed in November 2012 and 2014, before or at planting at 720 g ha^{-1}, with excellent control of volunteer crops and annual weeds. Perennial weeds

Table 2. Germinable weed seedbank in 6 sites in September 2012, 2013 and 2014, prior to no-till planting of bread wheat or barley + pea in Oued Zem, Morocco.

Weed species	Barley/weedy fallow rotation	Rotation 1 Wheat (2012-13) followed by barley + pea (2013-14)		Rotation 2 Barley + pea (2012-13) followed by wheat (2013-14)	
	September 2012	September 2013	September 2014	September 2013	September 2014
	Soil seedbank in September 2012 after conventional barley harvest in May 2012	Soil seedbank in September 2013 after no till bread wheat harvest in May 2013	Soil seedbank in September 2014 after barley + pea harvest in April 2014	Soil seedbank in September 2013 after no till barley + pea harvest in April 2013	Soil seedbank in September 2014 after no till bread wheat in May 2014
	Germinable seeds m^{-2} (mean of 6 sites)				
Diplotaxis assurgens (Delile) Thell.	1008	198	242	156	165
Papaver rhoeas L.	154	393	308	443	295
Plantago afra L.	100	213	71	182	85
Lysimachia arvensis (L.) U.M & A.	192	79	71	61	110
Calendula stellata Cav.	54	30	38	200	10
Medicago polymorpha L.	154	129	79	201	55
Misopates orontium (L.) Raf.	42	4	88	45	35
Sonchus oleraceus L.	33	38	17	32	20
Glebionis coronaria (L.) Spach	13	24	8	14	0
Scorpiurus muricatus L.	108	29	54	0	65
Lolium rigidum Gaud.	17	15	29	0	20
Others	479	568	1233	679	660
Total	2354	1720	2238	2013	1520
Standard error	1862	872	1850	773	800

such as *Arisarum simorrhinum, Bunium fontanesii, Launaea nudicaulis, Mandragora officinarum,* and *Silene vulgaris* were partially controlled.

Florasulam + 2,4-D was used at the tillering stage of bread wheat, providing excellent control of annual broadleaf weeds in year 2 (2013-14) and year 3 (2014-15). Weed densities in bread wheat, after weed control, showed 82, 8, and 14 weeds m^{-2} in April 2013, 2014, and 2015, respectively (Table 3). In two years, reductions in weed density were therefore 83% as compared to the initial situation (2012-13). Such low weed densities found in April, prior to wheat harvest in May, produced a small quantity of seed that shattered onto the soil and contributed little to germinable seedbank. In fact, herbicide use was found to be the main factor reducing arable seedbanks, because it limits both weed growth and weed seed production (Chauhan et al., 2006; Jose Maria and Sans, 2011; Kleemann et al., 2016).

Before applying glyphosate and planting the herbicide-free forage crops, weed densities were 432, 43, and 226 plants m^{-2} in November 2012, 2013 and 2014, respectively (Table 4). As mentioned for bread wheat, the low density of weed seedlings observed in the fields in

Table 3. Weed density in 6 no-till bread wheat fields in Oued Zem, Morocco, from 2012-13 to 2014-15.

Weed species	2012-13			2013-14*			2014-15		
	3 November 2012	10 February 2013	12 April 2013	10 November 2013	17 February 2014	17 April 2014	16 November 2014	9 February 2015	10 April 2015
	Weed density, mean of 6 sites (plants m^{-2})								
Diplotaxis assurgens (Delile) Thell.	236	13	6	42	7	1	13	1	0
Medicago polymorpha L.	17	3	4	0	6	0	58	17	0
Scorpiurus muricatus L.	7	1	1	0	2	0	123	1	1
Bunium fontanesii (Pers.) Maire	0	37	14	0	11	2	0	7	2
Silene vulgaris (Moench) Garcke	7	3	0	4	0	0	26	0	0
Papaver rhoeas L.	0	18	12	0	1	0	0	1	1
Lolium rigidum Gaud.	0	2	0	0	1	1	0	0	1
Sonchus oleraceus L.	10	1	1	2	1	0	0	1	0
Plantago afra L.	22	5	2	3	1	1	0	0	0
Calendula stellata Cav.	15	7	1	0	8	0	0	0	0
Glebionis coronaria (L.) Spach	10	2	1	0	0	1	0	0	0
Rhagadiolus stellatus (L.) Gaertn.	0	6	2	0	2	0	0	0	0
Vicia benghalensis L.	1	0	0	1	1	0	0	0	0
Centaurea maroccana Ball	3	0	0	1	0	0	0	0	0
Astragalus hamosus L.	1	0	0	0	1	0	0	0	0
Lysimachia arvensis (L.) U.M & A.	9	12	3	0	0	0	0	0	0
Others	104	29	35	15	5	2	41	5	9
Total	442	139	82	68	47	8	261	33	14
Standard error	24	4	2	3	2	1	10	1	1

*No glyphosate applied before planting in November 2013.

November 2013 was due to low rainfall (32.6 mm in the whole month, Table 1) and low weed emergence; thus, no glyphosate was sprayed. Glyphosate, used in November 2012 and 2014 before or at planting at 720 g ha^{-1}, provided excellent control of volunteer crops and annual weeds.

In the fields of hay forage, weed densities in herbicide-free barley + pea were 76, 109, and 34 plants m^{-2} in April 2013, 2014 and 2015, respectively (Table 4). The high density observed in April 2014 (109 plants m^{-2}) was due to glyphosate not used pre-planting. After three growing seasons, weed management and crop rotation (barley + pea/bread wheat/barley + pea) reduced weed density by 55%, as measured in April 2015, as compared to April 2013. Most of the weeds at haying had some mature seed. Therefore, a proportion of mature seed shattered on the soil onto the soil, which enabled weeds to re-infest bread wheat the next year. Hill et al. (2016) found that viable seed production was reduced by 64 to 100% when weeds were mowed with immature seeds present as compared to when plants were mowed with mature seeds present.

Weed species richness

In this 3-year study, a total of 68 weed species in wheat fields: 49 in 2012-13, 36 in 2013-14, and 40 species in 2014-15 were identified (Table 5). That is, weed management reduced weed species richness by 27% in one year and 18% in 2 years. The five major weed species were *Diplotaxis assurgens, Medicago polymorpha, Scorpiurus muricatus, Bunium fontanesii* and *Calendula stellata*.

The total number of weed species associated with barley + pea mixture during 3 growing seasons was 79 species: 68, 51 and 36 species in 2012-13, 2013-14 and 2014-15, respectively (Table 5). Species richness was reduced by 25% the first year and 47%, the second years. The 5 major weed species were similar to those found in bread wheat fields.

Conclusion

On-farm measurements from 2012-13 to 2014-15 showed

Table 4. Weed density in 6 no-till barley + pea fields in Oued Zem, Morocco, from 2012-13 to 2014-15.

Weed species	2012-13			2013-14*			2014-15		
	3 November 2012	10 February 2013	12 April 2013	10 November 2013	17 February 2014	17 April 2014	16 November 2014	9 February 2015	10 April 2015
	Weed density, mean of 6 sites (plants m^{-2})								
Diplotaxis assurgens (Delile) Thell.	90	15	5	21	14	10	8	1	0
Medicago polymorpha L.	78	5	5	0	26	19	48	2	10
Scorpiurus muricatus L.	38	1	0	0	2	1	60	3	0
Bunium fontanesii (Pers.) Maire	0	18	6	0	11	2	0	8	5
Silene vulgaris (Moench) Garcke	4	5	1	5	1	2	41	0	0
Papaver rhoeas L.	2	10	5	0	1	1	0	1	5
Lolium rigidum Gaud.	9	1	0	0	7	9	4	1	1
Sonchus oleraceus L.	4	1	2	2	0	1	0	1	0
Plantago afra L.	22	4	1	6	3	2	0	1	0
Calendula stellata Cav.	7	3	0	0	10	9	9	0	0
Glebionis coronaria (L.) Spach	6	2	2	1	1	17	0	0	0
Rhagadiolus stellatus (L.) Gaertn.	0	4	4	0	1	5	0	0	0
Vicia benghalensis L.	2	6	3	0	1	1	0	0	0
Centaurea maroccana Ball	6	1	1	3	1	1	0	0	0
Astragalus hamosus L.	3	0	0	1	1	0	0	0	0
Lysimachia arvensis (L.) U.M & A.	18	10	1	0	0	0	0	1	0
Others	136	50	40	3	9	28	16	4	13
Total	432	136	76	43	89	109	226	23	34
Standard error	10	2	1	2	2	2	7	1	1

*No glyphosate applied before planting in November 2013.

Table 5. Weed richness in 6 no-till wheat and 6 no-till barley + pea fields in Oued Zem, Morocco, from 2012-13 to 2014-15.

	2012-13	2013-14	2014-15	Total number of weed species
Number of weed species in 6 wheat fields	49	36	40	68
Number of weed species in 6 barley + pea fields	68	51	36	79

that integrated weed management combining glyphosate before no-till planting, post-emergent herbicide use in bread wheat, haying barley + pea mixture and crop rotation (barley + pea/bread wheat) reduced the weed seedbank by up to 35%, species richness by up to 47%, and weed density prior to harvest by up to 83%. Such changes suggest that integrated weed management practices in no-till system must be continued for more than three growing seasons to greatly reduce weed seedbanks and weed densities. In order to achieve good weed management as well as an integration crop/livestock production system, grazed weedy fallows should be replaced by forage crops for hay in the form of barley + pea forage mixtures. It is concluded that weed seedbanks and weed densities can be managed effectively when a multi-year weed management program is appropriately implemented into a no-till system.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Full Length Research Paper

Evaluation of root-knot nematode resistance in sweetpotato

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Five healthy, vigorous cultivars of sweet potato [*Ipomoea batatas* (L.) Lam] were cultivated under root-knot nematode *Meloidogyne incognita* challenge to distinguish levels of resistance to infection. Roots and soil samples were collected 84 days' post-infection and evaluated for specific host responses to nematode infection by visual screening analysis and quantitative assessments of symptoms of infection. Resistant control cultivar Nugget showed the highest degree of resistance manifested in lower necrosis and galling, high fresh root weights and low nematode and egg counts. Necrosis and galling scores were highest in susceptible cultivars Georgia Jet and DMO1 where extensive root cracking was observed in Georgia Jet, and storage root development was severely restricted in DMO1 which also produced the highest egg counts. TUO2 and Whatley Loretan were considered intermediately resistant based on egg counts and necrosis and galling. Our results suggest that genotypic differences between cultivars were apparent in multiple host responses to root-knot nematode infection. We have also provided initial evidence to support the identification of newly developed sweet potato cultivars with intermediate resistance to root-knot nematodes.

Key words: Plant-parasitic nematodes, host plant resistance, *Ipomoea batatas*, *Meloidogyne incognita*

INTRODUCTION

Plant-parasitic nematodes cause devastating effects on important crops throughout the world. Root-knot nematodes (RKNs) are a group of well-documented parasites of a wide variety of crops and are considered economically important plant-parasitic nematodes (Calderón-Urrea et al., 2016; Moens and Perry, 2009).

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Table 1. Root necrosis, root galling, and egg and nematode soil counts produced by *Meloidogyne incognita* race 3 and fresh root weights from sweet potato cultivars.

Cultivar	Necrosis and galling	Egg counts	Fresh root weights	Nematode soil counts
Nugget	2c[z]	213[b]	18[a]	31[c]
Ga. Jet	4[ab]	346[b]	15[a]	101[bc]
TUO2	3[ab]	298[ab]	14[ab]	2065[a]
WLA	3[b]	306[b]	9[bc]	134[b]
DMO1	4[a]	481[a]	7[c]	386[ab]

[v]Necrosis and galling values are the mean of five replications of five plants each. Significant differences exist between all of the treated cultivars in this study (P≤0.0001). It is expressed as the percentage of necrotic and galled root tissue. 0= no galling or necrosis, 1 = 1-10% galling; 2 = 11-25% galling and necrosis; 3 = 26-50% galling and necrosis; 4 = 51-75% galling; and 5 = above 75% galling (1-highly resistant 2-resistant, 3-intermediately resistant, 4 -susceptible, 5-highly susceptible. [w]Egg counts/g *inplanta* are the mean of five replications of five plants each. It is expressed as the number of eggs found per gram of root tissue. Significant differences exist between all the treated cultivars in this study (P≤0.01). [x]Fresh root weights are the mean of five replications of five plants each. It is expressed as the total fresh root weights in grams for all five sweet potato cultivars subjected to the inoculation of root-knot nematodes. Significant differences exist between all of the treated cultivars in this study (P≤0.0003). [y]Nematode soil counts are the mean of five replications of five plants each. It is expressed as number of nematode larvae per 100 grams of infected soil from each of the cultivars. Significant differences existed between cultivars in nematode counts from soil samples collected from pots (P≤0.0033). [z]Values within a column followed by the same letter are not different (Fisher's protected LSD test (@=0.05). Transformation of the data used for corresponding ANOVA was log (x+1). Back-transformed data are presented in the tabular format.

When RKN infect sweet potatoes, symptoms are demonstrated as round, spindle-shaped galls and definitive cracks and necrosis in tubers resulting in severe yield losses of 10.2% and reduced marketability of storage roots (Suzuki et al., 2012; Nicol et al., 2011; Overstreet, 2009; Sasser and Frekman, 1987; Bonsi and Phills, 1979). Primary evidence of RKN resistance in sweet potato is often measured through assessments of root-gall indices, root-necrosis, and counts of eggs and egg masses (Piedra-Buena et al., 2011; Cervantes-Flores et al., 2002; Bonsi and Phills, 1979). To increase efficiency in the characterizations of resistant genotypes, primary phenotypic screening analyses should include additional assessments of physiological responses to nematode infection. The development of successful RKN resistant sweet potato breeding programs is dependent on the identification of new RKN resistant sweet potato cultivars (Cervantes et al., 2002; Piedra-Buena et al., 2011). The conserved use of existing resistant cultivars may contribute to increased pathogen aggressiveness resulting in epiphytotic conditions. In the southern U.S, three sweet potato cultivars were developed to be included into sweet potato breeding programs however; the levels of RKN resistance in each breeding line has not been characterized. The objective of this study was to distinguish these sweet potato cultivars for resistance to RKNs.

MATERIALS AND METHODS

Five sweet potato cultivars (Nugget, Georgia Jet, TU-02, Whatley Loretan, and DMO1) were evaluated for resistance or susceptibility to RKN infection. Nugget (Cervantes-Flores et al., 2002) and Georgia Jet (Overstreet, 2009) demonstrate unique host responses to M. incognita; and served as resistant and susceptible controls. 12-cm long cuttings were excised from healthy, vigorous sweet potato vines and allowed to root in tap water for of 4 days, and transplanted into 500 cm^3 Styrofoam food containers (Dart Container Co.® Mason, MI) containing a sterilized media of 4-parts coarse sand and 1-part field soil (88.9% sand, 8.3% silt 2.8% clay). *Meloidogyne incognita* race 3 was previously identified using the NC Differential Host test (Hartman and Sasser, 1985) and perineal pattern analysis (Taylor and Netscher, 1974). Nematode inoculum was cultured on susceptible peanut (*Arachis hypogea* L.) plants. RKN eggs were extracted from peanut roots using Hussey and Barker's (1973) NaOCL extraction technique and approximately 10,000 eggs were injected into the soil of each plant at day 14 post-planting for infestation. Inoculated plants and untreated controls were grown under controlled greenhouse conditions of 25-28°C, 16 h light and watered daily. Plants were harvested 84 Days after inoculation and fibrous and storage roots were visually rated for the percentages of total root system that were galled and necrotic based on the visual screening method developed by Bonsi (1982). Root samples were assigned index values using a 1-5 root galling and necrosis index similar to the root gall index previously described and also by Kinloch et al. (1987), where 1 represented highly resistant (0% no galling or necrosis) 2: resistant (2-25% galling and necrosis,) 3: intermediate resistant (26-50% galling and necrosis) 4: susceptible (over 50% galled and necrotic) 5: highly susceptible (over 75% galled, with larger than average size galls and severe necrosis). Fresh root weights were recorded at harvest. Nematode eggs were extracted from a maximum of 3 g of each root system and enumerated under an inverted microscope to estimate total eggs per gram of root system based on root fresh weights. Nematode larvae were obtained from 100 cc of soil obtained from treated plants using the Baermann funnel method (Baermann, 1917) and counted under an inverted microscope. All data were transformed by log (x+1) to standardize the variance and subjected to analysis of variance (ANOVA) using the SAS GLM Procedure (SAS Institute, Cary, N.C.). Treated and untreated samples were arranged in a completely randomized design with five replications per genotype. Treatment means were compared using Fisher's LSD procedure (α=0.05). Back transformed means are presented in tabular format in Table 1 for clarity.

RESULTS

There were significant differences among cultivars for necrosis and galling index scores (P <0.0001), nematode counts (P<0.0033) and root fresh weights (P<0.0003) and statistical differences in egg counts (P<0.01) (Table 1). The number of nematode eggs produced per gram of root tissue was lowest in Nugget in comparison to all cultivars. In comparison to Nugget, Georgia Jet, TUO2, WLA and DMO1 showed increased egg counts of 62.4, 39.9, 43.6 and 125.8%, respectively. While the roots of Nugget showed low galling and necrosis, moderate galling and less necrosis were shown in WLA and TUO2 roots in comparison to the susceptible cultivars which is indicative of a measurable degree of intermediate resistance. The highest incidence of necrosis and galling were shown in the susceptible control Georgia Jet and DMO1. Fresh root weights were highest in Nugget and Georgia Jet. In comparison to Nugget, mean root weight percentages decreased by 16.6, 22.2, 50 and 61.1% in Georgia Jet, TUO2, WLA and DMO1, respectively. Soil nematode counts were lowest for Nugget similar to that of Georgia Jet, while TUO2 had the highest. An increase in nematode soil counts of 225.8, 6561.2, 332.2 and 1145% was shown in Georgia Jet, TUO2, WLA and DMO1 respectively, in comparison to Nugget.

DISCUSSION

Resistance to RKN infection is manifested as a decrease or inhibition of nematode reproduction (Trudgill, 1991; Corbett et al., 2010) or prevention of feeding site establishment (Williamson and Kumar, 2006; Corbett et al., 2010). Although not significantly different from susceptible control cultivar Georgia Jet, Nugget was significantly different from DMO1 (P<0.01) which produced the highest egg counts per gram of root tissue and was considered the most susceptible cultivar, followed by susceptible control Georgia Jet. Decreased egg counts per gram of root tissue was shown in TU02 and WLA when compared to susceptible control Georgia Jet and DMO1 which suggested resistance. Reductions in the severity of root galling and necrosis is a primary attribute of resistance in sweet potato genotypes (Bonsi and Phills, 1979; Cervantes-Flores et al., 2002; Piedra-Buena et al., 2011). Root samples from DMO1 were severely necrotic with minor storage root development in comparison to Georgia Jet and others and a 2.5-fold decrease in root weights occurred between Nugget and DMO1. The ability to tolerate pathogen infection with minor effects on agronomic performance such as yield has been shown in earlier studies and may be attributed to genotypic specificities in resistance. Plant responses to biotic factors may be associated with alterations in root growth. Corbett et al. (2011) suggested increased root weights in resistant tomato genotypes when compared to un-inoculated resistant plants, to be associated with RKN challenge and further indicated that resistant plants displayed a measure of tolerance. A similar response was shown in resistant control Nugget, where root fresh weights were higher in treated plants compared to controls. Though not significant, there was a 31.3% difference in mean nematode counts between Nugget and Georgia Jet. Secondary metabolites produced by different plant species have shown antagonistic effects against plant parasites (Stahl et al., 2016; Mazid et al., 2011). Additional research is needed to identify any potential differences in secondary metabolite production between these genotypes which may correlate to decreased nematode soil numbers.

The evidence presented in this study strongly suggested a correlation between the amplitude of resistance and genotype specificity during the evaluation of multiple host responses to sweet potato cultivars under RKN burden. The resultant data from this analysis confirmed previous evidence of resistance and susceptibility to RKN in existing control varieties and provided new information on host-nematode reactions in three newly developed sweet potato cultivars. To fully assess differential expression patterns among different genotypes, a comprehensive evaluation of expressed transcripts of sweet potato roots under RKN challenge is currently underway in our laboratory. The identification of molecular pathways involved in the host responses, and the development of genetic markers for resistance will provide efficiency in successful RKN resistant sweet potato breeding programs and contribute to the limited genetic information of the discreet processes in sweet potato physiological development under biotic stress.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Potential hydrogen ion of Quartzarenic Neosol with joint application of lime and gypsum

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The objective of the study is to investigate the interaction of lime and gypsum during incubation of soil samples in response to pH and also check the possibility of using more than one treatment for a single model by model identity method. The analysis of the potential hydrogen ion (pH) was performed in the Laboratory of Agricultural Chemistry, Federal Institute Goiano, Campus Rio Verde - GO, Brazil. The soil used is classified as Quartzarenic Neosol loam sandy and its corresponding soil taxonomy is Quartzipsamment. The experiment was completely randomized with four replications in a factorial 5 × 3 design, five doses of lime ($CaCO_3$) - DL (0, 1, 2, 3 and 4 t ha^{-1}) and three doses of gypsum ($CaSO_4$) - DG (0, 0.7 and 5 t ha^{-1}). The sources of $CaCO_3$ and $CaSO_4$, dolomitic lime and gypsum were used, respectively. Incubation was done in plastic containers with 20 ml of soil, and pH analysis was done every two days for 10 days. In general, it was frequently observed that reducing the pH of the reaction in the soil was attributed to higher DG applied together with $CaCO_3$. Due to the difference in all models tested by identity models, it is explained that the range of $CaSO_4$ doses and incubation periods promoted major change in the model either by interception, angulation or both. The reduction of pH in the soil is attributed to higher rates of gypsum used together with lime.

Key words: Active acidity, identity models, incubation period.

INTRODUCTION

One of the factors that limit crop productivity is the acidity of the soil, once liming is used for its correction (Fageria, 2001). Several authors have identified the importance of using lime together with gypsum to treat soil acidity profile (Caires et al., 2008; Maschietto, 2009; Dalla Nora et al., 2013; Zandoná et al., 2015).

Alone, liming raises the potential hydrogenion (pH) of the soil, neutralizes Al^{3+} toxicity, adds Ca^{2+} and Mg^{2+},

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provides favorable conditions for root growth and water and nutrient uptake by plants (Zandoná et al., 2015).

Gypsum is used in tillage system to minimize acidity problems. Low Al^{3+} toxicity is precipitated when it reacts with gypsum (Zambrosi et al., 2007). This causes it to be less toxic ($AlSO^{4+}$) and increases the Ca^{2+} concentration and S underground (Neis et al., 2010). This can make the lime to be active on the surface and subsoil soil (Caires et al., 2003). The main criticism is it results in the imbalance between the soil bases, considerably raising the exchangeable calcium content (Araujo et al., 2015).

When there is an interest to study quantitative variables, a variable response (pH) using one or more explanatory variables such as combined application of gypsum and lime, the regression analysis is a data analysis technique widely used. Due to different explanatory variables, regression analysis may be applied separately to obtain different results according to the number of situations.

Typically, the response variable Y and the set of factors, called the regression variables X_i, i = 1, 2, ..., n are measured in separate groups, to compare the resulting models if they differ (Seber, 1977).

Knowing the importance of identity models, it is important to apply this method on the response of soils to potential hydrogenionic based on the explanatory variables (gypsum doses and periods of incubation under lime function). This is because acidity limits the productivity of crops in sandy soils, beyond the limited studies on the reaction of gypsum and lime.

The hypothesis of this work is that gypsum and lime doses applied together has effect on soil pH at different periods of incubation, and further on, identity model was used for gypsum doses alone and for both lime and gypsum doses.

MATERIALS AND METHODS

The analysis of the potential hydrogenionic (pH) was performed in the Laboratory of Agricultural Chemistry from the Federal Institute Goiano, Campus Rio Verde - GO, Brazil. The soil used is classified as a Quartzarenic Neosol loam sandy and its corresponding one in soil taxonomy is Quartzipsamment (Embrapa, 2013).

The experiment was completely randomized with four replications in a factorial 5 × 3 design, five doses of lime ($CaCO_3$) - DL (0, 1, 2, 3 and 4 t ha^{-1}) and three doses of gypsum ($CaSO_4$) - DG (0, 0.7 and 5 t ha^{-1}). The sources of $CaCO_3$ and $CaSO_4$ were used as dolomitic lime and gypsum, respectively. Incubation was done in plastic containers with 20 ml of soil and pH analysis was done every two days for 10 days, totaling five periods of incubation periods.

During the incubation period of the soil, moisture was maintained at 60%, by measuring the mass of the sample units. At 2, 4, 6, 8 and 10 days after the beginning of the incubation period, the samples were placed in a forced-air oven at 45°C for 24 h. Subsequently, the soil subsamples were withdrawn for determination of pH potentiometrically in soil suspension solution of ratio 1:2.5 in distilled water, KCl 1 mol L^{-1} and $CaCl_2$ 0.01 mol L^{-1}. The suspension was read, with contact time (Embrapa, 2011).

The variance of interactions was analyzed ($p<0.05$) and identity of models ($p<0.10$) by the F test, set models linear as a function of the DL and represented the behavior of pH in surface graphs. After characterizing with F test ($p<0.10$), the combination of the models between the DG for each incubation periods (IP) and between the IP for each DG, it was considered that the hypothesis of similarity of the parameters a's and b's for the two sets of observations is rejected when Fc > Ft.

RESULTS AND DISCUSSION

The application of $CaSO_4$ and $CaCO_3$ together led to significant changes ($p <0.05$) in potential hydrogenionic (pH). At a dose of $CaCO_3$ (DL) 4 t ha^{-1}, the absence of $CaSO_4$ led to higher pH (H_2O) in relation to DG 5 t ha^{-1} and the incubation periods (IP) of 4, 6 and 10 days. The differences were 0.68, 1.89 and 1.87, respectively (Figure 1B, C and D).

According to Araujo et al. (2015), elementary sulfur and calcium sulfate positively affected the chemical properties of the soil, improving its fertility. Moreover, the elementary sulfur is more efficient than calcium sulphate in decreasing alkalinity. However, the use of this product requires the application of additional calcium in the soil.

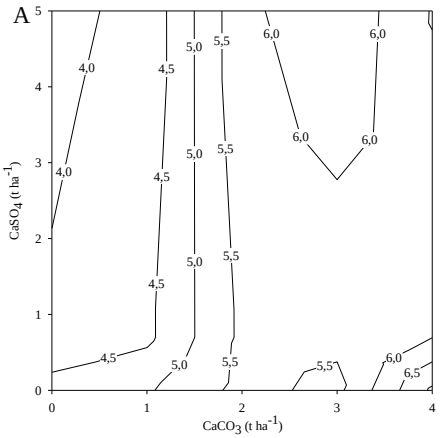
From DL 2 t ha^{-1} regardless of DG, pH proved to be appropriate for the soil in 2 and 4 days IP (Figure 1A and B), but at 6, 8 and 10 days of incubation, the pH was maintained from appropriate DL 2 t ha^{-1} without application of $CaSO_4$ (Figure 1C, D and E). Frade Junior (2013) verified that the average values of pH in water, which increased with the increasing doses of $CaCO_3$, raised the pH to approximately neutral ground for the three soils (Spodosol Humilúvico, Yellow Latosol and Ultisol Alítico Plinthic).

The pH given in water showed a linear polynomial fit in each DG depending on the DL in all IPs (Figure 1). The greatest increases in pH of DL function were checked at DG 5 t ha^{-1} IP in 2 and 4 days; there was an increase of 0.55 and 0.61, respectively in response to an increase of 1 t ha^{-1} $CaCO_3$ (Figure 1A and B).

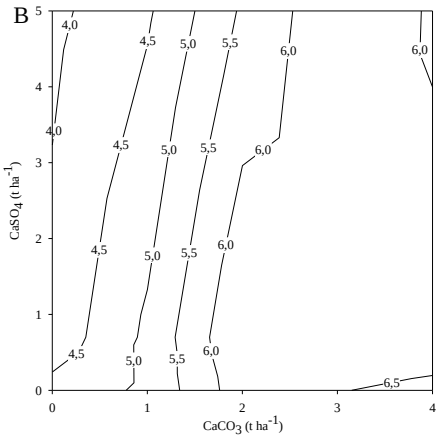
Costa (2015) observed that the surface application of limestone, with or without gypsum, were effective in reducing soil acidity to a depth of 0.20 m

The greatest increases in pH at IP of 6, 8 and 10 days were observed in the absence of $CaSO_4$; there was an increase of 0.71, 0.48, and 0.68, respectively in response to an increase of 1 t ha^{-1} of $CaCO_3$ (Figure 1C, D and E). According to Natale et al. (2007), with increased doses of correctives, there was a linear increase of pH effect on dystrophic Red Latosol.

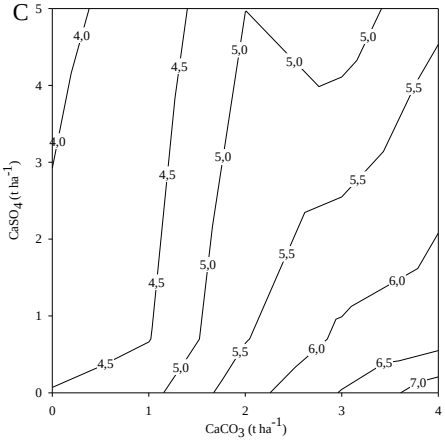
In 2 and 4 days IP (Figure 2A and B), the pH showed higher values in higher DL and DG based on the methodology for determining KCl; unlike IP 6, 8 and 10 days (Figure 2C, D and E) that showed higher pH values in greater DL and absence of $CaSO_4$. Chi et al. (2012) found that gypsum was effective in improving the physical and chemical properties of the soil, increasing the aggregate stability.



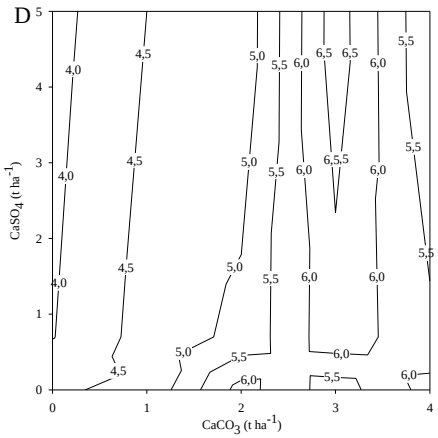
pH ($CaSO_4$ = 0) = 0.52* ($CaCO_3$) + 4.52; R^2 = 0.77
pH ($CaSO_4$ = 0.7) = 0.50* ($CaCO_3$) + 4.1; R^2 = 0.89
pH ($CaSO_4$ = 5) = 0.55* ($CaCO_3$) + 4.05; R^2 = 0.62



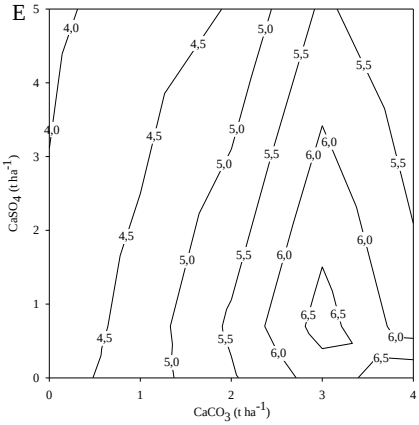
pH ($CaSO_4$ = 0) = 0.53* ($CaCO_3$) + 4.77; R^2 = 0.89
pH ($CaSO_4$ = 0.7) = 0.49* ($CaCO_3$) + 4.61; R^2 = 0.68
pH ($CaSO_4$ = 5) = 0.609* ($CaCO_3$) + 4.02; R^2 = 0.84



pH ($CaSO_4$ = 0) = 0.72* ($CaCO_3$) + 4.37; R^2 =0.98
pH ($CaSO_4$ = 0.7) = 0.59* ($CaCO_3$) + 4.09; R^2 = 0.96
pH ($CaSO_4$ = 5) = 0.36* ($CaCO_3$) + 3.92; R^2 = 0.85

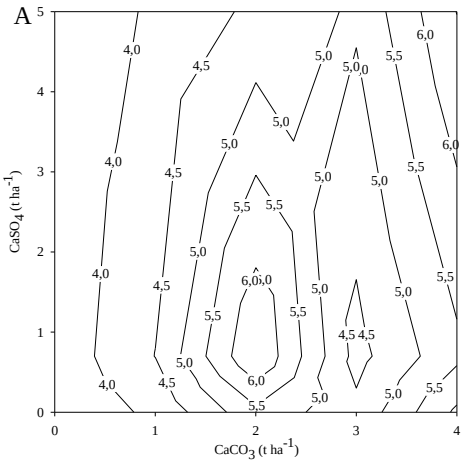


pH ($CaSO_4$ = 0) = 0.41* ($CaCO_3$) + 4.51; R^2 = 0.61
pH ($CaSO_4$ = 0.7) = 0.49* ($CaCO_3$) + 4.17; R^2 = 0.74
pH ($CaSO_4$ = 5) = 0.48* ($CaCO_3$) + 4.00; R^2 = 0.50

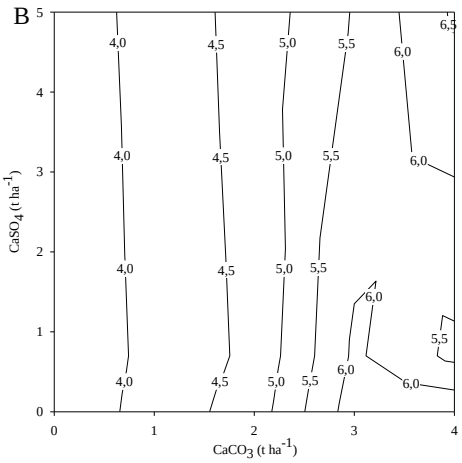


pH ($CaSO_4$ = 0) = 0.68* ($CaCO_3$) + 4.17; R^2 = 0.99
pH ($CaSO_4$ = 0.7) = 0.52* ($CaCO_3$) + 4.33; R^2 = 0.68
pH ($CaSO_4$ = 5) = 0.37* ($CaCO_3$) + 3.92; R^2 = 0.75

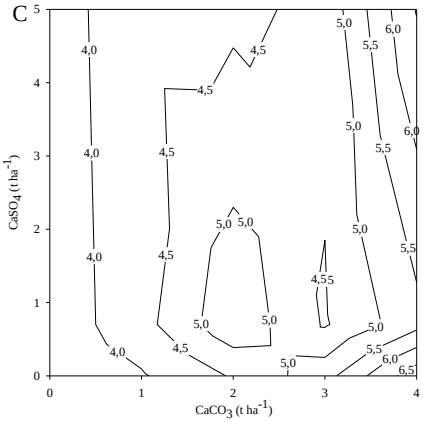
Figure 1. pH behavior water determined according to the relation of the doses of $CaCO_3$ and $CaSO_4$ in the incubation period two day (A), four days (B), six days (C), eight days (D) and ten days (E).



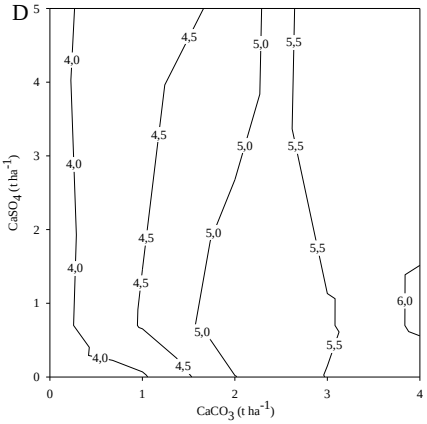
pH ($CaSO_4$ = 0) = 0.53*($CaCO_3$)+3.7; R^2 = 0.76
pH ($CaSO_4$ = 0.7) = -0.265*($CaCO_3$)^2 +1.384*($CaCO_3$) + 3.69; R^2 = 0.43
pH ($CaSO_4$ = 5) = 0.67*($CaCO_3$)+3.44; R^2 = 0.92



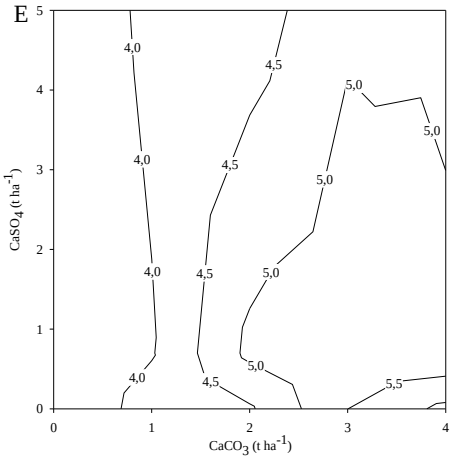
pH ($CaSO_4$ = 0) = 0.76*($CaCO_3$)+3.51; R^2 = 0.94
pH ($CaSO_4$ = 0.7) = 0.55*($CaCO_3$)+3.65; R^2 = 0.78
pH ($CaSO_4$ = 5) = 0.71*($CaCO_3$)+3.51; R^2 = 0.97



pH ($CaSO_4$ = 0) = 0.77* ($CaCO_3$) + 3.32; R^2 = 0.92
pH ($CaSO_4$ = 0.7) = 0.35*($CaCO_3$)+3.94; R^2 = 0.57
pH ($CaSO_4$ = 5) = 0.58*($CaCO_3$)+3.56; R^2 = 0.76



pH ($CaSO_4$ = 0) = 0.51*($CaCO_3$)+3.76; R^2 = 0.91
pH ($CaSO_4$ = 0.7) = 0.55*($CaCO_3$)+3.95; R^2 = 0.96
pH ($CaSO_4$ = 5) = 0.50*($CaCO_3$)+3.87; R^2 = 0.81



pH ($CaSO_4$ = 0) = 0.65*($CaCO_3$)+3.46; R^2 = 0.97
pH ($CaSO_4$ = 0.7) = 0.42*($CaCO_3$)+3.77; R^2 = 0.77
pH ($CaSO_4$ = 5) = 0.34*($CaCO_3$)+3.70; R^2 = 0.93

Figure 2. pH behavior KCl determined according to the relation of the doses of $CaCO_3$ and $CaSO_4$ in the incubation period two day (A), four days (B), six days (C), eight days (D) and ten days (E).

In DL 4 t ha^{-1}, DG 0.7 t ha^{-1} gave higher pH values (KCl); at DG 5 t ha^{-1} and IP in 4, 6 and 10 days, the differences were 1.01, 1.47, and 1.05, respectively (Figure 2B, C and E). Although at DL 4 t ha^{-1}, the absence of $CaSO_4$ gave pH (KCl) of 1.17, more than that of DG 5 t ha^{-1} with a difference of 1.17 at 10 days IP (Figure 2E).

According to Ebeling et al. (2008), the lowest pH values in KCl solution may result from KCl solution, which when in contact with the ground, induces the exchange of cations, releasing H^+ and Al^{3+} ions, due to the higher concentration of K^+ ions.

The pH determined by KCl presented polynomial fit of the second order DG 0.7 t ha^{-1} under DL at IP of 2 days and the other set of linear regression (Figure 2). The highest pH of function DL was checked at DG 5 t ha^{-1} and 2 days IP, with an increase of 0.67 when 1 t ha^{-1} of $CaCO_3$ was raised (Figure 2A). The surface application of gypsum reduced the exchangeable acidity (Al^{3+}) and increased the levels of Ca^{2+} and S-SO_4^{2-} in surface and subsurface, but reduced the surface levels of Mg^{2+} (Costa, 2015).

The pH determined by KCl presented polynomial fit of the second order DG 0.7 t ha^{-1} on DL function of the IP 2 days and the other set of linear regression (Figure 2). The greatest increases in pH of DL function were checked at DG 5 t ha^{-1} and 2 days IP, with an increase of 0.67 when 1 t ha^{-1} of $CaCO_3$ (Figure 2A) was raised. Ferreira et al. (2013) verified that soil Ca content showed a linear relationship with soil C under gypsum+lime ($p<0.0001$) and gypsum applications ($p<0.0001$).

In IP 4, 6, 8 and 10 days, the largest estimated values of the pH being 6.39, 6.82, 6.10, and 6.12, respectively were found at DL 4 t ha^{-1} and without $CaSO_4$ (Figure 2). Several authors found high yields of annual crops due to the increase in pH in the soil, as Fageria et al. (2005) found that increased productivity of beans in 6.4 pH water content in distrophic Red Latosol (Oxisol) which initially had a pH of 5.7. Fageria and Baligar (2003) found that higher yields with respect to the appropriate pH are through adequate supply of Ca and Mg and balance between basic cations.

In DL 2 t ha^{-1}, DG 0.7 t ha^{-1} gave higher pH values ($CaCl_2$) with respect to DG 5 t ha^{-1} and IP of 2, 4 and 8 days, the differences were 0.92, 1.43 and 2.06, respectively (Figure 3A, B and D). In DL 3 t ha^{-1} and IP of 2 days, the absence of $CaSO_4$ resulted in higher pH values being 1.54 and 0.98 higher than the DG 0.7 of 5 t ha^{-1}, respectively (Figure 3A); in the same DL, DG 0.7 t ha^{-1} gave higher pH values than the DG of 5 t ha^{-1} in IP of 6 and 10 days; an increase of 1.06 and 1.01, respectively (Figure 3C and E).

Rosa et al. (2012), evaluating lime and gypsum in distroferric Red Latosol, found that the application of increasing doses of lime caused an increase in pH and also the use of gypsum influenced positively the culture only at 871.3 kg ha^{-1} and lime of above 2000 kg ha^{-1}.

The pH given in $CaCl_2$ showed second order polynomial fit of the DG 0.7 to 5 t ha^{-1} on DL function of the IP 8 days and the other set of linear regression (Figure 3). The greatest increases in pH at IP of 2, 4 and 6 were recorded in the absence of $CaSO_4$ 0.63, 0.58, and 0.71, respectively, an increase of 1 t ha^{-1} of $CaCO_3$ (Figure 3A, B and C). The greatest increases in pH of DL function were checked at DG 0.7 t ha^{-1} and IP of 8 to 10 days; there was an increase of 0.56 and 0.67, respectively; increased by 1 t ha^{-1} $CaCO_3$ (Figure 3D and E).

Studying the effect of adding lime and gypsum on the base in soil columns, Dal Bó et al. (1986) found that treatment with gypsum isolated accelerated magnesium and calcium displacement in depth, so it is likely that this change in calcium and the changes introduced by incubation periods justify the rejection of the identity of tested models.

In IP of 2, 4 and 6 days, the largest estimated values of the pH being 6.26, 5.74, and 6.43, respectively, were found at DL 4 t ha^{-1} and without $CaSO_4$; but 6.27 and 6.30 values after 8 and 10 days of incubation of the samples were superior to the others (Figure 3).

The results obtained by the interaction of gypsum and lime doses applied jointly in the soil samples are related to the results of Carvalho and Nascente (2014)'s study on the influence of lime and gypsum in the production of biomass and cycling millet nutrients. It was found that there were increases of dry biomass production with the application of lime and gypsum provided there were no changes in the production of dry biomass of millet.

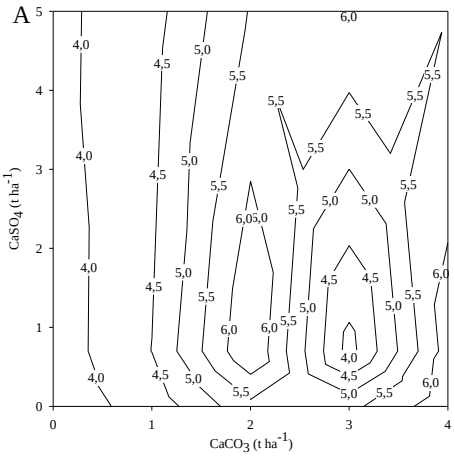
In general, it was frequently observed that reducing the pH of the reaction fixed in the soil was attributed to higher DG applied together with $CaCO_3$.

According to the analysis of variance F ($p> 0.1$), it was found that the adjusted models of 0, 0.7, and 5.0 t ha^{-1} were similar between the IP and dose (Table 1) of both models set under DL (Table 2). Based on Regazzi and Silva (2004), the approximated F should be preferred, since it provides a rate of type I error, regardless of the sample size.

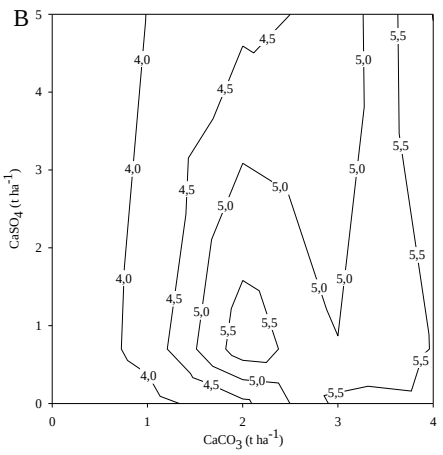
Due to the differences in all models tested by identity models, it is explained that the range of $CaSO_4$ doses and incubation periods promoted major change in the model either by interception, angulation or both. Ribeiro et al. (2005), comparing all adjusted equations, found that the various ions present statistical difference between the adjusted models, except for sodium, and significant among some equations adjusted per time, with the largest differences observed for chloride and calcium with 46.15 and 40.66%, respectively.

Conclusion

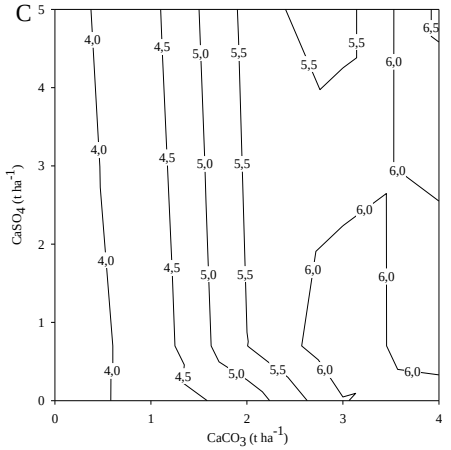
The reduction of pH correction in the soil is given to higher rates of gypsum used together with lime. Some parameters of the hypothesis models in some combination of gypsum doses within a period of



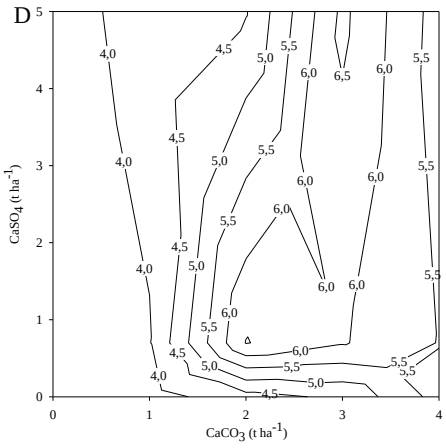
pH ($CaSO_4$ = 0) = 0.63*($CaCO_3$)+3.73; R^2 = 0.94
pH ($CaSO_4$ = 0.7) = 0.43*($CaCO_3$)+4.08; R^2 = 0.30
pH ($CaSO_4$ = 5) = 0.50*($CaCO_3$)+4.05; R^2 = 0.75



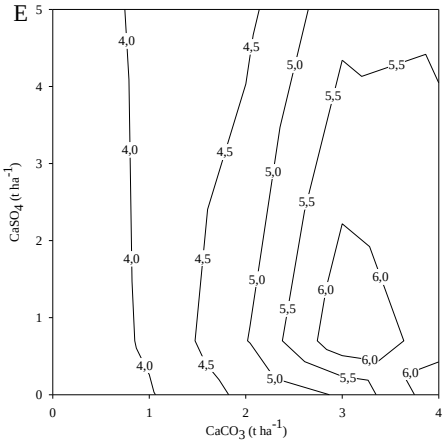
pH ($CaSO_4$ = 0) = 0.58*($CaCO_3$)+3.43; R^2 = 0.91
pH ($CaSO_4$ = 0.7) = 0.47*($CaCO_3$)+3.86; R^2 = 0.65
pH ($CaSO_4$ = 5) = 0.55*($CaCO_3$)+3.42; R^2 = 0.89



pH ($CaSO_4$ = 0) = 0.72*($CaCO_3$)+3.56; R^2 = 0.97
pH ($CaSO_4$ = 0.7) = 0.58*($CaCO_3$)+3.90; R^2 = 0.72
pH ($CaSO_4$ = 5) = 0.66*($CaCO_3$)+3.82; R^2 = 0.90



pH ($CaSO_4$ = 0) = 0.47*($CaCO_3$) + 3.49; R^2 = 0.88
pH ($CaSO_4$ = 0.7) = -0.33*($CaCO_3$)^2 + 1.9* ($CaCO_3$) + 3.35; R^2 = 0.75
pH ($CaSO_4$ = 5) = -0.11*($CaCO_3$)^2+0.97*($CaCO_3$)+ 3.58; R^2 = 0.62



pH ($CaSO_4$ = 0) = 0.65*($CaCO_3$)+3.4; R^2 = 0.95
pH ($CaSO_4$ = 0.7) = 0.67*($CaCO_3$)+3.62; R^2 = 0.84
pH ($CaSO_4$ = 5) = 0.46*($CaCO_3$)+3.66; R^2 = 0.93

Figure 3. pH behavior $CaCl_2$ determined according to the relation of the doses of $CaCO_3$ and $CaSO_4$ in the incubation period two day (A), four days (B), six days (C), eight days (D) and ten days (E).

Table 1. F calculated in the model identity to the combinations of the incubation periods as a function of lime and gypsum in each dose for the different method of determining pH.

Relation [1]IP (days)	[2]$CaCl_2$	[2]H_2O	[2]KCl
Dose $CaSO_4$ (0 t ha^{-1})			
2 - 4	24.05	53.09	127.65
2 - 6	11.27	47.41	69.01
2 - 8	34.34	52.68	65.39
2 - 10	10.07	30.48	24.31
4 - 6	31.83	30.39	37.31
4 - 8	41.43	106.53	53.55
4 - 10	29.99	41.17	9.65
6 - 8	107.38	214.80	70.00
6 - 10	29.69	19.93	8.27
8 - 10	33.31	120.39	11.03
Dose $CaSO_4$ (0.7 t ha^{-1})			
2 - 4	56.71	42.56	39.64
2 - 6	138.80	14.04	7.87
2 - 8	151.92	41.78	16.34
2 - 10	240.60	38.33	19.58
4 - 6	41.29	43.01	96.40
4 - 8	58.96	74.27	28.71
4 - 10	93.60	35.06	33.68
6 - 8	29.22	33.42	30.60
6 - 10	8.93	22.97	36.50
8 - 10	125.19	11.48	30.52
Dose $CaSO_4$ (5.0 t ha^{-1})			
2 - 4	135.05	11.47	8.27
2 - 6	40.72	52.65	18.55
2 - 8	40.68	43.57	110.32
2 - 10	116.57	45.83	112.99
4 - 6	49.73	58.09	25.74
4 - 8	93.88	52.67	38.11
4 - 10	33.84	53.62	78.62
6 - 8	76.80	65.50	112.63
6 - 10	75.98	13.34	84.83
8 - 10	44.43	25.70	57.55

[1]Incubation time of soil samples; [2]Method of determining the pH; tabulated F ($p<0.1$) = 1.8125.

Table 2. F calculated models of identity for the combinations of doses of $CaSO_4$ due to the $CaCO_3$ doses of each incubation period and method for measuring the pH.

Relation dose $CaSO_4$	[1]$CaCl_2$	[1]H_2O	[1]KCl
Incubation period - 02 days			
0 - 0.7	44.0	57.9	12.6
0 - 5.0	18.1	119.8	66.8
0.7 - 5.0	173.3	30.5	35.6
Incubation period - 04 days			
0 - 0.7	70.6	18.2	30.3
0 - 5.0	33.1	75.7	13.9
0.7 - 5.0	68.2	43.6	40.5

Table 2. Cont'd.

Incubation period - 06 days			
0 - 0.7	32.2	80.3	123.7
0 - 5.0	27.1	147.6	25.8
0.7- 5.0	36.1	52.2	84.8
Incubation period - 08 days			
0 - 0.7	365.9	191.7	15.6
0 - 5.0	115.5	243.3	24.1
0.7 - 5.0	115.8	22.8	24.3
Incubation period - 10 days			
0 - 0.7	86.0	48.7	23.6
0 - 5.0	45.6	106.2	22.8
0.7 - 5.0	88.4	53.8	31.7

[1]Method for measuring the pH; F tabulated ($p < 0.1$) = 1.8125.

incubation were discarded.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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