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Review

Fertilizer micro-dosing in West African low-input cereals cropping: Benefits, challenges and improvement strategies

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Soil amendments are often unavailable in adequate quantities for increased crop production in smallholder cereal-based cropping systems in Africa. In order to increase crop yields and encourage farmers to apply inorganic fertilizers, fertilizer micro-dosing technology was developed. Fertilizer micro-dosing or "micro-fertilization" consists of the application of a small quantity of mineral fertilizer together with seeds of the target crop in the planting hole at sowing or 2-4 weeks after sowing. The objective of this paper is to review literature concerning crops responses to fertilizer micro-dosing in West Africa. The review also evaluates the benefits and challenges associated with nutrient management under fertilizer micro-dosing and supportive strategies for further improvement in the efficient use of limited nutrient sources of smallholder farmers were suggested. Recent scientific developments on fertilizer micro-dosing revealed that this technology has given promising results in respect of crop yields improvement, fertilizer use efficiency and economic returns. Other studies have, however, indicated that fertilizer micro-dosing increases the risk of soil nutrient imbalances due to low-input. For this reason, we suggest that fertilizer micro-dosing should be used in concert with organic amendments to optimize productivity of smallholder farmers in West Africa.

Key words: Fertilizer micro-dosing, smallholder farmer, crop yield, farmer's income.

INTRODUCTION

During the past three decades, the paradigms underlying the use of fertilizers and soil fertility management research and development efforts have undergone substantial change due to experiences gained with specific approaches and changes in the overall social, economic, and political environment (Sanchez, 1994). Contrary to conventional knowledge, it is vital to acknowledge that the farmers' decision making process is not merely driven by the soil and climate, but by a whole set of factors cutting across the biophysical, socio-

economic, and political domain (Izac, 2000). In the light of this consideration, food production for the expanding world population has required the development and application of new technologies, and an intensification management to produce more food per unit of land (Stewart et al., 2005). Currently, a holistic approach in soil fertility research and strategy focus on the new paradigm of Integrated Soil Fertility Management (ISFM) which embraces the driving factors and consequences of soil degradation – biological, chemical, physical, social, economic, health, nutrition and political (Bationo et al., 2006). The ISFM defined as the application of soil fertility management practices and the knowledge to adapt these to local conditions, which maximize fertilizer and organic resource use efficiency and crop productivity (Sanginga and Woomer, 2009) has been promoted and advocated to preserve soil quality while promoting its productivity. Earlier, this soil fertility management practice had been considered as a prerequisite for the achievement of productive and sustainable agricultural production systems (Akponikpe, 2008).

Mineral fertilizer has been recognized as an entry point for sustainable agriculture production systems. However, the use of mineral fertilizer is still very low in most smallholder cropping systems in West Africa. According to the African Fertilizer Summit (2006), the fertilizer consumption in 55% of SSA countries is less than 5 kg ha^{-1}. In order to encourage farmers to use mineral fertilizer, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and partners have developed fertilizer micro-dosing technology. This technology has been found as an appropriate precision agriculture for smallholder farmers in the sahelian region of Africa.

This paper, focusing on recently published data, gives emphasis on (1) what has been done at research level on fertilizer micro-dosing technology (2) what can be learnt from the adoption of this technology and (3) what can be suggested for the effective use of this technology to enhance the productivity of smallholder cropping systems in the Africa.

FERTILIZER MICRO-DOSING

Fertilizer micro-dosing technology consists of the application of a small quantity of mineral fertilizer together with seeds of the target crop in the planting hole at sowing or few weeks (3 to 4) after planting (Hayashi et al., 2008; ICRISAT, 2009). In the Sahelian countries, fertilizer micro-dosing relies on smaller quantities (2 to 6 g $hill^{-1}$) of placed mineral fertilizers targeting in priority the most limiting element, phosphorus (Buerkert et al., 2001; Tabo et al., 2007). Micro-dosing decreases substantially the recommended amount of fertilizer that smallholder farmers need to apply per hectare, that is, from 200 to 20 kg ha^{-1} in the case of di-ammonium phosphate (Hayashi et al., 2008).

The techniques of applying fertilizer vary depending on soil and climatic conditions. For instance, in southern Africa, farmers use fertilizer measured out in an empty soft drink or beer bottle cap, while in western Africa, the farmers measure fertilizer with a three-finger pinch (ICRISAT, 2009). A three-finger pinch is equivalent to 6-g doses in the case of NPK 15:15:15 fertilizer which is about a full soft drink bottle cap. With ammonium nitrate fertilizer for instance, a beer bottle cap is equal to 4.5 g which is equivalent to 17 kg N ha^{-1} (Twomlow et al., 2010). Farmers in the Sahel use a soda bottle cap to allocate fertilizer, hence fertilizer micro-dosing is popularly known as the Coca-Cola technique (Tabo et al., 2006). Applying fertilizer in micro-doses permits more precise and better timed fertilizer placement and hence appropriate management of fertilizer (Sanginga and Woomer, 2009). This technology has also been strategically combined with other practices such as seed priming (Aune and Ousman, 2011), water harvesting, or application of manure, crop residues, and compost prepared from household and garden wastes (Sanginga and Woomer, 2009).

ORIGIN OF FERTILIZER MICRO-DOSING TECHNOLOGY

Fertilizer micro-dosing or hill placement of mineral fertilizer is a technology originally developed by the International Crops Research Institute for the Semi-Arid Tropics, Sahelian Center (ICRISAT-SC) with partners in Germany (Rebafka et al., 1993). In 1999, ICRISAT began a series of modeling workshops in conjunction with the International Maize and Wheat Improvement Center (CIMMYT) and the Agricultural Production Systems Research Unit (APSRU) in which research and extension officers used a simulation model (APSIM - Agricultural Production Systems Simulator Model) (Keatinge et al., 2003) to evaluate the type of resource allocation questions faced by resource-poor farmers in the semi-arid regions of southern Africa. A common theme started from the proposition that farmers may, at best, initiate investments in small quantities of fertilizer (Rohrbach, 1999). The robustness of the simulated responses to small quantities of N fertilizer was surprising, and contrary to much of the documented soil fertility research

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results in the region which started with at least 25 kg N ha^{-1} (Mushayi et al., 1999; Mafongoya et al., 2006). Simulation results for 1951 to 1999 rainfall period in southern Zimbabwe, suggested that farmers could increase their average yields by 50 to 100% by applying as little as 9 kg N ha^{-1}. These results indicated that farmers were better off applying lower rates of N on more fields, than concentrating a limited supply of fertilizer on one field at the recommended rates (Carberry et al., 2004).

On-farm experimentation was then initiated with farmers on micro-dosing alone or in combination with available animal manures (Ncube et al., 2007). The on-farm trial results confirmed that farmers could increase their yields by 30 to 100 % by applying approximately 10 kg N ha^{-1} (Rusike et al., 2006). Scaling out of micro-dosing was initiated in 2003/2004 with support from the Department for International Development (DFID) and the European Commission Humanitarian Aid Office (ECHO) which encouraged the application of the micro-dosing of ammonium nitrate fertilizer by more than 160,000 farmers (Rohrbach et al., 2005; Twomlow et al., 2007). Currently, fertilizer micro-dosing technology has reintroduced fertilizer use by smallholder farmers in Zimbabwe, Mali, Burkina Faso, Niger, and Mozambique and in the southern part of the African continent (ICRISAT, 2009; INERA, 2010; Twomlow et al*.,* 2010). This technology establishes a pattern for future productivity as farmers become accustomed to increasing their investments in inputs in order to generate increased returns. It is therefore an entry point for increased use of fertilizers in farmers' fields, which can lead to more sustainable development (Tabo et al., 2008). In fact, micro-dosing has been identified as a climate smart technology (The Montpellier Panel, 2013), and a pathway for the intensification of agricultural systems in Sub-Saharan Africa (Murendo and Wollni, 2015).

BENEFITS OF FERTILIZER MICRO-DOSING

Fertilizer affordability

Fertilizer micro-dosing was developed in an attempt to increase the affordability of mineral fertilizer while giving plants enough nutrients for optimal growth (Hayashi et al., 2008). High rates of fertilizer input have been recommended to farmers for a long time to increase yields, but smallholder farmers could not afford to apply such fertilizer quantities. Small amounts are more affordable for farmers (Bationo and Buerkert, 2001) because of reduced investment cost (Tabo et al., 2006, 2007). For example, the application of 20 kg ha^{-1} of diammonium phosphate instead of 200 kg ha^{-1} recommended to smallholder farmers reduces input cost and investments risk while increasing crop yields (Bielders and Gérard, 2015).

Increase in crop yield

Several studies have examined the immediate millet or sorghum response to fertilizer micro-dozing in the Sahel. Muehlig-Versen et al*.* (2003) showed that hill application of 5 kg P ha^{-1} led to 65% of the yield increase obtained with 13 kg P ha^{-1} broadcast, resulting therefore, in a significant increase in fertilizer-use efficiency. Abdou et al*.* (2012) reported consistently significant increase in pearl millet yield following strategic placement of 4 kg P per hectare as NPK 15-15-15 or DAP (di-ammonium phosphate) at planting. Similar effects have been previously reported from the application of 4 kg P ha^{-1} as compound NPK fertilizer (Bationo et al*.*, 1998). Other studies in Niger have shown that application of 6 g NPK fertilizer per hill can more than double millet yields (Bationo and Buerkert, 2001) and there is a positive economic return to the use of fertilizer (Hayashi et al*.*, 2008; Tabo et al*.*, 2011).

Having tested this technology in three Sahelian countries (Niger, Mali and Burkina Faso), Tabo et al*.* (2007) showed that the average grain yields of millet and sorghum were greater by 44 to 120% while the farmers' income increased by 52 to 134% when using hill application of fertilizer compared to the earlier recommended fertilizer broadcasting methods and farmers' practice. However, the application of these rates of fertilizer needs one additional person at the time of sowing for fertilizer application. The labour demand at sowing period is high which can lead to delayed sowing thereby resulting in yield decrease. Recent on-farm research in Niger has shown that farmers can delay the application of fertilizer under micro-dosing technology from 10 to 60 days after sowing without significantly reducing the yield and the economic returns (Hayashi et al*.*, 2008).

Recently, Okebalama et al. (2016) reported optimal maize grain yield with $N_{20}P_{40}K_{20}$ and $N_0P_{40}K_{20}$ fertilizer micro-doses. Also, Ibrahim et al*.* (2014) showed that increasing the depth of fertilizer micro-dosing application from 5 to 10 cm results in a marked increase in millet yields. Crops under micro-dosing have been observed to perform better under drought conditions because the crops' larger root systems are more efficient at exploiting moisture at greater depth later in the season when soil moisture at the surface of the soil is low (ICRISAT, 2009). The positive effect of fertilizer micro-dosing in increasing millet yield has been attributed to the better exploitation of soil nutrients due to early lateral roots proliferation within the topsoil (Ibrahim et al*.*, 2015). In the early development, it was postulated that the positive effect of fertilizer micro-dozing can probably be attributed to a root-growth stimulating effect of phosphorus fertilization as previously reported by Aune and Bationo (2008) and Buerkert and Schlecht (2013).

Questions on whether these results could be replicated across different soil types, agro-ecological zones and

climates have been raised by many researchers. Tabo et al. (2008) confirmed that fertilizer micro-dosing has the potential to greatly increase yields across a range of agro-ecological zones and rainfall situations in West Africa, from the drier Sahelian zone to the wet Sudano-Guinean environment. In Ghana, NPK fertilizer micro-dosing increased maize yields by 99% in the humid forest zone (Okebalama et al., 2016). In Zimbabwe, wide-scale testing of the micro-dosing (17 kg N ha^{-1}) consistently showed increased grain yields by 30 to 50% across a broad spectrum of soil, farmer management and seasonal climatic conditions (Twomlow et al., 2010). Also, the findings of Hayashi et al. (2008) showed that fertilizer micro-dosing improved the harvest index of millet crop.

Farmers' income increases due to fertilizer micro-dosing application

More important than yield increases are the financial returns and risk associated with the adoption of a technology. Generally, the application of reduced dose of mineral fertilizer increases the economic return of applied technology. Profitability of maize to low rates of N fertilizer has been reported (Twomlow et al., 2010). Millet under micro-dosing gave net monetary gains which were 68 % higher than the net returns from the traditional practice and 33% higher than the net gain from the conventional recommended practice (Tabo et al., 2008). Okebalama et al. (2016) showed that $N_{20}P_{40}K_{20}$ micro-dose under continuous sole maize cropping was more profitable than the recommended fertilizer rate by about GH¢1,000.00 (about $350.00) net return difference per ha. In Mali, Burkina Faso, and Niger, ICRISAT (2009) reported an increase in sorghum and millet smallholders' family incomes by 50 to 130%.

Recently, Bielders and Gérard (2015) reported a value-cost ratios (VCR) of less than 1 from 36% of 276 fertilizer micro-dosing demonstrations setup across a 3-year period in the Fakara region, western Niger. However, the economic risk associated with micro-dosing (2 g DAP $hill^{-1}$ for instance) appears higher than has hitherto been reported and widespread adoption may not be warranted without institutional support. In practice, one generally considers that the VCR should be at least 2 for adoption in developing countries, but VCR values of 3–4 may be required in risky environments (CIMMYT Economics Program, 1988). This means that yield increases following micro-dose application should be at least twice, but ideally 3 to 4 times. Better economic returns of micro-dosing have been obtained in Mali (Aune et al., 2007) and Sudan (Aune and Ousman, 2011) with 0.3 g fertilizer per hill, which was achieved by mixing seeds and fertilizer in a 1:1 ratio before sowing. In addition, the latter practice saves on labor costs at sowing, since seed and fertilizer can be applied simultaneously, something that is not feasible with the rate of 2 g fertilizer per hill. Aune et al. (2007) observed higher yield increases with the application of 6 g than 0.3 g per pocket with a VCR of 0.43 to 1.17 and 3.4 to 11.9, respectively. Accordingly, the application of 0.3 g of fertilizer appealed to farmers because of the good return on investment, low financial risk, low cash outlay and low workload required.

Increase in nutrient use efficiency (NUE)

Tabo et al. (2006) noted that micro-dosing optimizes NUE, while Zougmoré et al. (2004) found that the combination of water harvesting technologies and fertilizer improved water and NUE by crops. Okebalama et al. (2006) observed that micro-dosing increased N, P and K use efficiency of maize in rotation with cowpea than in sole cropping. Reports from ICRISAT (2009) showed that implementation of micro-dosing technology enhanced NUE and improved productivity relative to spreading fertilizer over the field. Small amounts of applied fertilizer give an economically optimum (though not biologically maximum) response, and if placed in the root zone of widely-spaced crops rather than uniformly distributed, result in more efficient uptake (Bationo and Buerkert, 2001). The efficient use of fertilizer by plants depends on mode of application, with the most efficient method being hill placement (Bationo and Waswa, 2011).

Technology adoptability

By using much lower rates of fertilizer than the recommended rate, in more efficient ways that deliver economically optimum returns, farmers are much more able and inclined to adopt the practice. Surprisingly, the adoption rate of micro-dosing, like many other intensification techniques in sub Saharan Africa (SSA) is generally considered to be low (Liverpool-Tasie et al., 2015). In northwest Benin, Natcher et al. (2016) found that both adoption and project awareness of micro-dosing were low following two years of field trials. However, recent survey on 415 smallholder farmers in eight semi-arid districts of Zimbabwe reported 47%micro-dosing adopters and 53% non-adopters (Murendo and Wollni, 2015). Among the adopters, the adoption of micro-dosing increased the likelihood of being food secure by 47 percentage points compared to the counterfactual case. Fertilizer micro-dosing adoption is also gender specific. Winter-Nelson (2014) reported that female-headed households were significantly less likely to adopt micro-dosing than others, possibly due to labour shortages or difficulties in accessing fertilizer. Across four countries in West Africa, a baseline study has shown that women are 25% more likely to adopt a combination of micro-dosing and rainwater harvesting compared to men (Abdoulaye et al., 2014).

To increase fertilizer micro-dosing adoption rate, it is very important to understand the constraints that farmers face and ascertain the technique adaptability to the farmers' existing farming practices. This is very important because insufficient adaptation of technologies to farmers' condition among others had been recognized as a major constraint to adoption (Sanginga and Woomer, 2009). Other identified major constraints to the widespread adoption of micro-dose technology include access to fertilizer, access to credit, insufficient flows of information and training of farmers, and inappropriate policies (ICRISAT, 2009). Winter-Nelson (2014) reported that training in micro-dosing raised the probability of adoption by 30 to 35% points in the semi-arid areas of Zimbabwe. Experiences from both western and southern Africa have shown that adoption of micro-dose technology requires supportive and complementary institutional innovation as well as input and output market linkages (Bationo et al., 2006).

Increased food security

Micro-dosing has the potential for improving food security. The over 100% yield increases of cereal crops produce of smallholder farmers (Tabo et al., 2006, 2007) suggests increased food security and less need for food aid. The findings of Twomlow et al. (2010) provided strong evidence that N micro-dosing has the potential for broad-scale impact on food security for a large section of the rural poor across dry regions of southern Zimbabwe. Rohrbach et al. (2005) reported that the estimated DFID's support for the distribution of 25 kg of ammonium nitrate fertilizer to each of 160,000 farm households contributed 40,000 additional tons of maize production, valued by the World Food Programme at 5 to 7 million USD. Murendo and Wollni (2015) found that micro-dosing improved household food security of smallholder farmers in the semi-arid areas. That notwithstanding, with the predicted significant reduction in productivity of the major crops because of future warming and shift in precipitation patterns in West Africa (Ahmed et al., 2015), the resilience of soils under fertilizer micro-dosing to climate change for improving food security becomes questionable.

With the projected decrease in the productivity of certain crops due to climate change (IFPRI, 2007) one wonders if fertilizer micro-dosing technology could be considered a climate change adaptation strategy that would replace nutrient losses via leaching and erosion resulting from high rainfall. This is important because without technology adaptation, the long-term mean of crop yield has been projected to decrease in most West African countries (despite some projected increase of precipitation) by the middle of the century, while the inter-annual variability of yield increased significantly (Ahmed et al., 2015).

FERTILIZER MICRO-DOSING CHALLENGES AND POSSIBLE SOLUTIONS

Labour intensive

Farmers have reported that micro-dosing is labourious, time consuming and difficult to ensure each plant gets the right dose of fertilizer (ICRISAT, 2009). In an attempt to address these issues, ICRISAT collaborates with private fertilizer companies in eastern and southern Africa, to identify appropriate fertilizer types and promote the sale of small packs suited to the resource constraints and risk preferences of small-scale farmers. ICRISAT is also exploring the use of seed coating (with fertilizer) as another option of further reducing the quantity of fertilizer to be used as well as the labour constraint. In addition, researchers are looking at packaging the correct dose of fertilizer as a tablet that aids in application (ICRISAT, 2009). Alternatively, with the development of labour-reducing equipment, precise plant hill fertilizer micro-dosing would complement farmers' efforts (Tabo et al., 2007).

On the other hand, Liverpool-Tasie et al. (2015) found no empirical evidence that micro-dosing is more labor intensive than traditional methods of fertilizer application as is conventionally thought. Hayashi et al. (2008) opined that delayed fertilizer application strategy for micro-dosing would enable farmers to better manage available labour and also have some flexibility and an additional option in investing in inorganic fertilizer. Accordingly, delayed application allows farmers to push labour usage to later in the season, after planting, when the labour pool is not as limited, thereby reducing the chance of bad results by applying fertilizer after crops have emerged. On the contrary, delayed application may attract additional labour cost thereby increasing the overall production cost and hence, becomes a constraint. Even though delayed fertilizer application to emerged crops may be cost effective, matching fertilizer nutrient requirement of crops during growth phase is dynamic. This is vital because the mismatching of nutrient availability with crop needs may probably contribute to nutrient losses or reduced nutrient use efficiency by crops. Plant responsiveness to micro-dose fertilizer nutrients availability depends partly on the fertilizer placement method and time. The fertilizer placement method reduces nutrient losses, while on time placement helps the plant to have early established roots that can explore for more nutrients deeper down the soil (Ibrahim et al., 2014). Be that as it may, delaying application in order to save the amount of fertilizer applied to the crops could be beneficial if the fertilizer is strategically applied to synchronized nutrient availability and crop demand, particularly when applications are timed to moisture availability. Therefore, obtaining maximum profitability lies not only in reducing the amount of fertilizer use per unit area but also in reducing costs per unit crop produce

through higher nutrient use efficiency and yields.

Financial constraints

Most farmers are faced with lack of financial means at the onset of the rainy season. Abdoulaye and Lowenberg-DeBoer (2000) pointed out that local farmers cannot afford to invest in the purchase of inorganic fertilizer prior to the cropping season due to an insufficient food supply for the household and the need to use cash to purchase family food. Nevertheless, delayed fertilizer application can lessen the financial burden of the local farmers during the sowing period. Delayed fertilizer application offers smallholder farmers opportunity to raise the cash needed to purchase and apply fertilizers only to established plants, thus increasing their chance of producing more grain and economic returns (Hayashi et al., 2008). Also, farmers' supportive groups (co-operative) or warrantage/inventory credit strategy as practiced in West Africa aims to resolve the farmers' capital constraint. Organized farmer groups provide access to post-harvest credit provided on the basis of storage of grain as collateral (warrantage), enabling farmers to sell crops later in the season for higher prices and higher profits (Bationo et al., 2006).

Nutrient mining

The possibility of soil nutrient mining arising from fertilizer micro-dosing technology has raised much concern. Researchers have questioned the logic of micro-dosing, claiming that the use of such a small quantity of fertilizer is not sustainable (Twomlow et al., 2010). Singh and Ajeigbe (2007) noted that the average use of 10 kg/ha/yr of fertilizers in West Africa leads to a negative balance of nutrients in the soil and continuous decline in crop yields, which perpetuates malnutrition, hunger and poverty through the vicious circle of 'low input-low production-low income' and food insecurity. No doubt, as grain yields increase per unit area and very little organic matter, including crop residues, are put back into the soil, there is the risk that nutrient imbalances will inevitably develop with time (Tabo et al., 2007). As such practising fertilizer micro-dosing alone may lead to soil degradation on the long term. Accordingly, it is important to ensure that organic matter is added and incorporated into these soils to improve their structure and enhance their capacity to store adequate moisture and nutrients even after crops are harvested (Tabo et al., 2007). Soil organic matter plays a central role in maintaining tropical soil fertility and its conservation and maintenance in tropical cultivation systems is imperative if soil degradation is to be halted and cropping made sustainable (Ross, 1993). The quantity of soil organic matter in the soil has been found to depend on the quantity of organic material which can be introduced into the soil either by natural returns through roots, stubble, slough off roots nodules and root exudates or by artificial application in the form of organic manures (Adebola et al., 2012).

As opinioned by Gambo et al. (2008), the most satisfactory method of increasing crop yields are by judicious use of organic manures in combination with little portions of inorganic sources for nutrient use efficiency. This is factual because unlike organic fertilizer, mineral fertilizer does not improve aggregation of soil particles; neither does it activating soil microbial activities. The use of organic manures has been recommended for long term cropping in the tropics as slow mineralization of these manures promote crop yield for a long period of time (Gambo et al., 2008). Organic fertilizers are known to improve soil organic matter, and macro- and micro-nutrients qualities of the soil. Organic manures sustain cropping systems through better nutrient recycling and improvement in soil physical, chemical and biological properties (Ojeniyi, 2000). Hence, the principle that makes organic manure useful and important in soil fertility maintenance is their impact on soil fertility supplies, moisture holding capacity and structural characteristic (Udoh et al., 2005).

Finally, it appears that most micro-dose fertilization studies were mainly based on sole cropping of cereals such as maize, wheat, millet and sorghum. However, the effect of inter-cropping, strip-cropping, or mixed cropping of these crops and micro-dosing of fertilizer are yet unknown. In most African countries, most food crop farms are intercropped because of the predominance of smallholder farm holdings of less than 2 ha and the need to achieve household food security. As such, while monocropping is uncharacteristic of smallholder farming, intercropping/mixed cropping is typical to most smallholder farmers even though traditionally practiced with little or no application of fertilizers. Intercropping of cereals will encourage growing of traditional crops (millet, sorghum etc) in order not to be completely replaced by introduced high value crops such as maize (Bandyopadhyay et al., 2007). Also, little is known about the impact of fertilizer micro-dosing on performance of other crops such as cowpea, garden eggs, cucumber and amaranth. Research on these crops should be considered as the findings could benefit smallholder farmers, considering their nutritional advantage.

CONCLUSION

Fertilizer micro-dosing technique is useful in the management of fertilizer for cereal crop production, particularly under smallholder farming in most West and Southern African countries. The technology has led to high reduction in the recommended rate of fertilizer application to crops. Small quantities of fertilizer (about 9g per plant) placed with seed at sowing or at the base of

each plant 3-4 weeks after sowing increases crop yield by 30 to 100%. Increase in nutrient use efficiency of crops and farmers income are added benefits associated to fertilizer micro-dosing. Despite its potential for broad-scale impact on food security, the technology adoption is low, in addition, soil nutrient mining and labour intensive challenges have raised concerns of some researchers and farmers, respectively. Nonetheless, fertilizer micro-dosing can contribute usefully to sustainable agricultural development, especially when integrated with organic fertilizer application.

Conflict of Interests

The authors have not declared any conflict of interest.

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Response of Wheat (*Tritium aestivum* L.) to Variable Seed Rates: the Case of Hawassa Area, Southern Ethiopia

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Wheat serves as a staple food and feed for all over the world. In Ethiopia, wheat is one of the most important cultivated cereals ranking 4th in area coverage, 3nd in total production and 2nd in yield. A wheat seed rate of 125 kg ha^{-1} is usually used across most wheat growing areas of Ethiopia in any production conditions. In order to optimize seed rate and plant density of wheat, an experiment was conducted at the agronomy experiment field, College of Agriculture, Hawassa University, Ethiopia, with variable seed rates of 100, 125, 150 and 175 kg ha^{-1}. For this purpose, an experiment was laid down using a randomized complete block design with three replication and four treatments. The result of the current experiment showed significant difference for number of tillers, plant height, grain yield, and 1000-grain weight whereas non-significant difference were witnessed for spike length. Result of this particular study indicated higher number of effective tillers (10.27), spike length (8.9 cm), 1000-grain weight (41.7 g) and grain yield (25.78 qtha^{-1}) production with 125 kg ha^{-1} seed rate. On the basis of results obtained from this experiment, it is concluded that wheat should be sown with 125 kg ha^{-1} seed rate in the current study area provided that all other agronomic management practices is kept optimal.

Key words: Grain crop, planting density, seed rate, *Tritium aestivum* L. and yield.

INTRODUCTION

Wheat (*Tritium aestivum* L.) is a staple food of the masses and feed for animals all over the world (Abbas et al., 2009). This important cereal crop serves as a stable food for one-third of the world's population (Hussain and Shah, 2002). It is grown on 220 million hectares in almost all countries and climatic regions (Singh and Trethowan, 2007) constituting 15.4% of the world's arable land (Curtis, 2002). According to FAO (2005) report, about 620 million metric tonnes of wheat is produced from 217 million hectares of land. The crop utilizes about 30% of the land area under cereal cultivation and account for about 27% of the world cereal production.

Ethiopia is the second largest producer of wheat in sub-Saharan Africa following South Africa and covering about

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1.61 million hectare of land under rain-fed production system. In Ethiopia, wheat is one of the most important cultivated cereals ranking fourth after teff (*Eragrostis teff*), maize (*Zea mays*) and sorghum (*Sorghum bicolor*) in area coverage (CSA, 2007), 3rd in total grain production after Maize and Teff and 2nd in yield next to Maize (Assefa et al., 2015; FAO, 2005). The crop is cultivated by 4.746 million farmers accounting for more than 16.3% of the total cereal production (CSA, 2014). Moreover, the crop plays an appreciable role in supplying the population with carbohydrate, proteins and minerals (Schulthess et al., 1997; Hayatulah et al., 2000). The average per capita consumption of wheat in Ethiopia estimated to be 39 kg year^{-1} during the year 1994 - 1999 (CIMMYT, 2000).

However, the mean national average yield of wheat is 2.45 tonnes ha^{-1} which is 24 and 48% below the African and world average, respectively (FAO, 1994), and 3-4 tonnes ha^{-1} far below the research station yields of over 6 tonnes ha^{-1} in the country (Assefa et al., 2015). This low yield in the country is on account of many production limiting factors, among these planting density of wheat, which determines the proper stand establishment of the growing crop and crop vigor through balancing competition for production resources among plants, which ultimately affects the yield of the crop (Korres et al., 2002) is amongst the important agronomic factors. Thus, keeping the importance of optimal seed rate in view, a research trial was undertaken to optimize this factors for a wheat crop under the agro-climatic conditions of Hawassa southern Ethiopia in 2016 cropping season.

MATERIALS AND METHODS

The experiment was conducted in Southern Ethiopia, Hawassa University College of Agriculture agronomy experimental field during 2016 cropping season with seeding rates of 100, 125, 150 and 175 kg ha^{-1}. The site is located at 7°4' North latitude and 38° 31' East longitude. The altitude of the area is 1700 m.a.s.l. The soil of the site is sandy loam, with 7.9 pH value, which is volcanic origin and described as flovisol. The average rain fall of the area is 900-1100 mm annually, whereas annual maximum and minimum temperature are 27 and 12°C respectively. The experiment was laid out in randomized complete block design with three replications using a total plot size of 49.5 m^2 (5 m width and 9.5 m^2 length) with a spacing of 1 m, 0.5 and 0.2 between blocks, plots and rows respectively.

The data were recorded for effective number of tillers, spike length (cm), plant height (cm), 1000-grain weight (g), grain yield (kg ha^{-1}) and analyzed statistically using SAS computer software version 9.2 (SAS, 2008). Tiller number was counted by considering the fertile tiller per plant randomly taking five plants from each plot of the middle three rows at maturity. Spike length was measured taking five plants from each plot of the middle three rows at maturity. Plant height was measured from ground level to tip of the plant taking five plants from each plot of the middle three rows per plot at maturity. Grain yield was weighted taking five plants from each plot of the middle three rows at maturity and using a sensitive balance. Thousand grain weight was also determined by counting seeds randomly taken from each plot and weighing using sensitive weighting balance.

RESULTS AND DISCUSSION

Number of tillers

The economic yield of most cereals is determined by the number of tillers the plant produces. It has the great agronomic importance as this may compensate the difference in number of plants across plots (Acevedo et al., 1998). The current experiment showed that different seed rate revealed significant difference in number of tillers among the treatments, wherein, the use of 125 kgha^{-1} seed rate produced higher number of effective tillers (10.27) compared with (7.67 and 6.00) tillers produced using a seed rate of 150 and 175 kgha^{-1}, respectively (Table 3), which showed that a lower effective tiller production occurred with an increasing seed rate. This is because the higher the seed rate might increase the competition for space thereby result in lesser number of tillers per plant. Baloch et al. (2010) also reported that, lower seed rates significantly increased the number of fertile tillers produced per plant. However, effective tiller number decreased with increasing seed rate beyond 125 kgha^{-1} while higher number of effective tiller is obtained at 125 kgha^{-1} seed rate. This can be explained that, as the seed rate is higher it result with narrow free space which limit the tillering capacity of the plant. Similar result also reported by Hayatullah et al. (2000), stating a decreasing number of effective tillers beyond the optimal seed rate. This is because, the process of tillering is mainly controlled by genetic and environmental factors (Longnecker et al., 1993; Zencirci 2008), and plant population density can regulate the microenvironment of the production system through affecting the competition for space and production resources. Ozturk et al. (2006), in his work on growth and yield response of facultative wheat to winter sowing, freezing sowing and spring sowing at different seeding rates also demonstrated that, reducing seed rate result in more tillers and spike per plant. Alemayehu et al. (2015) also presented that, the number of fertile tillers showed a decreasing pattern moving from 100, 125 to 150 seed rate ha^{-1} (Table 1).

Days to seedling emergence

In the current experiment, planting with a seed rate of 100, 125 and 150 kg ha^{-1} showed similar and later emergence date as compared to 175 kg ha^{-1} seed rate. However, statistically significant early emergence date was recorded when crop was sown using 175 kg ha^{-1} seed rate. Planting with a seed rate of 125 kg ha^{-1} gave emergence relatively late followed by 100 and 150 kg ha^{-1} seed rate, respectively although the difference was not statistically significant (Table 2).

Table 1. Mean square of ANOVA's of number of tillers, spike length, plant height, 1000-grain weight and grain yield in wheat.

Sources of variation	Degree of freedom	Number of effective tillers	Spike length	Plant height	1000-grain weight	Grain yield
Replication	2	0.15	0.33	5.49	1.00	6.74
Treatment	3	9.24*	0.22**	24.40*	8.72*	148.20*
Error	6	1.04	0.13	1.53	0.98	22.40
Total	11	3.12	0.19	8.48	3.09	

Table 2. Effects of seed rates on germination in wheat

Treatments	Days to germination
Seed rate (kgha^{-1})	
100	6.00^{a}
125	6.33 ^{a}
150	5.67 ^{a}
175	4.33^{b}
LSD	1.04
CV %	9.38%

Table 3. Mean number of effective tillers and plant height as affected by variable seed rates.

Treatments	Number of effective tillers	Plant height (cm)
Seed rate (kgha^{-1})		
100	8.40^{ab}	66.47^{c}
125	10.27^{a}	69.13b
150	7.67^{bc}	70.73^{b}
175	6.00^{c}	73.27^{a}
LSD	2.04	2.47
CV %	12.6%	1.8%

Means sharing the same superscript letter do not differ significantly at P = 0.05 according to the LSD test.

Plant height (cm)

Plant height is regulated by the genetic makeup of the plant and the environmental factors (Shahzad et al., 2007), and planting density determines the growing situation by affecting the competition for space and production resources. The current experiment showed significantly different plant height with varying seed rates, the maximum plant height of 73.27 cm was obtained from 175 kgha^{-1} seed rate followed by 150 kgha^{-1} seed rate which was resulted with a plant height of 70.73 cm (Table 3). Minimum plant height was obtained with the lowest (100 kgha^{-1}) seed rate which produced a plant height of 66.47 cm (Table 3). Increased plant density resulted in increased height of the plants, this is because high plant density remains with minimum space for horizontal expansion of the plant and increase the competition for light interception between plants drives upward growth. The result of the current experiment, is in agreement with Suleiman (2010), who reported that, increase in the seeding rate resulted in a slight increment in the height of plants. Alemayehu et al. (2015) also reported an increasing pattern in plant height with increasing seed rate ha^{-1}. This result, however, did not coincide with Baloch et al. (2010) and Abbas et al. (2009) who reported that increase in the seeding rate resulted in a slight decline in the heights of the plants. This could be because of variable environmental conditions and genetic makeup of the genotypes used in all these studies.

Spike length (cm)

The length of spike plays a vital role in wheat towards the

Table 4. Mean spike length and grain yield as affected by variable seed rates.

Treatments	Spike length (cm)	Grain yield (qtha^{-1})
Seed rate (kgha^{-1})		
100	8.6	18.26^{ab}
125	8.9	25.78^{a}
150	8.5	14.70^{b}
175	8.27	8.99^{b}
LSD	NS	9.5
CV %	4.22%	27.99%

Means sharing the same superscript letter do not differ significantly at P = 0.05 according to the LSD test.

Table 5. Mean 1000-grain weight as affected by variable seed rates.

Treatments	1000-grain weight (g)
Seed rate (kgha^{-1})	
100	39.7^{b}
125	41.7^{a}
150	38.50^{b}
175	38.00^{b}
LSD	1.97
CV %	2.5%

Means sharing the same superscript letter do not differ significantly at P = 0.05 according to the LSD test.

grain per spike and finally the yield (Shahzad et al., 2007). The analysis of variance for spike length shows no significant difference among the treatments (Table 4); however, a relatively higher spike length of 8.9 cm was recorded from 125 kg ha^{-1} seed rate closely followed by 100 kg ha^{-1} seed rate which produced 8.6 cm long spike. Further increase in planting densities beyond 125 kg seed rate ha^{-1} resulted in slight decline in the length of spike (Table 4). Longer spike at 125 kgha^{-1} seed rate can be referred to the ideal plant population in this treatment, which resulted in optimum crop plant competition. This finding is in-line with the findings of Abbas et al. (2009) and Suleiman (2010), who reported a decreasing trend in spike length in both increasing and decreasing direction from the optimal plant population. Similarly, Ozturk et al. (2006) reported more spike production per plant with reducing pattern of seed rate.

Grain yield (qtha^{-1})

The data showed statistically significant difference for grain yield. 125 kg ha^{-1} seed rate produced a higher grain yield of 25.8 qtha^{-1} followed by 100 kg ha^{-1} seed rate which produced a grain yield of 18.28 qtha^{-1} (Table 4). The lowest grain yield was obtained from 175 kg ha^{-1} seed rate followed by 150 kg ha^{-1} seed rate which produced 8.99 and 14.7 qtha^{-1} grain respectively (Table 4). Further decrease in planting densities beyond 125 kg seed rate ha^{-1} resulted in slightly lower grain yield although not statistically different, whereas an increase in seed rate beyond 125 kg ha^{-1} resulted with statistically lower grain yield (Table 4). This finding is in agreement with the finding of Baloch et al. (2010), who reported a decline in yield of wheat with increasing seed rate, this might be explained that dense wheat population creates keen competitions between plants for production resources leading to a decreasing grain yield. Similar finding also reported by Hayatullah et al. (2000), which presented a decreasing pattern in grain yield of wheat with increasing seed rate. This might be due to shrivelled grain and lodging of crop which results from having higher plant population ha^{-1}.

1000-grain weight (g)

The current experiment showed that, variable seed rates has a significant effect on 1000-grain weight. Among seeding rates, the maximum 1000-grain weight (41.7 g) was obtained with 125 kgha^{-1} seed rate followed by 1000-grain weight (39.7 g) obtained from a 100 kgha^{-1} seed rate (Table 5). The relatively lower 1000-grain weight (38 g) was obtained from a seed rate of 175 kgha^{-1} followed by a 1000-grain weight of (38.5 g.) which was obtained from a seed rate of 150 kgha^{-1} (Table 5). This experiment shows that 1000-grain weight declines with increasing seed rate. This might be due to higher planting density on account of higher seed rate used that increased plant competition and eventually declined the grain weight. The result of the current study is in agreement with Shahzad et al. (2007) and Baloch et al. (2010), who reported the negative effect of increasing seed rate on 1000-grain weight. Alemayehu et al. (2015), in his research on effect of seed sources and rates on productivity of bread wheat (*T. aestivum* L.) varieties at Kersa, eastern Ethiopia also reported the production of highest 1000-grain weight at the lowest seed rate. Thus, there is a negative relationship between plant density and 1000-grain weight. This is because at higher plant density most grains would fade at early stage because of competition between growing grains to absorb preserved matters and as the result low grains would produced (Rahim et al., 2012). However, a different result was found by Hayatullah et al. (2000), showing the absence of significant effect of seed rate on 1000-grain weight.

CONCLUSION AND RECOMMENDATION

In the present study, wheat produced higher number of

tillers, plant height, 1000-grain weight and grain yield when 125 kg ha^{-1} seed rate is used. Therefore, with the optimal application of all the recommended agronomic practices for the same crop, 125 kg ha^{-1} seed rate can be suggested to achieve better crop performance and higher grain yield of wheat in the study area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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The performance of Soybean using *Moringa* as alley to improve soil productivity in North-Central Nigeria

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This study assesses the performance of intercropping *Glycine max* (Soybean) and *Moringa oleifera* under alley cropping in Abuja, Nigeria during the 2014 and 2015 cropping season. The field experiment was laid out in Randomized Complete Block Design (RCBD) with 3 replications. The replicates contained five plots each, separated between blocks by 1 m pathway and 0.5 m within the blocks. The plot measured 5.0 m × 4.0 m each. Thus, a total of 15 (treatments) plots were used. Results showed that total nitrogen, like all other elements observed, significantly reduced during first cropping season (0.03%) but improved significantly at P<0.05 (0.07%) after the second trial except Sodium. Sodium concentration reduced to 0.38 Cmol^{-1} in Glycine max/Moringa inter crop plots of 50,000 stands of Soybean and 10,000 stands of *M. oleifera* in 2015. Highest Soybean seed yield (0.68 tha^{-1}) in 2015 cropping season and Maximum Land Equivalent Ratio (23 and 68% more yield in 2014 and 2015 respectively) were obtained from 50,000 Soybean stands + 10,000 stands of *M. oleifera*. The intercropping showed positive relationship between the two plants in terms of yield and the improvement of Nigeria Guinea Savannah soil fertility through organic biomass.

Key words: Alley cropping, fertility, Moringa, soybean, yield.

INTRODUCTION

Alley cropping, an agroforestry approach where annual (agricultural) or perennial (horticultural) crops are cultivated between widely spaced rows of economic woody species (Kang, 1997). Alley cropping, over the years, is a system that increased agricultural productivity, improved livelihoods through income diversification, enhanced conservation strategies, promoted organic waste recycling and induce landscape aesthetics value among others over the continuous mono cropping (de la Fuente et al., 2014; Stoltz and Nadeau, 2014; Kang, 1997). The system had the challenge to increasing the sustainability of agricultural yield with limited inputs to meet the growing human needs, while maintaining the natural resource base and reducing environmental degradation through recycling of organic matter (Cardinael et al., 2015, Ramsdale et al., 2006).

The continuous monocropping without adequate fertilization has led to soil fertility depletion and

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subsequent low crop yields in the savannahs of Nigeria (Esu, 2010). However, in recent times, the awareness of farmers in Nigeria to adopt alley cropping over monocropping due to its importance, increased in rate by 55% in the zone (Alene et al., 2015; Ajeigbe et al., 2010; Inaizumi et al., 1999). The adoption is as a result of efforts by agricultural extension services agents and short-term trainings occasionally organized by Nigeria's Ministry of Agriculture and International Institute of Tropical Agriculture (IITA) (IITA, 2008). This becomes necessary due to geometric increase in human population and urbanisation coupled with other climate change drivers such as deforestation thereby reducing arable land size and its fertility over time (Magigi and Drescher, 2010; Peter and Runge-Metzger, 1994). Hence, alley cropping as an alternative to maximize land use output, improve agro-ecosystem resilience and enhance sustainable livelihoods (Glover et al., 2012).

Soybean (*Glycine max*) is one of the commonly grown legumes under sole cropping system in Nigeria, the largest producer of the crop in Sub-Saharan Africa (ACET, 2014; Kamara, 2009). The tremendous potential to improve incentive of resource-poor farmers, particularly in a developing country like Nigeria can be achieved through adoption of innovative agricultural land use approach (ACET, 2014; Alene et al., 2015; Danshiell, 1994). Land-use conflict has been recognized as one of the challenges leading to unproductive yield in the savannah zones; others are soil fertility decline, poor seeds and low input (Cardinael et al., 2015; Peter and Runge-Metzger, 1994). Soil fertility have increased overtime on fields that soybean are grown through nitrogen fixing.

Meanwhile, in the Guinea Savannah region of Nigeria, farmers (including women) are growing *Moringa oleifera* as the woody perennial crop on their farmlands is increasing (Abdullahi et al., 2014; Torimiro et al., 2009). The tree plant, though not a nitrogen-fixing plant, is among the known promising exotic multipurpose tree species recommended for fuel wood, fodder, food, medicinal value, and soil fertility improvement (Edward et al., 2011; Goss, 2012). The tree also provides semi-shade, thus useful in alleys where intensity of direct sunlight exposure could damage crops (Nouman et al., 2013). However, there is the need to experiment the integration of *Moringa* with soybean as an alley crop in the Guinea savannah farmlands of Nigeria to examine the high leguminous crop productivity in terms of yield and its effects on soil properties. Hence, the study aims at evaluating the effect of *Moringa* intercropping on the yield components and growth performance of Soybean, and to determine the mixture productivity of intercropping soybean and *M. oleifera* on same field plots.

MATERIALS AND METHODS

The Federal Capital Territory (FCT), Abuja, lies in the transect between Southern Guinea and Derived Savannah agro-ecological zones of Nigeria, majority of the farmers in the zone grow similar arable crops under monocropping system (Figure 1). Field trails were conducted in two cropping (rainy) seasons (2014 and 2015) at the Teaching and Research Farm of Faculty of Agriculture, University of Abuja, Nigeria, located on latitude 8°98' East and longitude 7°19' North. Average annual temperature in the area is 33°C with 14% humidity during planting season and an annual rainfall of 1,200 to 1,600 mm.

A Randomized Complete Block Design (RCBD) with three replicates was used in the two trials. Each replicate (block) contained five plots and separated from the other block by one meter pathway. The plot measured 5.0 m × 4.0 m each and 0.5 m alley was used to divide the plots within the block. Thus, a total of 15 plots were used in the trials. The treatments of which effects were assessed include 40,000 stand/ha Soybean only, 62,500 stand/ha Soybean and *Moringa* stand, 50,000 stand/ha Soybean and *Moringa* stand, 40,000 stands/ha Soybean and *Moringa* stand and Sole *Moringa* stand. All *Moringa* stand were at 10,000 stand/ha each.

Seedlings raised from *Moringa* seeds (Bauchi Local) under a nursery were transplanted after 3 weeks to the field. Planting of Soybean (TGX 1440 – 7t) seeds and *Moringa* seedlings were done simultaneously at the first week of August. *M. oleifera* seedlings were planted at spacing of 1 m × 1 m giving a population of 10000 stands ha^{-1}. Plant population of soybean were varied; 50 cm × 50 cm (40,000 stands ha^{-1}), 40 cm × 40 cm (62,500 stands ha^{-1}), and 50 cm × 40 cm (50,000 stands ha^{-1}) respectively. Beating up and supply was done on *Moringa* seedlings and soybean seeds respectively after 7 days from the initial establishment on the field. The plots were manually weeded at 21 and 47 days after planting.

Data collected on Soybean include, number of leaves/plant, plant height (measured at a distance between the soil surface to the tip of the apical bud), number of pods/plant (determined by counting the number of pods-containing at least two seeds from 10 plants selected at random and dividing the total number of pods by 10), number of seeds per pod (obtained by counting the number of seeds from 10 pods selected at random and dividing the total by 10. Others are the biomass yield of *M. oleifera* and Soybean, Land Equivalent Ratio (LER) and Land Equivalent Coefficient (LEC). The following formula were used to determine LER and LEC as described by Willey (1985):

$$\text{LER} = \frac{\text{Intercrop yield of crop A}}{\text{Sole crop yield of A}} + \frac{\text{Inter crop yield of crop B}}{\text{Sole crop yield of crop B}}$$

$$\text{LEC} = \frac{\text{Intercrop yield of crop A}}{\text{Sole crop yield of A}} \times \frac{\text{Inter crop yield of crop B}}{\text{Sole crop yield of crop B.}}$$

Data on all the parameters were subjected to Analysis of Variance (ANOVA) using Randomized Complete Block Design (RCBD), $Xij = \mu + Ti + \beta j + \sum ij$; where Xij = Trial SS, μ = Population mean = 0, Tij = Experimental Treatment effects, βij = Block effect and ∑ij = Error terms. Treatment means were separated with Least Significant Difference (LSD) (Gomez and Gomez, 1984).

Using the methods described by Reeuwijk (1992), the Bouyoucus hydrometer method was used in determining soil textural analysis. The soil pH soil was measured using a glass-calomel electrode (MP 220 AFAB Lab, LLC) (IITA, 2008). Soil organic matter and carbon as well as Total Nitrogen were all determined using titration and kjedahl digestion (1992). The soil available phosphorous content was also analysed by 0.5 M sodium bicarbonate extraction solution at pH 8.5 using the methods in Reeuwijk (1992). Exchangeable cations (Ca2+, Mg2+, K+ and Na+) were determined by using atomic absorption spectrometry (AAS) and the soil sample

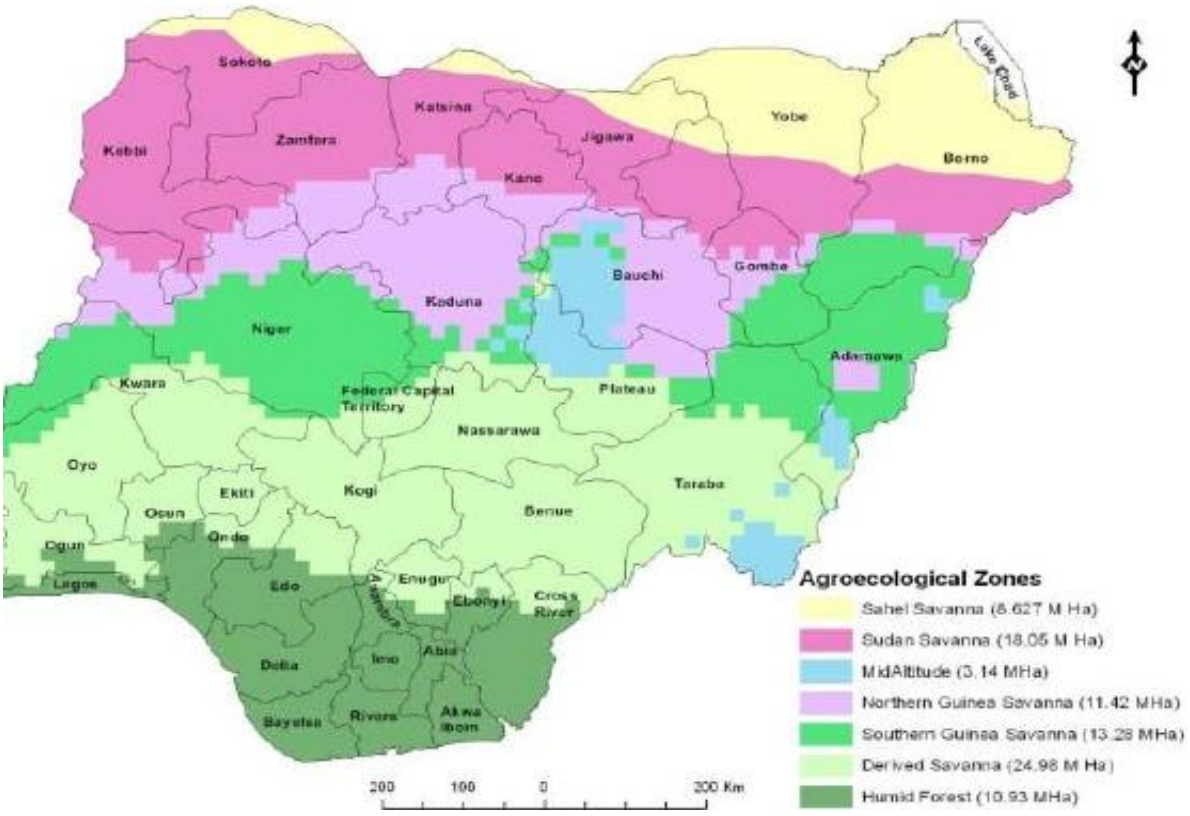

Figure 1. Map of Nigeria showing the agroecological zones, in Federal Capital Territory lies the transect between two savannah agroecological zones (Source: www.iita.org).

saturated with 1N NH4OAc solution at pH 7 (Chapman, 1965).

RESULTS AND DISCUSSION

Tables 1 and 2 showed the soil physicochemical properties of the field before and after planting in each cropping season respectively. The Guinea savannah soil classified as Alfisols and mostly well drained (Salako, 2003; Nwaka, 2012; Esu, 2010) is strongly acidic with pH values of 5.20 prior to planting in 2014 but became moderately acidic after the second trial in 2015 with pH ranges from 5.8 in sole crop plot of *Glycine max* to 6.8 in soybean/*Moringa* inter crop plots. This is an indication that the Moringa alley cropping reduces soil acidity as reported in Undie et al. (2013).

There was a significant reduction (0.03%) in total nitrogen after the first cropping season in 2014, but the trend reversed with a significant improvement (0.07%) after the second cropping season trial in 2015 (Table 2). The trend was similar for all the basic cations being assessed except the elemental sodium value that drastically reduced from 0.90 to 0.38 $Cmol^{-1}$ in *G. max/Moringa* inter crop plots of 50,000 stands of *Glycine* and 10,000 stands of *M. oleifera* in 2015. The result indicated that nutrient loss in the first year of planting, thus the plants did not make significant impact on improving soil fertility. The M. oleifera stands at early stage requires soil nutrients for absorption and adsorption to aid rapid growth, contributing significantly to the soil fertility loss (Undie et al., 2013, Emmanuel et al., 2011).

Table 1. Pre-planting physicochemical properties of the soil in the experimental site.

Elements	Values
Clay	40%
Silt	17%
Sand	43%
Textural class	Clay loam
pH (ratio 1:2:5)	5.80
Organic carbon	2.4%
Total nitrogen	0.05%
Available phosphorus	19.3 ppm
Exchangeable cations	**$cmolkg^{-1}$**
Ca	2.60
Mg	2.22
K	0.63
Na	0.90

Table 2. Physicochemical properties of the soil as influenced by Soybean/*Moringa* intercrop after harvest in 2014.

Year	Treatment		Elements					Exchangeable cations					
	Soybean (Pltsha^{-1})	Moringa (Pltsha^{-1})	Clay	Silt	Sand	pH	Organic	Mg	K	Na	N	P	Ca
2014	40,000	0	40	17	43	5.6	2.5	0.04	15	2.0	2.05	0.62	0.74
	62,500	10,000	40	17	43	5.6	2.4	0.03	18	2.5	2.12	0.62	0.70
	50,000	10,000	40	16	43	5.8	2.5	0.04	15	2.5	2.50	0.65	0.78
	40,000	10,000	39	17	43	5.7	2.5	0.05	15	2.0	2.50	0.65	0.70
	0	10,000		17	43	5.8	2.6	0.05	15	2.0	2.00	0.62	0.79
2015	40,000	0	40	17	43	5.8	2.5	0.05	15	4.0	3.05	0.62	0.54
	62,500	10,000	40	17	43	5.6	2.4	0.03	18	2.5	2.12	0.62	0.70
	50,000	10,000	40	16	43	6.8	2.8	0.07	09	3.5	4.50	0.65	0.38
	40,000	10,000	39	17	43	6.7	2.7	0.06	11	4.0	3.50	0.65	0.40
	0	10,000		15	43	6.6	2.7	0.05	18	4.0	4.00	0.62	0.59

Pltsha^{-1}, Plants/ha.

Although Abdullahi et al. (2014) and Nouman et al. (2013) reports contradicts M. oleifera inhibiting soil fertility improvement and maintenance in alley cropping at first growing season. The contribution within the short period from the Soybean component did not replenish the soil at the first cropping season as reported in Table 2. Alene et al. (2013), Ajeigbe et al. (2010) and Ouédraogo et al. (1999) had reported that Soybean cultivation gradually expands as a result of its nutritive and economic importance and soil fertility enhancement through nitrogen fixation over time. The clay, sand and silt composition of the soil did not change even after the trials, indicating negligible effect on the physical component of the soil by the practice. Thus, the intercropping is not only environmentally friendly but also sustainably maintains the soil fertility and acidity of the experimental area. Matusso et al. (2012) and Okoruwa (2001) reported that inter cropping involving legumes reduces soil temperature and moisture loss, which favours multiplication and growth of some soil microorganisms that will enhance foliage multiplication

In 2014 cropping season, the seed yield of soybean was not significantly (P>0.05) influenced by *Moringa* intercropping (Table 4) except in the intercrop plot of 62,500 stands of soybean/10,000 stands of *Moringa*. The seed yield of soybean was fairly the same in both sole crop plots and inters crop plots in 2014. This non-significant difference in seed yield of soybean in both cropping systems in 2014 confirmed the fact that *Moringa* component in the first few months of its establishment was unable to contribute to the soil fertility or its neighbourhood effect on the component crop. However, in 2015 cropping season, the seed yield of *G. max* in the intercrop plots was 30% greater than that obtained from the sole crop plot.

While the highest (P>0.05) seed yield of *G. max* was obtained from inter crop plot of 50,000 Soybean stands and 10,000 stands of *M. oleifera*, the lowest seed yield was recorded in sole crop plots of soybean in 2015. In both years of cropping, the seed yield of soybean in the intercrop plot of 62,500 of soybean and 10,000 stands of *Moringa* was relatively low, being reduced by 12% in 2015 (Table 3). Also, the leafiness and height of the alley species planted within rows of soybean of 2015 season was slightly higher than the previous season as stated in Table 3. This reduction showed that crop competition in the affected plots was very aggressive indicating that a plant population of 72,500 ha^{-1} will encourage serious crop competition for Soybean and *Moringa* inter cropping venture. Hussain et al. (2013) and Nabavi and Mazaheri (1998) reported decrease in the formation of nitrogen at the nodules on soybean roots was attributed to competition and shading effects under inter cropping situation and the phenomenon consequently reduced the seed yield of soybean. The trend in the seed yield of soybean as influenced by *Moringa* inter cropping was the same as those of its growth characters and yield components.

In the 2015 cropping season, seed yield of soybean, irrespective of treatments was 20% greater than the previous cropping season. During the second year of cropping, the *Moringa* component was well established and the non-harvestable portion were pruned and spread as green manure. The exercise thus may have improved the fertility status of the soil to the benefit of the companion crop as discussed in Larwanou et al. (2014) and Hussain et al. (2013) that *Moringa* leaves extract improves growth and productivity.

Meanwhile the biomass yield of *M. oleifera* in both cropping systems were fairly the same in both 2014 and 2015 respectively (Tables 5 and 6). The result on biomass weight was the same as those of the dry matter and crude oil content of *Moringa* plants. The above results on *Moringa* showed that intercropping with Soybean has no negative effect on its growth and development rather it improved as shown in the inter-crop

Table 3. Growth performance and yield of Soybean intercropped with *M. oleifera.*

Season	Moringa (Popn/ha)	Soybean (Popn/ha)	No. of leaves/plant	Height (cm)/plant	No. of pods/plant	Seed yield (t/ha)
	40,000	-----	25.33	44.51	23	0.32
	62,500	10,000	23.21	38.23	18	0.26
2014 season	50,000	10,000	25.23	45.60	24	0.38
	40,000	10,000	25.45	45.34	24	0.36
	-------	10,000	------	-----	----	------
Mean			**24.81**	**43.42**	**23.25**	**0.33**
LSD P >0.05			**1.09**	**2.43**	**1.87**	**0.021**
	40000	-----	28.45	45.21	18	0.20
	62500	10000	24.81	34.87	25	0.42
2015 season	50,000	10000	27.33	48.45	35	0.68
	40,000	10000	27.67	46.34	35	0.64
	-------	10000	------	-----	----	------
Mean			**27.07**	**43.72**	**28.25**	**0.49**
LSD P>0.05			**1.55**	**1.22**	**4.37**	**0.04**

Popn/ha, Population/ha.

Table 4. Biomass yield (t ha^{-1}/harvest) of *Moringa* intercropped with Soybean, 2014.

Harvest period	Soybean (Popn/ha)	Moringa (Popn/ha)	Fresh biomass	Dry biomass	Crude protein
	40,000	-----	---	-----	
	62,500	10000	5.4	1.54	0.33
70 days	50,000	10000	7.7	2.03	0.38
	40,000	10000	6.7	1.88	0.46
	-------	10000	6.7	1.97	0.43
Mean			**6.63**	**1.86**	**0.40**
	40,000	-----	----	-----	
	62,500	10000	6.0	1.63	0.28
115 days	50,000	10000	4.6	1.32	0.30
	40,000	10000	7.8	2.27	0.26
	-------	10000	7.6	2.19	0.25
Mean			**6.50**	**1.60**	**0.27**
160 days	40,000	-------	---		
	62,500	10000	4.8	1.22	0.13

Table 4. Contd.

	50,000	10000	5.8	1.53	0.12
	40,000	10000	6.4	2.12	0.10
	--------	10000	5.5	2.01	0.13
Mean			**5.63**	**1.72**	**0.12**

Popn/ha, Population/ha.

Table 5. Biomass yield (t ha^{-1}/harvest) of *Moringa* intercropped with Soybean, 2015.

Harvest period	Soybean (Popn/ha)	Moringa (Popn/ha)	Fresh biomass	Dry biomass	Crude protein
	40,000	---	---	----	
	62,500	10000	5.4	1.54	0.33
70 days	50,000	10000	7.7	2.03	0.38
	40,000	10000	6.7	1.88	0.46
	-------	10000	6.7	1.97	0.43
Mean			**6.63**	**1.86**	**0.40**
	40,000	-----	----	-----	
	62,500	10000	6.0	1.63	0.28
115 days	50,000	10000	4.6	1.32	0.30
	40,000	10000	7.8	2.27	0.26
	-------	10000	7.6	2.19	0.25
Mean			**6.50**	**1.60**	**0.27**
	40,000	-------	---		
	62,500	10000	4.8	1.22	0.13
160 days	50,000	10000	5.8	1.53	0.12
	40,000	10000	6.4	2.12	0.10
	--------	10000	5.5	2.01	0.13
Mean			**5.63**	**1.72**	**0.12**

Popn/ha, Population/ha.

plots of 50,000 stands of soybean crops and 1000 stands of *Moringa* plants per hectare. There are several reports on *Moringa* biomass productivity increase during intercropping with other crops, producing mutual relationship that enhances yield and repairs degraded soil through nutrient fixing (Abdullahi et al., 2014; Larwanou et al., 2014; Nouman et al., 2012; Goss, 2012).

The mixture productivity of soybean and *M. oleifera* in the trial is shown in Table 6. The Land Equivalent Ratio (LER) values for intercropping systems (except in 62,500 stands of soybean/ 10000 stands of *M. oleifera* in 2014), were greater than unity in all seasons. Anyaegbu (2014) reported that intercropping *Solanum tubersum*

Table 6. Mixture productivity of Soybean and *M. oleifera* Intercrop in 2014 and 2015.

Soybean population (ha^{-1})	Moringa population (ha^{-1})	2014		2015	
		LER	LEC	LER	LEC
40,000	---	1	---	1	---
62500	10000	0.87	0.23	1.12	0.26
50000	10000	1.23	0.45	1.68	0.78
40000	10000	1.25	0.44	1.57	0.65
---	10000	1	---	1	---

LER, Land equivalent ratio; LEC, land equivalent coefficient.

and *Abelmoschus esulentus* is more advantageous in terms of land economy and productivity when induced with organic manure of *Moringa* leaf extract. Averagely, over the two seasons, the LER was maximum in the intercropping system of 50,000 stands of soybean and 10000 stands of *Moringa* plants. Thus, in this cropping system, inter cropping produced 23 and 68% more yield in 2014 and 2015 than the sole crop yields of both crops. This implied that efficient use of the land using mixture population increased yield and sustain organic fertilization than other treatments. The poor results obtained under 62,500 stands of Soybean and 10000 stands of *Moringa* mixture showed that competitive pressures among the component crops were high. The evidence of the good performance of the crops under the cropping system (50,000 stands of soybean and 10,000 stands of *Moringa*), was shown by their individual Land Equivalent Coefficient, (LEC). A LEC of 78% as recorded in 50,000 soybean/10000 stands of Moringa ha^{-1} in 2015 showed that the neighbourhood effects between and among the crops were significantly beneficial and involved full complementary. Optimization of land resources as evaluated in LEC is developed to assess the interaction and productivity potential of crop mixtures and formulating proportions consists of economic and ecological optimization (Keesman et al., 2007; Yanfang et al., 2002). Amanullah et al. (2016), Yilmaz et al. (2008), and Odhiambo and Ariga (2001) also reported greater LER as greater than 1.0 in maize-common beans intercropping, another leguminous cash crop with high protein value. Intercropping has also been proven to result in efficient use of natural resources (Li et al., 2006). As farmers in semi-arid regions of Kenya have embraced the system over monocropping due to its profitability (over 40% net profit) (Rao and Mathuva, 2000). Hussain (2013) in his paper discussed the advantages of intercropping not only in terms land equivalent ratio but also in weed suppression and sustainable land fertility.

Conclusion

The result of the experiment showed that the total nitrogen and all other basic cations assessed reduced after the first cropping season but improved significantly at the end of the second trial in 2015. The effect of *Moringa* intercropping did not influence seed yield of soybean at the first cropping season until the second cropping season in 2015, the seed yield of all the inter-crop plots was 30% higher than that obtained from the sole crop plot. Highest seed yield (0.68 tha^{-1}) of Soybean and maximum Land Equivalent Ratio (LER) with 23 and 68% more yield in 2014 and 2015 than the sole crop yields of both crops were obtained from treatment plot of 50,000 Soybean stand + 10,000 stand of *M. oleifera*. The relationship between the two plants as assessed by LEC was cordial and complementary. There is the need to investigate the socio-economic analysis of intercropping the two species, especially at this period of price fluctuations and hyperinflation in Nigeria. Thus, an intercropping system involving 50,000 stands of *G. max* and 10,000 stands ha^{-1} of *M. oleifera* is recommended for good seed yield of *G. max* and biomass productivity of *M. oleifera* in the Guinea savannah zone of Nigeria.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Embryo-like structures and root regeneration induced by 2, 4-dichlorophenoxyacetic acid in twenty African sweet potato cultivars

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Embryo-like structures were induced from 'whole leaf' explants of sixteen of the twenty investigated sweet potato cultivars on Murashige and Skoog (1962) medium supplemented with 2,4 Dichlorophenoxyacetic acid (0.2 mg/L) at the Makerere University Agricultural Research Institute Tissue culture laboratory. Shoot and root regeneration was possible in the non-African cultivar, Jonathan, whereas only root regeneration was successfully induced in five African cultivars. Cultivar type had a highly significant ($P < 0.001$) effect on frequency of embryo-like structures and efficiency of both shoot and root regeneration. The embryo-like structures induced could be useful for initiation of cell suspensions to enable genetic transformation of African cultivars. The method for *de novo* regeneration of roots has potential application in the regeneration of plants or hairy root cultures for cultivars that are recalcitrant to shoot regeneration.

Key words: 2, 4-dichlorophenoxyacetic acid, cultivar, *in vitro* recalcitrance, regeneration efficiency, somatic embryogenesis.

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is mainly cultivated for its expanded edible roots which contain high carbohydrate and beta-carotene, depending on cultivar. The total sweet potato production for the world is 103.1 million metric tons (USDA, 2015). However, the potential production for sub-Saharan Africa is not

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attained, as yields are very low at about 5 t/ha (Luo et al., 2006). The low yields are mainly due to various challenges including high incidence of virus disease and increasing attacks by sweet potato weevils (Fuglie, 2007).

For a long time, traditional breeding has contributed to the improvement of crop traits. Nevertheless, due to the high male sterility, self and interspecific incompatibility and hexaploid (2n = 6x = 96 chromosomes) nature of sweet potato, conventional hybridization is still difficult, and hybrid progenies undergo segregation leading to loss of many valuable traits (Yang et al., 2011). Genetic transformation is a promising tool to improve sweet potato traits like nutrition and resistance to weevils and viruses (Ghislain et al., 2008; Kreuze et al., 2008; Shimada et al., 2006). Genetic transformation enables access to genes from unrelated species thereby enabling breeders to improve plants with traits that are not available in the crop germplasm (Ghislain et al., 2008).

Efficient development of transgenic plants requires optimal transformation and regeneration conditions. Although, successful induction of somatic embryogenesis has been reported in sweet potato, most of the published protocols are cultivar-specific (Santa-Maria et al., 2009). Low embryogenic frequencies and complete recalcitrance has been reported for most important and adapted sweet potato cultivars (Al-Mazrooei et al., 1997; Liu et al., 2001; Zang et al., 2009), particularly for African cultivars (Luo et al., 2006). The non-African cultivars that have been transformed so far, including Jewel (Luo et al., 2006), Huachano (Kreuze et al., 2008), White Star (Gama et al., 1996), Kokei 14 (Otani et al., 1998), Yulmi (Choi et al., 2007) and Lizixiang (Yu et al., 2007), can only be used for research purposes in Africa. These are not farmer-preferred cultivars due to several drawbacks, such as low dry matter and vulnerability to diseases and pests (Yang et al., 2011). Hence, the development of an efficient *in vitro* regeneration and transformation method for farmer-preferred sweet potato varieties in sub-Saharan Africa is important and urgently required.

The objective of the study reported here was to screen a large number of farmer-preferred sweet potato cultivars for the response to somatic embryogenesis induced by 2,4-Dichlorophenoxyacetic acid (2,4-D). Published reports show that there is a correlation between the quality and quantity of embryo-like structures and subsequent plant regeneration in sweet potato (Al-Mazrooei et al*.,* 1997).

MATERIALS AND METHODS

Twenty farmer-preferred Ugandan sweet potato cultivars and one USA cultivar, Jonathan, were used in this study. These cultivars as shown in Table 1 were selected based on preferred attributes among consumers in Uganda, including high dry matter content (Mwanga et al., 2007; Mwanga et al., 2001). The non-African cultivar, Jonathan, was previously reported to regenerate through somatic embryogenesis (Cipriani et al., 1999). The cultivars were grown in a screenhouse at Makerere University Agricultural Research Institute, Kabanyolo. Vines from the screen house grown plants were grafted on *Ipomoea setosa,* an indicator plant for virus infection (Figure 1). Cuttings from those sweet potato plants corresponding to scions whose indicator plants were asymptomatic were used in establishment of virus-free *in vitro* cultures.

Cuttings containing five to eight nodes were taken from the greenhouse to the laboratory. The cuttings were washed for fifteen minutes with flowing tap water and then immersed for 20 min in 39% (v/v) commercial JIK containing 3.85 % sodium hypochlorite (NaOCl) and 0.03% Tween 20®. Thereafter the vines were submerged in 70% ethanol for 2 min. After surface sterilization, the vines were transferred to sterile water and rinsed three times. They were then each cut into one or two nodes and inserted into sweet potato propagation medium.

The propagation medium was composed of MS (Murashige and Skoog, 1962) salts premix (4.3 g l^{-1}), sucrose (30 g/L), myo-inositol (0.1 g/L), 5 ml/L sweet potato vitamin stock comprised of 40 g/L ascorbic acid, 20 g/L L-arginine, 4 g/L putrescine HCl, 0.01 mg/L gibberellic acid (GA_3) and 0.4 g/L calcium pantothenate (Kreuze et al., 2008). All reagents used in this study were sourced from Sigma-Aldrich. The medium was adjusted to pH 5.8 before adding 3 g/L phytagel and autoclaved at 121°C for 15 min under 15 kPa.

Four-week-old whole leaves (petiole with lamina) from the *in vitro*-grown plants were placed on callus induction medium (CIM) under dark conditions at 28°C. The cut end of the petiole was slightly pushed into medium. CIM was made of 4.3 g/L MS premix (without vitamins), 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 1 ml/L Vitamins stock (0.5 mg/ml nicotinic acid, 0.1 mg/ml thiamine and 0.5 mg/ml pyridoxine), 100 mg/L myo-inositol, 30 g/L sucrose and 7 g/L agar. The cultures were transferred to fresh media every two weeks. A total of ten to fifteen explants were used per cultivar in each petri dish, and this was replicated three times and arranged in a completely randomized design. After twelve weeks the callus formed was transferred to embryo initiation medium (EIM) under a photoperiod of 16 h, regardless of it being embryogenic or non-embryogenic. EIM was made as CIM but 2, 4-D was replaced with 1 mg/L abscisic acid (ABA). After four weeks on EIM, the callus was transferred to plant regeneration medium devoid of plant growth regulators. Fully developed plants were transferred to soil in a screen house where they developed normally.

Data on number of explants forming callus and those forming embryo-like structures were collected after twelve weeks. Data on shoot and root regeneration was collected every week since some callus formed roots within three weeks of culture initiation. Cultures were discarded after twenty-four weeks since at this stage most calli lost their embryogenic capacity and could not regenerate shoots. Frequency data was transformed using the arcsine square root before analysis to stabilize the variance. The table presented in this paper shows non-transformed data expressed as percentages. GenStat statistical package was used to analyse the effect of cultivar on total callus proliferation and formation of embryo-like structures, which was performed using general linear model of analysis of variance (ANOVA) at 5% level of significance. Mean values were compared using the least significance difference (LSD) method at the 5% level.

RESULTS

In the present study, explants placed on callus induction medium responded by producing callus within three days. The callus was not only on the wounded tip of the petiole but also on the intact leaf lamina (Figure 2B). Two types of callus were induced; embryogenic and non-embryogenic. The embryo-like structures were bright

Table 1. Some attributes of Ugandan cultivars selected for *in vitro* regeneration and genetic transformation.

Cultivar	Flesh color	SPVD resistance	Maturity (Days)	Dry matter (%)	Year of release and specific location of collection/release in Uganda
Kakamega	Intermediate orange	M	135	33	2004 in Uganda. Kenya landrace
NASPOT 1	Cream	M	135	32	1999 (bred clone)
New Kawogo	Cream	R	140	32	1995 (Landrace), Germplasm (1988)
Bwanjule	White/Cream	M	135	30	Landrace/ 1995, Germplasm (1988)
Araka	Cream/white	F	105	32	Landrace: Germplasm 2005
NASPOT 8	Pale orange	M	120	32	2007
Dimbuka-Bukulula	Cream	S	128	32	2001, Germplasm from Masaka
Silk omupya	White	R	100	34	Germplasm from Pallisa
Kyebandula	Cream	M	120	32	Landrace: Germplasm -Mbale
Munyeera	Cream	R	165	33	Landrace: Germplasm from Mpigi
Ssemanda	Cream/white	R	120	33	Landrace: Germplasm from Mpigi
Kisakyamaria	Cream	S	120	32	Landrace: Germplasm from Mbarara
Luwero Silk	Cream	S	120	34	Landrace: Germplasm -Luwero
Namusonga	Cream	F	120	34	Landrace: Germplasm from Pallisa
Magabali	Cream	R	165	33	Landrace: Germplasm-Kabale
Kigaire	Cream	M	120	32	Landrace: Landrace Soroti/Serere
Nyidoyamulalo	Cream	R	120	32	Landrace: Germplasm-Kamuli
UNK-Luwero 2 (Uknown)	Cream/white	S	120	33	Landrace: Germplasm -Luwero
UNK Luwero (Unknown)	White	M	165	30	Landrace: Germplasm -Luwero
Jamada	Cream	M	150	32	Landrace: Germplasm -Luwero

Note: all cultivars are adapted to Uganda with potential for adoption in other areas of East Africa. SPVD sweet potato virus disease resistance M = Moderate resistance, R = resistant, S= Susceptible, F=Fair, Dry matter content (%) = per cent of fresh weight.

Figure 1. Preparation of plant material for somatic embryogenesis experiments. **A**, sweet potato cultivars growing in greenhouse ready for bio-indexing. **B,** Bio-indexing of sweet potato (scion) by grafting on *Ipomoea setosa* (rootstock) indicator plants. **C,** *In vitro* cultures growing from virus-free and decontaminated plants. **D,** "Whole leaves' used for callus induction on medium supplemented with 2, 4-D.

yellow or orange in colour and compact in appearance, while non-embryogenic callus was white and friable and proliferated earlier and more rapidly than embryo-like structures. The embryo-like structures were induced in sixteen of the twenty investigated African cultivars cultured on media with 0.2 mg/L 2,4-D. Cultivar type had a highly significant ($P < 0.001$) effect on embryo-like structures induced (Table 2). The frequency of embryo-like structures ranged from zero to 25% depending on cultivar (Table 2). Embryo-like structures started developing within six weeks of initiation of cultures.

Somatic embryos of cv. Jonathan developed into shoots after three weeks on ABA-containing media. Cultivar type also had a highly significant ($P < 0.001$) effect on the ability to regenerate shoots (Table 2). Regeneration of roots was achieved from callus of five African cultivars. Contrary to the regeneration of shoots which occurred through somatic embryogenesis, the regeneration of roots appeared to be so through organogenesis as they were formed directly from callus without somatic embryos. In addition, the roots were regenerated earlier than shoots, sometimes, as early as three weeks after culture initiation on 2, 4-D-containing media. The emergence of new roots was sustained throughout the culture period. The type of cultivar showed

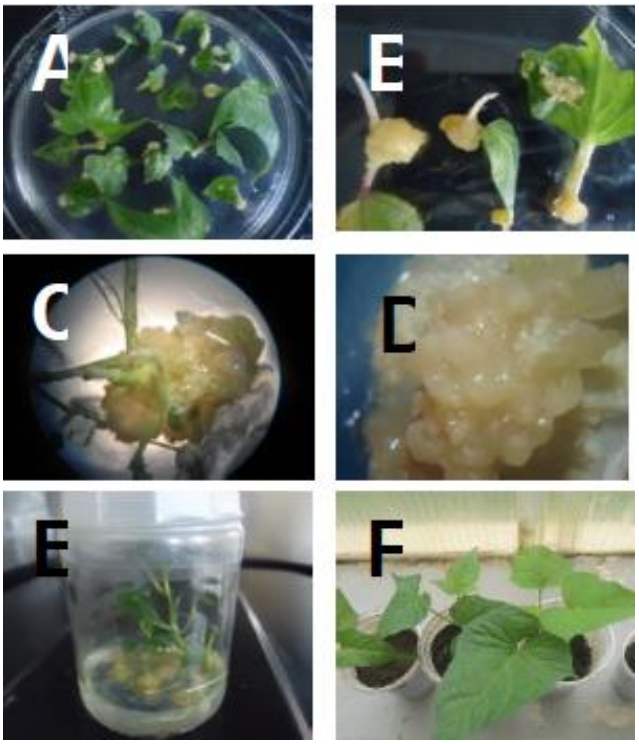

Figure 2. Induction of embryo-like structures and regeneration of shoots and roots in African sweet potato cultivars. **A**. Induction of callus from the base of petioles attached to leaves. **B.** Root regeneration from callus before development of embryo-like structures. **C.** regeneration of multiple roots from callus after 5 weeks on callus induction medium. **D.** Development of embryo-like structures after placement of embryogenic callus on ABA-supplemented medium. **E.** Regeneration of shoots from callus of cv. Jonathan after transfer to plant regeneration medium. **F.** Survival of plants after transfer from in vitro cultures to soil in screen house.

a highly significant ($P < 0.001$) effect on the ability to regenerate roots (Table 2).

DISCUSSION

Sweet potato has for long been considered recalcitrant to somatic embryogenesis (Yang et al., 2011). Most of the reported protocols are cultivar specific, and have low regeneration frequencies which are also difficult to reproduce (Santa-Maria et al., 2009; Yang et al., 2011; Yu et al., 2007). In the present study, the auxin 2, 4-D was used for callus induction and embryo-like structures was achieved in sixteen of the investigated twenty African cultivars. These cultivars have not been reported to respond to somatic embryogenesis in previous publications. Luo et al. (2006) reported that African cultivars were difficult to regenerate, but did not name the cultivars tested or their geographical location.

The induction of somatic embryogenesis with 2, 4-D appears to be preferable in view of existing literature (Yang et al., 2011). Liu et al. (2001) induced embryo-like structures from shoot apices of fifteen cultivars from China and Japan on medium supplemented with 9.05 mM 2, 4-D. The embryogenic response of these cultivars was low and so variable ranging from 6.7 to 85.2% (Liu et al., 2001). Similarly, Sihachakr et al. (1997) found large differences and low frequency of embryo-like structures among ten sweet potato cultivars exposed to auxin (10 μM 2, 4-D) treatment. Frequencies ranged from 0 to 17% response when using lateral buds as explants (Sihachakr et al., 1997). The frequency of embryo-like structures in the current study ranged from no response at all to 25% depending on cultivar (Table 2). In contrast to somatic embryogenesis protocols, most auxin-cytokinin shoot organogenesis protocols have replaced 2, 4-D with other auxins in order to avoid the callus stage and achieve rapid regeneration (Luo et al., 2006, Gong et al., 2005; Santa-Maria et al., 2009).

Although, somatic embryogenesis is a complicated process to manage than shoot organogenesis (Luo et al., 2006; Newell et al., 1995), the regeneration of sweet potato through somatic embryogenesis is still a preferred option than shoot organogenesis (Yu et al., 2007). This is mainly because somatic embryogenesis leads to high efficiency of selection of transgenic plants on medium and avoids the regeneration of chimeric plants after genetic transformation (Song et al., 2004; Yang et al., 2011). Recently, there have been some reports of applying somatic embryogenesis using cell suspensions (Yu et al., 2007; Zang et al., 2009). However, other reports suggest that the use of liquid cultures is extremely complicated and depends on availability of cell suspension cultures (Song et al., 2004; Zhai and Liu, 2003). By opting for solid medium in this study, it was possible to deploy explants which are easy to access and are readily available as noted in previous reports (Cipriani et al., 1999; Kreuze et al., 2008; Song et al., 2004). The use of solid medium for somatic embryogenesis has allowed many researchers to use various plant organs as explants for example, leaf discs (Newell et al., 1995), stem internodes (Song et al., 2004), root explants (Liu and Cantliffe, 1984), root discs (Newell et al., 1995) and lateral buds (Cavalcante Alves et al., 1994).

The regeneration of roots in this work could be useful for the initiation of root cultures, which could serve as a source of tissue for regeneration of plants or hairy root cultures (Jones et al., 2007; Dodds et al., 1991). However, it remains difficult to explain this type of morphogenesis which was evident as early as three weeks after culture initiation on 2, 4-D-supplemented medium. It is likely that the explants of the cultivars had high levels of endogenous auxins. If this is the case then the exogeneous auxin 2, 4-D only accelerated the root regeneration process (Becerra et al., 2004; George et al., 2008). Many factors have been implicated in the unpredictable responses in culture, including variations in the developmental and physiological stage of *in vitro*

Table 2. Embryogenic callus formation from 'whole leaf' explants of 21 sweet potato cultivars and regeneration of roots and shoots.

Cultivar	Explants tested (No.)	Explants inducing callus		Explants Inducing embryogenic callus		Explants regenerating roots		Explants Regenerating shoots	
		No.	%	No.	%	No.	%	No.	%
Naspot 1	13.3 ±0.3	10±1.5	75.0±10.4	0±0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Dimbuka	13.7 ±0.7	12.3±0.7	90.2±4.8	1.3±0.7	9.8±4.8^{bcde}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Semanda	14.3 ±0.3	12.3±1.8	86.0±10.9	0.3±0.3	2.3±2.2^{ab}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
New Kawogo	13.3 ±1.2	12±1.5	90.0±3.8	3.0±0.6	22.5±6.7^{ef}	1.0 ± 0.6	7.5±3.9^{b}	0.0 ± 0.0	0.0 ± 0.0^{a}
Bwanjule	14 ±0.6	12±1.0	85.7±4.3	1.3±0.9	9.5±6.8^{bcde}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Namusoga	14.7 ±0.3	11.3±0.3	77.3±2.0	0±0.0	0.0±0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Jamada	13.7 ±0.9	10.7±1.5	78.0±5.8	0.3±0.3	2.4±2.8^{ab}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Magabali	14.7 ±0.3	12.7±1.3	86.4±10.2	3.7±0.3	25±1.7^{f}	2.0 ± 0.6	13.6±3.7^{cd}	0.0 ± 0.0	0.0 ± 0.0^{a}
Kyebandula	14 ±0.6	12.3±0.3	88.1±2.1	2.7±0.3	19.0±3.0^{def}	1.3±0.9	9.5 ± 5.9^{bc}	0.0 ± 0.0	0.0 ± 0.0^{a}
Jonathan	14 ± 0.6	11±0.6	78.6±7.4	2.3±0.3	16.7±1.8^{cdef}	3.0±0.6	21.4 ± 3.9^{d}	1.3 ± 0.9	9.5 ± 6.8^{b}
Kakamega	14.7±0.3	11.3±2,2	77.3±13.5	0.0 ± 0.0	0.0±0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Araka red	13.7±0.3	10.7±1.3	78±10.8	0.7±0.7	4.9±4.8^{ab}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Naspot 8	14.3±0.7	12.0±0.6	83.7±2.0	0.0±0.0	0.0±0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Munyeela	13.7±0.9	11.0±1.0	80.5±7.7	0.7±0.7	4.9±4.4^{ab}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Kisakyamaria	13.3±0.9	9.7±0.9	72.5±3.0	0.0 ±0.0	0.0±0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Silk omupya	13.7±1.3	9.7±1.7	70.7±9.7	1.3±0.7	9.8±5.4^{bcdef}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Luwero Silk	14.3±0.3	10.3±0.7	72.1±4.2	0.0 ±0.0	0.0±0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Kigaire	13.7±0.9	9.7±0.7	70.7±5.7	0.7±0.3	4.9±2.5^{abc}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Nyidoyamulalo	14.3±0.3	10.7±0.7	74.4±5.7	0.3±0.3	2.3±2.4^{ab}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}
Luwero 2 (uknown)	14.7±0.3	13.3±0.7	90.9±2.5	1.3±0.9	9.1±5.9^{abcd}	1.0 ± 0.6	6.8 ± 4.1^{b}	0.0 ± 0.0	0.0 ± 0.0^{a}
Luwero (uknown)	14.3±0.7	10.0±1.0	69.8±3.9	1.0±0.6	7±4.5^{abcd}	0.0 ± 0.0	0.0 ± 0.0^{a}	0.0 ± 0.0	0.0 ± 0.0^{a}

F-test	Explants inducing callus (%)	Explants inducing embryogenic callus (%)	Explants regenerating roots (%)	Explants regenerating shoots (%)
Cultivar	ns	< 0.001	< 0.001	< 0.001

Data represent means ± standard errors for three replicates (10 to 15 explants each). Values followed by the same letter in each column are not significantly (ns) different at the P ≤ 0.05 level (LSD test).

plants (Triqui et al., 2007). Previous reports indicate that ABA is required for induction, maturation and synchronization of somatic embryos leading to elongation into shoots (Anwar et al., 2010; Song et al., 2004; Triqui et al., 2007). However, in the current study, two Ugandan cultivars, Magabali and Kyebandula that induced more embryo-like structures than the embryogenic callus reported for the USA cultivar, Jonathan, did not regenerate shoots. The failure of ABA to induce somatic embryos or promote development of preformed somatic embryos in the sixteen African cultivars that successfully induced embryo-like structures could be attributed to cultivar differences in the response to ABA or the sustained effect of 2,4-D as reported previously (Becerra et al., 2004; George et al., 2008; Santa-Maria et al., 2009).

In conclusion, this study has identified up to sixteen African sweet potato cultivars which were able to induce embryo-like structures on medium supplemented with the potent auxin 2,4-D (0.2 mg/L). The embryo-like structures induced in this study could be useful for the initiation of embryogenic cell suspensions which are not readily available for transformation of most sweet potato cultivars (Yang et al., 2011). The method

for *de novo* regeneration of roots may be applicable in the regeneration of plants or hairy root cultures. A possibility exists that the use of other types of plant organs as explants and various concentrations of 2, 4-D or other auxins could improve somatic embryogenesis in the cultivars investigated. This view is now being pursued as a follow-up study. The final goal of this work is to use the somatic embryogenesis protocol for *Agrobacterium*-mediated transformation in order to improve sweet potato traits.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Maturity indices for tomato (*Solanum lycopersicum* L.), cv. Ghalia 281 in Central Uganda

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Application of maturity indices and optimal harvest time improves handling and marketing operations and minimizes pre and postharvest losses for tomato products. Growth patterns of tomato (*Solanum lycopersicum* L.), cv. Ghalia 281, were therefore analyzed to determine nondestructive maturity indices for optimal harvest regimes. Propagation experiments were run in central Uganda in 2015. A total of 216 tomato fruits were tagged and their diameter, height and color recorded daily prior to physico-chemical and nutritional analyses at Makerere University Food Science and Technology laboratory. The longest fruit (5.55 cm) was from breaker, while the shortest (4.95 cm) was in light red samples. Total soluble solids were highest at breaker (5.40 °Brix) and red (6.00 °Brix) and was lowest at turning stage (4.00 °Brix). Green tomatoes had the highest carbohydrate content of 5.99 g/100 g, followed by breaker tomatoes with 5.71 g/100 g, while the lowest CHO (4.17 g/100 g) was observed from tomatoes at turning stage. The pH decreased from 4.98 (green) to 4.60 (light red). Protein content was highest (13.05%) from red tomatoes and lowest in pink samples (10.22%). Fruit diameter was negatively correlated with fruit color (r = -0.748, *P*≤0.05) and °Brix (r = -0.787, *P*≤0.05). A highly negative correlation occurred between fruit age and pH (r = -0.949, *P*≤0.05). There was a high positive correlation between fruit diameter and total titrable acidity (r = 0.959, *P*≤0.05). Optimal harvesting should occur at breaker for distant markets and fruit with red outer colour be proposed for local consumers. Maturity indices for determining harvest time of tomato is a combination of fruit age, diameter and color, because these correlate significantly with physico-chemical and nutritional characteristics including total soluble solids (TSS), total titrable acidity (TTA) and protein content.

Key words: Color, fruit diameter, maturity index, *Solanum lycopersicum*, total soluble solids, vegetables.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the highly valuable horticultural crops in the world (Pinho et al., 2011; Caron et al., 2013; Araujo et al., 2016). According to the Food and Agricultural Organization of the United

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Nations (2014), the world production of tomato reached 161.8 million tons representing 33.7 t/ha of cultivated area in the year 2012. Tomatoes are preferred for consumption due to a low acid index, high reducing sugars content, suitable fruit flavour and great culinary versatility (Junqueira et al., 2011; Beckles, 2012). In addition, Carvalho et al. (2005) consider tomato fruit as the main source of lycopene in the human diet because of its strong antioxidant action that helps prevent degenerative and heart diseases and some types of cancer.

In Uganda, there has been a shift from production of traditional staple food crops to high value quick maturing crops like tomatoes (IPC, 2017). Tomato has highly important commercial and subsistence values in Uganda (Muzaale, 2014; YAP, 2016). However, tomato is a climacteric fruit. This implies that harvesting at an optimal stage gives the productive and commercial sectors greater flexibility for its management (Caron et al., 2013). Tomatoes develop their full characteristic flavor, taste and color during storage if picked during an optimum period. Tomatoes harvested at an early stage of maturity are susceptible to shrivelling and mechanical damage and develop poor flavor and taste, despite having long storage life (Mattheis and Fellman, 1999; Beckles, 2012). Harvesting at an advanced stage of maturity produces fruit that have good taste and flavor but have a short storage life and are not suitable for transporting for long distances (Dadzie and Orchard, 1997).

Thus, farmers ought to schedule the harvesting at optimum maturity periods to ensure quality and obtain good market price, followed by correct handling and packing of fruit (Rajkumar et al., 2012). Despite this, most farmers, especially those operating on a small scale, face challenges, among which are choice of right varieties, ineffective transport to distant markets and high perishability of tomatoes exacerbated by harvesting at improper maturity stages due to farmers' limited knowledge of maturity indicators (Kato, 2011; Muzaale, 2015).

Even then, there have been attempts (Wanitchang et al., 2011; Rajkumar et al., 2012) to examine non-destructive indices for assessing maturity of fresh fruit. Matsuda et al. (2010) studied optimal harvest time for transgenic tomatoes and Zhang and McCarthy (2012) used magnetic resonance imaging to evaluate tomato maturity. However, these methods could present high technical and financial burden to the small scale farmers in Uganda. Additionally, studies on vegetables in Uganda have focused on pests and diseases (Tushemereirwe et al., 2000, 2004) and postharvest losses. Studies on optimum harvest regimes for vegetables have not been accorded much attention. This could partly explain the increasing postharvest losses, low income and food insecurity in Uganda (IPC, 2017).

This study was therefore aimed at investigating the maturity indices for optimal harvest of one of the newly introduced but commercially viable tomato (*Solanum lycopersicum* L.) c.v. Ghalia 281 to enable small scale farmers determine harvest time based on technically and financially feasible maturity indices. The specific objectives of the study were to (i) analyze the morphological development of tomato fruit, (ii) assess the maturity and ripening stages of tomato fruit and (iii) determine the nondestructive maturity indices for optimal harvest of tomato in Central Uganda.

MATERIALS AND METHODS

Experimental site

The tomato cv. Ghalia 281, was propagated at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) which is located at 0°27'60''N, 32°36'24''E at an altitude of 1,250 to 1,320 m above mean sea level (Figure 1). The area receives an average annual precipitation of 1,218 mm and slightly drier periods in June to July and December to February (Komutunga and Musiitwa, 2001). The mean annual temperature is 21.5°C.

The Kabanyolo soils are formed on residuum and colluvium from quartzites, gneiss and basement complex rocks. Colluvium enriched with lateritic gravel is common on the side slopes of MUARIK (Yost and Eswaran, 1990). The soils are clayey, acidic (pH of 6.08 to 6.12) with low organic matter content and deficient in most minerals including P, Ca, K, Mg, Na and N. MUARIK is reported to be suitable for growing cabbage and pumpkin. However, for improved growth, yields and maturity of tomato, the Kabanyolo soils require amendments with lime (Okiror et al., 2017). In this study, the soils were not amended in order to mimic the practices of small scale farmers in Uganda (Nyombi et al., 2010).

Tomato propagation and sampling

Seedlings were produced following the methods described by Pinho et al. (2011). The soils were ploughed and loosened using hand hoes. Manual watering was done on days in which the site did receive rains as done by most small-scale farmers in Uganda (Kato, 2011). The trials were run between May and August 2015. Tomato seedlings were transplanted manually using hand hoes into pre-made holes in 3 randomized blocks on 26 May 2015. As is the case with most local small scale farmers (Muzaale, 2014, 2015; Ogundare et al., 2015), tomatoes were established in the field without application of any fertilization at transplanting. There is generally low use of chemical fertilizers by the Ugandan smallholder farmers mainly due to perceived high cost, poor availability, and lack of knowledge related to their use (Nyombi et al., 2010).

Two (2) healthy growing plants were tagged in each of the three slope points of the block (shoulder, back-slope and foot-slope) and data on leafing, root collar diameter, plant height, pest and disease incidence and weeding and pruning were recorded until flower emergence that occurred between 15-16 weeks after transplanting. After flower emergence, fruit development was monitored in all the 18 tagged plants. On the first and second branch of the tagged tomato plants, fruits were tagged and fruit diameter, height, color and pest and disease incidence were recorded daily. This was because each branch could yield 4 to 8 fruits. From the selected branches, two (2) tomato fruits were harvested from each of the 6 fruit maturity stages, that is, green, breaker, turning, pink, light red and red, and labelled and packed in a cooler box to avoid manual contact. Tomato fruit selection was based on the external colour classification for fresh tomatoes by the United States Department of Agriculture (USDA, 1991). Therefore, 216 fruits were sampled

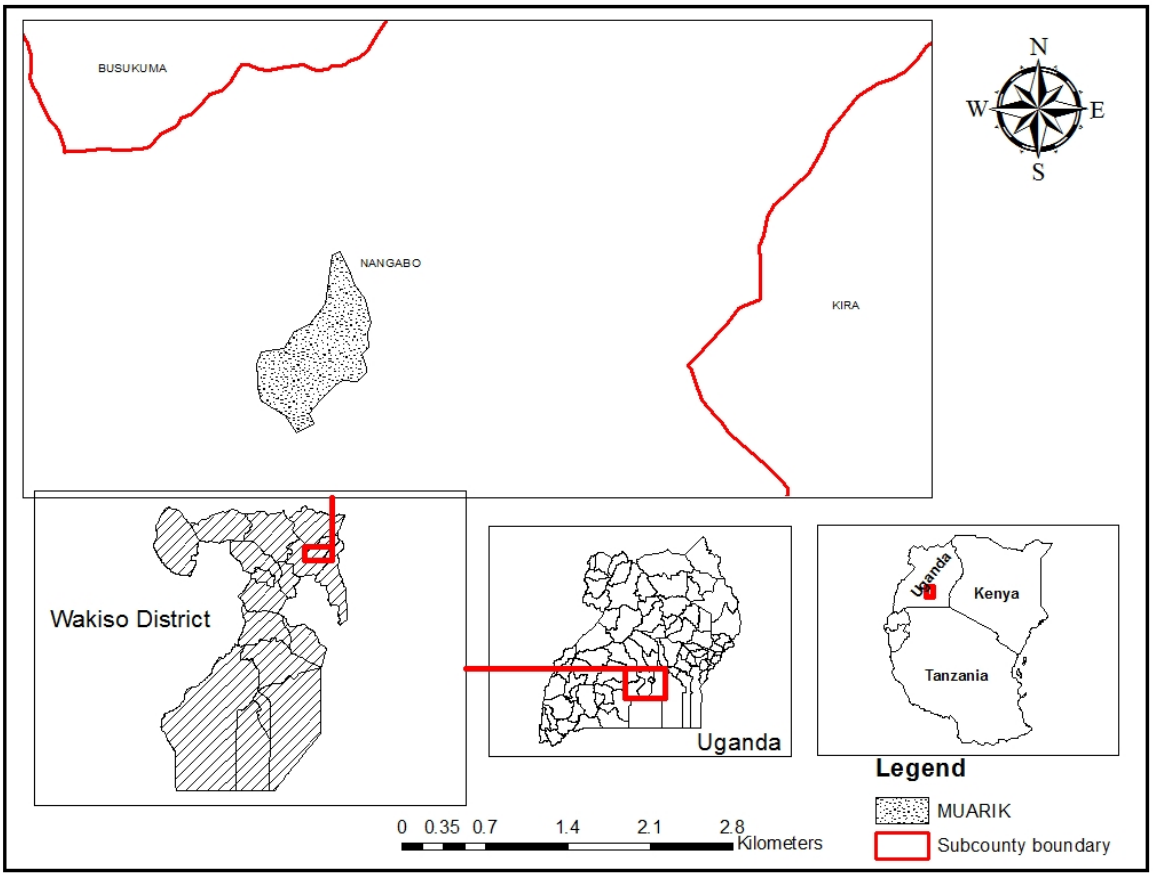

Figure 1. Map of East Africa showing the location of the study site at MUARIK, Wakiso district, Central Uganda.

representing 2 replicates of 6 maturity stages and 2 plants for 3 slope positions in 3 blocks. Fruits were sampled in the morning following the uniformity of color, size and absence of disease and injury. Samples were delivered to Makerere University Food Science and Technology laboratory for physico-chemical and nutritional analysis.

Laboratory analyses

Physico-chemical (pH, moisture and dry matter content) and nutritional (protein, total soluble solids, carbohydrates, total titrable acidity, Fe, Mn, Ca and K) composition were determined according to the methods described by AOAC (2000) and Okalebo et al. (2002).

Internal colour of tomato samples was determined using the Lovibond apparatus. The red, yellow and blue colour units were adjusted until a perfect colour match was obtained. The value of the colour with the lowest unit was subtracted from the rest of colours leaving two units which were then used to describe the colour of the sample. Colour was described using the nomenclature, notably; red, orange (combination of red and yellow), yellow, green (combination of yellow and blue), blue and violet that is derived from red and blue (Okia et al., 2013).

Data were entered in Microsoft Excel and subjected to analysis of variance (ANOVA) comparisons at 5% significance level. The Pearson's correlations (r) were run to establish the relationships between morphological, physico-chemical and nutritional maturity indices and to ascertain the optimal maturity indices.

RESULTS AND DISCUSSION

Fruit development

The fruit development curve for tomato cv. Ghalia 281 was determined (Figure 2). Following flower emergence, there was a rapid increase in fruit diameter and length in the first week, before it decreased in week 2. Further increase in fruit length occurred in week 3, reaching maximum towards the end of week 3 of fruit maturation. Diameter increased at a lower rate and plateaued within week 3 before harvest (Figure 2). Samples were harvested within 8-11 weeks after the transplanting date.

According to Wu and Kubota (2008), tomato fruit enlarge with time after anthesis during the green stage, reach maximum size at around the end of the green stage and hardly change in size after the breaker stage through the red stage as demonstrated in this study. This implies that tomato cv. Ghalia 281 can be promoted for large scale production by farmers in Uganda, thereby bridging the widening income and food security gaps in the country. Chester (2004) and Lovejoy (2016) indicated that several tomato varieties are ready for harvest between 6-11 weeks following transplanting. This study indicates that fruit development trends for cv. Ghalia are

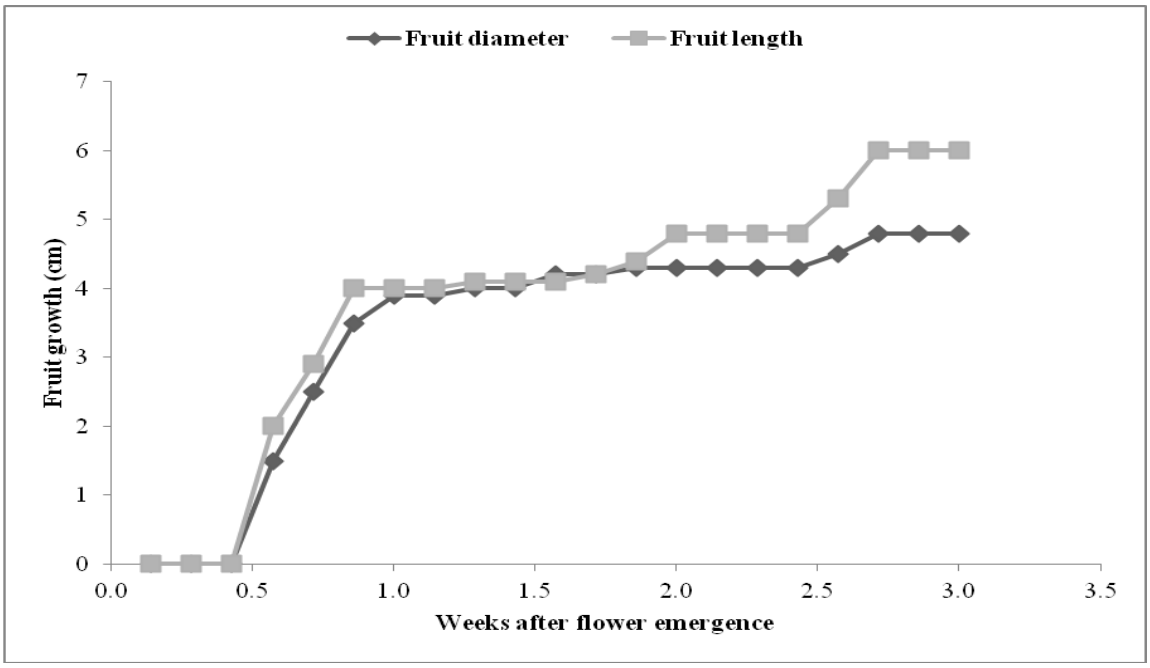

Figure 2. Fruit development of tomato, cv. Ghalia 281, in 2015.

Table 1. Maturity indices of tomato, cv. Ghalia 281, at different harvest stages[a].

Parameter	Unit	Harvest stage					
		Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Fruit diameter	(cm)	4.35±0.25	4.30±0.20	4.70±0.10	4.65±0.05	4.40±0.10	4.30±0.10
Fruit length	(cm)	5.30±0.20	5.55±0.25	5.15±0.05	5.15±0.05	4.95±0.15	5.10±0.10
TSS[b]	(°Brix)	5.00±0.59	5.40±0.59	4.00±0.59	5.00±0.59	5.00±0.59	6.00±0.59
MC[c]	(%)	93.72±0.22	92.73±0.03	94.44±0.02	93.95±0.08	93.79±0.12	93.40±0.43
CHO[d]	(g/100g)	5.99±0.20	5.71±0.26	4.17±0.64	4.32±0.16	4.36±0.01	5.26±0.01
Color	(Lovibond scale)	Yellow/green 4.00±0.00	Yellow/green 4.00±0.00	Yellow/green 3.35±0.35	Yellow/orange 3.07±0.09	Red/orange 4.97±0.21	Red/orange 5.47±0.24
pH	(-)	4.98±0.01	4.85±0.01	4.69±0.01	4.65±0.01	4.60±0.03	4.64±0.00
Protein	(%)	11.05±2.47	10.89±0.00	11.32±0.13	10.22±0.50	10.51±0.02	13.05±0.66
TTA[e]	(g/100 g)Citric acid	0.43±0.07	0.45±0.01	0.55±0.10	0.57±0.09	0.46±0.05	0.41±0.03
Maturity stage	Observed color[f]	Green	Breaker	Turning	Pink	Light red	Red

[a]Data means ± standard deviation; [b] TSS = total soluble solids; [c] MC = moisture content; [d] CHO = carbohydrates; [e] TTA = total titrable acidity; [f]USDA (1991)

consistent with earlier findings by Robinson (1996), Dadzie and Orchard, (1997) and Mattheis and Fellman (1999). Suryawanshi (2014), indicated tomatoes to be ready for harvest in at least 8.5 weeks following transplanting. Study samples were harvested within 8-11 weeks after transplanting pointing to the fact that cv. Ghalia 281 can be harvested within the generally acceptable time frame. The variation in times of harvest may be explained by the differences in soil, weather and pest and disease incidence across study sites. This study therefore recommends wide scale production of cv. Ghalia 281 to address the increasing demand for vegetables in Uganda (IPC, 2017).

Maturity and ripening stages

As tomato fruits progressed in maturity, they took on a bell shape with a peak at turning but at breaker and red the diameters were equal (Table 1); the second greatest diameter was observed at pink stage. Fruit length was highest in breaker, followed by green and lowest in light red stage. Mean TSS values at turning were lower than at breaker and red. Tomato moisture content was similar

from breaker to pink. The highest carbohydrate content was obtained from green followed by breaker, and lowest at turning. The pH of tomato was generally acidic, decreasing from green, through light red. The highest protein content occurred at red and the lowest was from pink tomatoes. Total titrable acidity was highest in pink and red followed by turning. The morphological traits namely; color, diameter and length, and physico-chemical characteristics including pH, total soluble solids, total titrable acidity, carbohydate and protein content were best at breaker and red (Table 1). According to Zhang and McCarthy (2012), tomato maturity relates with quantifiable parameters including firmness and color which reflect the biochemical changes during ripening. In this study, breaker tomatoes had yellow green color, while the fully mature tomatoes were red. Usually, tomatoes are harvested at breaker stage for distant markets and fully ripe for local markets because the right stage of maturity influences storage life and quality and acceptance of fruit by consumers (Dadzie and Orchard, 1997).

The changes in fruit diameter and length are expected as tomatoes mature. Zhang and McCarthy (2012) stated that tomato ripening is usually associated with a number of variations: the cellular structure and internal structure of the fruit. In addition, the most significant visual changes in the morphological characteristics of the fruit during maturation occur in the size, shape, length and volume of the fruit as it advances in age (Dadzie and Orchard, 1997). This therefore means that fruit length and diameter are important indices for determining tomato maturity.

According to Gould (1974), fruit with pH values of 4.5 have appreciable aroma and taste. The optimal harvest regimes had TTA content generally decrease from turning to red samples. Titratable acidity decreases with ripening of tomato fruit (Gautier et al., 2008). Changes in pulp pH and total titratable acidity during maturation have been reported by Dadzie and Orchard (1997). However, the trend tends to vary with cultivars, in that, some cultivars are characterized by a decrease in pulp pH and increase in titratable acidity as fruits advance in age, while in some cultivars, there are no significant changes in pulp pH and titratable acidity during fruit maturation. Thus, pulp pH and titratable acidity could be used as indicators of maturity for tomato cv. Ghalia 281. The determination of pH and TTA however requires intricate and destructive laboratory procedures that may not be suitable for small scale farmers in Uganda.

The optimal harvest total soluble solids (TSS) values at breaker and red (Table 1) agree with Pinho et al. (2011) that observed between 4.0 (early harvest) to 6.00 Brix (late harvest) from tomatoes grown in both organic and conventional fields. Total soluble solids include sugars, acids, vitamin C, amino acids and some pectins (Dadzie and Orchard, 1997). These soluble compounds form the soluble solids content of the fruit. In most ripe fruits including tomato, sugar forms the main component of soluble solids. The TSS is an important postharvest quality attribute in the screening of tomato. Since the amount of TSS in fruits usually increases as they mature and ripen, the soluble solids content of the fruit can be a useful index of maturity or stage of ripeness for tomato.

This study generally revealed increasing protein values with fruit maturity which concurs with Carrari et al. (2006). More still, Faurobert et al. (2007) and Matsuda and Kubota (2010) found more total soluble protein in red stage than breaker fruit. The decreasing trend of carbohydrates with fruit maturity in this study however disagrees with Gautier et al. (2008). The variation in carbohydrates content could be attributed to differences in soil properties and agronomic practices such as fertilizer application, weeding, pruning and mulching (Fungo et al., 2011; Okiror et al., 2017).

Maturity indices

Fruit diameter was negatively associated with fruit color (r = -0.748, $P \leq 0.05$) and total soluble solids (r = -0.787, $P \leq 0.05$) (Table 2). Fruit age was negatively correlated with pH (r = -0.949, $P \leq 0.05$). There was a positive correlation between fruit diameter and TTA (r = 0.959, $P \leq 0.05$) and fruit diameter and moisture content (r = 0.817, $P \leq 0.05$). There was a moderate positive correlation between fruit color and protein content (r = 0.630, $P \leq 0.05$). A weak positive correlation occurred between fruit age and fruit diameter (r = 0.256; $P \leq 0.05$). Therefore, the maturity indices of tomato fruit are morphological features including diameter, age and color and the physico-chemical and nutritional parameters notably, pH, TSS, carbohydrate and protein content (Table 2).

There is a debate regarding effectiveness of color as a maturity index. Zhang and McCarthy (2012) recognized outer color as an index for maturity of tomato fruit, but dismissed it as unreliable. Zhang and McCarthy (2012) stated that during tomato processing, the fruit fed to the processing line are usually a mixture of tomatoes of multiple cultivars. Thus, much as colour is a significant index for cv. Ghalia 281, it may not be reliable for a mixture of cultivars. Molyneux et al. (2004) reported tomato skin color to vary between cultivars despite the cultivars falling within the same maturity stage. Dadzie and Orchard (1997) urged that because external color is noninvasive and nondestructive, it can be used to assess fruit maturity in the field or inspection points.

Previous studies including that of Carvalho et al. (2005) demonstrated that L* value decreases as tomatoes ripen and turn red because carotenoid synthesis and loss of green color reduces fruit brightness. Caron et al. (2013) describe tomato as a climacteric fruit and assert that harvesting at the light red stage would give the productive and commercial sectors greater flexibility for its management. The arguments by Carvalho et al. (2005),

Table 2. Pearson's correlation coefficients (r) and analysis of variance for maturity indices of tomato, cv. Ghalia 281, at different harvest stages.

Maturity index	Correlation coefficient (r)	Analysis of variance	
		p-value	Significance
Fruit age vs. fruit diameter	+0.256	1E-10	**
Fruit age vs. fruit color	+0.374	1E-10	**
Fruit diameter vs. fruit color	-0.748	0.44	-
Fruit length vs. fruit color	-0.327	0.021	*
Fruit age vs. fruit length	-0.829	1E-10	**
Fruit age vs. TSS	+0.173	1E-10	**
Fruit diameter vs. TSS	-0.787	0.05	*
Fruit length vs. TSS	+0.134	0.644	-
Fruit age vs. MC	+0.350	1E-06	**
Fruit diameter vs. MC	+0.817	6E-22	**
Fruit length vs. MC	-0.646	8E-22	**
Fruit age vs. pH	-0.949	1E-10	**
Fruit diameter vs. pH	-0.381	0.013	**
Fruit length vs. pH	+0.764	0.001	**
Fruit age vs. protein	+0.296	4E-10	**
Fruit diameter vs. protein	-0.389	2E-08	**
Fruit length vs. protein	-0.091	5E-08	**
Fruit color vs. protein	+ 0.630	2E-07	**
Fruit age vs. TTA	+0.194	7E-11	**
Fruit diameter vs. TTA	+0.959	2E-13	**
Fruit length vs. TTA	-0.170	1E-13	**

*, ** Significantly correlated maturity indices at 0.05 and 0.01 alpha level, respectively.

Dadzie and Orchard (1997) and Caron et al. (2013) seem valid for the case of small scale farmers in Uganda. Thus, this work retains color as an important index for maturity of tomato c.v. Ghalia 281.

The pH, TSS, TTA, carbohydate and protein contents are possible physico-chemical and nutritional maturity indices. According to Zhang and McCarthy (2012), characterization of the intricate process of maturity and ripening poses a challenge to fruit farmers and processors as well as scholars. The pH, TSS, TTA, protein and carbohydrate contents vary as fruit mature (Carrari et al., 2006; Faurobert et al., 2007; Gautier et al., 2008; Matsuda and Kubota, 2010), making these parameters indispensable maturity indices. However, determination of pH, TSS, TTA, protein and carbohydrate content requires expensive and technically intricate destructive sampling (Matsuda et al., 2010; Pinho et al., 2011). Noninvasive and nondestructive indicators which can show compositional changes or structural variations as fruit mature, is preferred (Zhang and McCarthy, 2012). It may be inevitable to undertake invasive and destructive maturity assessments especially to corroborate morphological indices and/or in determining optimal levels of carbohydrates, protein, MC, TTA and TSS required in foreign markets and in the pharmaceutical industry (Matsuda et al., 2009).

Conclusions

This study reveals that, there was a rapid increase in fruit diameter and length in the first week, before it decreased in week 2. Further increase in fruit length occurred in week 3, reaching maximum towards the end of week 3 of cv. Ghalia 281 fruit maturation.

Tomato should be harvested when the colour turns breaker and/or red. At these stages, tomato fruit would have attained acceptable physico-chemical (pH, moisture content and colour) and nutritional (TSS, TTA, protein content) qualities for distant and adjacent markets, respectively.

A combination of fruit diameter, color and age are reliable nondestructive and noninvasive indices for determining optimal time of harvest. Use of TSS, TTA and protein content are reliable indices but may not be suitable for small-scale growers because their determination requires skilled staff and expensive laboratory procedures.

It is recommended that low cost but technologically effective non-destructive tools such as colour charts, diameter tapes and Calipers be developed, in consultation with small-scale farmers, to monitor fruit maturity and determine optimal harvest times for tomato. Further studies are recommended to determine physico-

chemical and nutritional values at different storage conditions such as temperature and relative humidity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Full Length Research Paper

Toxicity of neonicotinoids used in melon culture towards *Apis mellifera* L.

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The cultivation of melon (*Cucumis melo* L.) is of great importance to the Brazilian economy, especially the semiarid regions of the Northeast region. Damage caused by pests have hindered the production, requiring that control measures be adopted, among them applications of chemical insecticides, including neonicotinoids. Studies have shown collateral damage to beneficial insects such as bees, important pollinators for 90% of angiosperms, especially melon. The objective of this study was to evaluate the toxicity on melon crops of neonicotinoids used to control pests related to honeybee, *Apis mellifera* L. Bioassays were performed in the laboratory. The mortality of specimens over time when contaminated with the products thiamethoxam, midaclopride and acetamiprid (two commercial products by different companies) was evaluated. The exposure of bees to the compounds was performed by food ingestion (sugar candy) contaminated with the lowest and highest doses recommended by the manufacturers. Regardless of the dose, all insecticides were toxic, decreasing up to 11 days the useful life of bees as compared to the control (water + sugar candy), which survived 18 days on average.

Key words: Contamination, pollinators, bees, *Cucumis melo* L.

INTRODUCTION

The cultivation of melon (*Cucumis melo* L.) is of great importance to the Brazilian economy. In recent years, it has the highest export volume to the international market. In 2014, 196,850 tonnes of the product were exported

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(Reetz et al., 2015). Northeastern semiarid regions are the main productive centers for its favorable climate and soil conditions (Lopes et al., 2012). However, such conditions have also favored pest insect attacks, hindering the production (Fernandes, 2016).

Control of melon pests is usually done with the use of chemicals, due to immediate positive results regarding the suppression of population of such organisms.

However, if used improperly, they cause major environmental problems such as food contamination, environmental pollution, poisoning and death of farmers, as well as extinction of animals (Ferrari, 1986). In many cases, they promote a selection of resistant population lineages, causing a greater crop dependence on pesticides, committing the fauna and in many cases decreasing the number of beneficial organisms (Chagas, 2016) such as pollinating insects, bees and wasps (Barbosa, 2016).

Several authors have investigated the deleterious effects of insecticides, mainly neonicotinoids, on pollinating insects, including bees (Bortolotti et al., 2003; Barnett et al., 2007; Thompson and MAUS, 2007; Schneider et al., 2012; Palmer et al., 2013; Sandrok et al., 2014; Barbosa, 2016; Pacífico da Silva et al., 2015). However, there are few studies focusing on the Brazilian Northeast region and more specifically on melon crops. The effects of insecticides on bees vary, including changes in the olfactory system, flight disorders, impaired immune response and reduced survival rate (Pacífico da Silva et al., 2016).

Considering the environmental impact the improper use of such insecticides may cause and considering bees as pollinators important to melon plants, although easily affected by pesticides in crops, the main objective of this study was to evaluate the toxic effects of neonicotinoids insecticides, used in a melon crop to control pests, on worker *Apis mellifera* L. via ingestion of food contaminated with the lowest and the highest doses of insecticides recommended by manufacturers.

MATERIALS AND METHODS

The bioassay was conducted at the Entomology Laboratory of the Center for Agricultural Sciences of the Federal University of Campina Grande (UFCG), Campus de Pombal, Paraíba State.

Bee specimens were previously collected in beehive frames from colonies of the Central Apiary of Entomology Laboratory (UFCG) located at the experimental farm of that institution in the city of São Domingos de Pombal.

In this apiary, hives are installed in rational, wooden Langstroth structures kept in the natural environment of the Caatinga semiarid, typical of the region. Before the collection of the specimens, the hives went through a "preparation" process: the collection and selection of insects for experimentation. This preparation consisted of managing the supply of energy of colonies using an artificial diet comprising water and sugar syrup in a 1:2 proportion enriched with 2.5% of Glicopan® (free amino acids) and 2.5% of Aminomix® (vitamins, minerals and amino acids). The supply was weekly provided in individual feeders with a capacity of 500 mL in order to stimulate the mass production of young bees in hives, reaching a population suitable for bioassays. The hives were analyzed to select bees for the experiment. The general appearance of the population was taken into account. Hives that had all nest spaces configured for the production of juveniles, number of adult bees able to cover 2/3 of the breeding area and a food collection activity that resulted in a return flow from the field above one hundred (100) bees per minute were considered suitable.

Among the frames containing bee nests from selected hives, the worker bees were removed during the pre-emergence to adulthood stage in order to obtain newly emerged adults. The frames were packed in a wooden box adapted to a motor vehicle to transport them to the laboratory. For toxicity testing, four neonicotinoids insecticides were selected. The products were applied at the lowest and highest concentrations recommended by the manufacturers for the control of insect-pests in melon crops. Insecticides and their respective trade names, active ingredients and concentrations used (doses in grams of the product commercial per liter – g.p.c./L) are described in Table 1. Each neonicotinoid dose represented one treatment. The control consisted of distilled water only.

For the contamination of food, a chemical solution of each product, with its respective doses, was incorporated into the honey using a dosage calculated based on weight/volume. Then, powdered sugar (100 g) was added to prepare the sugar candy, obtaining a homogeneous diet for each treatment: 30% of chemical solution in 100 mL of honey. The treatments were arranged in a completely randomized design with nine treatments and five replications. Each experimental unit consisted of 20 adult worker bees up to 48 h old. To constitute the plots, bees were transferred to wooden boxes (cages) (11.0 cm long x 11.0 cm wide x 7.0 cm high) containing holes on the sides and sealed with nylon screens for ventilation. The boxes were previously covered on its internal base with filter paper and had a transparent glass lid on the top.

For each cage, contaminated sugar candy (honey + sugar) (10 g) was offered, varying according to the treatment, but always in a makeshift feeder (sterilized pet bottle caps), covered with a steel mesh facilitating the access of bees and avoiding death by drowning. Beside the feeder, a cotton swab soaked in distilled water was added. It was also packed in sterilized pet bottle caps and used as a source of water. For the negative control, sugar candy without contamination by insecticide was used associated with the water source mentioned above. The bioassay was kept in a room at 25 ± 2°C, RH of 70 ± 10% and 12 h photoperiod. At 1, 2, 3, 6, 12, 24, 48, 72 and 96 h after the beginning of exposure of bees to the products, the toxicity of the insecticides was observed on the individuals until their death. For each treatment, the evaluated biological parameter was the mortality rate of the specimens by counting dead insects over time. Only bees that did not move, even when lightly touched with a slim-tip brush, were considered dead.

Statistical analysis was performed using the software GraphPadPrism (v.5 for Mac). The median of the survival time with a 95% confidence interval and the significant differences between groups were estimated using the Kaplan-Meier test followed by a log-rank test. A significance level of $p<0.05$ was adopted.

RESULTS AND DISCUSSION

After one hour with contaminated food supply, it has been found that highest dose (3.0 g.p.c./L of water) of the neonicotinoid insecticide thiamethoxam caused highly toxic effects to bees, presenting mortality of insects in all experimental plots (Figure 1A). The lowest dose of thiamethoxam (0.3 g.p.c./L of water), added to the sugar candy offered to bees, also showed a toxic effect, but only up to 24 h (Figure 1B).

Similar effects were found by Carvalho et al. (2009),

Table 1. Trade names, active ingredients and doses of insecticides from the neonicotinoidsgroup evaluated in toxicity tests using *A. mellifera* L. worker bees under laboratory conditions.

Trade name	Active ingredient	Dose (g.p.c./L of water)	
		Lowest	Highest
Actara 250 WG	Thiamethoxam	0.3	3.0
Evidence 700 WG	Imidacloprid	1.0	1.5
Mospilan	Acetamiprid	0.25	0.3
Orfeu	Acetamiprid	0.25	0.3

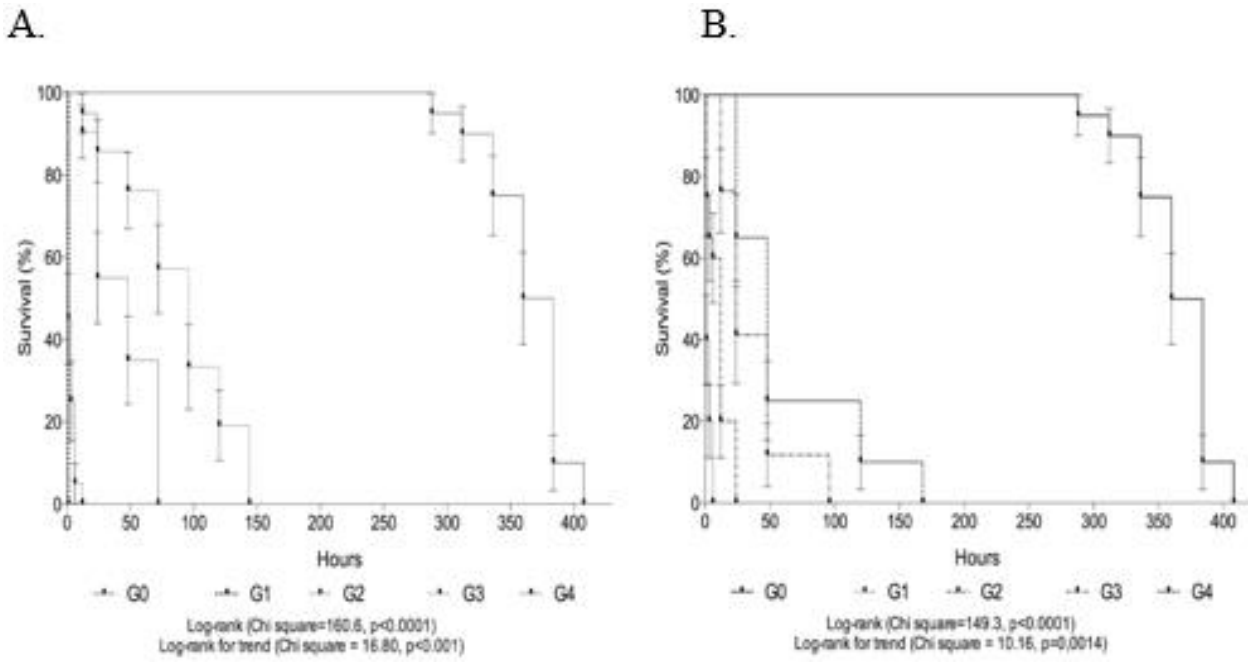

Figure 1. Survival rate (%) of *A. mellifera* L. worker bees fed with sugar candy contaminated with the highest or the lowest dose of thiamethoxam, imidacloprid and acetamiprid recommended by the manufacturers to melon crops. A) Highest doses. B) Lowest doses. Note: G0: control, sugar candy without insecticide + distilled water; G1: sugar candy + thiamethoxam; G2: sugar candy + imidacloprid; G3: sugar candy + Mospilan®/acetamiprid; G4: sugar candy +Orfeu®/acetamiprid

according to whom the movements of bees were disordered and shaking, and they died a few hours after being exposed to sub-lethal doses of thiamethoxam (150 g/L H_2O). Antunes-Kenyon and Kennedy (2001) observed mortalities in less than 3 h. Thiamethoxam caused an 89% mortality of individuals exposed to contaminated food and spraying of sugar candy containing the insecticide.

These results corroborate those found by Laurino et al. (2011), showing that, in addition to direct damage, the effects caused by thiamethoxam chemical molecules may have a cytotoxic action, causing vacuolation, that is, formation of clear vacuoles in the midgut of bees. This was also reported by Oliveira et al. (2013) on evaluating the deleterious effects of chemical molecules on the midgut of bees. Catae et al. (2014), after exposing *A. mellifera* L. to a diet containing a sub-lethal dose of thiamethoxam 1/10 of CL_{50} (0.0428 ngi.a./L of diet) for up to 8 days, reported that the continuous exposure to a sub-lethal dose of thiamethoxam may damage organs responsible for the metabolism of the insecticide molecule.

The neonicotinoid insecticide Evidence 700 WG®, which contains the active ingredient imidacloprid, at the highest and the lowest dose, 1.5 and 1.0 g.p.c./L, respectively, proved to be toxic to bees when applied together with food, similar to thiamethoxam. However, the mortality of bees occurred within 48 h (Figures 1A and B). Thirty minutes after an oral treatment with imidacloprid, Decourtye et al. (2004) found a deficiency in the olfactory

learning of bees contextualized by a proboscis extension response, that is, the chemical molecule affected the functioning of the insect's brain, causing mortality. Such toxicities significantly reflect on the life of these organisms, decreasing considerably as compared to their useful life, which may have an average of 960 h, equivalent to 40 days (Rocha, 2008). In this study, the control treatment had an average useful life of 18 days under laboratory conditions. The insecticides Orfeu® and Mospilan®, both with the same active ingredient (acetamiprid), at concentrations of 0.25 and 0.3 g.p.c./L , also proved toxic to bees. However, they were toxic after 78 h, reducing up to 11 days the useful life of bees as compared to the survival time of the control treatment.

Experimentally, chemical molecules caused early death of bees assuming that these organisms, if contaminated in field, would not return to their colony. It is assumed they would be vulnerable to attack from predators because of intoxication symptoms observed in this study.

The evaluation of toxicity by the tested insecticides to bees, with their respective doses, showed that if exposed to contaminated food, there is a fast death of some specimens. It would justify the non-return of bees to colonies because of a possible suppression of mass of these organisms in the area when such insecticides are used.

Pesticide poisoning may occur during the collection of nectar and contaminated pollen grains since neonicotinoids are chemicals with a contact and systemic action. They can be dispersed internally or deposited in different parts of the plant, consequently appearing in the floral components of melon when sprayed with such insecticides, since the systemic insecticide circulates with the sap (Mariconi, 1977).

Medrzycki et al. (2003), evaluating the effect of sub-lethal doses of imidacloprid on the behavior of bees, observed a decrease in mobility and communication among insects, which hindered their social behavior. Tome et al. (2012) also found changes in the behavior of stingless bees when fed with a diet contaminated with imidacloprid.

This is in line with reports by Freeman and Doherty (2006), who described the occurrence of toxicity of imidacloprid on adult bees, consequently decreasing activities such as pollen conduction frequency during foraging and formation of a number of operculated cells. Such events were probably caused by the effects (hyperarousal/tremors) of agonist acetylcholine molecules, active ingredients of neonicotinoids. Possibly, the side effects compromise the growth of colonies in the field by affecting the ability to search for food and exerting direct effects on the propagation of new individuals, as reported by Whitehorn et al. (2012).

Considering the above, the treatments with thiamethoxam and imidacloprid caused symptoms of poisoning shortly after the beginning of the intake of contaminated food, inducing a poor motor coordination (tremors) and prostration of intoxicated individuals at the bottom of the cages. The presence of regurgitations of food consumed was noted. It is evidenced by regurgitated remains in the glass plate of the boxes where the bees were accommodated. Such symptoms are supposedly a result of the action of the neurotoxic insecticides under evaluation. They act as acetylcholine agonists on the central nervous system of insects in nicotinic post-synaptic receptors (Nauen et al., 2001). However, they are not degraded by the enzyme acetylcholinesterase, which causes hyperarousal (tremors) and consequently insect death (Carvalho et al., 2009).

All treatments at their lowest doses showed symptoms of poisoning six h after exposure to contaminated food. However, they also decreased the survival time of bees, with average values of 6, 96 and 168 h (Figure 1B), related to intake of thiamethoxam, imidacloprid and acetamiprid, respectively. These were different times from those observed for the highest doses.

According to Iwasa et al. (2004), the neonicotinoid thiamethoxam has a nitro group (N-nitroguanidine), making this molecule on average 192 times more toxic to bees than molecules with a cyano group (N-cyano-amidine) such as acetamiprid. This probably justifies the difference in toxicity considering bee lethal times. For the authors, the low toxicity of acetamiprid in relation to other neonicotinoids studied in this work could be associated with the high detoxification capacity of such molecules mediated by enzymes dependent on the cytochrome P450 of nerve cells. The authors reported that the difference in toxicity, even between active ingredients of a same chemical group, such as imidacloprid, is approximately 400 times as compared to acetamiprid. The importance of toxicity studies using different plant protection products are thus evidenced even if they have the same mode of action on organisms in general.

Brunet et al. (2005) studied the metabolism of acetamiprid in *Apis mellifera* worker bees and reported that, when adult workers received acetamiprid containing radio isotopemarkers orally, more than 50% of the acetamiprid were metabolized in different parts of the insect's body (head, thorax, abdomen, hemolymph, midgut and rectum) in less than 30 min, indicating a very short half-life. During the first hours, the authors also found a higher frequency of acetamiprid on the nicotinic receptors of acetylcholine in the abdomen, thorax and head. This implies another hypothesis regarding the differences in toxicity responses of plant protection products of a same chemical group given to the bee population of this study.

For Iwasa et al. (2004), the products of the acetamiprid metabolism by *A. mellifera* do not have a high toxicity. This could explain the significant difference among insecticides of a same chemical group, a result of this study, and the toxic effects of acetamiprid, thiamethoxam and imidacloprid. This discrepancy may also be

elucidated by the lower affinity to the nicotinic acetylcholine receptors of acetamiprid as compared to imidacloprid (Tomizaw et al., 2000; Tomizawa and Casida, 2003).

Based on this information, it is assumed that differences in lethal times of the products evaluated in the present study indicate that lower concentrations may be less toxic to bees. However, there is a question regarding the toxic viability to insect-pests.

There are many challenges to be faced in this field in order to analyze the real situation of the insect and pesticides relationship. However, according to the results obtained and analyzed in this study, it is suggested that the application of neonicotinoid insecticides be performed not coinciding with the melon flowering period, so that there is no contamination of the plant reproductive parts and consequently pollinating bees, concomitantly present at this phenological stage of the crop.

Conclusions

Regardless of the dose of insecticide added to the diet (sugar candy), thiamethoxam, imidacloprid and acetamiprid are toxic to *A. mellifera* L. worker bees.

The insecticide, thiamethoxam is the most toxic to worker bees as compared to the other active ingredients evaluated, imidacloprid or acetamiprid, regardless of the dose added to the food (sugar candy).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Full Length Research Paper

Evaluation of aggregate distribution and selected soil physical properties under maize–jack bean intercropping and gypsum rates

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Adequate soil management can create favourable conditions to increase aggregation and porosity of the soil, resulting in better aeration of the soil and water infiltration. Consortia of maize and other species have been used in no-till systems to increase dry matter production, and consequently, the soil cover. This study aimed to evaluate the effects of consortia of maize and jack bean (*Canavalia ensiformis)* on aggregate stability and soil physical properties at different depths and with different rates of gypsum. The experiment was conducted in Paraná State, Brazil. The experimental design consisted of completely randomised blocks arranged in a split-plot design with four replications. The main plots consisted of maize intercropped with jack bean or not intercropped, and the subplots consisted of six rates of gypsum (0, 1, 2, 3, 4 and 5 t ha^{-1}). After maize cultivation, soil samples were collected for analysis of macroporosity, microporosity, total porosity, and soil bulk density at three depths. Monoliths were collected at 0-0.15 and 0.15-0.30-m depths for aggregate stability analysis. The application of gypsum promoted higher aggregate stability at the 0-0.15-m depths, but there was no effect on macroporosity, microporosity, total porosity, or soil bulk density. Maize intercropped with jack bean promoted more stable aggregates and increased macroporosity and total porosity. The use of gypsum in maize intercropped with jack bean promoted amelioration of the soil structure. The intercropping system increases the aggregate stability and macroporosity. The intercropping system also offers farmers the opportunity to improve the physical properties of the soil to benefit plant growth. In addition, intercropping maintains soil function, such as aeration, water infiltration and retention, and nutrient availability. In the long term, intercropping systems may be more stable than monocultures.

Key words: Bulk density, calcium sulphate, consortium, cover plants, cropping system, macroporosity, management system, soil sustainability, *Zea mays.*

INTRODUCTION

Inadequate soil management due to intensive machinery use, lack of crop rotation, and low input of organic matter

cause the breakdown of soil structure and consequently lead to soil compaction. Soil compaction is characterised by the compression of soil aggregates, resulting in reduced pore volume (Batey, 2009), increased bulk density, decreased porosity (macroporosity), and reduced water infiltration into the soil (Meyles et al., 2006). Soil compaction also increases resistance to erosion, but soil compaction decreases the water storage capacity of the soil.

Soil aggregate stability represents an important attribute for evaluating the soil structure and its physical quality; the evaluation of soil aggregate stability can be performed using indirect measurements that assess the amount of water-stable aggregates (Salton et al., 2008). An enhanced aggregate stability decreases the losses of soil carbon, nitrogen, and phosphorus (Kasper et al., 2009). Soil aggregation occurs when soil particles approach one another due to the action of roots and fungal hyphae (Six et al., 2006), wetting cycles (Bastos et al., 2005), and electrostatic attraction between soil particles. These structures are then stabilised by cementing agents, such as clay, iron oxide, and aluminium (Salton et al., 2008); organic matter (Noellemeyer et al., 2008; Chieza et al., 2013); exudates; mucilage; roots; and polyvalent cations. The aggregation and stability directly affect the plant growth, since these properties regulate the supply of oxygen and water in the soil, in addition to being an important factor in controlling erosion in tropical acidic soils. A promising approach to increasing stability of soil aggregates is the use of polyvalent ions such as calcium (Becher, 2001).

The use of agricultural gypsum affects soil water characteristics (Escudero et al., 2015) by increasing surface soil water permeability, reducing soil compaction, and increasing hydraulic conductivity (Nan et al., 2016), particularly in soils with a high sodium content (Vasconcelos et al., 2013).

Most studies emphasise the indirect action of gypsum that improves soil chemical properties, favouring root growth in deeper soil layers (Serafim et al., 2011) and conditioning soil biological activity. This improved root growth favours greater absorption of water by plants and minimizes the effects of drought on the crops (Pauletti et al., 2014; Zandoná et al., 2015). However, studies conducted by Rosa Junior et al. (2006) showed that the use of gypsum increased the amount of aggregates larger than 1.0 mm in diameter and flocculation of clays, which increased the content of larger-diameter aggregates. Other physical soil properties such as soil bulk density and porosity can be affected by the combination of soil management and gypsum use (Bonini et al., 2012).

The soil management regime affects the aggregate stability, especially aggregates with significant organic carbon content. Therefore, establishing a management system aimed at producing and improving the physicalqualities of the soil is of great interest to the western region of Paraná. One of the alternatives is a consortium of species, and the maize crop is well adapted for this system. The consortium with maize can be with *Brachiaria* or with other species that initially experience reduced growth due to competition. The use of plants intercropped with maize can be an excellent option to increase soil organic content and sometimes to provide nitrogen to the crop (Corrêa et al., 2014). Among the plants of the Fabaceae family that are capable of fixing nitrogen, jack bean (*Canavalia ensiformis*) can be satisfactorily intercropped with maize (Paz et al., 2012) due to its rapid initial growth, even under diffuse light. This protects the soil from erosion and weeds.

In view of the aforementioned discussion, the present study aimed to assess the physical properties of an oxisol soil at different soil depths following intercropping maize with jack bean with additions of agricultural gypsum.

MATERIALS AND METHODS

Description of study site

The experiment was conducted at the “Núcleo de Estações Experimentais of UNIOESTE”, Campus de Marechal Cândido Rondon, in southern Brazil. The average altitude of the site of the experiment is 420 m above sea level, and the geographic coordinates are 24°31’S latitude and 54°01’W longitude. The soil of the region was characterised as an oxisol (LVef), with clayey texture (Santos et al., 2013).The local climate was subtropical Cfa according to the Köppen climate classification, with rainfall distributed evenly throughout the year and with hot summers. The average temperatures ranged from 17 to 18°C during the coldest season of the year and from 28 to 29°C during the hottest season. Rainfall in the region ranged from 1600 to 1800 mm, with the wettest months (December to February) ranging from 400 to 500 mm (Caviglione et al., 2000) (Figure 1).

Treatments

The experimental design consisted of completely randomised blocks arranged in a split-plot design with four replications. The main plots consisted of maize intercropped with jack bean or not intercropped, and the subplots consisted of six rates of gypsum (0, 1, 2, 3, 4 and 5 t ha^{-1}). This experimental design was used to facilitate the mechanical sowing of maize (Figure 2). Each subplot

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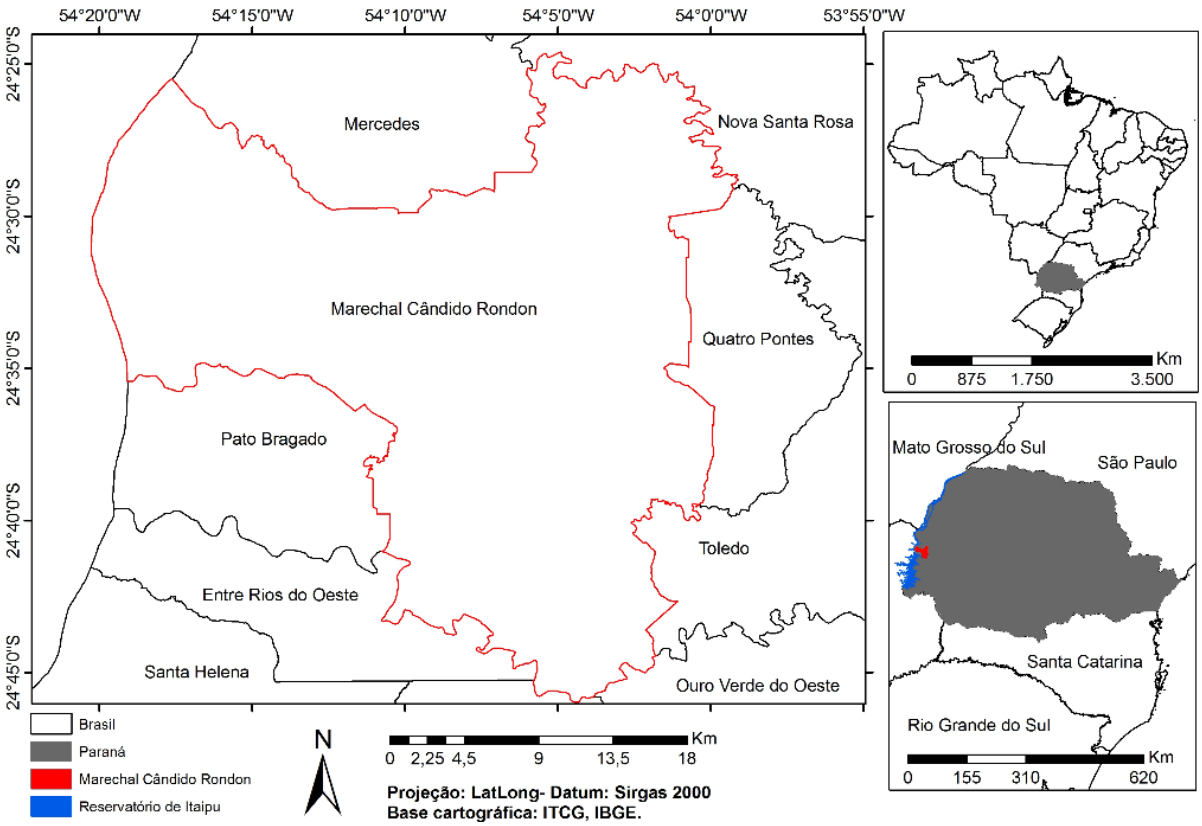

Figure 1. Location of Marechal Cândido Rondon/Paraná/Brazil.

Block I	D1	D3	D4	D5	D2	D6	Block III	D4	D1	D2	D5	D6	D3
	D6	D2	D5	D1	D3	D4		D3	D4	D5	D6	D2	D1
Block II	D2	D3	D6	D4	D1	D5	Block IV	D1	D5	D2	D4	D6	D3
	D5	D6	D3	D4	D2	D1		D5	D6	D4	D3	D1	D2

Maize + jack bean Maize not intercropped D = Rates

Figure 2. Experimental design.

had a total area of 31.5 m² (4.5 m wide by 7 m long). Thirty days before maize seeding, gypsum was manually applied to the soil surface. Maize hybrid 30F53 was mechanically seeded in a no-till system. The spacing was 0.70 m, with 4.2 seeds sown m^{-1}. The base fertiliser was 300 kg ha^{-1} of a 10-20-20 formulation of N, P_2O_5, and K_2O, respectively. Two days after sowing the maize, jack bean was seeded manually between the maize rows at a rate of 12 kg ha^{-1}.

Soil sampling and analyses of chemical properties

Before the implementation of the experiment, soil samples were collected at a 0.0-0.20-m depth for determination of the chemical properties and particle size characteristics. Chemical analyses were performed according to the methodology proposed by Raij et al. (2001). The results of chemical analysis of the soil are as follows: pH ($CaCl_2$) = 6.05; organic matter = 24.61 g dm^{-3}; P = 2.36 mg dm^{-3}; Ca^{2+} = 6.61 $cmol_c$ dm^{-3}; Mg^{2+} = 1.77 $cmol_c$ dm^{-3}; K^+ = 0.25 $cmol_c$ dm^{-3}; Al^{3+} = 0.00 $cmol_c$ dm^{-3}; $H^+ + Al^{3+}$ = 2.54 $cmol_c$ dm^{-3}; and base saturation (V%) = 77.26%.

Soil sampling and analyses of physical properties

After maize harvest, undisturbed soil samples were collected with

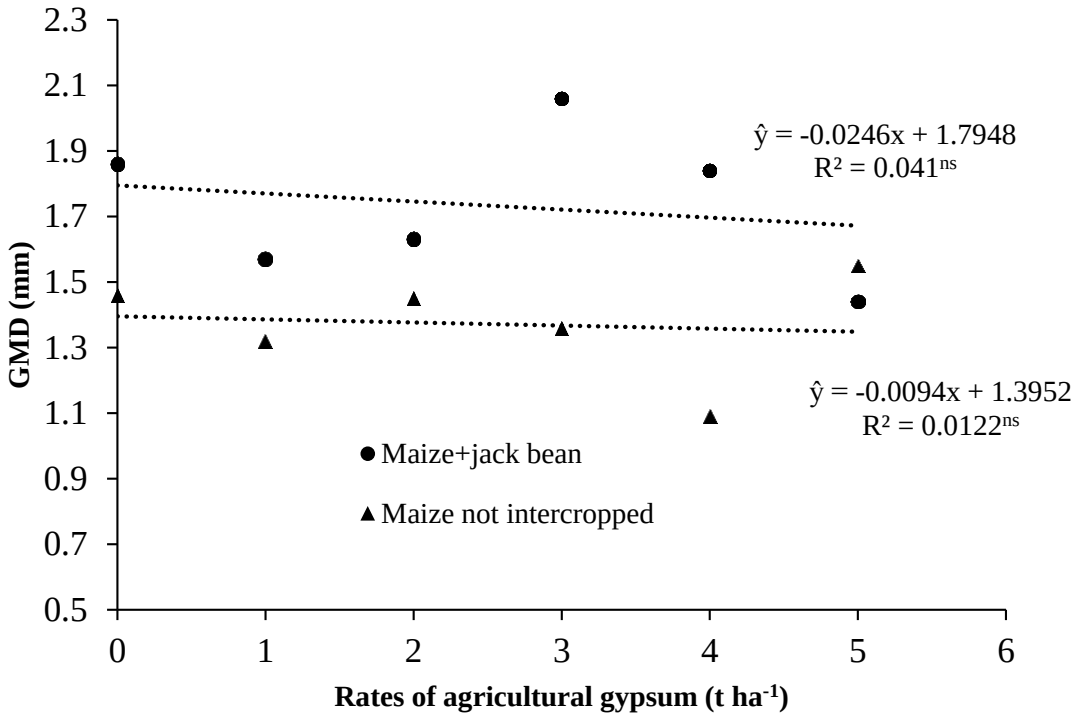

Figure 3. Geometric mean diameter (GMD) of aggregates in oxisol soils under different managements and rates of agricultural gypsum.

metal rings 0.047 m in diameter and 0.025 m in height at 0.0-0.10-, 0.10-0.20-, and 0.20-0.30-m depths for assessments of macroporosity, microporosity, total porosity, and soil bulk density. Initially, these samples were saturated by capillarity in trays until they reached about two-thirds the height of the samples, for 48 h. The total porosity was calculated as saturated soil water content. Quantification of macroporosity values (pores ≥ 50 µm) and microporosity (pores ≤ 50 µm) was obtained by subjecting all of the samples to a pressure of -0.006 MPa using a voltage table. The macropores were estimated as the difference between the saturated soil water content and the soil water content after application of -0.006 MPa of pressure. The soil bulk density was determined by the volumetric ring method, in which undisturbed soil samples were oven-dried at 105°C for 24 h (EMBRAPA, 1997).

Soil sampling and analyses of aggregate stability

Undisturbed soil samples (monoliths) were randomly collected at 0-0.15 and 0.15-0.30-m depths for analysis of aggregate stability. At the time of sampling, the soil consistency was friable. At the laboratory, the sample was passed through a #4 mesh (4.76 mm) sieve and retained on a 2-mm sieve. Plant fragments, other waste, stones, and gravel retained on the sieve were excluded. The analysis of aggregate stability was determined using the method described by Kemper and Chepil (1965), with three replications. In this method, soil aggregates are sieved in water with vertical oscillation for 15 min. Thirty grams of each sample were then placed on the top sieve of a set of sieves with mesh openings of 2.0, 1.0, 0.5, 0.25 and 0.10 mm. The water level of the apparatus was adjusted to the top level of the sieves so that water reached only the bottom of the 2-mm sieve.

Statistical analyses

The effects of treatments were analysed by analysis of variance (ANOVA) using the SAEG statistical program package (Saeg, 2007). For qualitative factors, the Tukey procedure was used where the ANOVA was significant, and regression analysis was used for quantitative variables. Differences were considered statistically significant at a p-value less than 0.05.

RESULTS

Soil aggregate stability

The results obtained from the ANOVA showed effects of the interaction of both gypsum rate and crop management system on the geometric mean diameter (GMD) of soil aggregates. A greater soil aggregate stability occurred in maize intercropped with jack bean. Based on the regression analysis of the rates of agricultural gypsum, there was no adjustment for either equation (Figure 3).

Analysis of the post-effects of the gypsum rates at the two soil depths assessed for aggregate stability showed a significant effect. More stable aggregates were observed at the 0-0.15-m depth with a rate 3 t ha^{-1} (2.06 mm). At this depth, the GMD was, on average, 1.73 mm, while at the 0.15-0.30-m depth the GMD was 1.37 mm; otherwise,

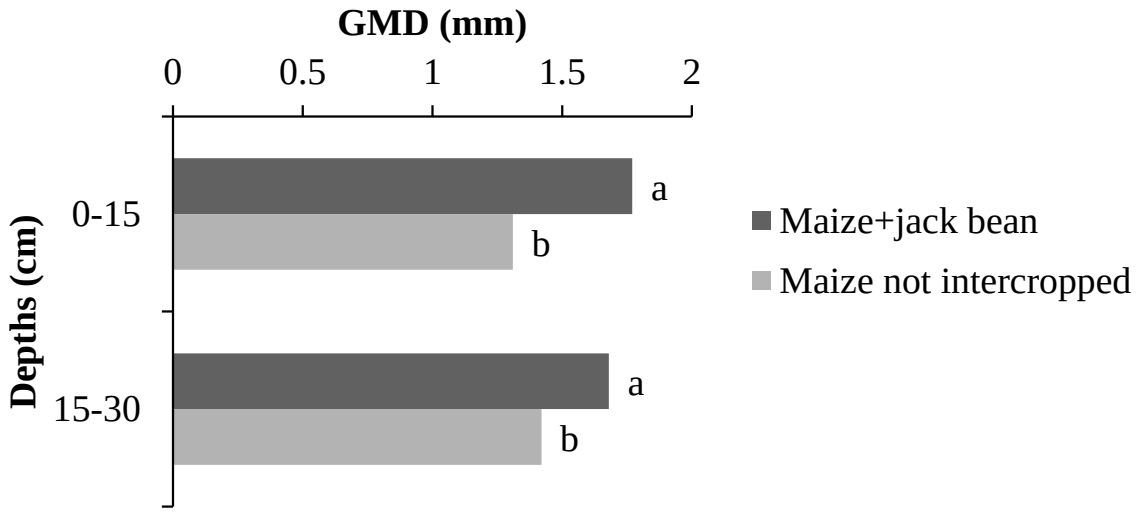

Figure 4. Geometric mean diameter (GMD) of aggregates in oxisol soils under two crop management systems. Bars followed by the same lowercase letter within a soil depth are not significantly different according to the Tukey test ($P < 0.05$).

Table 1. Average results for microporosity, macroporosity, total porosity, and soil bulk density at different soil depths after maize was intercropped and not intercropped with jack bean.

Depths (cm)	Macroporosity (m^3 m^{-3})			Microporosity (m^3 m^{-3})		
	Maize + Jack bean	**Maize not intercropped**	**Average**	**Maize + Jack bean**	**Maize not intercropped**	**Average**
0-0.10	0.130^{Aa}	0.053^{Ab}	0.09^{A}	0.445^{ns}	0.462^{ns}	0.45^{ns}
0.10-0.20	0.067^{Bb}	0.059^{Aa}	0.06^{B}	0.441	0.477	0.46
0.20-0.30	0.064^{Bb}	0.041^{Bb}	0.05^{B}	0.440	0.465	0.48
Average	**0.087^{a}**	**0.051^{b}**		**0.442^{a}**	**0.468^{a}**	
Depths	**Total porosity (m^3m^{-3})**			**Bulk density (Mgm^{-3})**		
0-0.10	0.575^{Aa}	0.515^{Ab}	0.54^{ns}	1.31^{ns}	1.34^{ns}	1.33^{ns}
0.10-0.20	0.508^{Bb}	0.536^{Aa}	0.52	1.36	1.43	1.40
0.20-0.30	0.504^{Bb}	0.506^{Ab}	0.53	1.40	1.39	1.40
Average	**0.53^{a}**	**0.52^{a}**		**1.36^{a}**	**1.39^{a}**	

Means followed by the same lowercase letters on the same line and the same uppercase

at the surface layer, aggregation increased by 21%. Regardless of the gypsum rate and crop management used, most organic matter accumulation occurred at the soil surface layer (Figure 3).

Figure 4 shows the average results for the GMD of the aggregates in the two crop management systems and depths. At the two assessed depths, there was greater aggregate stability in the crop system of maize intercropped with jack bean; the reported increase was 26% at the 0-0.15-m depth and 15% at the 0.15-0.30-m depth.

The greater root growth observed in maize intercropped with jack bean contributed to this result, as the two crops formed a tangled root system that involved soil particles, increasing the soil aggregation and stability.

Soil physical properties

Regarding macroporosity, microporosity, total porosity, and soil bulk density, the results obtained showed no effect of gypsum rate as well as of the interaction between gypsum rate and management system (Table 1). When the effect of the management alone was evaluated, without the interaction between management and depth, a significant effect was found for

macroporosity and microporosity, but there were no effects on total porosity or soil bulk density (Table 1). The maize intercropped with jack bean showed higher macroporosity (0.087 m^3 m^{-3}) compared to maize not intercropped (0.051 m^3 m^{-3}); otherwise, in this system, macroporosity was 41% higher than in maize not intercropped. This result is related to the greater stability of the aggregates (GMD) found in this treatment, as shown in Figure 4.

Assessment of the interaction between the crop management system and depth showed that the average macroporosity (0.09 m^3 m^{-3}) and total porosity (0.57 m^3 m^{-3}) were higher at the 0-0.10-m depth. At this depth, the macroporosity was 63% higher than the average value of the other depths, and total porosity was higher, reaching 12% (Table 1).

DISCUSSION

Soil aggregate stability

The greater stability observed in the area planted with maize intercropped with jack bean, unique to the soil surface layer, occurred because in this system there was more input to the organic matter in the soil, such as leaves, plant roots, root exudates, dissolved organic matter, and bioturbation. In previous studies (Onweremadu et al., 2007; Shaoshan et al., 2010; Six and Paustian, 2014), it has been shown that organic matter influences the soil structure and stability by binding soil mineral particles.

The improved soil aggregation resulting from the accumulation of organic matter in the soil surface layer occurs due to the high specific surface area and cation exchange capacity of this layer. This allows more electrostatic bonds between soil particles, facilitating the formation of microaggregates (0.20 to 0.25 mm) and macroaggregates (> 0.25 mm) (Six et al., 2004). These findings corroborate the results obtained by Silva et al. (2013), who reported higher aggregation at the 0.15-m depth with gypsum rates of 28 and 56 t ha^{-1}. According to those authors, this higher aggregation was explained by the higher concentration of divalent cations (Ca^{+2}) that promoted flocculation of clays.

Root growth also affects soil structure. As roots grow, they compress the soil near the growth zone, which forces clay particles together, thus favouring aggregate formation (Brandão and Silva, 2012). The roots release exudates and mucilaginous materials near the rhizosphere, which directly or indirectly affect soil structure. In addition, fungal mycorrhizae is often associated with the root system, resulting in greater aggregation. Finally, when roots decay, the decomposition products contribute to the soil aggregation and aggregate stability.

Gypsum is the most commonly known and used calcium compound for studying the amelioration of soil structure (Gupta and Khan, 2015). However, in this study, only the use of gypsum did not promote amelioration of the stability of aggregates. These results are corroborated by Bennett et al. (2014), who found no effect of gypsum rates on the stability of soil aggregates.

When the agricultural gypsum was applied together in maize intercropped with jack beans, the aggregate stability increased. This increase occurred because the root systems promote an input of large amounts of organic matter in the soil, which increases the physico-chemical bonding between the organic colloids, calcium, and soil minerals (Norambuena et al., 2014).

The results concerning the use of agricultural gypsum are contradictory in this study. Sometimes the use of lower rate agricultural gypsum caused favourable effects on soil structure, while higher rates caused unfavourable effects. Therefore, in the regression analysis, there was no adjustment for any equation.

Soil physical properties

The previously mentioned finding was corroborated by Bertollo (2014), who found no significant differences in soil chemical properties in a study with gypsum and limestone rates in a no-till system. Results that diverged from the findings of this experiment were observed by Müller et al. (2012), who reported significant differences in macroporosity and microporosity at the 0-0.075-m depth with the use of agricultural gypsum at a rate up to 12 t ha^{-1}; however, these authors also did not find any effect on total porosity and soil bulk density.

This increase in macroporosity was related to the addition of organic matter by plants in the soil surface layer as well as the large surface area of the roots of most plants. The labile organic matter affects aggregation and therefore is the physical property most affected by soil management.

The average macroporosity values were found to be below the critical value of 0.10 m^3 m^{-3}. Soil macroporosity less than this value affects plant growth due to the decreased availability of oxygen, which reduces the supply of oxygen for respiration to the roots and thus the generation of energy for mineral nutrient absorption. The average values for soil bulk density were below the critical values for crop growth in clayey soils of 1.45 Mg m^{-3} (Reichert et al., 2009).

Corroborating these findings, Lanzanova et al. (2010), in a study of the physical properties of an Argisol soil following cultivation of different green fertilisers in rotation with soybean; did not find a significant difference in macroporosity, microporosity, total porosity, or soil bulk density. Before the 16 years of cultivation with green fertilisers in rotation, differences only in the macroporosity

and soil bulk density in the area of uncovered soil were found.

Conclusions

The use of agricultural gypsum promoted greater aggregate stability at the 0–0.15-m depth and did not affect macroporosity, microporosity, porosity, or soil bulk density. More stable aggregates result in higher soil resistance to erosion and better aeration. The use of intercropping systems promotes improvements in aggregate stability, soil macroporosity, and total porosity, and intercropping optimises the use of land. The intercropping system offers farmers the opportunity to improve physical properties of the soil that promote plant growth and to maintain soil function, such as aeration, water infiltration and retention, and nutrient availability. In the long term, intercropping systems can be more stable than monocultures.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Allometric relationships and carbon content for biomass-carbon estimation of East African Highland Bananas (*Musa spp*. AAA-EAHB) cv. *Kibuzi, Nakitembe, Enyeru* and *Nakinyika*

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Globally, interests to increase carbon stocks have gained momentum in both woody and non-woody ecosystems. Despite efforts made to generate appropriate methods to estimate these stocks, most equations developed do not cater for intraspecific variabilities across e.g. species, regions or growth stages; especially in the case of bananas. Therefore, there is need to develop more robust equations to improve on the precision of biomass-carbon prediction especially at local scales to facilitate estimation of specific carbon stocks often lost in global assessments. This study aimed at developing cultivar-specific biomass estimation relationships and determining carbon content of EAHB cultivars at two growth stages. Plant data were collected purposively using destructive sampling techniques on farmers' plots for 4 cultivars (*Kibuzi, Nakitembe, Enyeru* and *Nakinyika*) in two agro-ecological zones: the L. Victoria crescent and the South-western farmlands in the districts of Lwengo and Mbarara respectively. Results show that biomass differed across cultivars (P<0.001); hence four equations (*Enyeru*, *Nakinyika, Kibuzi_Nakitembe* and Generic) were developed following an exponential function, y=Aexp(ax), using diameter at breast height (DBH) as the predictor variable with an R^2 range of 82-94%. EAHB mean carbon content varied significantly with growth stage (P<0.05) (47.6% for maiden plants before flowering and 48.8% for mature plants with a developed bunch). This study concludes that it is important to develop cultivar-specific equations for biomass-carbon estimation of EAHB cultivars to help assess their contribution to the carbon cycle especially in future studies.

Key words: East African Highland Bananas (EAHB) cultivars, allometric equations, total plant biomass, carbon content, growth stage.

INTRODUCTION

Globally, interests to enhance carbon stocks in the biosphere have gained momentum in both woody and non-woody ecosystems as a means to address global climate change (Nair et al., 2009; Anthony et al., 2011;

Lal, 2011). However, considering the continuous shortage of land available for production of woody ecosystems (Henry et al., 2009), the need to find accommodative alternatives to deal with increasing atmospheric GHGs without compromising food production and economic development has to be addressed, e.g. through use of perennial crops like banana. The approach has since then received attention despite that pre-requisites to actual implementation of such initiatives require accurate verifiable methods developed to estimate biomass, carbon content and carbon stocks especially in agricultural landscapes (Singha et al*.,* 2011; Shem et al*.,* 2013) which remains a big challenge.

Nevertheless, efforts to estimate species biomass in both natural and agricultural ecosystems have been realized especially for crops like coffee, banana, commercial tree species, cocoa, etc., whose allometric equations have been globally developed (Hairiah et al., 2001; IPCC, 2003; Nyombi et al*.,* 2009; ICRAF, 2011). This has mainly been attributed, for example, to the need to explore the role of such species in the global carbon cycle through carbon sequestration monitoring, as well as for their sustainable management (Eamus et al*.,* 2000). In spite the importance of appropriate methods to estimate carbon stocks, these equations do not cater for intraspecific variabilities across e.g. species, regions or growth stage. Hence the need to develop more robust and viable equations to accurately capture the impact of region-specific and species-specific carbon contents and stocks of ecosystem components which are in most cases lost in global assessments (Hutchinson et al*.,* 2007).

Uganda is one of the largest national producers of bananas (*Musa* spp.) in the world; and is recognized as a secondary center of diversity with high levels of different cultivars observed on individual farms (Suzanne and Emile, 1999; Edmeades et al*.,* 2005; FAO, 2009). Over 75% of the cultivars are East African Highland Bananas (EAHB) (Karamura, 1998; Nantale et al., 2008). The perennial crop is an important food security crop cultivated in a wide range of agro-ecological zones and readily available throughout the year (NARO, 2001; Eledu et al*.,* 2004; Wairegi, 2010). Though the potential of bananas to sequester carbon has been reported (e.g. Rodel et al., 2000; Christina, 2004; Oliver, 2009), there is limited knowledge on how much different cultivars contribute despite their high morphological and physiological differences. This could perhaps be attributed to the lack of cultivar-specific methods to estimate their biomass. This is because existing equations widely used in carbon studies were developed by Arifin (2001) using bananas grown in Indonesia that perhaps exhibit different morphological traits as compared to EAHB.

Nevertheless, efforts made by Nyombi et al. (2009) to develop such equations for EAHB are worth appreciating though they did not explore the use of Diameter at Breast Height (DBH) to predict plant biomass, a commonly used predictor variable in many carbon related studies (e.g. Amy et al., 2010; Arias et al., 2011 and Adeline et al., 2013 among others). In addition, DBH has been considered as the best explanatory variable for biomass prediction of several species, but also given its ease to measure and high accuracy (Shem et al., 2013). Key variables commonly used for bananas have mainly been the pseudo-stem girth-at-base, its diameter at 100cm, and or plant height (Nyombi et al*.,* 2009; Wairegi et al., 2009); hence the need to explore the use of DBH as a predictor variable for biomass of EAHB cultivars was worth considering in this study.

On the-other-hand, carbon content values are an important element to consider in any carbon related study. Though scarce, information on local carbon content values is more important than generalized ones as recommended by Timothy et al*.* (2005). This is because such data on various species e.g. bananas are essential for accurate assessment of their carbon stocks (Arias et al., 2011). However, the conversion coefficient of biomass to carbon stock of 50% that has been universally accepted and promulgated by scientific bodies, e.g. IPCC (Timothy et al., 2005; West, 2009), is subject to debate given that it perhaps does not cater for intraspecific variabilities across species, different growth stages, or even regions.

But also, other studies have proposed the use of a default carbon content conversion value of 0.46 for trees (Hairiah et al., 2010), lower than one recommended by IPCC. However, a study by Thomas and Malczewski (2007) found out that coniferous trees had a higher carbon content value of 50.9% than other hardwoods in China, while others like (Gifford, 2000) actually noted a 54.1% content for *Pinus radiate* in Australia, all higher than the 50% value. This therefore shows great uncertainties in the use of one carbon content value as opposed to another; hence a great need to estimate species-specific carbon content values to better estimate their carbon stocks. This could also be considered for different growth stages for species like banana with different development stages that exhibit several carbon content potential components. Therefore, this study also

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determined the actual carbon content value of EAHB to minimize over or under estimation of carbon stocks that could perhaps be brought about by the use of general values.

MATERIALS AND METHODS

Study area

Plant biomass-carbon data were obtained in 2013 from two distinct agro-ecological zones; that is, the Lake Victoria Crescent and South-western Grass Farmlands in Kisekka and Nyakayojo sub-counties of Lwengo and Mbarara districts respectively. These were classified as potential banana production areas by Eledu et al. (2004). Mbarara district lies at a high altitude of about 1400 m above sea level (0°20.5'S 30°31'E) and Lwengo at a low altitude range of 1080-1330 m above sea level (00°24'S 31°25'E) (Nantale et al., 2008; Kemigabo and Adamek, 2010). Both areas experience a bimodal mean annual rainfall range of about 1000-1500 mm (Lwengo) and 1000-1200 mm (Mbarara). Their mean annual temperature range lies between 20-25°C. According to the 1998 FAO soil classification, the soil types are acric ferrasols, and dystric regosols and lixic ferrasols for Kisekka and Nyakayojo respectively. However, to minimize variability across zones, all farms selected were comprised of the ferralsol soils given that they are deep in nature and cover about 60% of the potential banana production area for Uganda (Eledu et al., 2004).

Farm site selection

Based on the preliminary findings of the reconnaissance survey conducted in December 2012 and with the aim of minimizing the effect of potential confounding factors, participating farmers were purposively selected following a set of criteria: i) The farm had all the cultivars of interest; ii) The plantation was mature (20 to over 50 years); iii) All farms in a given region existed in a similar soil type classification and relatively same altitude range; iv) The farmer was willing to participate fully in the study. (ii) and (iv) were also considered for the same reason in other studies (e.g. Nantale et al., 2008; Wairegi et al., 2009). In total therefore, 14 farmer plantations (7 in each area) were considered since they were the only ones meeting the criteria; but also considering the availability of resources. Four cultivars (*Kibuzi* and *Nakitembe* existing in both sites, and *Enyeru* and *Nakinyika* being unique to Mbarara and Lwengo respectively) were selected because they had a higher population density than others identified, similar to observations by Wairegi et al. (2009); and their total biomass allometric relationships had not been developed before.

Biomass estimation

In each sampling plot, all individuals belonging to the cultivars of interest were inventoried *in-situ* before destructive sampling (ICRAF, 2011). Estimation of total plant biomass therefore included non-destructive sampling measurements (Height and Diameter) of individual banana stems important for use in the allometric models generated for this study as suggested by Wairegi et al. (2009) and ICRAF (2011). To minimize bias and cater for variability, six individual mats, two for each cultivar, were purposively identified anywhere on the same farm. These were then tagged for excavation for dry weight biomass and oven-dry carbon content determination. Care was taken to ensure that mats selected had at least two individuals at different growth stages; that is, H1 (maiden plant before flowering, at least half or more the height of H2) and H2 (plant at true phonological maturity with a developed bunch). Therefore, a total 84 mats (14 per cultivar per site) were sampled. For every mat, total plant biomass (TPB) of selected plants was obtained and used to develop cultivar specific allometric relationships. In general, allometric relationships for trees are best taken at DBH (1.3m) in reference to Brown et al. (1989). However, a number of studies such as Wairegi et al. (2009) and Nyombi et al. (2009) have developed similar relationships for EAHB bananas considering Girth at base (G^{Base}) and Diameter at 1 m (D^{100cm}); and height. Therefore, in this study, all the three diameter levels and height were considered to find out which one best predicts the relationship for a specific cultivar. Girth at base was calculated as πD.

Individual plants were then carefully dug out from the soil and prepared following procedures detailed in Nyombi et al. (2009). Sub-samples of each part (pseudo-stem, leaves, corm, peduncle and fingers), 250 g each, were weighed, bulked and carried to the Soil Science Laboratory in Kawanda. These were oven dried to constant weight at approximately 70°C for 48 h (Timothy et al., 2005). In total, 1001 sub-samples representing all plant parts for all cultivars were obtained (that is, 420 for H1 class and 581 for H2; each class comprising of 5 and 7 samples per individual respectively). Total plant part dry mass (biomass) was then calculated based on an equation obtained from Timothy et al. (2005); where:

$$DryMass(kgs) = \left[\frac{\text{Subsample Dry Mass}}{\text{Subsample Fresh Mass}}\right] \times Fresh\ Mass\ of\ Whole\ Sample$$

Biomass data was then regressed with all the diameter levels and or plant height as explanatory variables to develop power equations; and one with the best explanatory power was selected and linearized (Nyombi et al., 2009) as below:

$$In(y) = c + aIn(x)$$

Where: *y* is the total dry plant biomass (Kg) (corm, pseudo-stem, leaves (H1) or corm, pseudo-stem, leaves, peduncle and fingers (H2)); *c* a constant; *a* the equation parameter; and *x* the explanatory variable (diameter, girth or height). The choice to estimate total plant biomass as opposed to several other carbon studies was due to the morphological nature of bananas where the corm remains the true stem of the plant not the pseudo-stem (UNCST, 2007). All data collected by destructive sampling was used for model calibration and validation.

EAHB carbon content determination

Out of all the plant individuals obtained through destructive sampling, six cultivar specific individuals with their sub-samples (corm, upper stem, middle stem, lower stem, leaf, and or fruit and peduncle), originally dried for biomass determination were randomly sampled following a sampling design of (3 cultivars×2 sites×7 (or 5) parts×6 replicates). A total of 432 sub-samples (H2:252 and H1:180) were selected for plant part carbon content determination following procedures laid out in Okalebo et al. (2002) for plant carbon content analysis.

Data analysis

All data were statistically analyzed using GenStat software

Table 1. Regression analysis of biomass across variables variate: ln_total_biomass.

Variable	*Enyeru*				*Nakinyika*				*Kibuzi_Nakitembe*				*Pooled*			
	v.r.	***F pr***	***SE.***	***R^2***	***v.r.***	***F pr***	***SE.***	***R^2***	***v.r.***	***F pr***	***SE.***	***R^2***	***v.r.***	***F pr***	***SE.***	***R^2***
ln_G_Base	3.34	0.079	0.501	0.080	3.29	0.081	0.563	0.078	20.50	<.001	0.535	0.151	48.76	<.001	0.533	0.223
ln_D_100	81.16	<.001	0.263	0.755	149.82	<.001	0.219	0.851	540.97	<.001	0.237	0.833	760.01	<.001	0.247	0.823
ln_DBH	168.20	**<.001**	0.093	**0.933**	214.51	**<.001**	0.187	**0.891**	762.88	**<.001**	0.205	**0.876**	1123.36	**<.001**	0.210	**0.873**
ln_H	57.20	<.001	0.299	0.684	192.01	<.001	0.197	0.88	351.15	<.001	0.282	0.764	446.99	<.001	0.303	0.732

G_Base was the girth at base; D_100 the diameter at 100 cm; DBH the diameter at 130 cm and H the height. N for *Enyeru*, *Nakinyika*, *Kibuzi_Nakitembe* and Pooled data were 28, 28, 112 and 168 respectively.

(v.13.3.5165). Descriptive statistics used to explain the distribution of biomass across cultivars were obtained for region specific and pooled data. ANOVA was run to test for any significant differences, if any, in biomass across the factors (cultivar type and growth stage) considering the l.s.d of their means at a 95% confidence level. Prior to equation development, simple linear regressions were run across cultivars for all variables (DBH, Height, Girth at base and Daimeter_100 cm) with biomass as the response to obtain a predictor variable (s) with a better explanatory power to predict biomass. Following results of Anderson-Darling normality test, data used in the generation of the equations were *log* transformed to fit a linear equation because the raw data were not symmetrically distributed; but also to increase on the sensitivity of the statistical tests (Seth, 2008). To develop the allometric relationships, half the data were used for equation calibration and the other half for validation.

One-way ANOVA was also performed to test for any significant differences in carbon content of cultivars as well as growth stages at a 95% confidence level. Mean values of the carbon content for the various plant parts were also determined. However, given that the degree of freedom for growth stage was 1 (very small to base a decision on), the difference in carbon content across growth stages was also tested using a two sample T-test assuming equal variance at a 95% confidence level (details of the analysis not presented in this document).

RESULTS

The average total dry biomass amounts across all cultivars sampled in Mbarara were generally higher (*Kibuzi*, 8.13±4.68; *Nakitembe*, 7.98±3.91 and *Enyeru* 9.15±4.58) than those in Lwengo (*Kibuzi*, 5.69±2.60, *Nakitembe* 5.59±2.98 and *Nakinyika* 4.89±2.45). Therefore, the relatively high average biomass amounts for pooled data (6.89±3.95) could perhaps be explained by the biomass amounts resulting from data obtained from Mbarara. The standard errors across all cultivars were high. The variation could be attributed to the differences in biomass that was obtained from plant individuals growing at different stages (H1 and H2). ANOVA results showed a significant difference in biomass for both factors (cultivar type and growth stage) with $P<0.001$. However, basing on the l.s.ds of the means, biomass was different for cultivars *Enyeru* and *Nakinyika*, and similar for *Kibuzi* and *Nakitembe*.

Therefore, it was on this basis that three allometric relationships were developed for biomass prediction of the cultivars (that is, *Enyeru, Nakinyika and Kibuzi_Nakitembe*). Also, a generic equation for EAHB was developed to ascertain how best it could predict biomass for other cultivars. Regression results for all cultivars as well as pooled data showed that DBH was highly correlated with a coefficient of determination (R^2) of above 87% compared to others (Table 1). These results were based on all the data for a specific cultivar or set of cultivars. It was therefore on this basis that DBH was selected as a better explanatory variable for biomass prediction of EAHB cultivars.

All equations were highly correlated with DBH ($P<0.001$) with R^2 between 82-94% being higher in cultivar specific equations of *Enyeru* and *Nakinyika* compared to a set of cultivars (that is, *Kibuzi_Nakitembe* and the Generic equation) (Figures 1 and 2, and Table 2).

A generic equation was also developed for use in similar studies in future for EAHB given that its predictions were highly correlated across all cultivars giving an R^2 of 82, 90 and 88% for *Enyeru*, *Nakinyika* and *Kibuzi_Nakitembe*; respectively (details of analysis not presented in this document). These were not significantly different from those predicted by the specific or a combination of cultivars as shown in Figures 1 and 2 above. Therefore, the linear equations that were developed for predicting total plant biomass of specific cultivars were as follows:

Carbon content of EAHB

On average, carbon content of EAHB across parts followed the pattern: fruit>leaf>corm>stem>

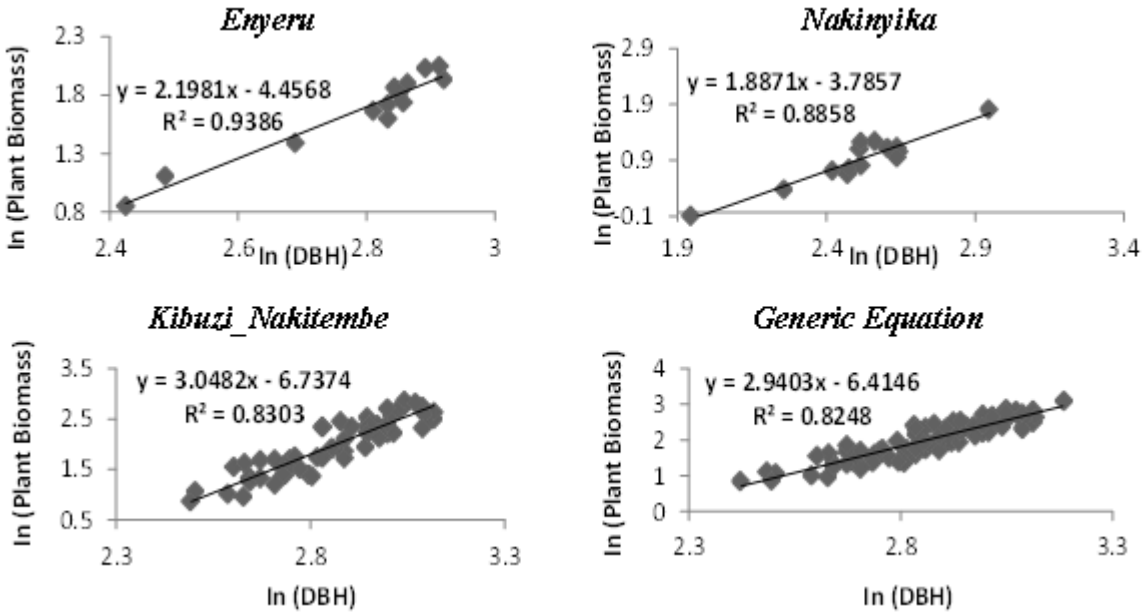

Figure 1. Calibrated allometric relationships for EAHB cultivars.

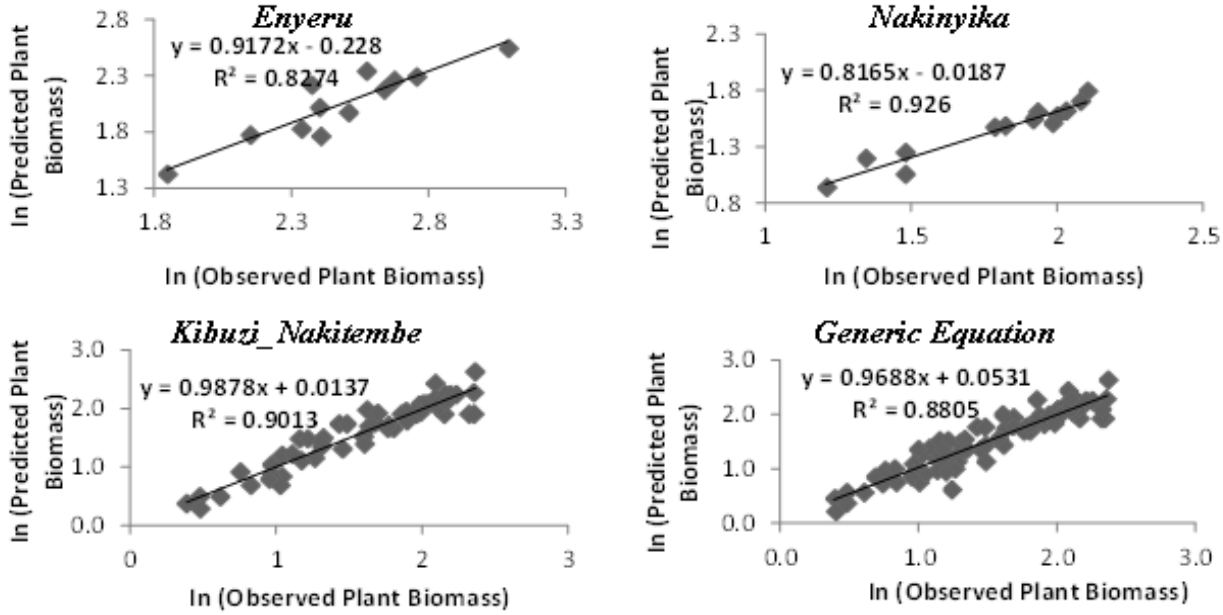

Figure 2. Validated allometric relationships for EAHB cultivars.

peduncle for H2; and leaf>corm>stem for H1 (Figure 3). However, in the interest of this study, focus was put on the carbon content of cultivars and or growth stages. Results from One Way ANOVA showed no significant difference in carbon content across cultivars (P>0.05) but growth stages (P<0.05). The later was also confirmed by the results obtained from the T-test (P<0.05). Therefore, 47.6 and 48.8% were the means of the carbon content

Table 2. Summary of equations developed for total biomass estimation of EAHB cultivars.

Cultivar	n	Model	c	a	S.E (a)	S.E (c)	R^2	R^2 (adj.)	*P*
Enyeru	14	$In(y)=c+aIn(x)$	-4.457	2.198	0.170	0.473	0.939	0.933	0.000
Nakinyika	14	$In(y)=c+aIn(x)$	-3.786	1.887	0.196	0.493	0.886	0.876	0.000
Kibuzi_Nakitembe	56	$In(y)=c+aIn(x)$	-6.730	3.048	0.189	0.540	0.830	0.827	0.000
Generic	84	$In(y)=c+aIn(x)$	-6.415	2.940	0.151	0.432	0.825	0.823	0.000

a and *c* are regression coefficient constants, *y*, dry plant biomass (Kg), and *x,* the explanatory variable DBH.

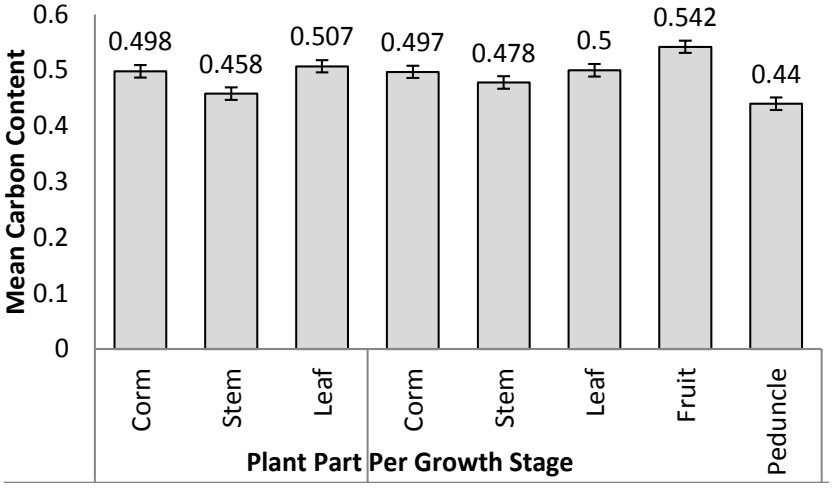

Figure 3. Carbon content means of plant parts.

considered in this study for H1 and H2; respectively as obtained from the T-test. Nevertheless, in studies where these growth stages are not considered e.g. at flowering, then the mean value of 48.2% can be used as the carbon content of EAHB.

DISCUSSION

Allometric relationships for biomass estimation of EAHB cultivars

The allometric equations developed in this study were cultivar specific (Figures 1 and 2; and Table 2) though results showed that total dry biomass was significantly different across both cultivars and growth stages. These findings are in line with Nyombi et al. (2009) suggestion on the need to develop growth stage (or cultivar) specific allometrics given that dry biomass of EAHB differs across ontogeny. This was also evident considering the differences in the means of dry biomass across cultivars in the different regions except for cultivars *Kibuzi* and *Nakitembe* whose biomass was not significantly different. Similarities exhibited in biomass obtained from *Kibuzi* and *Nakitembe* cultivars could perhaps be attributed to the fact that *Kibuzi* shows some similar traits as those of the *Nakitembe* clone set where *Nakitembe* cultivar belongs (Karamura, 1998). However, in the interest of this study, growth stage specific allometrics were not developed since focus was put on developing cultivar specific equations using data obtained from both stages; hence their applicability to all stages of growth considered in this study.

Linear regressions run on all potential total plant biomass predictor variables (height, girth at base, DBH and diameter at 100 cm) revealed that DBH was the best predictor variable with an R^2 ranging between 87-93% across cultivars (Table 1) similar to observations made for *Eucalyptus* in Kenya (Shem et al., 2013). Results are also in line with the predictor variable used for above ground biomass estimation of bananas developed in Indonesia by Arifin (2001) a widely used allometric relation for bananas in carbon studies (e.g. in Oliver, 2009; Henry et al., 2009; Hariah et al., 2010 among others) though DBH was taken as 135 cm. The variable is also commonly preferred for other perennial crops like

trees, coffee, cocoa, etc (Arifin, 2001; Basuki et al., 2009; Amy et al., 2010; Twongyirwe, 2010; Michiel et al., 2011; Sirike, 2012; Mugasha et al., 2013); hence making it a key variable to consider in such a study.

Girth at base however, emerged the weakest of all variables across cultivars (except *Kibuzi_Nakitembe* whose R^2 was very small) not being significantly related to biomass; results deviating from those obtained by Nyombi et al. (2009). This could perhaps be attributed to the fact that DBH has not been explored before for biomass estimation of EAHB among other factors. Important to note however is that the equations developed in this study (Table 2) cater for intraspecific variabilities that could perhaps be brought about by the type of cultivars used, age, and site conditions (edaphic and climatic variability) as noted by Juan et al. (2010). But also such variabilities could be as a result of increased variance in total dry biomass of individuals due to growth stages that resulted in high standard errors across all cultivars as well as pooled data (Table 2) (Nyombi et al., 2009) including on-farm variations and management among others.

Despite that the 3 parameters (DBH, height and diameter at 100 cm) gave high R^2 values, all could not be included in the model as this would be considered inappropriate; but also to eliminate cases of redundant parameters with high co-linearity in one equation function (Montgomery and Peck, 1992). However, in cases where DBH data is not available (e.g. when a plant is still young), height can be used as an alternative parameter for plant biomass estimation (Nyombi et al., 2009; Mugasha et al., 2013) though it is relatively difficult to measure as well as time consuming compared to DBH.

The fact that biomass quantities were significantly different for region specific cultivars but similar for common ones was proof enough to generate specific equations instead of a generalized one given that the former has the potential to improve the accuracy of prediction (Wairegi, 2010). However, developing such equations for more than 80 EAHB cultivars could be challenging due to limited resources (Karamura, 1998; Gold et al., 2002; Wareigi, 2010). Therefore, in cases where a cultivar specific equation is absent, the generic equation developed in this study could perhaps be applied on cultivars of more or less similar origin after all its prediction gave significantly high R^2 values for all cultivars ranging from 82-90%; not very different from specific ones.

Carbon content of EAHB

In general, the average carbon content of EAHB was found to be 48.2% relatively lower than the recommended value of 50% (Timothy et al., 2005; IPCC, 2006). Results are in line with those obtained for broadleaf tree species whose average C. content was minor than 50% for the whole plant (Arias et al., 2011) and among plant parts as reported in coniferous species in a study by Yen et al. (2009). Also, the value is very close to the 48% C content value that was used in a study by Shackleton and Scholes (2011) but slightly higher than the 46 and 47.9% values used for the conversion of dry wood biomass to carbon (Hairiah et al., 2010; ICRAF, 2011). All in all, results obtained in this study fall in the range of 46-49% carbon content values recommended for use in the tropics for tree species with DBH >10 cm (IPCC, 2006) considering that all individuals used for carbon content determination in this study had a DBH value >10 cm (Figure 3).

The difference of 1.2% in C content between growth stages could be as a result of one stage (H1) lacking both the fruit and peduncle components present in the other (H2) given that the components common to both show no significant difference across stages (Figure 3). Therefore, to obtain relatively accurate estimates for carbon stocks of cultivars in this study, it was considered prudent enough to use the growth stage specific C. content values, that is, 47.6% (H1) and 48.8% (H2) since they were locally available as recommended by Timothy et al. (2005).

In comparison with say tree components, generally bananas have more C. content in leaves at any stage (50-50.7%) compared to tree species like *V. guatamalensis* (41.0%) but not far from *P. caribeae* (49.6%) as observed in a study by Arias et al. (2011). This could be attributed to the fact that banana as a whole possesses large leaves as compared to any broad leaved tree species. However, comparing stems, EAHB contain less carbon content (45.8-47.8%) than one observed for tree species (e.g. *P. caribeae* with a 50.8% content). This could be explained perhaps by the pseudo-stem nature of banana stems containing high moisture content (Jing et al., 2010) as opposed to wood deposit present in trees. Therefore, considering these results, the 50% carbon content coefficient would be a relatively high estimate for species like EAHB (48.2%) but could be a fair rule of thumb in cases where the specific carbon content is missing (Arias et al., 2011).

CONCLUSIONS

Banana biomass can be accurately estimated using an exponential function ($y=Ae^{xp(ax)}$). The values of the constants tend to vary from one cultivar to another. The use of DBH as the best predictor variable for biomass of EAHB cultivars was confirmed as recommended for use in most carbon related studies. Carbon content was significantly different across growth stages ($P<0.05$) and not cultivars ($P>0.05$). The mean carbon content of EAHB is 48.2% slightly higher than the carbon content value

(47.6%) of banana plants before flowering and lower than those at maturity with a content value of 48.8%. All the values were found to be lower than the globally recommended 50% value by IPCC.

RECOMMENDATIONS

Generally, the allometric equations developed for biomass estimation of EAHB cultivars (*Enyeru, Nakinyika, Kibuzi and Nakitembe*) cater for intraspecific variabilities, growth stage, cultivar type and site conditions considering DBH as a key predictor variable as observed in other carbon related studies. Also, the determination of the actual carbon content of these bananas was timely as this was used to relatively estimate the actual plant carbon stock of the cultivars that would perhaps be lost in the use of readily available values. Therefore, more biomass prediction equations should be developed for other banana categories like plantains to ascertain the contribution of the entire banana cropping system to the global carbon cycle given that EAHB cultivars are not grown in isolation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Impact of crop rotation sequences on potato in fields inoculated with bacterial wilt caused by *Ralstonia solanacearum*

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The potato industry in Kenya is threatened by bacterial wilt because most production areas are infested with the wilt-causing *Ralstonia solanacearum* and over 50% yield losses have been reported. Continuous cultivation causes soil physical and biological constrains that greatly affect the crop performance and increase proliferation of the bacterium. Rotation with non-host or suppressant plant species could contribute to considerable reduction of bacterial wilt in the subsequent potato crops. This study tested the effect of different crop sequences on *R. solanacearum* population in the soil, wilting incidence and yield of potato. Two season field experiments were conducted at two sites (Egerton University, Njoro and National Agricultural Research Laboratories (NARL), Kabete) with 17 different crop sequences. Rotations involving brassica and legumes with potato gave a higher emergence percentage compared to the other sequences. The bacterial population was significantly influenced by the different environments from the first season to the third season; *F* (1, 102) =53.2, *P*<0.001, *F* (1, 102) =12.5, *P*< 0.001 and *F* (1, 102) =236.8, *P*<0.001 respectively. There was a significant effect *F*(16,119)=7.063, *P*<0.001 of the crop rotation sequences on the wilting incidence of potato. Pre cropping potato with spring onion and barley resulted to a significantly lower wilting incidence compared to all the other crop rotation sequences with a mean of 8.3% across sites. The results showed that Potato-Lablab-Potato and Cabbage-Lablab had the highest yield with 19.9 and of 19.7 tons/ha in the one crop rotations and pre crops to potato respectively. A Genotype x Environmental means versus IPCA scores showed that the yield due to barley-spring onion, spring onion-barley and wheat-spring onion as pre crops were more stable in both locations compared to the other cropping sequences. The study indicates that rotations involving spring onion with the locally grown cereals such as barley and wheat can be utilized in curbing bacterial wilt. Rotations involving lablab and cabbage may also be used to increase the yield of potato in bacterial wilt infested fields. These crops should be used in rotations involving more seasons so as to achieve better effects.

Key words: Bacterial wilt, crop rotation, potato yield, *Ralstonia solanacearum*, wilting incidence.

INTRODUCTION

Potato crop is the second most important staple food crop after maize in Kenya and an important food and cash crop in the medium and high rainfall areas where it has a comparative advantage over maize. Continuous cultivation on agricultural land causes soil physical constraints in the form of high bulk density, low water conductivity and reduced effective rooting depth of crops, which can greatly affect crop performance and increase nematodes infestation (Akanni and Ojeniyi, 2008). Bacterial wilt caused by *Ralstonia solanacearum* is reported to be one of the major challenges affecting potato farmers in Kenya (Ateka et al., 2001; Kaguongo et al., 2009; Nyangeri, 2011; Kwambai et al., 2011). Small scale farmers own small land parcels that limit them from practicing crop rotation and therefore practice continuous cultivation of crops in bacterial wilt infested soils. The question is therefore; what kind of cropping systems can be adopted by small scale farmers to reduce bacterial wilt infection in potato crop and thereby strengthen potato production?

Crop rotation as a cultural control method is applied with the objective of achieving maximum benefits due to the contribution of the crops to the soil and the crops effect to the pathogen population. The use of these crops is based on several principles such as use of non host crops, crops with suppressive effect due to their exudates and secondary metabolites, N fixing plants, plants with high residual matter and their adaptability to the specific environs. Plants release secondary metabolites in significant amounts at varying stages of plant growth. Some of the reported metabolites include; Benzoxazinoids: 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA) which is the most common benzoxazinoid found in wheat and wild barley among other cereals (Fall and Solomom, 2011). It is reported to cause allelopathy, repel insects and also resist pathogens. They are stored as inactive glucosides in the plant but become active upon tissue disruption. They cause mutagenic effects on the pathogens DNA and react with amino acids. Flavonoids are present in barley in the form of lutonarin, saponarin and isovitexin. Several phytoanticipans in the flavonoids group are reported to inhibit spore germination and the growth of *Xanthomonas oryzae* (Padmavati et al., 1997). UV-absorbing flavonoids in cotton leaf tissues have also been found to be antagonistic to *Xanthomonas campestris* (Edward et al., 2008).

Host exclusion or withdrawal is achieved by growing non hosts crops in any crop rotation regimes. The duration taken in the crop rotations highly determines the reduction of inoculum in the soil. Short term crop rotations have been shown to affect pathogen populations and disease status in the soil (Lemaga, 2001; Narayanasany, 2013). However research on long term rotations have shown that crop rotations require more time to significantly and effectively reduce soil borne pathogens (Larkin et al., 2010; Wright et al., 2015). Rotational crops are also used as cover crops to suppress disease both in the growing phase and in the decomposition phase by inducing an increase in soil microbial biomass and in soil biological activity. The nutritive contribution of the rotation crops as cover crops and as green manure is also another factor that is utilized in crop rotations. Residue quality is known to indirectly influence the organic matter content and aggregation. A marked difference has been observed in the levels of carbon in the soil after root and shoots were incorporated into the soil (Ball et al*.,* 2005). Shoots are considered to breakdown rapidly compared to the roots and therefore are a short term source of nitrogen to the subsequent crop. Decomposition of incorporated crop residues also vary depending on the C:N ratio and lignin content of the crop residues. Residues with a low C:N ratio (<25:1) are shown to decompose rapidly thereby creating a suitable substrate for microbial activity (Kriaučiūnienė et al., 2012). Dolichos bean has been found to decompose rapidly with a reduction of at least 25% to 63% of their initial dry weight within the first four weeks (Ibewiro et al., 2000; Ruiz-Vega et al., 2010) compared to the other crops in the study.

Other dynamics that influence the disease status in the soil as a result of crops grown include: Chemotactic effect of exudates, organic and amino acids from the different cover crops or their green manure (Yao and Allen, 2006). Chemotaxis as a factor that promotes the proliferation of the pathogen and contributes to the infection rate of the host plant in a crop rotation system. It is reported that *R. solanacearum* strain K60 was attracted both to plant root exudates of tomato which is a host plant and rice , a non-host plant to the pathogen, however tomato root exudates indicated three times stronger attraction compared to rice exudates at protein concentrations of 100 ug/ml (Yao and Allen, 2006). In this study, non-host acids and root exudates are reported to be less attractive or repellant to *R. solanacearum*. Proliferation of different microbes is also influenced by the different cover crops or green manure and has been observed to vary significantly according to the type of cover crop (Patkowska and Konopiński, 2014). Other factors such as the formation of DNA-containing extracellular traps by

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Table 1. Climatic and edaphic characteristics of the experimental sites.

Site	Altitude, Longitude and Latitude	Rainfall (mm)	Temp (°C)	Soil type	pH
Egerton	2225 m asl, 01° 13'S and 35° 30'E	1012	22	Sandy loam Mollic phaozems	5.5-6.0
NARL	1737m asl, 36° 41'E and 01° 15'S	980	23	Clay loam Humic nitisol	4.5-7.0

Source: Jaetzold and Schmidt (1993) and Oloo et al. (2011).

non host plants as observed by Tran et al. (2016) and Hawes et al. (2016) also plays a role in the proliferation of disease in the soil.

This study focused on the impact of crop rotation sequences of several cereals, legumes as well as spring onion on reducing bacterial wilt in infected soils and to improve potato yield. The efficacy of any crop rotation is determined by the type of soil, soil pH, soil moisture, weather and other abiotic factors and therefore crop yield and disease incidence may vary from one location to another and from one season to another (Adebayo and Ekpo, 2006). It is therefore important to consider crops for rotation in any specific environment based on their adaptability. The present study therefore attempted to use the mentioned crops grown by small scale farmers in medium to high altitudes areas to evaluate their impact on potato production and bacterial disease when utilized in crop rotations. This was carried out in fields inoculated with *R. solanacearum* to simulate farmers fields infested with bacterial wilt. The objective of this study was to identify alternative crops besides the potato monoculture that can be used to reduce the effect of bacterial wilt and consequently increased yields.

MATERIALS AND METHODS

Site description

Two season field experiments were conducted at two sites: Egerton University, Njoro and National Agricultural Research Laboratories (NARL), Kabete field stations in the years 2013 and 2014. The edaphic characteristics of these locations are indicated in Table 1.

Preparation of inoculum

For purposes of inoculum preparation, the procedures of Kinyua and Miller (2012) were followed. *R. solanacearum* isolated from infected tubers was grown on Tetrazolium Chloride (TZC) agar medium at 28°C for 48 h. Wild-type bacterial colonies (based on colony morphology) were harvested, suspended in CPG liquid culture (Casamino acids-peptone-glucose) and incubated for three days at room temperature, Kinyua and Miller (2012). The cultures were centrifuged at 10,000 rpm for 10 min at 10°C, suspended in distilled water and adjusted to 10^8 CFU ml^{-1}, as described by Yadessa et al. (2010).

Inoculation of experimental fields

The experimental fields were inoculated with *R. solanacearum* inoculum to simulate the farmers' fields which are infested with the pathogen. Disease infested plots were developed by growing a susceptible potato variety "Tigoni" for a season. Ten mililitres of the inoculum was sprayed to the rhizosphere of each plant at the 30th day after planting (Broekhuizen, 2002; Ayana et al*.,* 2011). All the potato plants were ploughed and incorporated into the soil after more than 50% of the plants showed wilting symptoms.

Treatments

The experiment had 17 crop sequences as the treatments using short season crops. Crops used were; spring onion var. Green bunching, Garden pea var. peas plum, Potato var. Tigoni, Wheat var. Kwale, Barley var, Sabini, Canola var. Tower, Lablab (Local variety) and cabbage var. Coppenhagen. The treatments were Potato-Cabbage-Potato (1), Lablab-Cabbage-Potato (2), Potato-Canola-Potato (3), Garden Pea-Canola-Potato (4), Potato-Lablab-Potato (5), Cabbage-Lablab-Potato (6), Potato-Garden Pea-Potato (7), Canola-Garden Pea-Potato (8), Potato-Spring Onion-Potato (9), Wheat-Spring Onion-Potato (10), Potato-Wheat-Potato (11), Spring Onion-Wheat-Potato (12), Potato-Barley-Potato (13), Barley-Spring Onion-Potato (14), Spring Onion-Barley-Potato (15), Potato-Potato-Potato (16) also referred to as monoculture in the text, Fallow-Fallow-Potato (17) – the plots were not ploughed or weeded in this treatment throughout the fallow seasons.

Experimental layout and agronomic practices

The experiment was laid out in a randomized complete block design with four replicates in plots of 3 m by 3 m. The two cropping patterns were considered. Rotation of potato with one crop termed as one crop rotation and use of two different crops before the main potato crop considered as pre crops to potato. The crops were planted in the long rains of 2013, short rains of 2013, and long rains 2014 in succession as indicated in the above treatment sequences. After every harvest, the above ground vegetative biomass was left on the ground and incorporated in the soil during the planting of the next crop in the next season using a hand hoe. The seed potato ("Var. "Tigoni") was planted at a spacing of 75 cm by 30 cm giving a total of 40 plants per plot in furrows in the last season. Hand weeding was done at 4 weeks after planting and ridging (earthing up) was done twice at four and eight weeks after planting. Late blight was controlled with Dithane-M45 and Ridomil-MZ 72 sprayed at alternating times at a rate of 50 g/10 L of water when it was necessary.

Data collection

Wilting incidence

Assessment of the bacterial wilt incidence started at the onset of wilt symptoms after which counting of wilted plants was done on a weekly basis. Plants that showed either complete or partial wilting were considered wilted and tagged to avoid double counting in subsequent assessments and also to avoid the possibility of missing out those completely killed early in the growth period. Wilt incidence for each treatment was calculated as number of wilted

plants expressed as a percentage of the total number of plants emerged.

Bacterial population in the soil

The population of *R. solanacearum* in the soil was established three weeks after planting in the first season and at 15 weeks after planting of each crop in the subsequent seasons. Four soil samples were randomly picked from each experimental plot at 20 cm soil depth and were mixed thoroughly to make one sample. Ten grams of soil from each sample was put in a flask with 30 mls of distilled water. The soil suspension was stirred on a rotary shaker at 150 rpm for 30 min. The soil suspension was allowed to settle and 1ml aliquot suspension was drawn out using a sterile pipette tip. This suspension was put in sterile Eppendorf tubes and formed the stock suspension. Serial dilution was carried out upto 10^{-4} suspension. An aliquot of 100 µl (0.1ml) of the soil suspension was lawn plated on Semi Selective Medium (SMSA) in a petri plate for 10^{-1} and 10^{-3} serial dilutions suspensions. The plates were incubated at 30°C for 48-72 h. The colonies that showed typical *R. solanacearum* characteristics (fluidal and irregular with a characteristic red or pinkish red centres and whitish periphery) were counted from the 10^{-3} serial dilution suspension. Data of bacterial population was considered after the second crop to evaluate the impact of the two crops before the main potato crop. The number of colonies per ml was calculated using the following formula

$$No.of\ bacteria/ml = \frac{CFU \times 1000}{0.1}$$

Log transformation of bacterial population data after the second crop in the rotations was done for the purpose of analysis of variance (Log10) cfu*10000).

Potato yield

Harvesting was done once at 110 days after planting. Total weight of all tubers; ware (>55 mm), seed (35-55 mm), and chatt size (<35 mm) in diameter was recorded for each plot.

Data analysis

Data was analyzed for the response variables (Emergence %, Wilting Incidence Days After Planting (WI DAP %) and yield (tons/ha) using two way analysis of Variance (ANOVA). Post hoc mean separation was done using Tukeys HSD whenever there were significant results. Spearman *rho's* correlation was done to examine the relationships between the response variables. IBM SPSS statistic software Version 20 was used for the analysis of this data. To determine the stability of the crop sequences in the two environments, AMMI analysis was done and an IPCA versus Genotype x Environmental means plot was generated to graphically visualize the mean performances and stability of the cropping sequences on the yield of potato (GENSTAT Version 15).

RESULTS AND DISCUSSION

Effect of the crop sequences on the emergence percentage of the potato

The crop sequences did not indicate any significant effect in the emergence of the potato crop grown in the third season of the crop rotation sequences. Rotations involving brassica and legumes gave higher emergence percentage compared to the other treatments in both rotation sequences and in both sites in the subsequent potato crop (Table 2). Cabbage-Lablab and Garden pea-Canola as pre crops recorded the highest emergence percentage in the subsequent potato crop. This may be attributed to the rapid decomposition and mineralization of vegetables and the contribution of *Dolichos lablab* to fertility in the soil as also observed by Aganga and Tshwenyaye (2003), Sanginga (2003) and Agneessens et al. (2014).

Rotations involving wheat and spring onion generally gave a lower emergence percentage compared to the brassica-legume rotations. This may be attributed to the allelopathic effect reported in wheat. It contains alleochemicals such as phenolics and alkaloids found in the leaves, roots, seeds and roots which have been shown to have suppressive effects in the germination of several crop seedlings. *In-vivo* and *in-vitro* trials have shown the efficacy of cereals such as barley in suppression of germination in most seedlings such as lettuce, bread wheat, cabbage and alfalfa (Kremer and Ben-Hammouda, 2009). Wheat straw has been known to have a positive allelopathic effect in the reduction of the density and biomass of weeds. It inhibits the growth and yields of other crops such as rice, barley, cotton and soybean (Lam et al., 2012). A secondary metabolite, benzoxazinoids found in wheat undergoes enzymatic and chemical degradation upon tissue disruption and their phytotoxic mechanism is attributed to mutagenic effects on DNA and their ability to react with amino acids and disrupt proteins of germinating seedlings (Fall and Solomon, 2011). These factors may have contributed to the differences observed in the germination of potato in the third season in the different crop sequences.

Bacterial density in the soil

The bacterial population was significantly influenced by the location from the first season to the third season; F (1, 102) =53.2, P<0.001, F (1, 102) =12.5, P< 0.001 and F (1, 102) =236.8, P<0.001 respectively which is attributed to the different environmental (rainfall, temperature and soil) parameters. A major factor that contributed to the significant effect of the location as a main effect was the soil pH (Table 1). The strongly acidic clay soils are reported to favour the survival of *R. solanacearum* (Sharma, 2004) and this contributed significantly to the high wilting index in NARL site.

There was a decreasing trend of the mean bacterial population from the first season to the third season in both crop rotation patterns (Figure 1). The mean wilting incidence (WI) and average bacterial population of *R. solanacearum* was high in crop sequences involving only one crop in rotation with potato as compared to the

Table 2. Effect of different crop rotation sequences on emergence, yield and WI (%) of potato in third season

Treatments	Emergence (%)		Yield (Tons/ha)		WI 5 WAP		WI 10 WAP		WI 15 WAP	
	Eger	NARL	Eger	NARL	Eger	NARL	Eger	NARL	Eger	NARL
Spring onion-barley-potato	86.3±8.2	63.1±16.7	29.6±4.5^{ab}	7.8±2.0^{a}	0.0±0.0^{a}	0.0±0.0	0.7±1.3^{a}	0.8±1.7^{a}	2.2±2.9^{a}	14.2±4.8^{a}
Spring onion-wheat-potato	80.6±11.3	65±6.1	27.5±5.1^{ab}	7.8±1.6^{a}	2.1±2.7^{a}	1.0±0.0	4.0±4.6^{a}	1.1±2.2^{a}	6.1±6.0^{ab}	14.1±8.6^{a}
Canola-garden pea-potato	79.4±12.5	65.6±10.8	30.9±2.6^{a}	7.1±0.25^{ab}	1.5±3.0^{a}	0.0±0.0	3.6±4.2^{a}	0.8±1.7^{a}	5.3±2.2^{ab}	15.0±11.5^{a}
Barley-spring onion-potato	80.6±6.9	72.5±10.2	28.9±2.3^{ab}	7.5±0.9a^{b}	0.7±1.5^{a}	0.0±0.0	3.0±2.4^{a}	0.8±2.5^{a}	6.1±4.1^{ab}	16.7±3.6^{a}
Lablab-cabbage-potato	84.4±4.5	70.6±8.7	31.4±3.1^{a}	6.5±1.4^{ab}	6.0±6.9^{a}	0.0±0.0	6.5±10.5^{a}	0.8±2.2^{a}	11.2±10.1^{ab}	13.4±3.7^{a}
Potato-barley-potato	84.4±5.5	61.8±10.1	32.0±4.3^{a}	4.3±2.4^{ab}	2.9±4.1^{a}	0.0±0.0	5.6±4.7^{a}	0.0±0.0^{a}	6.6±3.5^{ab}	18.5±8.1^{a}
Potato-lablab-Potato	83.3±7.5	69.4±14.2	33.2±3.6^{a}	6.7±2.3^{ab}	3.6±4.5^{a}	0.0±1.6	5.7±7.7^{a}	0.8±1.6^{a}	8.7±7.3^{ab}	17.1±12.0^{a}
Potato-wheat-potato	83.3±9.2	61.2±10.9	25.8±4.7^{ab}	5.4±3.8^{ab}	6.6±4.7^{ab}	0.0±0.0	10.8±9.1^{a}	4.3±6.3^{ab}	13.9±5.8^{ab}	12.8±5.4^{a}
Cabbage-lablab-potato	85.6±4.3	72.5±8.9	33.0±5.6^{a}	6.5±0.5^{ab}	9.7±4.3^{ab}	0.0±0.0	11.7±3.9^{a}	0.8±1.6^{a}	13.1±4.7^{ab}	17.1±12.0^{a}
Potato-garden pea-potato	80.0±16.2	60.6±8.8	28.7±2.7^{ab}	6.8±1.1^{ab}	4.7±2.8^{a}	0.9±1.8	4.7±2.8^{a}	0.9±1.8^{a}	12.0±7.0^{ab}	17.6±11.6^{a}
Potato-spring onion-potato	77.5±11.9	63.8±13.6	29.4±2.3^{ab}	4.8±3.2^{ab}	3.1±2.5^{ab}	0.0±0.0	5.6±2.8^{a}	1.9±2.3^{a}	8.3±4.2^{ab}	21.9±11.8^{a}
Wheat-spring onion-potato	71.9±4.3	69.4±9.4	29.2±2.9^{ab}	6.7±1.5^{ab}	6.4±6.1^{ab}	0.0±0.0	9.6±7.3^{a}	2.0±4.0^{a}	16.5±13.1^{ab}	15.0±6.0^{a}
Fallow-fallow-potato	70.6±12.3	73.1±14.8	28.5±4.2^{ab}	8.3±2.2^{a}	8.3±9.0^{ab}	0.8±1.5	8.3±9.0^{a}	0.8±1.5^{a}	15.1±9.9^{ab}	16.9±2.2^{a}
Garden pea-canola-potato	79.4±8.2	80.0±9.1	26.7±2..4^{ab}	8.9±1.2^{a}	6.9±5.4^{ab}	0.0±0.0	10.0±8.1^{a}	1.4±1.6^{a}	10.6±7.9^{ab}	15.9±5.3^{a}
Potato-canola-potato	80.6±7.7	78.8±4.8	28.7±3.2^{ab}	7.8±1.2^{a}	6.0±7.1^{a}	0.0±0.1	11.4±9.2^{a}	2.4±1.6^{a}	13.8±9.2^{ab}	24.3±9.3^{a}
Potato-cabbage-potato	78.1±8.3	77.5±6.1	26.4±6.3^{ab}	7.6±1.2^{ab}	11.8±3.3^{ab}	0.8±1.6	19.5±7.9^{ab}	2.4±2.9^{a}	23.4±10.4^{bc}	24.5±12.2^{ab}
Potato-potato-potato	79.4±8.3	70.6±12.9	20.7±3.1^{c}	2.7±2.3^{b}	22.7±15.8	0.8±1.6	36.5±9.9^{b}	10.5±4.8^{b}	39.4±10.4^{c}	45.5±2.4^{b}
Mean	**80.4**	**69.2**	**28.8**	**6.6**	**6**	**0.3**	**9.9**	**1.9**	**12.5**	**18.7**
Pvalues	**0.568**	**0.242**	**0.01**	**<0.01**	**<0.01**	**0.19**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

results from this study also confirm that cabbage is not a recommendable crop to use for rotations in bacterial wilt infected fields. The study also indicates that barley-spring onion has potential to suppress diseases and concurs with similar results from a previous study showing barley/clover causing a reduction of the fungal diseases in the short term (Larkin et al., 2010). The effect of barley and wheat in the suppression of disease in the subsequent crop and reduction of the bacteria population may be attributed to root exudates microbial interaction. The study also highlights a scientific question whether the order of the crops has an interactive effect on disease or pathogen inoculum. According to the study pre crops starting with spring onion resulted to a lower wilting incidence compared to the vice-versa.

Previous studies have shown a positive contribution of brassicas such as canola to reduction of fungal soil borne diseases (Bohinc et al., 2012; Boydston et al., 2011; Larkin et al., 2010; Bednarek et al., 2009). However, incorporation of canola as green manure in this study did not have a significant effect on bacterial wilt nor on the *R. solanacearum* population in the soil.

The bacteria population after the second season negatively correlated (spearman's rho) with the emerged plant stand of the potato crop in the third season (*rs*=-0.135). The emerged plant stand significantly correlated with the yield at (r_s= 0.565, p<0.001) and an increase in the wilting incidence resulted to a significant decrease in the yield (r_s=-0.380, p<0.001). This indicates the significance of the bacterial population on the potato crop from emergence to harvesting.

Effect of the crop rotation sequences on the yield of potato planted in the third season

There was a significant interaction between the

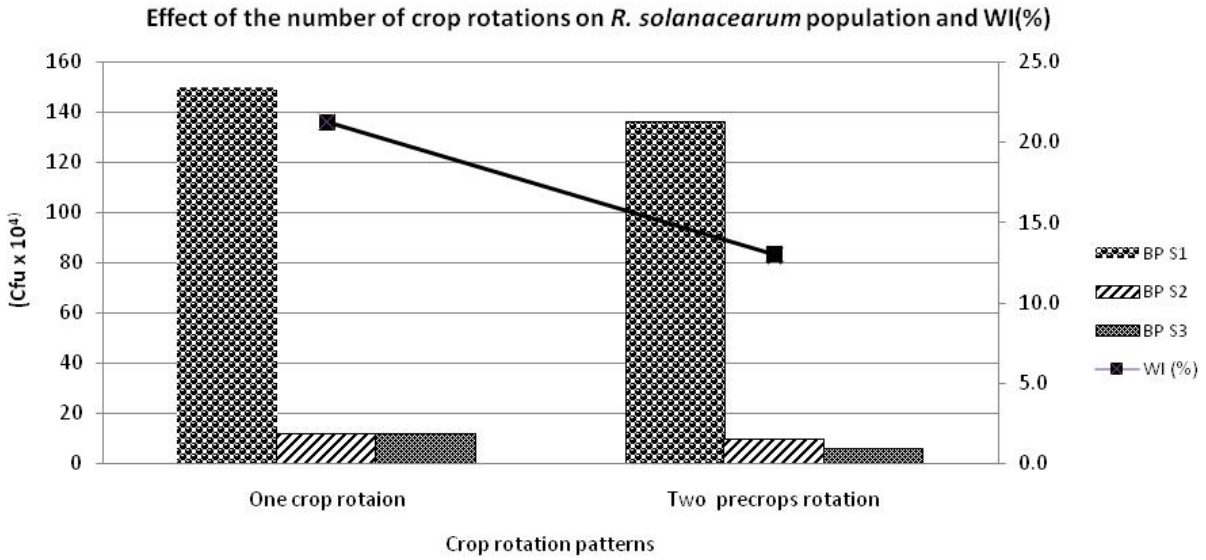

Figure 1. Effect of one crop rotation and pre crops sequences to potato on *R. solanacearum* population and WI% in the main potato season. Values are means of the cropping sequences in each cropping pattern. BP: Bacteria Population; S: Season; WI: Wilting incidence.

sequences involving two pre crops to potato. The mean yield was also higher in the two pre crops rotations compared to the one crop rotations with potato. The lower wilt incidence due to two seasons of rotation with different crops (Precrops) may be attributed to the break period which is a physical mechanism in controlling pathogen effect by withdrawal of the host. The absence of the host for two seasons in the two pre crop rotations resulted to a higher decline in the pathogen density compared to the one crop rotation sequences.

The bacterial population after the second crop was considered so as to evaluate the impact of the rotation crops on bacterial population in reference to the initial inoculation stage (Figure 2a and b). The pre crop of wheat-spring resulted to the highest decline of the *R. solanacearum* density in the soil (Figure 2b). A combination of the allelopathic effect of wheat and onion may have contributed to the reduced density of *R. solanacearum* in comparison to the other crop sequences. Flavonoids and benzoxazinoids are found in cereals such as wheat and barley and they have been reported to inhibit spore germination and growth of several pathogens such as *Xanthomonas oryzae* and *Xanthomonas campestris*, (Padmavati et al., 1997; Edward et al., 2008; Fall and Solomom, 2011).

Wilt incidence in the potato crop

The wilting progress significantly varied with the crop sequences. The progression of wilt in potato-cabbage-potato rotations did not significantly differ from the potato monoculture-no rotation (negative control) across all the three seasons as observed in Table 2. All the other one crop rotations had significantly lower progress in the disease development compared to the potato-cabbage-potato and the monoculture. There was a significant main effect of the crop rotation sequences on the wilting incidence; F (16,119) =7.063, P<0.001. Pre cropping potato with spring onion and barley resulted to a significantly lower wilting incidence compared to all the other crop rotation sequences with a mean of 8.3 and 2.2% wilt incidence respectively in both sites. Rotation of potato with wheat alone also had the least wilting incidence (12%) at NARL site. Spring onion-wheat-potato also had a lower wilting incidence of 10.1%, mean in both sites. Among the *R. solanacearum* non host bulb type crops recommended for crop rotation include spring onion according to Wang and Lin (2005). Potato-barley-potato rotation had the least WI in the one crop rotations with a mean of 12.1% across the two sites. Rotation of potato with cereals such as wheat and barley and spring onion was found to consistently influence the reduction of bacteria population and consequently reduce the wilting incidence (Table 2). Data in the table shows that the wilting incidence progress accelerated from the 10th week onwards.

Cabbage (*Brassica oleraceae* L.) is reported to be a common host plant of bacteria wilt and has also been found to be infected by *R. solanacearum* biovar 2 and 3 (Alvarez et al., 2008; Guidot et al., 2014; Nortj'e, 2015). Evaluation of different plants on wilt incidence and infection by *R. solanacearum* biovar 2 and 3 showed *Brassica oleraceae* var. capitata is a host to the bacterium whereas spring onion did not show any wilting nor was it infected by the two biovars (Nortj'e, 2015). The

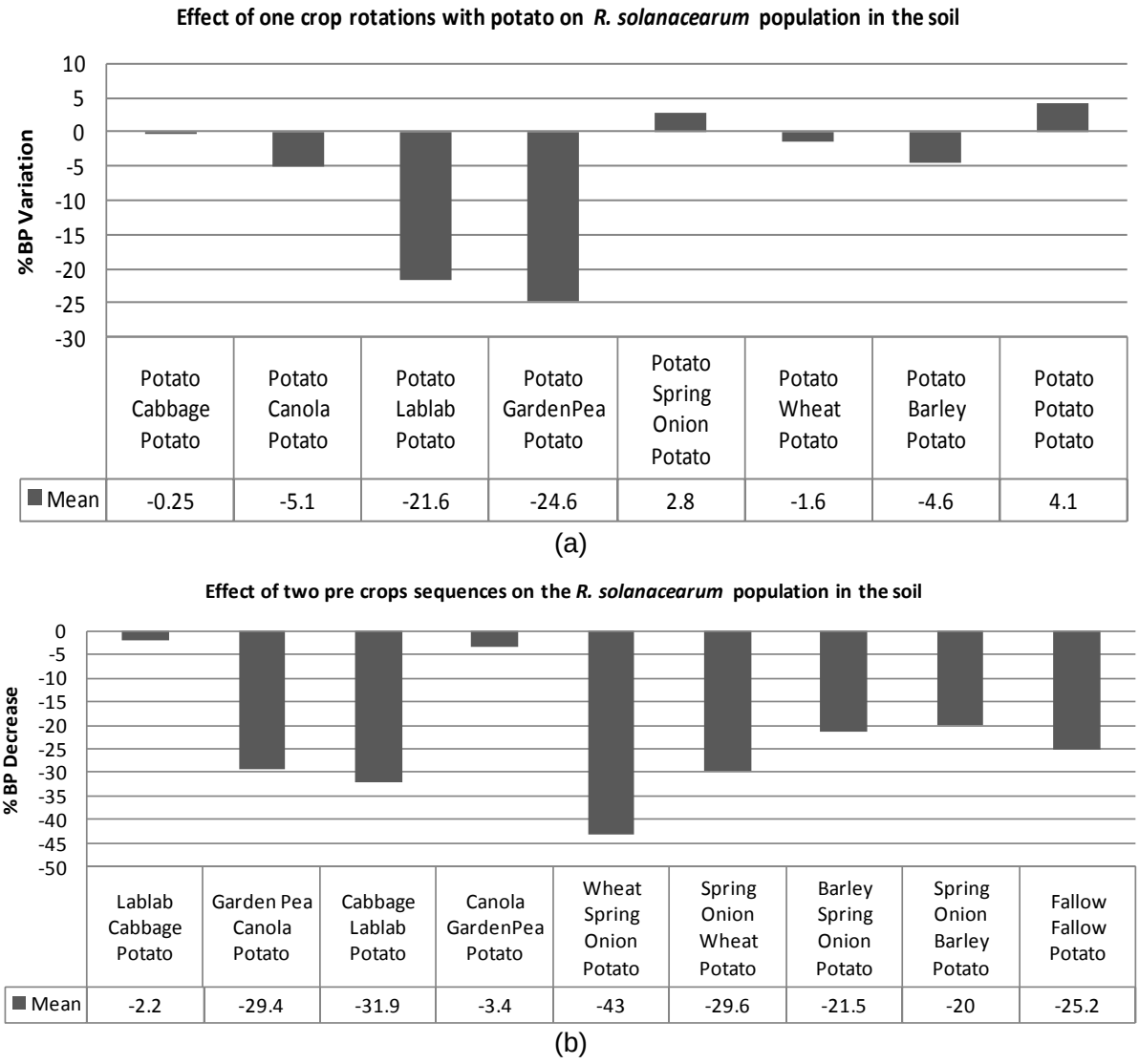

Figure 2. a) Effect of the different pre crops sequences on the % bacteria population in the soil after the second crop/season. b) Effect of the Pre crop rotations on the % bacteria population in the soil after the second crop % BP:% Bacterial Population % (Negative or positive figures denote a decline or an increase in the *R. solanacearum* population density respectively in reference to the bacterial population density after inoculation of the experimental field before the first crop was planted).

cropping sequences and the location $F(1,102)=1.9$, $P<0.025$ on the yields. The yield was significantly lower in NARL compared to Egerton site. This is attributed to the acidic soils (Table 1) found in NARL which favour proliferation of *R. solanacearum*. The mean yield in both sites showed that all other crop rotation sequences were significantly different from the monoculture except for the one crop rotation of potato with cabbage, wheat and spring onion. Potato-lablab potato and potato-canola-potato had the highest yield with 19.9 and 18.2 tons/ha respectively in the one crop rotations across the two sites. Cabbage-Lablab and Lablab-Cabbage as pre crops to potato also resulted to the highest yield of 19.7 and 19.0 tons/ha across the two sites. As shown in Table 2, rotation of potato with Lablab and canola yielded the highest in Egerton and NARL respectively in the one crop rotations with potato. Lablab is able to transport minerals from the depths of the soil to make it available to the plants due to its deep tap root and its active role in N fixation in the soil due to the presence of N fixing bacteria (Aganga and Tshwenyaye, 2003; Sanginga, 2003). Legumes are known to form symbiotic relationships with soilborne rhizobia known as plant growth promoting rhizobacteria (PGPR). These plant growth promoting bacteria produce plant growth regulators, are involved in symbiotic N fixation and solubilize minerals such as

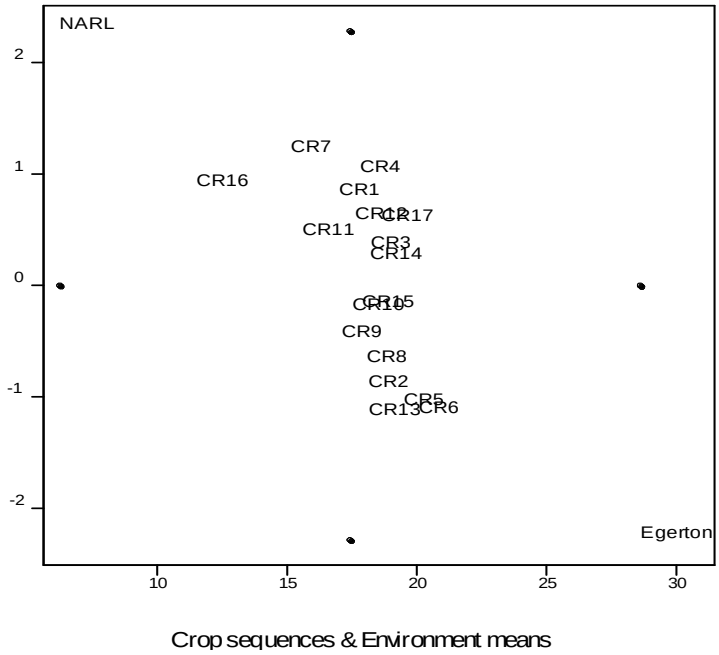

Figure 3. AMMI model for potato yield (t/ha) showing the genotype and environment means (X axis) against their respective IPCA 1 scores (Y axis).

phosphorus among other beneficial mechanisms that are important to crops (Montano et al., 2014; Cooper, 2008).

The presence of vegetable in the brassica legume patterns in the best yielding crop sequences may also have played a role to the yield increase due to the short duration taken by most vegetables for mineralization after incorporation into the soil surface (Agneessens et al., 2014). A study by Nyangeri (2011) indicated that cabbage recorded significantly marketable yields compared to crops such as maize and beans in one crop rotations with potato. Another research evaluating the rotation effects of canola, barley, and green beans to potato yield showed that canola yielded significantly higher yields compared to the other crops (Larkin et al., 2010). This study concurs with these previous results indicating the potential of brassicas in rotation with potato in increasing tuber yield. Residues with a high C:N ratio, high lignin and polyphenols content are known to immobilize inorganic N, resulting to reduced microbial activity and therefore reduced yields (Kumar and Gor, 1999). This may have contributed generally to the lower yields in the wheat and barley rotations with potato when compared to the rotations with legumes and brassicas.

Stability of the crop sequences

An additive main effects and multiplicative interaction (AMMI) model which combined the analysis of variance for the crop sequences and location main effects with the principal component analysis (PCA) of the crop sequences-environment interaction was performed on yield data of the potato (Figure 3). The effectiveness of crop rotation is highly dependent on the prevailing weather characteristics of any environment, adaptability of crops being used in the rotations and the soil environments which comprises of the physical and biochemical properties of soil. The potato yield after barley-spring onion (CR14), spring onion-barley (CR15) and wheat-spring onion (CR10) as pre crops was more stable in both locations compared to other cropping sequences. Rotation of potato with garden pea was more stable in NARL while cabbage-lablab and rotation of potato with lablab and barley were more stable in Egerton. The concept of environment specific system has also been demonstrated by several authors (Seremesic et al., 2013; Mrabet, 2011).

Conclusions

Rotations involving brassicas and legumes gave higher germination in the study. A higher emergence translated to a higher yield. Canola can be used in rotations with legumes with an objective of increasing the emergence percentage of potato. Two pre crops to potato will result to a decrease in bacteria population and at the same time reduce wilt compared to one crop rotation. The study also indicated that two successive crops planted as pre crops to potato are not adequate to destroy all the *R. solanacearum* population or eradicate the disease in the soil especially so if the inoculum is high. Rotation

involving spring onion and cereals such as barley and spring onion can be recommended to farmers in reduction of the bacteria population and wilting incidence in the subsequent potato crop but are however not recommended for boosting yield in potato rotation regimes. Lablab is a potential crop that can be used in rotation with potato to increase yields and can be recommended to farmers in Nakuru County while rotation of garden pea with potato can be recommended to farmers within Nairobi environs according to the stability studies. Short term evaluation of crop rotations are important predictors to crops that can be used to suppress bacterial wilt effect in farmers fields who are limited by land.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Elucidation of the betalainic chromoalkaloid profile of *Pilosocereus catingicola* (Gürke) Byles & Rowley subsp. *salvadorensis* (Werderm.) Zappi (Cactaceae) from Paraíba, Brazil

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The cacti are important plant genetic resources of the Brazilian semiarid region, with potential for the extraction of bioactive compounds such as flavonoids and alkaloids. The objective of this research was to characterize and quantify the chemical constituents of *Pilosocereus catingicola* (Gürke) Byles & Rowley subsp. *salvadorensis* (Werderm.) Zappi occurring in *caatinga* in the Westland of Paraíba, Brazil. We collected roots, stems and fruit of plants growing in populations at Arara, Areial and Boa Vista. Chemical characterization of the different plant tissues showed that roots and stems of *P. catingicola* contained steroids, flavonoids and saponins whereas fruit was dominated by high levels of betalainic chromoalkaloids (betalains). Tests performed to optimize extraction of betalains from *P. catingicola* fruit showed highest yields were from freeze-dried nuts extracted for 95 min, with the crude extract stored at -20°C for a maximum of 48 h. The betalains of all fruit samples were dominated by betacyanins with much lower amounts of betaxanthins observed in each population. Numerous betacyanin constituents were detected in fruit extracts, with the key constituents identified as betanin and phyllocactin. The Arara population yielded relatively more betaxanthins compared to plants from the Areial and Boa Vista regions.

Key words: Betacyanin, betanin, betaxanthin, caatinga, cactus, facheiro, phyllocactin.

INTRODUCTION

Plants in the Family Cactaceae (Order Caryophyllales) are indigenous to the Americas, possibly arising there some 30 million years ago during the mid-Tertiary period (Hershkovitz and Zimmer, 1997). The family is morphologically diverse, ranging from tree-sized specimens (e.g. *Pereskia sacharosa*) to creeping forms (e.g. *Ariocarpus retusus* subsp. *retusus*). Species occurrence is widespread throughout the tropical region

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of America, and in particular, Cactaceae members occupy vast areas in the semi-arid region of northeastern Brazil (Anderson, 2001).

An increasing variety of metabolites expressed by plants are targets of pharmacology studies due to their beneficial effects on living organisms such as antioxidant activity, whereby they prevent the oxidative stress caused by cellular metabolism, particularly by peroxidases and reactive oxygen species. Moreover, two classes of compounds extracted from within the Order Caryophyllales have received a great deal of attention due to their potential pharmaceutical applications - anthocyanins and betalainic chromoalkaloids (betalains), the latter restricted to a small group of only ten species (Chauhan et al., 2013).

Betalains are a group of nitrogen-based plant-products belonging to the alkaloid class and are biosynthesised via the shikimic acid pathway. They have known roles in preserving the integrity of cells by preventing oxidative processes caused by the action of free radicals that lead to apoptosis. Most reported betalains occur in flowers and fruits, but they can also be found in roots and leaves of plants belonging to the Order Caryophyllales where they effectively replace flavonoids such as anthocyanins in roles related to plant coloration (Gandía-Herrero and García-Carmona, 2013).

Betalains are characterized by vivid colors ranging from purple to violet or yellow and orange depending on the chains linked to the betalamic acid precursor of this metabolite. They are divided into two subgroups: the betaxanthins characterized by yellow, orange and red colours, which include the compounds vulgaxanthin, miraxanthin, portulaxanthin and indicaxanthin; and the purple-colored betacyanins found in certain vegetables as the compounds betanin, isobetanin, neobetanin, protobetanin and phyllocactin (Strack et al., 2003).

Several studies with cultured cacti have shown the nopal fruit (*Opuntia ficus-indica*) to be a promising commercial source of antioxidant compounds, particularly betalains (Stintzing et al., 2002; Wybraniec and Nowak-Wydra 2007; Castellanos-Santiago and Yahia, 2008; Jerz et al., 2008; Wybranec et al., 2009; Gandía-Herrero and García-Carmona, 2013). In addition, Anderson (2001) reported the presence of nitrogenous natural pigments, which include the betacyanins (violet color) and betaxanthins (orange), in *Lophophora williamsii* together with over 50 different types of other alkaloids.

The aforementioned studies were undertaken to characterise the diversity of alkaloids in plants within the genus *Opuntia*. Despite this work, there have been no such chemical studies of native cacti in Brazil. Surveys that have been done have related exclusively to the occurrence and distribution of plants in the genera *Cereus*, *Pilosocereus*, *Harrisia*, *Melocactus* and *Tacinga* in the arid zone of Paraíba. These plants have wide distribution in local ecosystems, but there has been a lack of investigations on their chemical constituents or potential uses and applications. It is likely that species in these genera may be important sources of functional compounds such as betalains.

The objective of this research was to characterize and quantify the betalainic constituents extracted from different tissues of *Pilosocereus catingicola* (Gurke) Byles & Rowley subsp. *salvadorensis* (Werderm.) Zappi occurring in populations within the *caatinga* areas of the state of Paraíba, Brazil.

MATERIALS AND METHODS

Sample collection

Plant samples were collected from three forest fragments of *caatinga* in the regions of Arara, Areial and Boa Vista, belonging to the middle Westland of Paraíba, Brazil (Figure 1). The three areas were chosen based on the criterion of having large number of specimens of *Pilosocereus* spp. reported by Barbosa et al. (2015).

Material collection and preparation of samples

Four samples were collected from each of the three populations (N = 12). The collection proceeded randomly at 8 am observing the cardinal points: North, South, East and West, where North was the reference point, and samples were harvested using a cleaned and sanitized knife hoe. The material was placed in a thermal box and taken to the Laboratory of Phytochemistry - Pharmaceutical Technology Laboratory (Prof. Raimundo Braz Filho) of the Universidade Federal da Paraíba, João Pessoa, Brazil and dried at 40°C for 120 h. After drying, samples were ground to a fine powder using a grinding mill (CQA m120) and then 50 g of each sample was packed into 250 mL plastic containers.

Extraction and determination of betalains in fruit

Dried samples were transported to the Plant Physiology Laboratory (Prof. Ian Woodrow), School of BioSciences, University of Melbourne, Australia. The protocol for extraction was adapted from Stintzing et al. (2002), with the modifications that 2 g of the dried pericarp was mixed with 5 mL of 50% MeOH and 50 mmol L^{-1} sodium ascorbate. Initially, the dried product was ground and then packed into 50 mL tubes. Each extraction was repeated five times. After solubilization with MeOH, the material was stirred by vortexing for 5 min at 25 °C, then centrifuged at 2000 rpm (25 °C). The supernatant was collected and concentrated under full vacuum centrifuge (SpeedVac) and then dissolved in 1 mL 100% MeOH and stored at -20°C.

For the hydrocolloid precipitation, 2 mL of 96% ethanol was added to 1 mL of the ground sample; the material was then left for 20 min for precipitation of proteins and mucilage. For separation, a 0.45 µM membrane was used (Phenomenex, Torrance, CA, USA) and samples were washed with 2 mL of ethanol/water (2:1, v/v). The ethanol was removed under reduced temperature using a SpeediVac at a temperature of 30°C and then the residue was redissolved in acidified water (pH 3).

The absorbance of extracts was measured using a spectrophotometer Mertek® SP-870 (600 nm; Sanford, North Carolina, USA) and the betalain content mg 100 g^{-1} = (A*F*MW*100/ε*l) where A = Absorbance; F = Dilution factor; MW = Molecular weight (indicaxanthin = 308 g mol^{-1} and betanin = 550 g mol^{-1}), ε is the molar extinction coefficient (indicaxanthin = 48,000 L

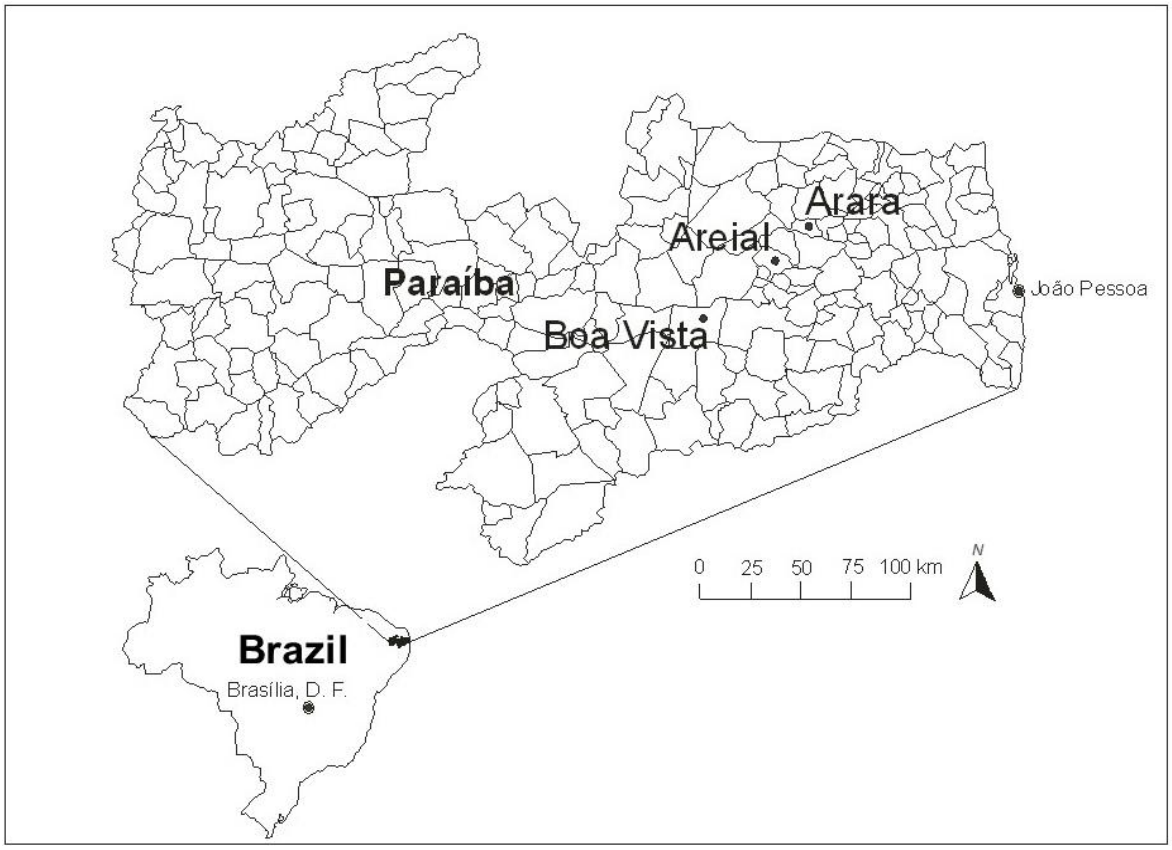

Figure 1. Location of sampled populations of *Pilosocereus catingicola* (Gurke) Byles & Rowley subsp. *salvadorensis* (Werderm.) Zappi within Brazil. (a) Map of Brazil showing the location of the region of Paraíba (box); scale bar represents 500 km. (b) Magnified map of the region of Paraíba showing the location of the three sampled populations; scale bar represents 50 km.

mol^{-1} cm^{-1} and betanin=60,000 L mol^{-1} cm^{-1}), and 1 = path length (1 cm) of the cuvette.

The separation of betalains was performed using reversed phase high performance liquid chromatography (RP-HPLC) and identification based on liquid chromatography mass spectrometry (LC-MS). We used an Agilent HPLC system (Santa Clara, CA, USA) 1200 with photodiode array detector and a LUNA C18 column (Phenomenex). Eluent A consisted of 0.2% trifluoroacetic acid (TFA) and 10% formic acid [HCOOH (65:35, v v^{-1})] and eluent B prepared with a mixture of 100% acetonitrile and 10% HCOOH (80:20, v:v). The system was coupled to an Agilent 6520 Quadrupole with time of flight mass spectrometer (QTOF MS) with electro-spray ionisation (ESI). The ESI-QTOF MS was operated in positive mode using the following conditions: pressure of nebulizer 35 psi, the gas flow 11 L min^{-1}, the gas temperature 325°C, capillary voltage 4000, Fragmenter 150 and skimmer 65 V. Data were collected based on three replicate runs and compounds identified based on UV absorbance, retention times and comparison of parent and MS2 fragment masses with literature values.

RESULTS AND DISCUSSION

Quantification and profile of betalains

The betacyanin concentration (mg 100 g^{-1} DM) of plants did not differ significantly between populations (Figure 2). The mean betacyanin contents were 17.4; 18.3 and 14.0 mg 100 g^{-1} for Arara, Areial and Boa Vista, respectively. Nevertheless, the betaxanthin content was significantly different between populations, with the highest content found in Arara (11.2 mg 100 g^{-1}) compared to Areial (8.1 mg 100 g^{-1}) and Boa Vista (6.3 mg 100 g^{-1}; Figure 2). The mean total content of betalains (betacyanins + betaxanthins) was 28.7; 26.4 and 20.4 mg 100 g^{-1} for populations of Arara, Areial and Boa Vista, respectively (Figure 2).

The betalain concentration found here for *P. catingicola* is quite similar to that found in other Cactaceae species such as *Opuntia stricta* (80.1 mg 100 g^{-1}), *O. ondulata* (19.6 mg 100 g^{-1}) and *O. ficus-indica* (15.2 mg 100 g^{-1}; Castellar et al., 2003), and the report by Castellanos-Santiago and Yahia (2008) of 81.0 mg 100 g^{-1} in *O. ficus-indica* varieties. However, the betalain concentrations in *P. catingicola* are lower than those of other groups such as *Beta vulgaris* plant varieties with 40-60 mg 100 g^{-1} (Von Elbe et al., 1981), and some Amaranthaceae reported by Cai et al. (2005) where values between 15.4 and 46.9 mg 100 g^{-1} were found in the dry tissue.

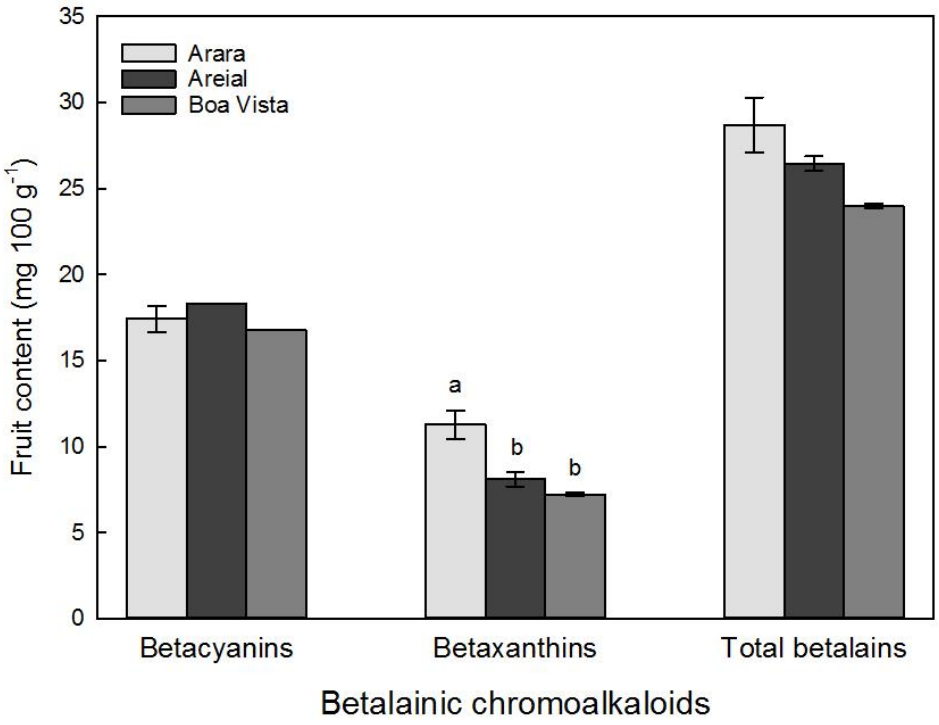

Figure 2. Total betalains (betacyanins and betaxanthins) quantified in extracts of *P. catingicola* subsp. *salvadorensis* fruit collected from three populations in Paraíba, Brazil. Data were analysed with one-way ANOVA with Tukey's post-hoc tests. The only significantly different result was observed for betaxanthins extracted from the Arara population (ARA, Arara; AR, Areial; BV, Boa Vista).

The betacyanin HPLC profile was qualitatively similar for all specimens studied (Figure 3); however, the relative amounts of some constituents differed between sampling sites, although no clear trend could be detected.

The first peak in the chromatogram was consistently the most abundant compound and was identified as the betacyanin betanin (Figure 3) at a retention time (RT) of 10.53 min. The compound had a parent mass of *m/z* 551.15 corresponding to $[M+H]^+$ $C_{24}H_{27}N_2O_{13}$ with key MS2 fragments of *m/z* 389, 309, and 507. This is identical to the fragmentation pattern found for betanin in numerous studies (e.g. Wybraniec and Nowak-Wydra (2007) in cactus fruit of *Mammilaria gender*; Strack et al. (2003) in Amaranthaceae plants; Stintzing et al. (2002) in fruits of *O. ficus-indica*; Jerz et al. (2008) in fruits of *Phytolacca americana*; and Wybraniec et al. (2009) in fruits of *Hylocereus polyrhizus* (Cactaceae).

The second peak was present in only trace amounts in all samples and was characterized as a 2'-O-apiosyl-isobetanin (RT 12.73 min; *m/z* 683.19 corresponding to $[M+H]^+$ $C_{29}H_{34}N_2O_{17}$ with fragment ions at *m/z* 551, 389 and 345; Figure 3). This betacyanin was previously unknown in Cactaceae, but recently it was elucidated by means of HPLC-ion interaction chromatography and mass spectrometry by Wybraniec et al. (2009) as an isomeric form of betanin.

The third peak was characterized as isobetanin (RT 12.96 min; *m/z* 551.15 corresponding to $[M+H]^+$ $C_{24}H_{26}N_2O_{13}$ with fragment ions 389, 507 and 344; Figure 3), and this matched the mass fragmentation pattern observed by Wybraniec and Nowak-Wydra (2007), Stintzing et al. (2002) and Jerz et al. (2008) in *Mammilaria*, *O. ficus-indica*, and *Phytolacca americana,* respectively.

The fourth peak was observed at low levels and was characterized as 17-decarboxybetanin (RT 13.51 min; *m/z* 507.16 corresponding to $[M+H]^+$ $C_{23}H_{26}N_2O_{11}$ with fragment ions of 345, 399 and 307 (Figure 3). This compound has also been characterized by Wybraniec-Wydra and Nowak (2007) in *Mammilaria*. Similarly, tests conducted by Jerz et al. (2008) using Ion-Pair High-Speed Counter-Current Chromatography (IP-HSCCC) analysis confirmed the presence of decarboxylated (2-decarboxy and 17-decarboxy) betanin/isobetanin and neobetanin, betanin derivatives in *P. americana* extracts. Such decarboxylated betacyanins can be produced as degradation products when alcoholic extracts are heated (Wybraniec et al., 2009). Therefore it is possible that the presence of decarboxy-betacyanins in the samples presented here may be indicative of decarboxylation occurring during the extraction process, but given extractions were performed under ambient temperature,

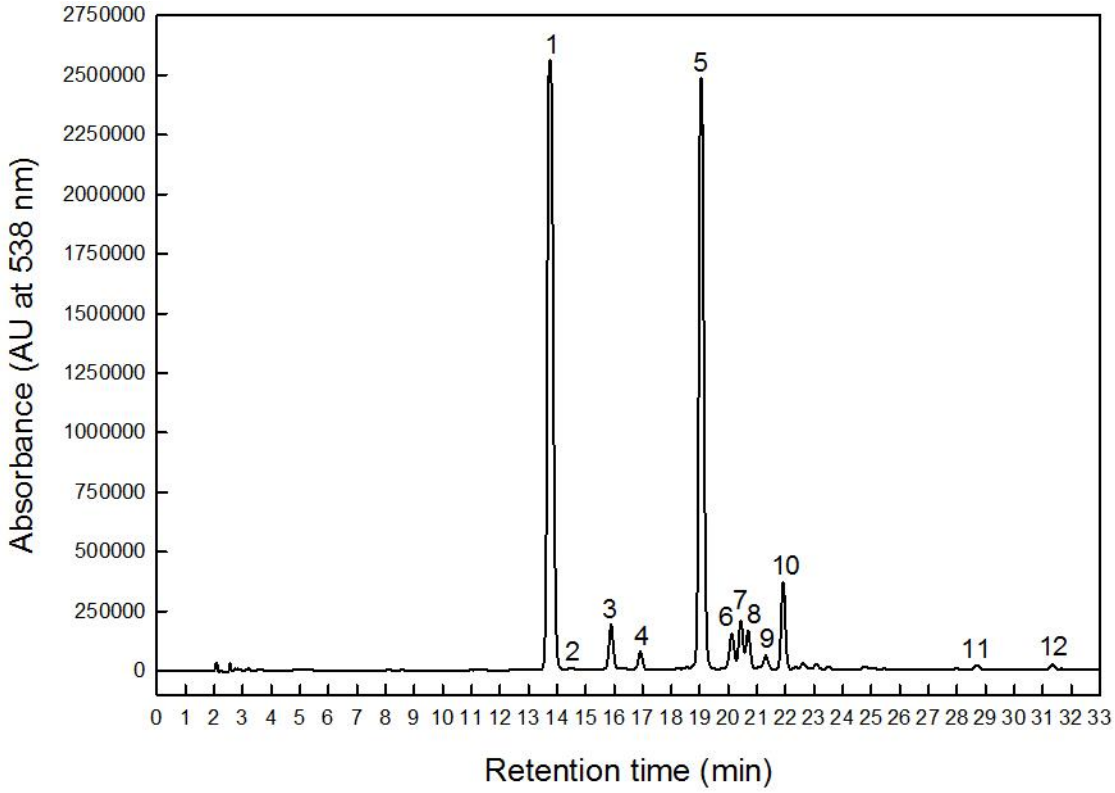

Figure 3. Representative HPLC chromatogram (λ 538 nm) of methanol extracted betalains from fruit of *P. catingicola* subsp. *Salvadorensis* collected from Paraíba, Brazil. Twelve betalainic chromoalkaloids were present in all samples collected from populations at Arara, Areial and Boa Vista. Compounds were identified by mass spectometry as: 1) betanin, 2) 2'-O-apiosyl-isobetanin, 3) isobetanin, 4) 17-decarboxybetanin, 5) phyllocactin, 6) 15-decarboxy-betanin, 7) isophyllocactin, 8) 6'-O-malonyl-2-decarboxybetanin, 9) unknown phyllocactin derivative, 10) 2'-*O*-apiosyl-phyllocactin, 11) 2'-(5''-O-E-Feruloylapiosyl)betanin, and 12) lampranthin II.

this is perhaps unlikely. Nonetheless, it has also been shown that decarboxylation of pigments can occur if the time between harvesting and freezing of the material is prolonged (Wybraniec and Nowak-Wydra, 2007). Although the time was minimised in this study, it remains possible that the observed decarboxy-betacyanins are artefacts of extraction.

The second most abundant compound in all samples was the fifth peak, identified as phyllocactin (RT 15.2 min, *m/z* 637.15 corresponding to $[M+H]^+$ $C_{27}H_{28}N_2O_{16}$ with MS2 ions 593, 389 and 551; Figure 3). Strack et al. (2003) characterised a compound with matching mass as a betacyanin from Cactaceae fruit. In that study, the connection of the malonyl residue of phyllocactin to glucose was deduced by analysis of permethylation and was confirmed by nuclear magnetic resonance (NMR) of phyllocactin isolated from *Schlumbergera buckleyi* flowers (Cactaceae; Minale et al., 1966; Kobayashi et al., 2000).

The sixth peak was characterized as 15-decarboxy-betanin (RT 16.06 min; *m/z* 507 corresponding to $[M+H]^+$ $C_{23}H_{25}N_2O_{11}$ with MS_2 ions 389 and 375; Figure 3). This compound has previously been described as being exclusive to *B. vulgaris* and its decarboxylated form as an artifact of extraction under elevated temperature (Herbach et al., 2004).

The seventh peak was characterized with an isophyllocactin (RT 16.06 min; *m/z* 637.16 corresponding to $[M+H]^+$ $C_{27}H_{28}N_2O_{16}$ with *m/z* 389 and 375 daughter ions; Figure 3) consistent with the molecular fragmentation found by Wybraniec and Nowak-Wydra (2007) and Wybraniec et al. (2009) in extracts from *H. polirhizus*.

The eighth peak was identified as 6'-O-malonyl-2-decarboxybetanin (RT 16.25 min; *m/z* 593.16 corresponding to $[M+H]^+$ $C_{26}H_{28}N_2O_{14}$ with *m/z* 345 and 389 daughter ions; Figure 3) with identical molecular fragmentation found by Wybraniec and Nowak-Wydra (2007) in *H. polirhizus* extracts.

A low abundance compound was observed as the ninth peak and remains unidentified. It is likely an unknown betacyanin (RT 16.89 min; *m/z* 785.24 corresponding to $[M+H]^+$ $C_{32}H_{36}N_2O_{21}$ with *m/z* 311 452 and 637 daughter ions; Figure 4). A search of the Scifinder database found

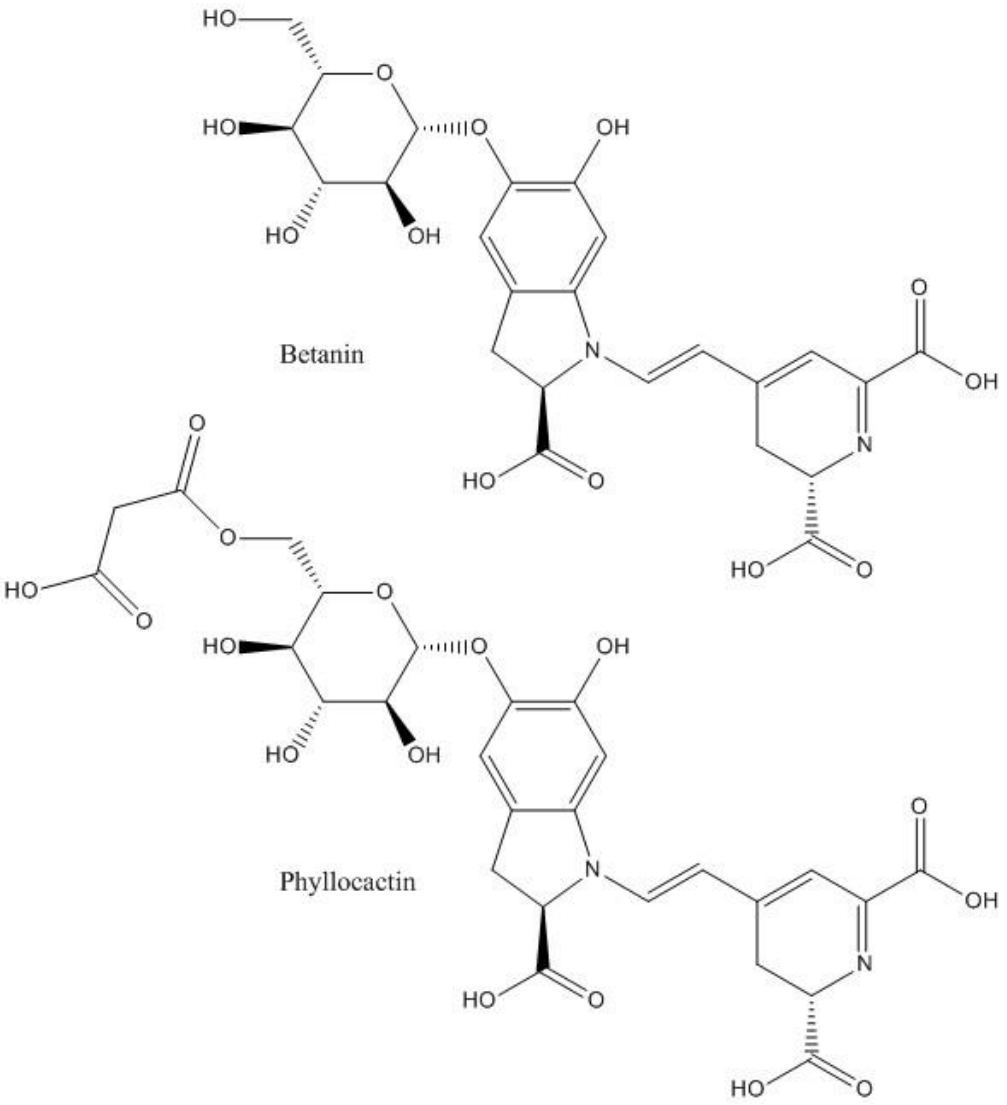

Figure 4. Chemical structures of the betacyanina betanin and phyllocactin, the two most abundant betalains found in fruit of *P. catingicola* subsp. *salvadorensis* collected from Paraíba, Brazil.

no known betacyanins matching that molecular formula. We suggest this compound is a novel betacyanin with a similar base structure to phyllocactin due to the 637 daughter ion, but with additional ornamentation. Further experiments using NMR spectroscopy are required to elucidate its structure.

The third most abundant compound in all samples was the tenth peak which was identified as 2'-*O*-apiosyl-phyllocactin (RT 17.5 min; *m/z* 769.19 corresponding to $[M+H]^+$ $C_{32}H_{36}N_2O_{20}$ with *m*/*z* 549, 637 and 389 daughter ions; Figure 3). This compound had previously been identified from Cacti by Wybraniec et al. (2010).

The eleventh peak was present in very low amounts, but could be identified as 2'-(5''-O-E-Feruloylapiosyl)betanin (RT 22.28 min; *m/z* 859.24 corresponding to $[M+H]^+$ $C_{39}H_{42}N_2O_{20}$ with *m*/*z* 333, 377 and 571 daughter ions). This compound has been reported by Strack et al. (2003) in experiments with plants of the Amaranthaceae family and by Kobayashi et al. (2000) examining betalains from Christmas cactus.

Despite also occurring in very low amounts, the twelfth peak was identified as lampranthin II (RT 30.3 min, *m/z* 727.2 corresponding to $[M+H]^+$ $C_{34}H_{34}N_2O_{16}$ with *m*/*z* 303, 585 and 389 daughter ions; Figure 3). Strack et al. (2003) analyzing the structure of lamprantin II (6'-OE-feruloyl-betanin) confirmed the presence of a pentose using NMR analysis. In addition, Vogt et al. (1999) observed accumulation of this compound in response to high light stress in *Mesembryanthemum crystallinum* epidermal layers (Aizoaceae).

Liquid chromatography coupled to mass spectrometry and photodiode array detector enabled us to successfully characterise the betalainic constituents of *P. catingicola* subsp. *salvadorensis* occurring in the *caatinga* areas in the state of Paraíba, Brazil. Similar approaches have achieved betalain separation and identification in fruits of *Opuntia* spp. (Castellano-Santiago and Yahia 2008) and fruits from *B. vulgaris* and *O. ficus-indica* (Stintizing et al. 2002). Another, potentially superior technique for identifying phytochemicals such as betalainic chromoalkaloids in extracts is LC-NMR, which can provide unambiguous structure of the molecule (Stintizing et al., 2003). For example, studies undertaken by Stintizing et al. (2005) analyzed betacyanin pigments by LC-NMR and 2D NMR spectroscopy in *Hylocereus polyrhizus* at neutral pH. Similarly, Wybraniec et al. (2006) and Wybraniec-Wydra and Nowak (2007) characterized phyllocactin, hilocerenin and betanin in *H. polyrhizus* and *H. mamilarinina* using 1H and 13C NMR spectroscopy.

In recent years there has been increasing interest in the development of natural dyes, mainly due to the apparent toxicity of synthetic dyes and their roles as environmental pollutants - this demand is being driven by strong consumer pressure. Consumer awareness is increasing day by day and is culminating in the interests of consumers to acquire natural food with integrity. Therefore, identification of natural alternatives to synthetic pigments is an important avenue of research for the food industry (Wang et al., 2006).

The cacti are prime candidates for providing natural dyes to replace synthetics. Among these plants, Park et al. (1998) and Stintizing et al. (2005) reported *O. ficus-indica* as a promising source of betalain compounds, not only as coloring agents but also an agents with the purpose of promoting cellular integrity through removal of free radicals and additional chemical reducing properties. Extracts of *O. ficus-indica* fruits exhibited antioxidant activity in various *in vitro* assays, including the oxidation of lipids in red blood cells of the blood and the oxidation of human LDL induced by copper and 2,2-azobis (2-amidinopropane) dihydrochloride (Tesoriere et al., 2004), and the ethanolic extract from cladodes show anti-inflammatory and analgesic effect (Park et al., 1998).

Natural dyes based on betacyanins and betaxanthins, such as those demonstrated in this work, have excellent nutritional characteristics, and also act as antioxidants and scavengers of free radicals (Strack et al., 2003), and their presence in the diet can reduce the risk of cardiovascular disease, cancer and age-related diseases (Delgado- Vargas et al., 2000). In addition, assays using betalains from *Rivina humilis* L. (Phytolaccaceae) have demonstrated effective action against lipid peroxidation and *in vitro* cytotoxicity towards cancer cells (Khan et al., 2012).

Conclusion

The fruits of *P. catingicola* subsp. *salvadorensis* growing in the *caatinga* areas of the state of Paraíba, Brazil contain high levels of betacyanins, particularly betanin and phyllocactin, together with lower levels of betaxanthin chromoalkaloids. Samples harvested from three different populations all showed similarly high abundances of betacyanins. Consequently *P. catingicola* has the potential for agricultural development and commercial harvesting as a source of antioxidants and natural dye compounds for use in in the pharmaceutical, cosmetic and food industries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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