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Full Length Research Paper

Alellopathic effects of aqueous extracts from *Bambusa vulgaris* Schrad. Ex J. C. Wendl. on seed germination and vigor from *Lactuca sativa*

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The aim of this work was to evaluate allelopathic effects of aqueous extracts from *Bambusa vulgaris* leaves and culm on *Lactuca sativa* L. germination. Plant material was collected at Dois Irmãos State Park in Recife, Brazil, and analysis was performed at Laboratory of Forest Seeds Analysis at Rural Federal University of Pernambuco. Experiments were conducted under natural conditions using leaf and culm extracts at concentrations of 100, 75, 50 and 25%, using distilled water as control. Seed germination was evaluated for seven days. Percentage of germination (%G), speed index of germination (SIG), mean time of germination (MTG), and mean speed of germination (v) were determined. Aqueous extract of *B. vulgaris* leaves negatively influenced percentage of germination and speed of germination index of *L. sativa* seeds. Control treatment showed the best means, being statistically superior than other treatments that did not differ among each other. Considering *L. sativa* as a bioindicator, it is possible that allelopathic effects might significantly interfere on other species establishment and germination. This is due to substances present on plant residues deposited or incorporated to the soil annually. Finally, the inhibitory allelopathic behaviour of the germination process of *L. sativa* seeds in response to aqueous extracts of *B. vulgaris* was observed in all parts and concentrations tested.

Key words: Allelopathy, allelochemicals, culm, bioindicator, *Lactuca sativa, Bambusa vulgaris.*

INTRODUCTION

In the last years, with traffic development, increase in human activity and strengthening of international business, communication between biological species from different habitats has become more frequent. Some

species were strongly adaptable to the environment and could grow and spread rapidly in the new environment, which affected local economy, ecology and society. Nowadays, allelopathy has been considered the main factor influencing invasion and dissemination of exotic plants (Chengxu et al., 2011).

Dois Irmãos State Park (PEDI) is a Full Protection Conservation Unit located in Recife/PE, Brazil. In the park, there are some species outside their natural habitat fixed on an Atlantic forest fragment. Among these, *Bambusa vulgaris* stands out. It is originated from Asia (Francis, 1993), popularly known as bamboo. The reasons why this species occur at PEDI are not certain, but according to Dechoum, their introduction happened years ago for ornamental and reforestation purposes and also by visitors and residents around the park.

Resistance or tolerance to secondary metabolites is a specific characteristic of this species, with presence of sensitive species referred as indicators of allelopathic activity such as *Lactuca sativa* L. (lettuce), *Lycopersicon esculentum* Miller (tomato) and *Cucumis sativus* L. (cucumber). To be indicated as a test plant, a species should present quick and uniform germination and sensitivity that allows expression of results under low concentration of allelopathic substances (Souza et al., 2007).

B. vulgaris Schrad. ex J.C. Wendl. belongs to the Poaceae family. It is a woody perennial plant with cylindrical stem, alternate leaves, parallel vernation and ligule between the lamina and the pulvinus (Lorenzi and Souza, 2005). Also, it is widely used in furniture, construction material, utensils, handcrafting, water pipes, etc. From an ecological point of view, it presents great efficiency in capturing CO_2 and thus can be used in recovery of degraded areas, erosion control and aggradation of water courses (Ribeiro, 2008).

Bamboo is quick and aggressive during colonization, impairing natural regeneration of other species (Ferreira, 2014). Impacts can be explained by possible allelopathic effects produced during secondary metabolism, which can be beneficial or prejudicial, direct or indirect, causing interference of one species on another (Fernandes et al., 2007; Souza et al., 2006).

According to Alencar et al. (2015), germination tests are the most commonly used analysis to evaluate the allelochemical potential of plant species and their effects on the community.

It is possible that the allelopathic effect of bamboo secondary metabolites might significantly interfere on germination and establishment of lettuce seedlings, but few information is available on this type of study.

Schulz et al. (2010) point out in their study that some species of bamboo are easily spreadable and act as homogenizer of the environments where they establish, demonstrating some allelopathic features. Based on these, their work focused on possible allelochemicals on aqueous extract from bamboo leaves on lettuce, which is a bioindicator.

Therefore, the present study aimed to evaluate allelopathic effects through germination tests of *L. sativa* seeds in aqueous extracts from *B. vulgaris* leaves and culm occurring in Dois Irmãos State Park, in Recife/PE, using different concentrations.

MATERIALS AND METHODS

This work was performed at Laboratory of Forest Seeds Analysis (LASF) from Department of Forest Science at Federal Rural University of Pernambuco (UFRPE). Plant material was collected at Dois Irmãos State Park (PEDI), located in the urban perimeter of the city of Recife/PE at Dois Irmãos neighbourhood, Brazil. PEDI is inserted in one of the largest Atlantic forest fragments of Pernambuco State, and it is a Full Protection Conservation Unit established in 1998 by State Law number 11.622/1998.

Lettuce seeds (*L. sativa* var. *crespa cinderela*) were bought from a local store, with germination rate of 98%.

Experiments were conducted under natural conditions (temperature 25°C and relative humidity 72%). *L. sativa* seeds were laid in gerbox boxes using germitest paper as substrate. Seeds were placed on the paper in five rows with ten seeds each, each row representing an experimental unit. 5 mL of the extract was added to the five experimental units using a pipette to equally distribute it. Distilled water was used instead of extract on the control group.

For extract preparation, material from *B. vulgaris* was separated in leaf and culm. 250 g of fresh plant material was weighted in a semi-analytical balance and ground in a blender with 1000 mL of distilled water, according to methodology described by Cruz et al. (2000). From this extract with concentration of 100%, dilutions were performed to make extracts with concentration of 75, 50 and 25%. Distilled water was used to represent concentration of 0%.

Individuals presented 5 mm of root projection used as criteria to identify germination. Germinated seeds were counted on a daily basis for seven days at the same time. For each aqueous treatment, four repetitions were used and the experimental design was entirely casual.

Analysed variables were:

(1) Germination percentage (%G), according to Standards for Seed Analysis (BRASIL, 2009), based on the following equation:

$$\%G = \frac{NG}{NT} \times 100$$

where NG = number of germinated seeds and NT = total number of seeds.

(2) Speed of Germination Index (SGI) was calculated using daily measurements occurring since the first day of germination until the

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Table 1. Effect of different concentrations of aqueous extract from *Bambusa vulgaris* leaves and culm on germination, speed of germination index, mean germination time and mean speed of germination of *Lactuca sativa* seeds.

Treatment (%)	Mean values							
	Aqueous extract from leaves				Aqueous extract from culm			
	G (%)	SGI	MGT (days)	v (day^{-1})	G (%)	SGI	MGT (days)	v (day^{-1})
T1-0	67.50^{a}	37.46^{a}	4.53^{a}	0.22^{a}	67.50^{a}	37.46^{a}	4.53^{a}	0.22^{a}
T2-25	39.00^{b}	18.45^{b}	4.97^{a}	0.20^{a}	27.00^{b}	14.73^{b}	4.50^{a}	0.22^{a}
T3-50	39.00^{b}	21.70^{b}	4.68^{a}	0.21^{a}	14.00^{b}	7.31^{b}	4.00^{a}	0.25^{a}
T4-75	23.50^{b}	11.56^{b}	4.62^{a}	0.22^{a}	24.50^{b}	19.57^{b}	3.88^{a}	0.26^{a}
T5-100	36.50^{b}	18.83^{b}	4.53^{a}	0.22^{a}	32.00^{b}	22.80^{ab}	3.84^{a}	0.26^{a}
CV	39.17	44.52	3.89	4.18	61.74	54.93	8.16	8.47

Mean values for germination (G%), speed of germination index (SGI), mean germination time (MGT) and mean speed of germination (v). Different lowercase letters indicate significant differences (Tukey test, $p > 0.05$).

last day of counting. Index was calculated with the equation described by Maguire (1962):

$$SGI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \ldots + \frac{G_n}{N_n}$$

where G_1, G_2, G_n = number of germinated seeds on daily counts and N_1, N_2, N_n = number of counting days.

(3) Mean germination time (MGT) is based on the equation by Ferreira and Borghetti (2004):

$$MGT = \sum_{i=1}^{k} n_i . t_i \; / \sum_{i=1}^{k} n_i$$

where n_i: number of germinated seeds in a certain time interval (t_i).

(4) Mean speed of germination (v): It is the opposite of mean germination time, which is another way to quantify germination from a kinetic point of view. The lower its value, the more vigorous the seeds (Ferreira and Borghetti, 2004).

$$v = \frac{1}{t} \text{ or } \sum_{i=1}^{k} n_i \; / \sum_{i=1}^{k} n_i . t_i$$

Data was transformed to $\text{arc sen}\sqrt{x/100}$ and submitted to ANOVA. Means were compared using Tukey test at 5% probability using BioEstat 5.0 programme.

RESULTS AND DISCUSSION

Variance analysis showed that aqueous extract from *B. vulgaris* leaves and culm presented significant negative effect on germination percentage and speed of germination index (SGI), but no significant effect on the remaining variables when compared with control (Table 1).

Aqueous extract from *B. vulgaris* leaves negatively influenced germination percentage and speed of germination index of *L. sativa* seeds, where control treatment showed the best means with statistically higher values. Other values were not different statistically. Based on regression analysis (Figure 1), concentrations of 70.22 and 69.36% promote maximum detriment of highlighted variables, respectively.

Schulz et al. (2010) analysed allelopathic effects of *Dendrocalamus giganteus*. They verified that aqueous extract of this species in various concentrations did not negatively influenced lettuce germination. However, Novais et al. (2017) analysed allelopathic effects of aqueous extracts from *Luetzelburgia auriculata* leaves on lettuce germination and found that this species had its germinative performance affected negatively while submitted to the extract in various concentrations. These effects were more evident as concentration increased.

Similar results were found in a study performed by Alencar et al. (2015) where they evaluated effects of aqueous extracts of *B. vulgaris* leaves on corn and beans seeds. The authors found that different concentration of the extract negatively affected both germination and SGI of the mentioned species. According to the authors, metabolites found on phytochemical analysis of *B. vulgaris* such as tannin, phenols, flavonoids, flavones, xanthones and catechins are probably related to the observed allelopathic action.

According to Pires et al. (2001), secondary metabolites responsible for negative allelopathic effect on different plant species are highly present on leaves.

Negative effects in germination percentage and speed of germination index were also observed when the effect of aqueous extract of *B. vulgaris* on the variables mentioned earlier. Control treatment showed the best means, all superior to the treatments with extract at concentrations of 25, 50 and 75% but not differing from treatment T5.

From regression analysis, maximum detriment of germination percentage and speed of germination index

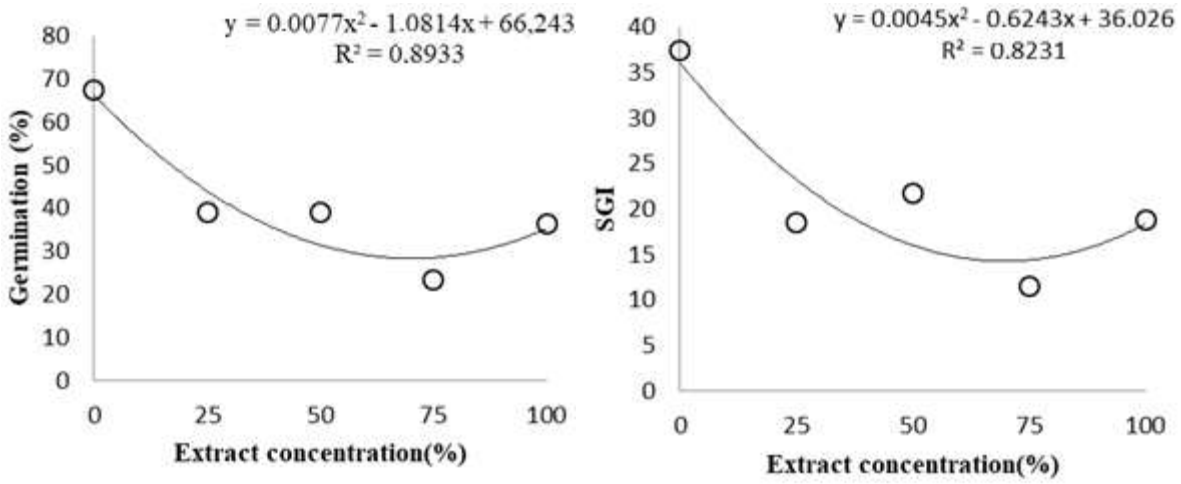

Figure 1. Effect of extract from *Bambusa vulgaris* leaves on germination and speed of germination index of *Lactuca sativa*

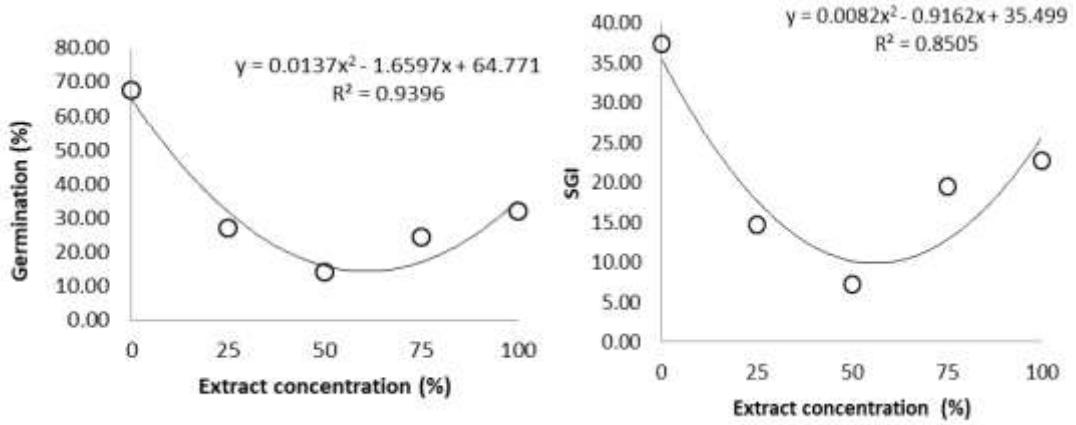

Figure 2. Effect of aqueous extract from *Bambusa vulgaris* culm on germination and speed of germination index of *Lactuca sativa* seeds.

occurred at estimated concentrations of 60.67 and 56.55% of culm extract, respectively (Figure 2).

Inhibition of seed germination can be one of the first physiological effects caused by allelopathic interactions of secondary metabolites produced by plants, being a secondary response of primary effects that happen in plant metabolism (Pedrol et al., 2006).

Works associated with allelopathic effects of aqueous extract from *B. vulgaris* culm are existing in the literature. However, the present study showed that this extract has the same allelopathic effects on lettuce, releasing phytotoxins capable of inhibiting germination and SGI.

The present study corroborates with Faria and Grombone-Guaratini (2011). The authors showed substances capable of inhibiting development and growth of other taxa by analysing aqueous and ethanolic extracts from a native bamboo from Atlantic Forest, *Merostachys pluriflora*, leaves, culm and rhizome. According to Putnan and Tang (1986), allelochemicals are present in all plant tissues, including rhizomes, roots, leaves, stems, fruits and seeds.

At the same time, these results differ from the findings of Sanquetta et al. (2013), that evaluate allelopathic potential of a native species of bamboo from Atlantic Forest *Merostachys skvortzovii*. No significant effects of aqueous extracts from leaves were observed on germination and SGI of *Mimosa scabrella*. Fernandes et al. (2007) also observed no significant effects of aqueous extract from *Merostachys multiramea* leaves on *Araucaria angustifólia* seeds germination or germination speed.

Regarding mean germination time and mean speed of germination, Silveira et al. (2014) tested allelopathic activity of *A. angustifólia* on *L. sativa*. They found that

aqueous extract from the Brazilian pine has inhibiting properties, thus showing allelopathic potential of this species.

Silva et al. (2010) demonstrated that, according to mean time and speed of germination of two forest species, there is a direct interference of their extract, therefore presenting its allelopathic potential on *L. sativa* germination independently of the concentration used.

The present study evidenced that aqueous extract of *B. vulgaris* leaves and culm has allelopathic effects on lettuce, releasing phytotoxins capable of inhibiting germination and SGI.

Considering that lettuce is a bioindicator, it is possible that allelopathic effects can significantly interfere on other species establishment and germination due to substances present on plant residues deposited on the surface or incorporated in the soil annually.

In this sense, new works evaluating allelopathic effects of aqueous extracts on species germination associated on *B. vulgaris* in natural environment are suggested. From such results, it is possible to propose management and conservation strategies for native species in natural vegetation fragments.

Conclusion

Based on the analysed results, the inhibitory allelopathic behaviour of *B. vulgaris* extract on *L. sativa* seed germination was found in all plant parts and concentrations tested.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Opportunities and challenges of Islamic microfinance in livestock production: An evidence from Syria

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Islamic rural microfinance represents the confluence of three rapidly growing activities: microfinance, Islamic finance and agricultural development. It has the potential to not only respond to unmet demand but also to combine the Islamic social principle of caring for the less fortunate with microfinance's power to provide financial access to the poor people involved in rural farming. This paper aims to analyze the governmental agricultural microfinance based on the Islamic principle of Murabaha, using the rural area of Hama government, Syria as case study. The qualitative approach and collected data from the microloan provider was used in this research. The main results show the success of this type of agricultural microcredit to develop the livestock production and its high likelihood of sustainability because it does not conflict with religious and social considerations of the targeted group as well as the high repayment, and it uses the participatory approach of the target group. It could play a very important role regarding the empowerment of rural women by establishing their own projects, owning shares in the village fund, obtaining annual profits, and household investment which can help to improve their family's living conditions. The risks to this type of agricultural microfinancing includes agricultural sector exposure to natural, productive, price and institutional risks.

Key words: Agricultural microcredit, microfinance, Murabaha, village funds, Syria.

INTRODUCTION

Achieving "*rural development, combating poverty and improving the living standard of rural families"* remains meaningless without a change in current modalities of thinking from bureaucratic approaches to a more pragmatic approach that helps implement and practice policy recommendations. A pragmatic approach may better tackle natural, political, economic, administrative and technological constraints as well as financial constraints. Therefore, it is necessary to think of other ways to finance the agricultural sector. Importantly, these methods must be accepted by target groups and rural residents and it should not contradict their beliefs. Agriculture is considered to be the backbone of the Syrian economy, as it accounts for the second largest share of the economy after oil (NAPC, 2013). It is an economic source for more than 46% of the population

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(Almohamed and Doppler, 2008). Therefore, agricultural development projects, in general, aim to combat poverty, reduce unemployment, and establish the principle of self-help with a participatory approach. Within this context, it aims to activate the participation of rural families to become economically viable and to achieve social and economic development of the community by various rural activities like the micro-foundation projects. The Credits are available to rural families from various institutions or persons, e.g. from the agrarian bank, agricultural cooperatives, relatives or private lenders. The first three sources of loans have an interest rate of between 0 and 8%, while the interest rate of lenders is about 25% (Almohamed and Doppler, 2008). Due to the Syrian crisis and the ensuing inflation, interest rate in the agricultural cooperative bank was raised to 10% in 2012 (NAPC, 2017).

Borrowing from an agricultural bank and cooperative associations remains as an option, despite low interest rates, due to bureaucratic difficulties and unfair conditions required by the agricultural bank. The Rural families find access to such institutions very complicated, since they need a lot of time to fulfill all the formalities. In addition, high formal and informal costs of governmental and money lender credits clearly affect the competitiveness of domestic commodities in global markets due to higher production costs. Furthermore, poor families cannot attain the necessary conditions to meet the required guarantee by the agricultural cooperative bank. The public opinion on the state banks in Syria is even among the individuals who have not interacted with them before, that they have a lot of requirements, too complex and too expensive, and even corrupt (IFC, 2008). Hence the need to find a new, innovative way to provide rural families with the capital necessary to cover the costs of the production process and the formation of fixed productive assets and this new method of finance should appropriate with the economic and social circumstances of the poor rural families. Sometimes the problem of not benefiting from available capital sources is the beliefs of rural farming families themselves. Therefore, many studies recommend that it is necessary to provide loans for the rural production process. At the same time, these studies indicate the need to refuge the rural families to recent forms of loans with interest rate because of their incompatibility with the Islamic religion legislation (Abdo, 2014; Alsawan, 2017). Therefore, there is an urgent need to develop essential programs for an efficient rural credit system. Crises are generally an opportunity for fundamental changes in existing institutions to achieve sustainable rural development, provide financial solutions consistent with religious beliefs, and overcome shortcomings of the previous financing system. Meanwhile, the current credit systems are characterized by administrative and institutional ineffectiveness and the efficiency of public banks has been hampered by institutional deficits, internal governance and lack of qualified staff, low levels of capitalism and statutory legislative impediments (IFC, 2008).

The Aga Khan development network and the World Bank, through the Consultative Group to Assist the Poor (CGAP) has been actively involved in bringing microfinance to the forefront of the Syrian government agenda and Kreditanstalt für Wiederaufbau (KfW) has also been active within the microfinance sector (CGAP, 2008).

Therefore, the main objective of this paper is to highlight the importance of microfinance based on the Murabaha principles as an alternative to current official funding, using the microfinance program in the Livestock development project in Hama as a successful pilot case. Actually, this microcredit system seeks to achieve sustainable development by adopting the principle of Murabaha and it also seeks to help create employment opportunities for target groups, improve the income of the beneficiaries and their participation in funds management, and promote practical ideas managed by the rural people themselves.

Objectives of the paper

The significance of this research is to highlight the importance of the microcredit system principle of Murabaha (sale for Profit) in the agricultural sector as an alternative for the current agricultural financing method and to discuss the system's strengths and weaknesses for the possibility of expanding it to all rural areas in the country and other developing countries that have the same social, economic and agricultural conditions.

The Murabaha (Cost-Plus Financing or sell for Profit) means sale on profit. Technically, it is a contract of sale in which the seller declares his cost and profit. As a financing technique, it involves a request by the client to the bank to purchase a certain item for him. The bank does that for a definite profit over the cost which is settled in advance. This research aims to study the mechanism of microfinance based on the Murabaha (resale for profit) by using data obtained from the Livestock Development Project (LDP), in the Governorate of Hama, Centre of Syria and finally this study aims to reach conclusions that can be applied and expanded in different rural areas and to ensure acceptance and participation by the target groups and their ability to repay. This research has a bird's-eye view that collects information, conceptualization, analyze and conclude on the problems of agricultural finance.

LITERATURE REVIEW AND MICROCREDIT EMPIRICAL STUDIES

Investment in microfinance is a multi-purpose investment aim towards social and financial achievements. There are

lots of studies on the role of microcredit in fighting hunger and advancing food security. Some relevant literatures will be used to build the conceptual framework of microfinance (CGAP, 2008, IFC, 2008; IFAD, 2009). Microfinance is a powerful tool to combat poverty. If the poor can reach financial services, they can gain more, build their assets, and protect themselves.

In China, Chinese Micro Finance Projects (MFPs) provided a very good case for studying microfinance outreach as they have overwhelmingly targeted the poor areas and households (the core poor) located in remote mountainous areas of northwest and southwest of China. In his study, Cheng (2007) advocates a strategic policy shift for donors and governmental institutions in China to redefine major clients of micro-loan services to those who have no access to formal loan services in the poor areas. However, the rich are effectively excluded, but among eligible households, rich and poor are equally likely to participate.

On the side of demand, farmers require credit for two reasons, the first is the acquisition of working assets to increase agricultural production, including simple inventory of goods to be resold through new marketing channels, and the second is credit as a liquidity reserve as Saris noted (Saris, 2001). These results are not out of line with the findings in Li et al. (2011a) as the microcredit program helped improve households' welfare such as income and consumption. Despite the optimistic findings on how microcredit has changed the rural households' living conditions, results show that the majority of the program participants are not poor, which represents some doubts on the social potential (such as poverty reduction) of China's microcredit programs. Li et al. (2011b) continued with microcredit research to examine the factors influencing the accessibility of microcredit by rural households using logistic regression and found that there are 12 household-level factors, including educational level, family size, income, and others such as interest rates and loan processing time.

An interesting paper about the microcredit in rural Morocco suggested that the demand for and use of microcredit is shaped not only by agro-ecological conditions, but by two major partially interrelated factors: debt-related norms articulated with the perception of the sanction in case of repayment default and the social life of microcredit, namely how social actors, credit officers, and local leaders engage with microcredit; the study argued that microcredit markets do not develop from demand-led supply, but instead, are historically, politically and socially constructed (Morvant-roux et. al., 2014). Amin et al., (2003) also evaluated whether microcredit programs reach the relatively poor and vulnerable farmers in two Bangladeshi villages. The study finds that while microcredit is successful at reaching the poor, it is less successful at reaching the group most prone to destitution, the vulnerable poor. It can be noted that there is an urgent need in microfinance programs to identify the main channels to reach real deserving people by involving the local communities in self-assessment process and identifying the groups with most needs for borrowing.

When talking about rural development, one should not lose sight of the role of rural women in development and their involvement in any program. A study about the gender and microcredit in India suggested that when agencies, governmental or non-governmental, in a developing country make credit available to low income women, they can reduce the costs of delivery, greatly increase repayment rates, and substantially improve the well-being of poor families (Elavia, 1994). Other study also suggests that such credit tends to increase women's participation in decision-making, reduce fertility, substantially improve household nutrition and raise awareness for children's education (Rosintan and Cloud, 1999). Thus, the microcredit for women is a commonly used strategy for women empowerment. The findings of a research about the empowerment effects of rural women's access to microcredit in Ghana confirmed that women are empowered as a result of their access to credit of an NGO-run micro-lending program. However, another study shows that some women have little control over the use of loans and are not better off; some are subjected to harassment and are worse off due to their inability to repay loans on time (Ganle et al., 2015). A study in Uganda also presents experimental evidence on gender and receiving micro-loan. Microenterprise owners were randomly offered either capital with repayment (discount loans) or without repayment (grants) and were randomly chosen to receive business skills training in conjunction with the capital. The research found no short-run effects for female-owned enterprise either from capital offered or training received. However, it found large effects on profits and sales for male-owned enterprise that were offered loans and this study suggested that repayment requirements increased the likelihood of profitable investment (Fiala, 2018).

To overcome farmer's inability to repay loans in low-productivity seasons, Akotey (2016) tried to provide micro insurance to solve this problem in a study conducted in Uganda. He examined the combination of microcredit and micro insurance and their potential to improve the well-being of low-income households. The study indicated that households using microcredit in combination with micro-insurance significantly gain in terms of welfare improvement. Microcredit alone may be good but its benefit to the poor is enhanced and sustained if the poverty trapping risks are covered with micro insurance. To this context, combining microcredit with micro insurance will empower the poor to sustainably combat against poverty (Akotey, 2016).

To confirm the significance of the microfinance, Chliova et al (2015) carried out a meta-analysis of empirical findings from 90 studies conducted to date about the microcredit and the main finding reveals the positive impact of microcredit on key development outcomes at

the level of the client entrepreneurs, additionally this study scrutinized that the microcredit generally has a greater impact in more challenging contexts. Over the past 30 years, the Islamic finance has grown markedly to become a global industry alongside other traditional types of financing. The only difference between traditional and Islamic credit is the Islamic financing based on the Murabaha principle.

Murabah is defined by (Skeck, 2015) as a sale on profit and technically a contract of sale in which the seller declares his cost and profit. This has been adopted as a mode of financing a number of Islamic banks. As a financing technique, it involves a request by the client to the bank to purchase a certain item for him. The bank does that for a definite profit over the cost which is settled in advance. The main findings of this study are that Islamic Relief follows a set of criteria that governs the size and type of fund to achieve the growth of the working capital. It also follows clear policies that encourage small investors and fulfill the requirements of several economic sectors using “Murabaha”, as a suitable alternative to the other conventional financial systems. It was also suitable because it agrees with the Islamic laws and regulations and helps to control and organize the relationship with investors.

An interesting study about the impact of Murabaha rate on the financial performance of Jordanian Islamic banks' (2000-2013) concluded that there was a significant impact of Murabaha rate on the Return of Assets (ROA) in the Jordanian Islamic Banks. This result has been a good indication for the increasing demand to finance Murabaha, which increases the volume of investments and assets of the bank and therefore is reflected in the returns achieved in the future. Also, the study showed that there was a significant impact of Murabaha rate on the Return on Equity. This means that the bank has the ability to use its assets properly, whereas the Murabaha rate has no significant effect on the earning per share. Another study by Mohieldin et al. (2011) confirms the same finding. The most important result of this study is that Islam has a rich non-traditional means and mechanisms, if they have been applied in a true way, it can lead to poverty reduction and inequality in Islamic countries with widespread poverty.

The paper of Farsca (2008) explores the use of Islamic finance instruments in MENA, arguing that the experiences of Islamic microfinance (MFIs) operating in the MENA in the last decade demonstrate that Islamic MFIs can be competitive with conventional finance in the region, and can address the basic financial needs of their clients in a cost-effective manner as well as can meet the reported demand of lower income groups for religiously tailored financial services. On both an ideological level and practical level, microfinance and Islamic finance complement one another. Islamic finance’s emphases on entrepreneurship, materiality, and risk sharing are reflected in microfinance basic model of joint-liability lending to the poor entrepreneur.

It must be noted, there are a few studies that cover the agricultural Islamic microfinance, hence the importance of this study as the first one in this Islamic agricultural microfinance research field. The Islamic microfinance for development of livestock production initiative established by the Syrian agricultural ministry is the second initiative at the Syrian-level after the Jabaal Al-Hoss Project in rural Aleppo governorate. This project focused on rural development in general and it does not focus on agriculture. It was established in 1999 by the Syrian government with corporation with UNDP (Farsca, 2011).

METHODOLOGY

Data sources

The data were obtained from the following sources:

(i) Statistical information published in the annual agricultural statistical books, obtained from the Ministry of Agriculture and Agrarian Reform, as well as statistical data provided by the Central Bureau of Statistics, the country study prepared by the research centers on financing and loans such as the National Centre for Agricultural Policies and the reports of international organizations.
(ii) Data provided by the LDP management and interviews conducted in the Department of Livestock Development Project in Hama governorate. A field visiting was also carried out in the targeted villages and discussions with agricultural extension staff and the committees responsible for managing the village fund.

Analytical method

In order to achieve the objective of the study, the qualitative approach was used. A descriptive method is one in which information is collected without changing the environment. It is used to obtain information concerning the status of the phenomena to describe "what exists" with respect to conditions in a situation.

Based on the available data, this research tries to highlight the importance of the agricultural microcredit based principle of Murabaha (sale for Profit) as an alternative for the current agricultural financing method and to discuss the new system’s strengths and weaknesses for the possibility of expanding it to all rural areas in the country that have the same social, economic and agricultural conditions.

Description of the research area and the studied villages

According to the classification of Wattenbach (2006), the research area is in the fourth farming system in the subsystem of “rain-fed middle plains”. 78% of the cultivated land is rain-fed, which explains the low productivity of crops. The research area has increased barley production by two-thirds of the wheat production area. The main cultivated crops in the study area are barley, wheat, olive and cumin. The second main economic activity of the area is animal husbandry with a focus on sheep rearing.

The income sources in the rain-fed agricultural system depend mainly on seasonal agricultural or non-agricultural employment. The reason for seeking non-agricultural work are mainly due to the low yields of agricultural crops in the low rainfall area (average of less than 250 mm per year), which is compounded by the scarcity of

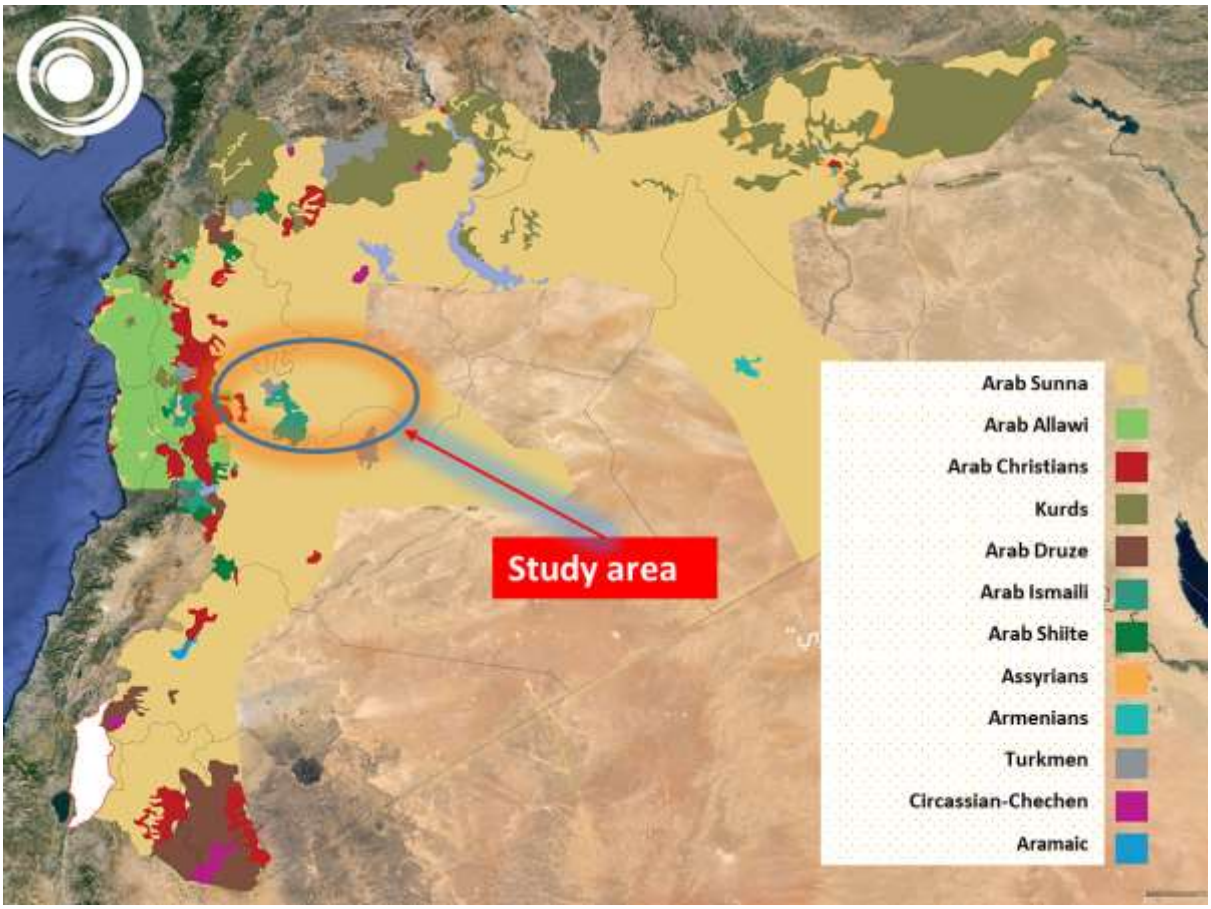

Figure 1. Demographic, ethnic and religious distribution in Syria, 2011.
Source: Website of the Blog NUSUH, translated from Arabic to English by the author.

other income sources. Rain-fed agriculture has been the result of the state's policies that prevent the drilling of wells in many regions to protect groundwater resources. According to the Wattenbach's classification, there are three types of families in this sub-system: rich families, who account for 10% of the total population in the region (own more than 60 dunums of land; 1 dunum is equivalent to 1000 m^2), medium families, who account for 30% (20-60 dunums), and the poor families, who account for 60% (less than 20 dunums) of the population. Poor and medium farmers are unable to obtain official loans because of their indebtedness to agricultural cooperative banks. Consequently, these farmers tend to borrow from the private sector with high interest rates. Thus, a big part of the governmental support to strategic crop goes to the traders who provide these loans. The poor farmers devote most of their production to household consumption, especially wheat, vegetables, and dairy products; however, they sell the surplus in good rainy years. It must be noted that the proportion of the rural population is 45% of the total population in Syria while the rural population in Hama Governorate is 63% (COS, 2013).

Actually, there is no official statistic on the religious demographic in the study area, but according to the US State Department's report on religious freedoms, the Sunni Muslim community in Syria is 77%, Allawi 10%, Druze 3%, Ismaili and Shi'a 8%, Christians and the Yazidi minority (US Department of the State, 2017). Figure 1 shows the demographic distribution in Syria. It is to note that the majority of the population in the study area regarding this figure is Arabic Sunnah, because of this fact, it is very important to carry out development program of credit based on the Islamic based principles.

RESULTS AND DISCUSSION

Following Prof. Muhammad Yunus' Nobel Peace Prize, microcredit lending has risen to prominence and the volume of microcredit loans substantially increased. Yunus' initiative is expanding in developing countries, as each country has tried to develop the idea of microfinance in line with its potential and its economic, social and institutional environment.

Describe the traditional official finance in Syria

Although the state seeks to harmonize the financial system with the overall developmental objectives, the official credit system is still characterized by its inflexibility to provide loans for production activities. It experiences liquidity shortages and its complex conditions to access loans make it difficult to reach the poorest people; in some cases, personal relationships play a crucial role in

obtaining access to credit. Many families are also unwilling to access bank loans for many reasons: religious factors that prevent borrowing for interest and the lack of experience required by the procedural requirements and guarantees necessary for the official credit; social factors also limit the access of rural families to official bank loans.

There is a great need to find an agricultural financing system that can overcome the above-mentioned problems of recent official credit system based on the principle of Murabaha which will be discussed later. The spread of the microfinance is expected in the agricultural sector and this approach is a socially and religiously acceptable solution to overcome the lack of liquidity by covering investment costs. It can be seen as a comprehensive approach due to its gender-neutral and participatory principle. It is also sustainable and has low risk of default for many reasons. Borrowers are shareholders at the same time and they manage the funds themselves. It is necessary to manage these funds effectively and for the credit to be repaid in monthly instalments. In addition, microcredit is used in animal production projects that are economically viable and have comparative advantage in the research area. The guarantee of each member in the funds reduces the risk of his inability to repay, which is considered as an additional advantage for this type of credit system. Unfortunately, the war in Syria since 2011 stopped production activities in most rural areas and the number of borrowers drastically decreased due to displacement of by the war.

Description of the agriculture and the livestock

Agriculture is considered as a significant sector of Syria's GDP and more than 35% of rural households have animals (NAPC, 2017). Therefore, the ministry of agriculture has set up a large development project, called Livestock Development Project (LDP), to invest in a livestock sector in areas with low potential for crop production. LDP is seeking to develop a microcredit program as part of its project, so that all investments in the agricultural sector will not depend on sources like personal savings nor from external sources such as private lenders. The LDP has many dimensions of development, economic, and social goals and extension services, so it provides components of an integrated rural development in Hama governorate. It aims to achieve the following goals (Syrian agricultural ministry, 2017):

(i) To increase incomes of poor families in rural areas and small breeders who depend on livestock production in various agricultural stabilization zones and Al Badia (the Step), which is estimated at 311,000 families distributed in 1,260 villages throughout the country and targeting about 100 villages in the project area in Hama branch (research area).
(ii) To apply the principles of herd management through guidance and training in livestock breeding and production and to provide veterinary services such as immunization, treatment and provision of veterinary medicines.
(iii) To supply feed and use feed as resources for rangeland rehabilitation in the steppe, pastoral seeds propagation, and pastoral shrubs cultivation.
(iv) To establish a microfinance fund for targeted poor villages and families that has the capacity to manage the funds in a legal manner.

Main components of the LDP

The project is concerning the integrated development of livestock in Syria and is implemented by the ministry of agriculture and the agrarian reform (MOAAR, 2015.) with funding from the Syrian government and a grant from IFAD. One of the LDP primary objectives is to establish sustainable local financial institutions that are owned and managed by the people themselves. The main components of LDP are shown in Figure 2. As shown in the Figure 2, LDP comprises many units regarding livestock production.

(i) Unit for increasing livestock production by supporting animal health and agricultural extension services. This unit supports projects for rural women, human and animal health care, literacy courses and courses on how to make dairy products. These projects were carried out during the last five years.
(ii) Unit for pasture improvement and feed resources development.
(iii) Unit of development for small and medium-sized enterprises and rural finance for livestock production. 42 finance funds were implemented with 1,911 small loans with a total value of 140,956,000 Syrian pounds (SYP) or 281,912 United States dollar (USD) (1USD=500SYP) (LDP website).

Figure 1 shows that the vast majority of the population of the governorate of Hama and its countryside are Muslims. Therefore, this type of small loans will definitely find a great demand in the rural areas. As previously stated that more than half of the rural population in the study area are poor, so the microcredit is a very good capital provider based on the principle of the Islamic principle Murabaha what is suitable for them regarding the believes and small agricultural projects and enterprises.

Working approach of the village microfinance funds

In the governorate of Hama, there were eight established

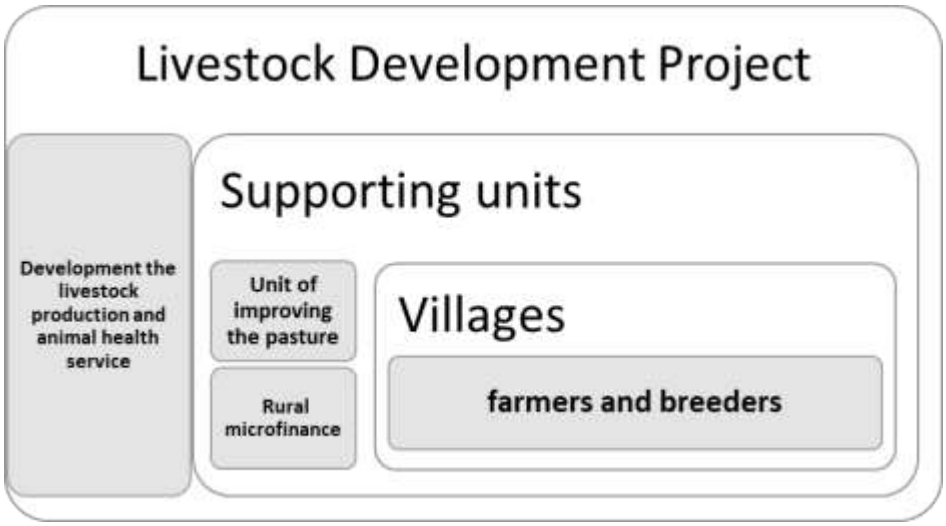

Figure 2. Main components of LDP.

Table 1. Demographic and natural characteristics of considered villages.

Village number	Stabilization zones	Size of area (ha)	cultivated land (ha)	Population (resident)	Number of animals			Illiteracy rate (%)
					Sheep	Cows	Goats	
1	3	3,769	2,223	13,238	5,000	100	468	2
2	2	2,550	700	700	2,900	20	60	3
3	1	1,934	1,509	2,800	2,430	138	576	6
4	2	1,246	1,246	7,466	3,478	876	0	2
5	3	3,331	2,865	5,216	3,658	70	500	1
6	3	88	83	2,200	7,146	91	576	2
7	3	10,396	8,785	4,500	9,217	96	1,328	5
8	2	1,909	1,871	2,700	8,272	85	1,027	10

funds by the end of 2014 with a total of 904 shareholders, of which 35% were women, and the loan amount totalled 1,775,000 SYP (MOAAR, 2015). Table 1 describes the demographic and characteristics of the eight villages. As it can be seen, the villages are in the first, second, and third settlement areas. These settlement areas vary in annual rainfall, 600, 350, and 250 mm/year, respectively (NAPC, 2017). Table 1 also shows that sheep breeding dominates the studied villages and there is a high percentage of cultivated land from the total area, which indicates the integration between livestock and crop production. In terms of illiteracy rate, all villages apart from three (6%) and Eight (10%) had relatively low illiteracy rate. Applying for microfinance funds from the LDP begins with the request of village residents to establish a village money box or fund (Figure 3). But the number of the members shall not be less than 100 persons and the contribution fee shall be set at 1,000 SYP per member. A three-member local committee is elected and trained to strengthen the participatory approach in managing the village fund; then, the three to six-month probationary phase begins. The evaluation of the village fund takes place after three years of lending. Figure 3 illustrates how microfinance works for a typical village in the research area.

The most important condition for obtaining the loan is that the applicant is a shareholder between 18-60 years' old who resides in the village and is supported by two individuals who contribute to the fund. In terms of the microfinance policy, the provided credits are repaid with the Murabaha (Farsca, 2008) till the end of the loan. Murabaha is something different from loan interest. It is established by the banks for religious reasons and beliefs. However, the loan with Murabaha value is paid in monthly instalments. There shall be no other costs paid by the beneficiaries such as fees or penalty interest. Murabaha is the bank's intermediary to buy a commodity at the customer's request and then to sell it at a price equal to the total cost of the purchase plus an agreed amount of profit between the bank and customers

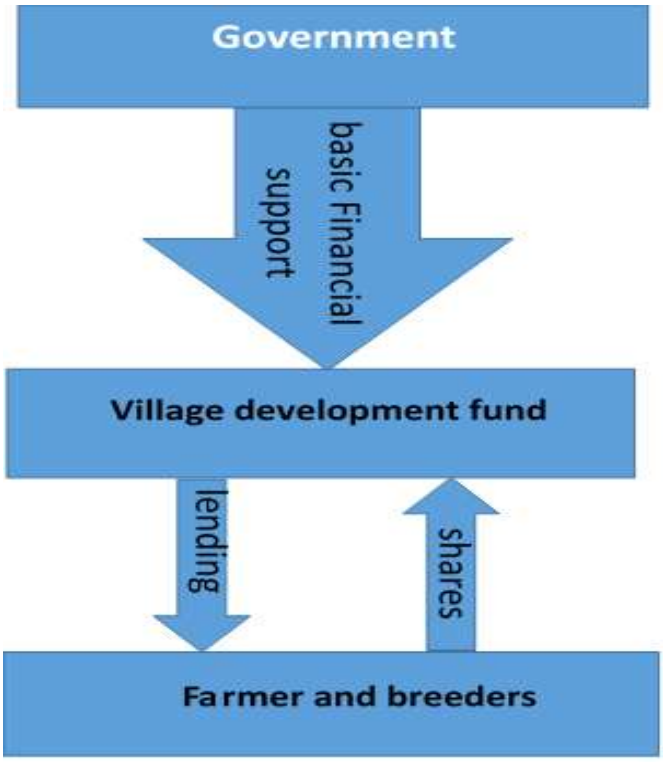

Figure 3. Community of village development fund.

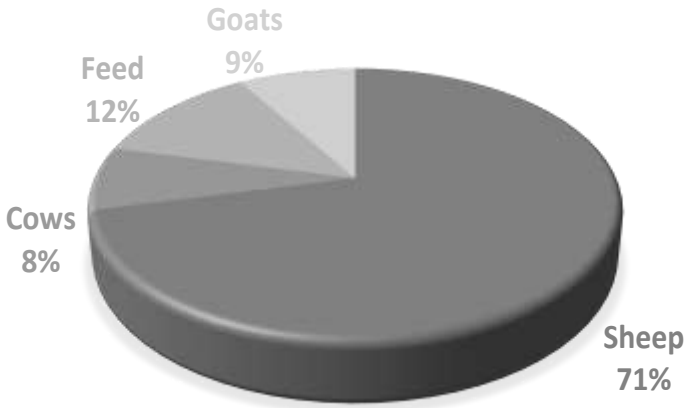

Figure 4. Use the provided loans in different activities related to livestock.

(Skeck, 2015). The total cost of the purchase is the purchasing price of the commodity plus all the expenses paid by the bank for purchasing the commodity, less any discount the bank receives from the seller. The Murabaha amount is, therefore, the total purchasing cost plus the bank's profit.

The Murabaha contract consists of three parties: the seller, the buyer, and the investment bank or the trader who is an intermediary between the seller and the buyer. However, the bank does not offer to buy the commodity until after the buyer has declared his desire to buy and promise to pay. In principle, Muslim scholars believe that Murabaha is allowed if it does not violate its conditions. It is to be noted that the principle of Murabaha is applied more in Islamic commercial and real estate banks.

The LDP provides about one million SYP in cash to each village. Once shareholders add funds to the village fund, the money value further increases. Loans are distributed to farmers who periodically contribute to the village fund. The farmers provide the required documents and receive the loan that will be repaid over a period of 12 months. As it can be seen in Figure 4, the majority of the loan is used to purchase sheep for breeding and milk production. Breeding Awassi sheep is widespread in Syria, which is favoured by consumers in importing countries, especially Saudi Arabia. The breeders also prefer Awassi sheep because they are adapted to harsh local conditions and environment. Sheep breeding is a profitable economic activity and it competes against crop production on labor and capital production factors even in regions with high potential of irrigated crop production (Almohamed and Doppler, 2008; Cheikh and Almohamed, 2017). The second biggest part of the loan is used to buy feed; funds are also used to buy goats and cows for breeding. Figure 5 indicates the percentage of men and women in the considered villages. The men's shares with 60% have a bigger part as the women only with 40%, but the difference is only about 10%. This shows the extent of women involvement in this microfinance program, which are almost completely unnoticed in short, medium and long-term loans provided by public banks such as the agricultural cooperative bank.

Table 2 shows there are differences in the amount of loans provided to each village without considering the population density of each village. Village Four had the largest share of loans with the biggest number of shareholders, and Village Eight had the lowest share of loans. In terms of the Murabaha, Village Three achieved the highest value of Murabaha while Village Five and Six achieved the lowest value. The project management had provided rewards for the villages with the highest value of Murabaha (Village Three and Eight, 6.76 and 5.02%, respectively). Table 2 also notes that the Murabaha rate is nearly equal to the interest rate of the agricultural cooperative bank (5%), which is less than the interest rate to non-cooperative farmers, which is usually 8%.

The high percentage of loan repayment and good rate of Murabaha in this successful microcredit example in Syria confirmed several advantages of this type of credit system, especially in rural development projects. Microcredit has the advantage of high demand and sustainability because it does not conflict with religious and social considerations of the rural target group and it is suitable to the poor farmer and breeders regarding its small volume. The active participation and contribution of

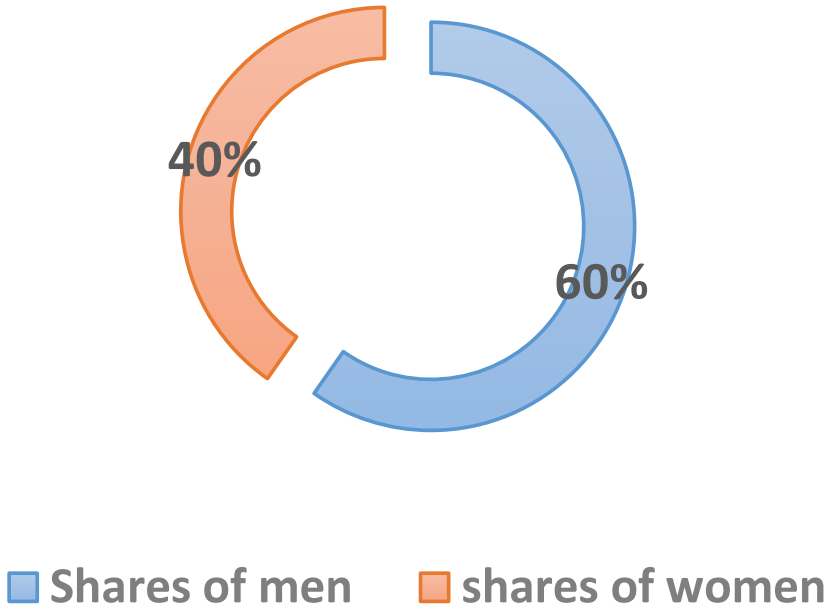

Figure 5. Percentage share of gender in the village fund.

Table 2. Total values of the loans for considered villages (1,000 SP) and the Murabaha.

Village number	Amount of loans (men)	Amount of the loans (women)	Total Murabaha	Rate of the Murabaha	Ratio of repayment (%)
1	1,395	945	112,320	4.80	100
2	2,105	665	135,360	4.89	100
3	1,565	820	161,280	6.76	100
4	3,764	2,189	295,334	4.96	100
5	1,140	270	67,680	4.80	100
6	1,866	398	108,670	4.80	100
7	2,100	1,300	170,400	5.01	100
8	1,315	900	111,120	5.02	100
Total	**15,250**	**7,487**	**1162,164**	**5.11**	**-**
Max	3,764	2,189	295,334	6.76	-
Min	1,140	270	67,680	4.80	-
Std.	831,90	600,45	68,782.21	0.66	-

rural women in the village's funds enhanced the role of rural women in development and decision-making process, which contributed significantly to increase the income and living standards of their families.

The high repayment rate was observed because the credit is paid back monthly and it was used in very profitable small businesses namely livestock production. This Islamic microcredit is also sustainable because it is based on a participatory approach and the funds are self-managed by the local community, which increases the confidence of the borrowers. The process of selecting the supervising committee of the funds is democratically carried out and a free election takes place; the simplicity of getting the loan regardless of the amount and the absence of administrative and bureaucratic complications boost trust and confidence from the borrowers. This type of microfinance system could also have some disadvantages such as the absence of continuous evaluation by government agencies and the risk of the sustainability like other state agricultural project due to lack of continuous evaluation and ongoing maintenance. The lack of governmental evaluation can have negative effects on the effectiveness and efficiency of borrowing over time, which is reflected in other Syrian governorates' weak performance and in some cases, personal relationships with the local supervising committee and project management play a major role in obtaining loans.

These advantages could encourage the private banks

to enter this market of Islamic microcredit. The private sector will be a strong competitor to the public sector because of its administrative efficiency and continuous assessment of the lending process, but that needs more investment in rural infrastructure and increase the number of branches in the rural areas. Despite these disadvantages, it is necessary to find a socially acceptable and viable credit system that can help rural families finance the needed capital for agricultural production, to improve their incomes and living standards, and enhance women's role in rural development.

Conclusions

Over the past 30 years, the Islamic finance has grown markedly to become a global industry beside other traditional types of financing. Therefore, Islamic finance and microfinance agree in essence, both of which are primarily concerned with providing social services and helping the neediest people. They also agree not to exploit the need of people and to profit from them but to call for social equality and to encourage the poor to get closer to the layers. The cooperative agricultural bank has played an important role in the Syrian agricultural sector. It provided subsidized loans with fixed interest rates as well as overseeing input provision and commodity purchase. In contrast, despite their needs, many rural residents have been unable to access this type of official funding because of the complex administrative and religious considerations. Thus, religious beliefs and social environment in rural areas have to be considered in any agricultural credit project to facilitate the establishment of a productive credit system as a means of continuously improving living conditions, distributing welfare benefits to all social groups and combating rural poverty. The credit system must also focus on the rural activities in which the target group has been successful in the past like the livestock production. Compared to crop production, livestock production has had lower vulnerability to external risks such as climate fluctuations and irregular rainfall; as a result, loans were focused on investing in livestock (Sheikh et al., 2017). To support income-generating activities for poor livestock breeders, government policies should focus more on the increased added value of milk products and milk processing, especially, Syria enjoys comparative advantage in the animal products in this sub-system that are mainly produced by poor households (NAPC, 2005).

It can be concluded that such an Islamic agricultural microfinance initiative can be considered as a successful one and an important innovation in a rural village of any Islamic developing country that can contribute to rural development, poverty reduction and creating jobs by providing financial services for the establishment of small enterprises. This type of credit can be considered as very suitable for rural areas in developing countries, fistful because it is directed to the small projects as is the agricultural sector based mostly on small family farms and it is centred on the principle of Islamic Murabaha, which corresponds the religious beliefs of the majority of the rural population. The Islamic microfinance has the potential to expand access to finance to unprecedented levels throughout the Muslim world. This type of credit uses the participation approach; the target group contribute to manage the village fund which strengthens farmers' confidence and contributes to the success of this initiative.

Unfortunately, in 2016 as a result of the war, this program stopped but there is hope it can be re-applied in the reconstruction phase in Syria because of its advantages, such as, to strengthen the role of rural women in improving the standard of living of their families and reducing poverty, which can be seen through the provision of about 45% of loans to rural women in the current research. The demand on this type of credit is high and does not conflict with the Islamic beliefs. There is a high potential of obtaining a legal formula for this type of micro financing since it is supported by a government institution which is the Syrian ministry of agriculture.

Agriculture financing is riskier than trade or industry finance. The village funds must therefore build up their institutional capacities to deal with the risks generally related to financial services provided to the poor and low-income families. The capacity-building of microfinance funds will therefore be strengthened by the project within the framework of improving management competencies at the microfinance management level and at the level of competency improvement among agricultural producers and livestock breeders, which are the actual clients of rural microfinance funds. The dependence of village funds on the financial nucleus is another risk to sustainability, so microfinance funds have to work to develop their own financial resources with the time.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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A study of poultry farms in Enugu State Nigeria and mapping of their mechanization needs using Global Positioning System (GPS) and Geographical Information System (GIS)

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Poultry has been identified by the World Bank funded Commercial Agricultural Development Program (CADP) as a value chain in the agricultural sector of Enugu State and thus has the potential for commercial agriculture. This work has investigated poultry farms in three production cluster areas in Enugu State namely: Emene (in Enugu East) located on 6.523815°N, 7.4151535°E and 1236 ft; Ngwo (in Enugu North/Udi) located on 6.523815°N, 7.57528°E and 572 ft and Nsukka located on 6.234754°N, 7.326473°E and 3422 ft. The study identified the position of each farm using Global Positioning System (GPS) hand held device; their specific mechanization needs and challenges limiting the performances of the farms using structured questionnaire, oral interviews and on-the-spot assessments. Position and mechanization data were represented in a map using ArcGIS version 10.3. The farms visited produce eggs, broilers or layers or combinations of them, and the sizes of the farms range from unit of thousands to tens of thousands of birds; these generally are commercial poultry farms but of low or medium scale levels. The survey reveals that most of the poultry farms are having the following mechanization needs: Power supply, egg handling, road network, waste handling, feed mill, pelleting machine, battery cage, automatic drinker, water pump, and automatic feeder; however the need depends on the level of production. Other problems or challenges facing the farms visited as observed during the survey are classified as management problems quantified as 60%, technical problems 30% and the problem of cost 10%. Result of this work is a step further in developing and building up the much talked about commodity-specific data base system for agribusiness decision support and development in the local area.

Key words: Mapping, poultry farms, mechanization needs, Global Positioning System (GPS), Geographical Information System (GIS).

INTRODUCTION

Poultry production is one of the major agricultural production activities going on in Enugu State of Nigeria. Poultry alongside with fruit trees (citrus, mango, guava, pineapple and cashew) and maize has been identified by the World Bank funded Commercial Agricultural Development Program as a value chain in the agricultural sector of Enugu State and thus has the potential for commercial agriculture (CADP-AB3498, 2009). Both rural

and urban dwellers are involved at varying levels. In urban or semi-urban areas, people operate poultry production systems at either subsistent, usually as a part time job or at commercial level. The level of production determines the land space, scale, technological application and other input resources. According to Adene (2010), commercial poultry production system is industrial in nature, large scale, dense and involves uniform stocks of modern poultry hybrids. It is capital and labour intensive as well as inputs and technology demanding. On the other hand, the rural poultry is by convention a subsistence system which comprises stocks of non-standard breeds or mixed strain, types and ages. It is generally of small scale, associated with household or grass root tenure and little or no veterinary inputs. The rural poultry sector is therefore in its original sense, a village-based, household or individual holding, an occupation which has however been extended to non-village settings in semi-urban localities, mainly by the middle class dwellers. However, between these two distinct systems, intermediate grades have evolved over time, in response to the national agro economy and consumer demands. This constitute what is now globally regarded as “family poultry” comprised of the rural or indigenous poultry types in some cases or a mixture of both indigenous and exotic hybrids or even totally exotic breeds. Available information shows that the scale of operation can range from stocks of a few units or a few dozens of a variety of poultry birds in the household poultry to tens or hundreds of thousands of chickens in the grades of commercial poultry (Adene, 2009).

Considering the contribution of poultry business to the economy of the state and the country at large, it is important to develop a good information network and data base system for decision support for poultry and allied business ventures. Also it is necessary to pursue full mechanization of the system in order to serve the growing population for both food and raw material for industries. Agricultural mechanization is the application of mechanical technology and increased power to agriculture, largely as a means to enhance the productivity of human labour and often to achieve results well beyond the capacity of human labour (UNIDO-FAO 2008). In Africa and specifically in Nigeria, record keeping and information management and retrieval systems are still generally unsatisfactory. Researchers, investors, government and farmers usually find it difficult to find or have access to needed information or data which may be necessary in different aspects of their specific interests. This research therefore seeks to contribute to solving this problem; and precisely in the aspect of poultry business in Enugu State by providing a map showing location of farms and their mechanization needs. The specific objectives include to identify the existing locations of some selected poultry farms in Enugu State using GPS, determine the mechanization needs and other challenges of these poultry farms through on-sight investigation, oral interviews and structured questionnaire and to represent the farms and their mechanization needs in a map using GIS software.

GIS and GPS as powerful information and management tools

Geographical Information System (GIS) is a high technological mapping system that integrates hardware, software and data for capturing, managing, analyzing and displaying all forms of geographically referenced information. GIS makes it possible to view, understand, question, interpret and visualize data in many ways that reveal relationships, patterns and trends in the form of maps, globes, reports and charts (ESRI, 2010). By using GIS, scientists can research changes in the environment; engineers can design road systems; electrical companies can manage their complex networks of power lines; government can track the uses of land; fire and police departments can plan emergency routes. Many private businesses have begun to use a GIS to plan and improve their services.

For agricultural purposes, GIS can be used to produce and read maps. Its major advantage is that it permits identifying spatial relationships between specific different map features. It can create maps in different scales, projections and colors. But it is not just a map making tool. It is primarily an analytical tool that provides new ways of looking at, linking and analyzing data by projecting tabular data into maps and integrating data from different, diverse sources. It accomplishes these by allowing creation of a set of maps, each with different theme such as soils, rainfall, temperature, relief, water sources, etc. From its early beginnings, GIS has been an integrating technology both from the point of view of its development as well as its use. This is because, once geographic information of any kind is translated into the digital form in a GIS, it becomes easy to copy, edit, analyze, manipulate and transmit (Maguire et. al., 1990). Some potential agricultural applications where GIS can lead to better management decision are: Precision farming, land use planning, watershed management, pest and disease management, irrigation management, resources inventory and mapping, crop area assessment and yield forecasting, biodiversity assessment, genetic resources management, etc. (Aronoff, 2010). Information

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Figure 1. Map of Enugu state showing the local governments.

and data on poultry business can be collated and/or accessed on the internet and used to create intelligent maps for better farm business practices (Xie and Wang, 2007). Khaleda and Murayama (2013) employed GIS in the delineation of the suitability of poultry sites in Gazipur, Bangladesh. Hassanein et al. (2012) applied GIS as a decision support system in planning the expansion of poultry farms in Egypt. Omodele et al. (2014) employed GIS in analysing the spatial distribution of poultry farms in Delta State.

On the other hand, GPS is applied in agriculture for guidance of equipment such as sprayers, fertilizer applicators and tillage implements to reduce excess overlap and skips. They can also be used to precisely locate soil sampling site, map weed, disease and insect infestations in fields and apply variable rate crop inputs, and in conjunction with yield monitors, record crop yields in fields (Liaghat and Balasundram, 2010; Dull, 2008). Possible agriculture related questions a GIS can answer the following questions: What is it (identification); Where is it (location); What has changed since (trends); What spatial patterns exist (patterns); What if (modeling) (ESRI, 2010). According to Stafford (2011), the advent of information technology has accelerated our ability to acquire large volumes of spatial data. It is widely acknowledged that the key technologies facilitating the modern precision agriculture are yield monitor, remote and proximal sensing, GPS and GIS.

MATERIALS AND METHODS

Description of the study area

Enugu State (Figure 1) was created on August 27, 1991 with the city of Enugu as its capital. The State derived its name from the capital city which was established in 1912 as a small coal mining town, but later grew to be capital of the former Eastern Region of Nigeria in 1967 when the Gowon administration created twelve states in Nigeria. Enugu State is located in a tropical rain forest zone with a derived savanna. The city has a humid tropical savanna climate with its humidity at its highest between March and November. The mean daily temperature of Enugu State is 26°C (80.1°F) as in the rest of West Africa; the rainy season and the dry season are the only weather periods that occurs in Enugu. The average annual rainfall in Enugu is around 200 mm which arrives intermittently and becomes very heavy in rainy season. Other weather condition affecting the city is Harmattan; a dry and dusty

northeast trade wind lasting a few weeks from end of November to January or February. Like the rest of Nigeria, Enugu is hot all year round. Enugu State occupies much of the highland of Udi, Awgu and Nsukka. The hills are flanked by the rolling lowlands of Orji-River, Adada and Anambra. The area contains about nine geological information and is made up of shale, sandstones and siltstone (Ofomata, 2002a). Enugu State is located at height of 200 to 500 meters above sea land (Ofomata 2002b).

In the west and south west of the escarpment, the Udi-Nsukka plateau, all fall gently towards the lowland along the Niger and Imo River. The Anambra main River drains extensively to the area of Uzo-Uwani local government area in North West and Awgu in South West. Ekjlu and Inyaba River is linked to Ebonyi and Cross-River basin (Wigwe, 1975). The vegetation of Enugu falls within the rain forest savanna acetone. Its floristic composition is consequence of its location in the transition zone between the tropical grassland. About 60 to 70% of the vegetation in the area is grasses and is predominantly *Hyparrhenum* species, androgen species, and *Penisetum prupereum* (Ajaero, 2008; Anyadike, 2002).

Agriculture is one of the major economic activities in Enugu. The major crops produced include yam, cassava, cocoa yam and vegetable. However, it is practiced on a subsistence level with continuous cropping. Other economic activities in Enugu State include palm-base works and designs, clay works and blacksmithing (Mbagwu and Obi, 2003). Enugu city serves as a host of various government institutions (Ajaero, 2008). According to 2006 Nigerian census, the Enugu metropolitan area has an estimated population of 722,664 persons. This is made up of 312,332 males and 410,332 females.

Research methodology

The research methodology is basically survey, involving on-site investigation, oral interviews and the use of structured questionnaire. Poultry farms investigated in this study were selected randomnly based on the zones where many clusters are located. Three of such zones were selected, namely: Emene (in Enugu East), Ngwo (in Enugu North/Udi) and Nsukka; which include rural and semi-urban settlements where there are clusters of poultry farmers. A total of eighteen poultry farms were visited during the survey. Primary and secondary data collected were used to analyze the mechanization needs of poultry farms in Enugu State. The primary data was generated through on-sight investigation, oral interviews and the use of structured questionnaire administered to the farmers, to find out their mechanization needs and other challenges facing poultry production in the area. Global Positioning System (GPS) hand held device was used to collect position data of the poultry farms. The secondary data were obtained from literature. Finally Geographical Information System (GIS) software (ArcGIS version 10.3) was used for data capturing, analysis, and mapping to show the different locations of the poultry farms on the study area and their mechanization needs indicated by different colour codes on the map.

RESULTS AND DISCUSSION

Poultry farms, locations and mechanization needs

The names of the poultry farms visited, their locations and mechanization needs are presented in Table 1. For easy mapping, the various farms have been coded as Pf 1 to 18, where Pf refers to poultry farm. It was found that the farms visited produce eggs, broilers or layers or combinations of them; and the sizes of the farms range from units of thousands to tens of thousands of birds. The poultry farms operate either battery cage or deep litter system or both. Usually, those just starting the business at commercial level prefer to start with deep litter because it is relatively cheaper compared to battery cage; unfortunately quality and durable battery cages are both scarce and expensive. ArcGIS version 10.3 was used to capture and represent the data shown in Table 1, and different maps were produced showing poultry farm locations and their mechanization needs (Figures 2 to 5).

Summary of other problems, needs and challenges faced by poultry farms visited

From on-sight investigations and interviews conducted at the various poultry farms visited, the following are the summary of challenges or problems faced by most poultry farms that either resulted in bankruptcy, total closing down of the farm or operating below profit margin which eventually would lead to gradual folding up. They include: (i). Poor record keeping and accountability; (ii) Lack of business honesty; (iii) Poor management; (iv) Pilfering and inadequate security (v) Inability to separate business from family affairs; (vi) Lack of sustained interest and commitment after the original owner is no more; (vii) Inadequate skills and experience in managing diseases especially at preventive level; (viii) Low level or complete absence of mechanization; (ix) Poor odour control and management; (x) Relatively unstable and high cost of input materials like feed, drugs, etc. Items one to six are classified as management problems which make up 60%, items seven to nine are technical problems representing 30% while item ten is the problem of cost representing only 10%.

DISCUSSION

Table 2 shows the identified mechanization needs and number of the poultry farms involved. The needs in descending order include: Power supply, egg handling, road network, waste handling, feed mill, pelleting machine, battery cage, automatic drinker, water pump, and automatic feeder. The observations as represented in Figure 6 show that about 56% of the poultry farms in the surveyed areas in Enugu State needed power supply. This result implies that the remaining 44% either depend on personal electricity generators or do not use electricity at all for any activity in the poultry pen. Due to the frequent power outage and erratic nature of electricity supply from the national grid, many of the farmers who can afford it depend more on electricity generating gasoline or diesel plants which come in different sizes. For most poultry farms electricity is used mainly for lighting and heating; only very few farms have equipment that require electric power for operating them. The cost of

Table 1. GPS reading and the address of poultry farms in Enugu State.

Notation	Name and address of poultry farms	Latitude (°N)	Longitude (°E)	Altitude (Ft)	Mechanization needs
Pf1	Bora poultry farm: km.5 old Enugu road, Enugu Ngwo	6.44602	7.451535	1236	Power supply, and egg handling machine
Pf2	Bora poultry farm: km.5 old road, Enugu Ngwo	6.44124	7.457445	1307	Power supply, egg handling machine, and fund
Pf3	Ozokwor poultry farm: Ugwuagbara in enugu north local government area near st Theresa	6.420118	7.456462	1323	Power supply, bad access road
Pf4	Nagnald poultry farm: km.5 Agu-Eke layout, hilltop Ngwo	6.442925	7.451552	1235	Battery cages and poor access road
Pf5	Engr Nwaeze poultry farm: km.8 Agu-Amabor Ngwo, Enugu Ngwo	6.413743	7441105	1293	Water pumping machine
Pf6	Parthy poultry farm: km.8 Agu Amabor Enugu Ngwo	6.41281	7.440473	1349	Feed mixer and automatic drinker
PF7	Educhi poultry farm: Onuagu Etiti village Enugu Ngwo	6.415112	7.45467	1310	Power supply and automatic feeder
Pf8	Beny's poultry farm: Ugwuomu Nike Emene, Emmanuel town	6.523815	7.577528	572	Power supply, egg handling machine and waste packing machine
Pf9	MIC poultry farm: Onuagu Etiti village Enugu Ngwo	6.527792	7.578562	1221	Power supply/ egg and waste packing machine
Pf10	Goodwill poultry farm: Phinomar Nig. LTD Enugu Ngwo. Box, 655 Enugu	6.425763	7.463465	1272	Waste packing device and egg handling equipment
Pf11	CADP Ministry of Agricultural Resources, World Bank assisted poultry, Ugwuomu Emene, Enugu East LGA	6.52607	7.577168	570	Power supply, truck for moving the waste and road network
Pf12	Nebo poultry farm: Ugwomu Nike Enugu state	6.523492	7.579607	557	Egg handling machine and pelleting machine
Pf13	Nebo poultry farm: Ugwuomu Nike, Emene, Enugu East LGA	6.523492	7.586053	525	Power supply, egg handling machine and bad road
Pf14	Odalije poultry farm: km.5 Agu-Eke layout Odalije street Enugu Ngwo	6.444258	7.45606	1285	Feed mill, power supply and battery cage
Pf15	Uncle's poultry farm: km.20 Ugwuomu Emene	6.527792	7.578562	562	Automatic drinker, road network
Pf16	Ibigbo poultry farm: opposite hill view sec. sch. Uwani Edem Nru Nsukka	6.844	7.377	1345	Power supply, feed mill, tractor for waste handling and egg handling machine
Pf17	Department of animal science, poultry farm: UNN	6.854	7.388	3422	Bad road, feed mill
Pf18	Department of Veterinary Medicine poultry farm: Old Vet, UNN	6.854	7.396	3756	Pelleting machine

fuel and maintenance of the plants invariably affect the total cost of production and hence places the farmer often at lower profit margins. About 44% indicated need for egg handling equipment or devices; most poultry farmers pick up eggs either from battery cage or deep litter systems by hand to a collection point where they are moved with

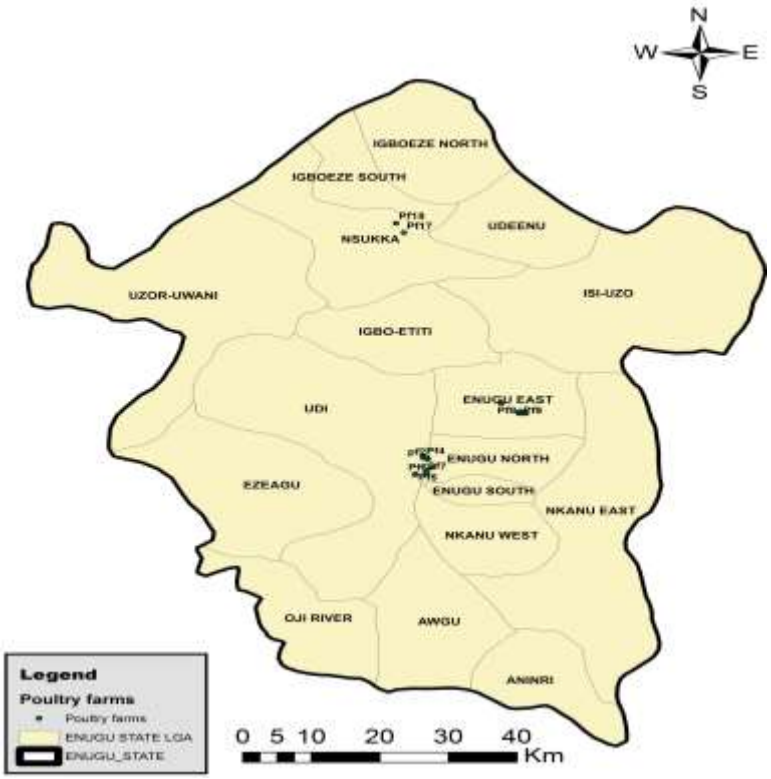

Figure 2. Map showing locations of surveyed poultry farms in Emene, Ngwo and Nsukka.

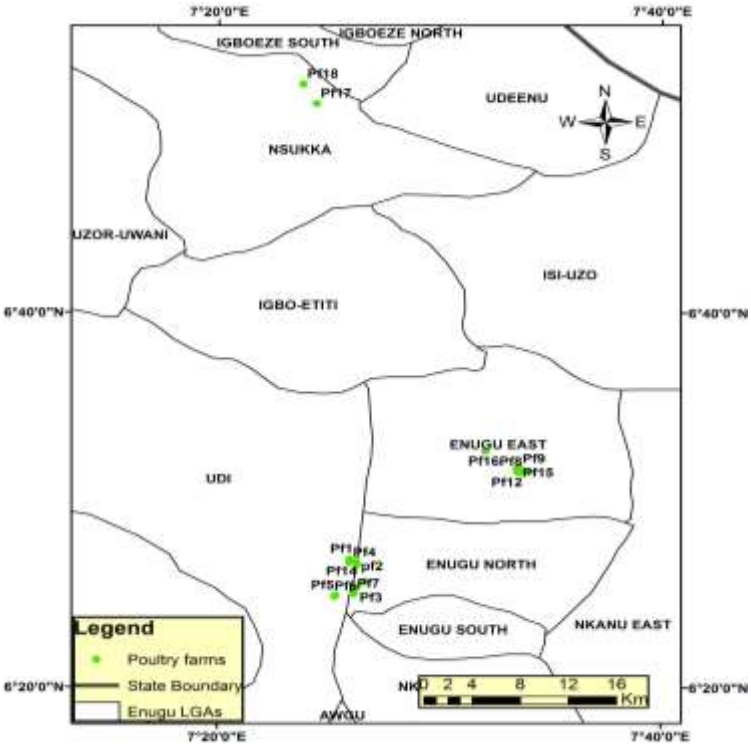

Figure 3. Enlarged map showing locations of surveyed poultry farms in Emene, Ngwo and Nsukka.

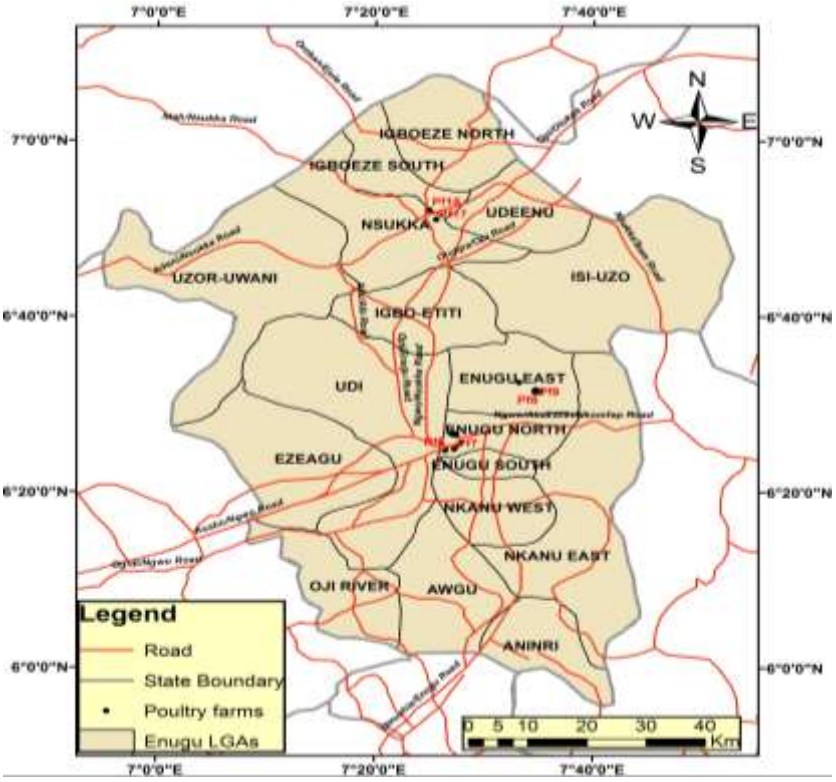

Figure 4. Map showing locations of surveyed poultry farms in Emene, Ngwo and Nsukka indicating the road network.

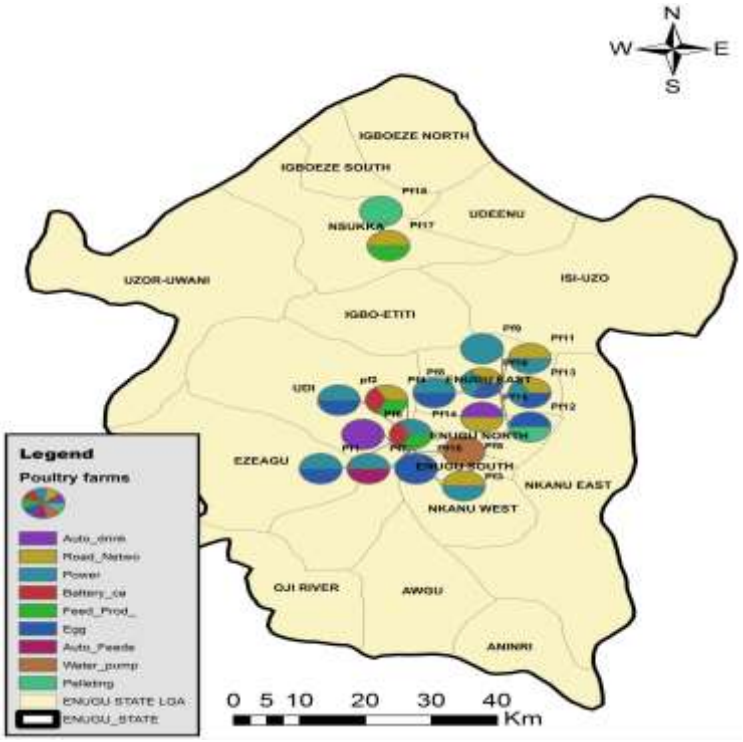

Figure 5. Map showing the location and mechanization needs of poultry farms at Emene, Ngwo and Nsukka.

Table 2. Identified mechanization needs and number of poultry farms involved.

S/N	Mechanization needs	Number of poultry farms
1	Automatic drinker	2
2	Road network	6
3	Power supply	10
4	Battery cage	2
5	Feed mill	4
6	Egg handling	8
7	Automatic feeder	1
8	Water pump	1
9	Pelleting machine	2
10	Waste handling	5

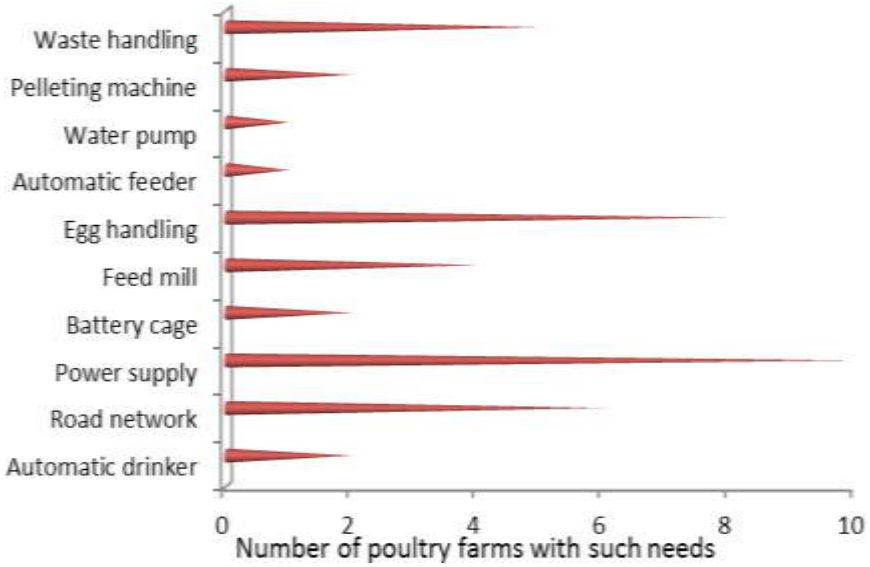

Figure 6. Mechanization needs of poultry farms in Enugu State.

either wheel barrows, hand trolleys or simply with plastic or metal containers/crates carried by hand to temporary storage rooms before they are moved to market. Significant damages are usually incurred depending on how careful the individual operators are, and this explains why up to 44% of farmers are requesting for better methods, tools or equipment for egg handling. 33% of the poultry farms indicated need for good road network; incidentally good roads are one of the major challenges facing the State as a whole. Responses from individuals interviewed during the survey revealed that many of the damages incurred on eggs usually occurred during transportation due to bad roads. This is so because majority of the poultry pens are usually situated in undeveloped areas of either semi urban or rural areas where the only access roads available are those initiated by the farmers themselves by just clearing the access lands with or without grading. Such roads are usually susceptible to erosion and quickly develop pot holes; and without any form of maintenance, which is usually expensive, become very bad and almost unusable. About 28% indicated need for better waste handling equipment. It was observed that most farms use mostly hand tools for waste removal from the poultry pens and then move them with either hand push machines or tractors to designated dumping sites. At the site, they may be bagged and stored for sale to crop farmers as organic fertilizers. Some use water to flush out the waste to certain channels and away to either open lands or prepared ponds. About 22% indicated need for feed mills. Interacting with the farmers revealed that most of them buy their poultry feeds from dealers representing animal feed companies; therefore only very few venture into personal feed formulation and milling. Furthermore, only very few of the farmers are aware or even think of the need for specialized equipment such as: Battery cage,

automatic drinker, pelleting machine, automatic feeder, water pumps. This result is in line with what was earlier stated by Adene (2010), that the level of production determines the land space, scale, technological application and other input resources. That there were no poultry farms among those visited that indicated hatchery equipment, incubators and other related technologies as their mechanization needs suggests the level and type of poultry production going on in Enugu State. It is actually commonly reported that producers of day-old chicks are either scarce or nonexistent in Enugu and the whole South Eastern Nigeria, unlike the South West where it is more common to find many producers of day-old chicks. The reason for this is not very clear; some elite poultry farmers suggest that it is more about attitude than capital; since hatchery and production of day-old chicks require huge capital investment on equipment, waiting time to patiently develop the skill and practice, and involves certain level of risk, especially at the beginning. Notwithstanding, the fact remains that day-old chicks are constantly in high demand by poultry farmers in the South East and other parts of the country, which is an indication of a potential market.

Conclusion

Poultry has been identified by the World Bank funded Commercial Agricultural Development Program (CADP) as a value chain in the agricultural sector of Enugu State and thus has the potential for commercial agriculture. This work has investigated poultry farms in three production cluster areas in Enugu State namely: Emene (in Enugu East), Ngwo (in Enugu North/Udi) and Nsukka. The study identified the position of each farm, their specific mechanization needs and challenges limiting the performances of these farms. Position and mechanization data were represented in a map for easy and quick information, understanding and decision support which are useful to the government, non-governmental organizations, investors, researchers, consumers, etc. for improving and enhancing poultry production business in Enugu State. The farms visited produce eggs, broilers or layers or combinations of them; and the sizes of the farms range from units of thousands to tens of thousands of birds; these generally are commercial poultry farms but of a low or medium scale levels. The survey reveals that most of the poultry farms are having the following mechanization needs: Power supply, egg handling, road network, waste handling, feed mill, pelleting machine, battery cage, automatic drinker, water pump, and automatic feeder; however this depends on the level of production. Other problems or challenges facing the farms visited as observed during the survey are classified as management problems quantified as 60%, technical problems 30% and the problem of cost 10%. It is therefore concluded that if these needs and challenges will become adequately tackled, poultry production in Enugu State will be greatly enhanced, thereby improving supply of egg, chicken meat, and layers for producers. Also more jobs will be created and this will positively impact on the economy of the State and the Country at large.

Recommendations

From the findings earlier stated, the following recommendations are made:

(i) There is need for continual workshops, training, enlightenment campaign and continuing education for poultry farmers; and this can be achieved through improved extension services by the various government and non-government agencies. This will help to address the management and technical problems/challenges encountered by the farmers.
(ii) The government should encourage the engineers and researchers through grants, incentives and favourable policies to develop indigenous technologies which will be affordable while adequately addressing the mechanization needs of the poultry farmers at their various levels of production, especially for egg and waste handling.
(iii) Necessary infrastructure such as good road network should be planned and implemented by the government to minimize all losses associated with bad roads and increase networking, marketing and associated business opportunities.
(iv) Considering that many of the machines used by high level commercial farmers are electrically powered, and the general need of electricity for lighting and simple heating; concerted efforts should be made by government and non-governmental organizations to provide steady electric power supply.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Analysis of a bacterial community structure and the diversity of *phz*F gene in samples of the Amazonian Dark Earths cultivated with cowpea [*Vigna unguiculata* (L.) Wald]

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It is important to understand the Amazonian Dark Earth (ADE) diversified microbial communities which colonize agricultural soils and interact with plants, allowing a more sustainable way of soil utilization. Genomic prospection of biotechnological interest, such as the phenazine biosynthesis genes, was carried out by the characterization of the bacterial community structure through the analysis of the 16S rRNA gene in ADE rhizospheric and bulk soil sampled in the forest, and in the agriculture managed soil, being subsequently cultivated with caupi bean. Additionally, the *phz*F gene coding for a key enzyme in the phenazine biosynthesis was detected and quantified. Gene polymophism (Terminal Restriction Fragment Length Polymorphism, T-RFLP) analysis revealed differences in the bacterial community structure between colonized rhizospheric and bulk soil, but there were no differences concerning the 16S rRNA gene copy number. Besides, the *phz*F gene copy number was higher in the rhizospheric than in the bulk soil, without any difference between forest and agricultural soils. This work confirms that the type of soil and the interaction between plants and microorganisms are key factors that shape the structure and diversity of bacterial communities and represent a biotechnological potential, with the possibility of finding natural compounds for use in biological control.

Key words: Amazonian Dark Earth, rhizosphere, quantitative polymerase chain reaction (PCR), terminal restriction fragment length polymorphism (T-RFLP), microbial diversity.

INTRODUCTION

Soil bacterial communities carry genes with yet unknown functions that could possibly be important concerning biotechnological applications. Plant commensal bacteria play an important role contributing to their protection from harmful organisms as also to their physiology (Berlec, 2012). To date, molecular techniques make possible to identify and characterize these microorganisms, particularly functional bacterial communities associated to plants. In this way, the Amazonian Dark Earth, also known as Amazonian Black Soil or “Terra Preta” (ADE),

represents a kind of soil that is colonized by bacterial communities with potential biotechnological contribution (Lehmann et al., 2003; Lima et al., 2002). These soils are characterized by a dark coloration due to the high charcoal concentration, originated from traditional burnings, utilized by the Amazonian ancient populations. It is found in diverse sites in the amazon biome, exhibiting elevated pH values, fertility and microbiological diversity (Navarrete et al., 2010). Due to these characteristics, the ADE soils behave as reservoir of specific bacterial genes participating in certain functions, e.g. in the nitrogen and carbon cycle, coding for proteins responsible for degradation processes and secondary compounds synthesis (Germano et al., 2012; Brossi et al., 2014; Nakamura et al., 2014; Lima et al., 2014). It is feasible that many genes could be expressed under the interaction between microorganisms and plants as their products are important sources of bioactive metabolites. Besides, bacterial organisms pose relevant role in the production of compounds utilized in the pharmaceutical, food and farming industry. Certainly, the detection of secondary compounds in the environment produced by prokaryotic cells has been increasing in face of the improvement of molecular tools that make possible their application in biocontrol of plant pathogens in commercial crops.

The phenazine, produced by bacterial organisms commonly found in plants rhizosphere, is a pigmented nitrogenated heterocyclic compound that exhibits an ample inhibitory activity against eukaryotic and prokaryotic cells (Mavrodi et al., 2010; Lovic et al., 1993). Microorganisms' competition and survival are the main factors involved in the phenazine synthesis, mainly in the root, in their natural habitat as their producers are allowed to carry out redox reactions, e.g. Nicotinamide Adenine Dinucleotide (NAD) oxidation and Reactive Oxygen Species (ROS) generation (Guttenberger et al., 2018).

The phenazine-1-carboxylic acid compound, produced by the *Pseudomonas fluorescens* strain 2-79, was initially reported by Mavrodi et al. (2010), in significant concentrations of 27 to 43 ng g^{-1} in rhizospheric soils, suggesting that this antibiotic exerts great activity in the root. The phenazine synthesis is carried out by an *Operon* composed of seven biosynthetic genes (*phz* ABCDEFG) in *Pseudomonas* species (Fitzpatrick, 2009). Through root exudates, plant adapts to the soil microbiota community shaping the surrounding rhizophere in a dynamic and vital biochemical relationship (Jacoby et al., 2017).

Despite scientific advances in soil microbiology, the majority of soil microorganisms are still unknown. Previously to molecular genetics methodology aiming to identify bacterial organisms, phenotypic identification was currently used, based on colony morphology, color and bacterium individual morphology and staining properties, and until now culture and staining techniques are coupled to genomic studies. The fact that every living unicellular or multicellular organism share common properties displaying unique profile coded in the DNA, the most conserved sequences that show specific variations among different organisms were selected and employed to distinguish and group bioorganisms in separate taxonomic units. Therefore, the most promising genes were those coding for ribosomal RNA, 5S, 16S and 23S rRNA and also interspace sequences of these genes. Nowadays, the DNA sequence coding for the 16S rRNA subunit is the most commonly employed for prokaryote phylogenetic studies (Palys et al., 1977; Pace, 1977; Suárez Moya, 2017; Larkin and Martiny, 2017; Fierer, 2017).

The frequency of bacteria and gene coding for the phenazine compound present in rhizospheric ADE soil was quantified and described, utilizing specific probes that determined conserved regions of the 16S rRNA bacterial gene and the *phz*F gene. Besides, it was possible to quantify the studied genes and to determine the molecular profile of bacterial communities in these soils. It is important here to inform about the role of functional bacterial community in rhizospheric and bulk ADE soils cultivated with the caupi bean [*Vigna unguiculata* (L.) Wald].

MATERIALS AND METHODS

This research work was conducted in the Laboratory of Phytopathology of the Instituto Nacional de Pesquisas da Amazônia-INPA, Manaus-AM and in the Laboratory of Cellular and Molecular Biology of the Centro de Energia Nuclear na Agricultura, Universidade de São Paulo (CENA)-USP. The organization of the experimental procedures is described in the flowchart (Figure 1).

Soil sampling

Samples of the ADE soil were collected in the year 2013, in the Hatahara site located in the Amazonas state, in the Iranduba county (Figure 2), in two distinct areas, the forest area (03°16'494''S 60°12'340''O) and the agricultural area (03°16'516''S 60°12'275''O). Sampling procedure was carried out in five points, starting in a central location in the North, South, East and West positions. Collection points were separated from each other by 15 m, and two samples were collected from each point at a depth of 20 cm in the soil.

The chemical analysis of soil attributes under the different studied environments was previously realized in the experimental

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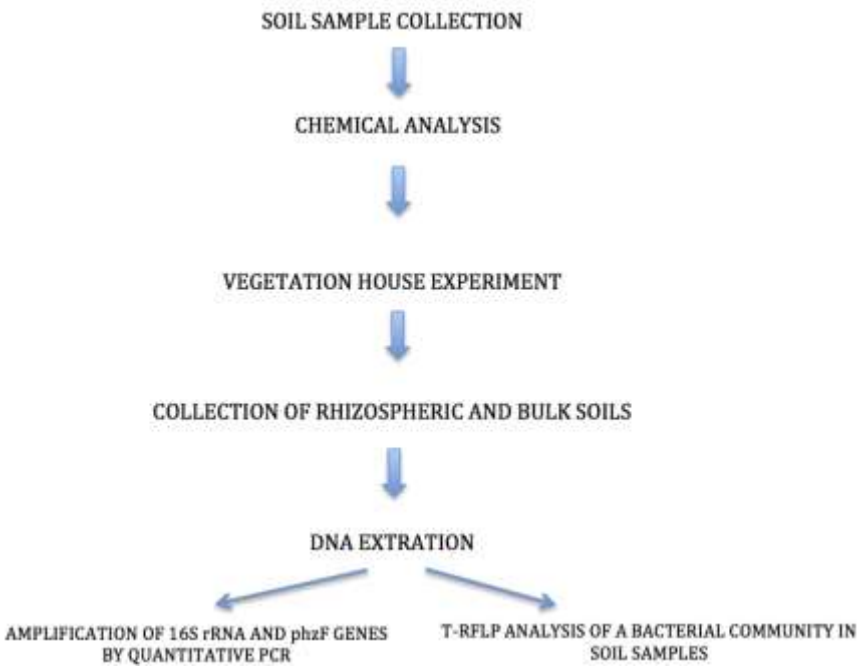

Figure 1. Flowchart of experimental procedures carried out in this research work.

Figure 2. Geographical location of Hatahara site, Iranduba county in the Amazonas state where the soil was collected.

set up of the mesocosm. From each area, forest and agricultural, 400 g of soil samples were collected. The analyses were carried out in triplicate, in the laboratory of the Department of Soil Science in the Escola Superior de Agricultura Luiz de Queiroz-ESALQ, Piracicaba-SP. For phosphorus (P) and Aluminum (Al) dosage, the extraction was done by calorimetry utilizing an ion exchange resin and potassium chloride, respectively. Acidity potential (H+Al) was determined with the Shoemaker Mac lean e Pratt buffer; organic matter (OM) and the sum of exchangeable bases (EB) were determined by the Walkley-Black method; Calcium (Ca), Manganese (Mg) and Iron (Fe) ions contents were determined by atomic absorption spectrometry, extracted with an ionic exchange resin (Raij et al., 2001).

The experimental design was totally random, with a factorial scheme of 2×2×2, being two soil samples, agricultural and forest; two systems, with and without plants; two genes, 16S rRNA and *Phz*F, with 15 repetitions. Collected samples at points of each area (forest and agricultural) were homogenized, yielding a composed sample representing each area, distributed in 10 pots of 3.6 kg capacity. After asepsis, by total immersion in 70% alcohol for 10 min, washing in *milli*Q water and dried in towel paper, 5 seeds of the caupi bean [*V. unguiculata* (L.) Wald] (Crioula-INPA variety) were sown in 5 pots containing soil sample of each area. After the germination period, 2 plants were kept until the end of the experiment. In order to control the experiments, 5 pots were kept exclusively with the collected soil, without seeds, under the same conditions of pots with germinated plants. The experimental design was completely randomized and carried out in the vegetation house

located at the Instituto Nacional de Pesquisas da Amazônia-INPA, Manaus-AM.

At 45 days post seeding, in the flowering period, rhizospheric soils were collected from each pot with 2 caupi bean plants, from both forest and agricultural area, and also from pots with just soil without plants, treated in the same conditions as earlier stated. The soil collection was done utilizing a sterilized firm bristle brush. From each pot with plants, 3 samples of rhizospheric soil were collected, as also from pots without plants but with soil only (bulk soil), without the influence of beans root. The soil samples were kept at -20°C until DNA extraction.

Metagenomics analysis

Genomic DNA was extracted from rhizospheric and bulk soil in triplicate, utilizing the Power lyzer DNA Extraction™ Kit *(*MoBio, Carlsbad, CA). In microtubes containing glass beads, 0.25 g of each sample was added and gently homogenized. After cell lysis, total DNA was extracted according to the manufacturer's instructions, ascertaining the DNA quality by measuring the sample in the spectrophotometer, Nanodrop 1000 (Thermo Scientific, Waltham, EUA).

The bacterial 16S rRNA gene segment was PCR amplified utilizing the primers U968F (5' AAC GCG AAC AAG CTT AC 3') and R1387 (5'CGG TGT GTA CAA GGC CCG GGA ACG 3') (Heuer et al., 1997), yielding an amplicon of 400 bp. The primers pair Ps_up1 (5'ATCTTCACCCCGGTCAACG3') and Ps_low1 (5'CCRTAGGCCGGTGAGAAC3') were applied for PCR synthesis of the *phz*F gene, generating a segment of approximately 427 bp (Mavrodi et al., 2010). The quantitative PCR reactions were run in a total volume of 10 µL containing 5 µL of the SYBR Green Rox (qPCR Kit, Fermentas, Brazil), 1.0 µL of each primer (5 pmol), 2 µL of *milli*Q water and 50 ng of the purified product. PCR conditions were optimized to each primer.

Also, for the 16S rRNA gene amplification, the following conditions were optimized: 94°C for 10 min, 40 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 40 s. At the end of the reaction, a melting curve was included in the following conditions: 95°C for 15 s, 56°C for 1 min with temperature of 95°C for 15 seconds, and collection at 0.7°C. For the *phz*F gene, the optimized conditions were: 94°C for 4 min, 94°C for 1 min, 62°C for 30 s, 72°C for 45 s, and the melting curve was obtained in the following conditions: 95°C for 15 s, 62°C for 1 min, 95°C for 15 s. The reactions were carried out in the StepOnePlus™ Real Time PCR System equipment (Applied Biosystems).

Specific and cloned fragments of the 16S rRNA and *phz*F genes were utilized to build the standard curve. The amplicon positive control was purified utilizing the GFX™ PCR DNA and Gel Band Purification kits (GE Healthcare), following the manufacturer's instruction, and quantified in the spectrophotometer model ND-2000 (Nanodrop Technologies, Walthan, EUA), calculating the gene copy number per gram of soil. The standard curves were generated applying 10 serial dilutions, varying from 10^2 to 10^7 gene copy per µL. The cycle threshold (Ct) values were utilized as normalizers, identifying the cumulative points of amplicons in each sample by the generated fluorescence in each cycle. Aiming to verify if there was difference between genes copy number found in soils of each area assayed, analysis of variance was conducted at significant level of $p<0.05$ using the Software Graphpad Prism version 5. The analysis data, when necessary, were transformed to $\log^{10}$ and analyzed according to the relative species abundance.

Terminal restriction fragment length polymorphism (T-RFLP) analysis of a bacterial community in soil samples

T-RFLP analysis was carried out to determine the bacterial community structure of agricultural and forest soils. The bacterial 16S rRNA gene was PCR amplified utilizing DNA samples obtained from rhizospheric and bulk ADE soils. The PCR was set up employing the primers: 27F AGAGTTTGATCMTGGCTCA and 1492R TACGGYTACCTTGTTACGACTT (Edwards et al., 1989; Woese et al., 1990). In order to subsequently detect the amplicons by the T-RFLP technique, the primer 27F was labeled with the 6-carboxyfluorescein (6-FAM) in its 5' terminal.

The amplified fragments were purified utilizing the GFX™ PCR DNA kit and the Gel Band Purification kit (GE Healthcare), following the fabricant's instructions. The purified amplicons were submitted to reactions with the *Msp*I restriction enzyme (Invitrogen, EUA), and the digested products were sodium acetate/EDTA and absolute ethanol precipitated. To carry out the analysis of the Terminal Restriction Fragments (T-RFs), the precipitated digested product was resuspended in highly deionized formamide (Hi-Di formamide) and GeneScan 500 ROX Size Standard (Applied Biosystems).

The T-RFLP data was analyzed in a file generated by the sequencer's Data Collection Software, in the Peak Scanner software v 1.0 (Applied Biosystems) to determine the length of restriction fragments termini by comparison to pattern of fragments termini size (Trotha et al*.,* 2002). The data were exported to an Excel electronic worksheet (Microsoft) and converted to a matrix for further multivariate analysis. The evaluation of the bacterial community structure obtained by T-RFs profiles was done by analysis in a multidimensional scale (multidimensional scaling, MDS) by stress levels of 0.06 and 0.01. The test was calculated based on the Bray-Curtis similarity coefficient, utilizing the Primer 6 software (Playmouth Marine Laboratory, Primer E, United Kingdom) (Clarke, 1993). Subsequently, a similarity analysis was run to determine statistical differences among the samples analyzed. Biological data were run in the Assistat Software version 7.7 beta (Silva, 1996) and submitted to variance analysis, and the means compared by the Duncan test at 5% probability. The redundancy and multivariate analysis were carried out to evaluate the correlation of bacterial communities' structural composition with chemical attributes of the agricultural and forest soil. The Canoco Software, version 4.5 (Biometris, Wageningen, Netherlands) was utilized.

RESULTS

Soil chemical analysis

There were significant statistical differences when comparing the chemical analysis data of collected samples in the forest and agricultural area (Table 1). Sulphur (S) and potassium (K) concentrations presented 64.2 and 33.6% differences in the forest and agricultural areas, respectively. Concerning Magnesium (Mg) and Calcium (Ca) contents, the differences were 31 and 0.7% respectively. There were no statistical significant differences in the parameters of Phosphorus (P) and Iron (Fe) content, acidity potential (H+Al), bases sum and ionic exchange capacity.

Bacterial and phzF gene diversity in rhizospheric and bulk soils

The gene copy number of the bacterial 16S rRNA in the forest soil determined by qPCR was 3.85×10^9 copies/g of soil in rhizospheric soils and 3.94×10^9 copies/g of soil in

Table 1. Chemical analysis of the Amazonian Dark Soil (ADE) in the forest and agricultural areas.

Area	Chemical parameters[1]										
	pH	P	S (**)	K (**)	Ca (*)	Mg (*)	H+Al	SB	CTC	Fe	M.O
Forest	5.5±011[a]	372±42.9[a]	4.3±0.57[b]	0.73±0.057[b]	158±16.7[a]	6.6±1.1[b]	32±1.7[a]	165.6±19.7[a]	197.5±19.7[a]	101±63.2[a]	67±3.6[a]
Agricultural	5.3±0.1[a]	409±61.77[a]	12±0.57[a]	1.1±0.057[a]	157±12.6b	9.6±0.57[a]	40±6.1[a]	168.1±6.7[a]	209.2±6.7[a]	79.3±4.5[a]	73.6±6.6[a]
CV%	1.99%	13.20%	6.90%	6.08%	9.39%	11.18%	12.36%	9.45%	7.20%	49.7%	7.60%
Differences forest vs. agricultural	3.7%	9.1%	64.2%	33.6%	0.7%	31%	20%	1.4%	5.5%	21.4%	8.9%

Calcium (Ca), Aluminum (Al), Magnesium (Mg); Acidity Potential (H+Al), Bases Sum (SB) and Cationic Exchange Capacity (CTC) are represented in mmolc dm^{-3}; organic matter (MO) in g dm^{-3}; Phospohorum (P), Iron (Fe) and Sulphur (S) in mg dm^{-3}. [1]Means with standard deviation. **Means followed by different letters differ statistically between them by the F test ($p<0.01$). *Means followed by different letters differ statistically between them by the F test ($p<0.05$).

bulk soils, while in the agricultural soils, there were 3.51×10^8 copies/g of soil in rhizospheric and 2.79×10^8 copies/g of soil in bulk soils. Despite numeric differences, there were no significant differences by the Duncan test at 5% probability concerning the gene copy number of the bacterial 16S rRNA between rhizospheric and bulk soils in the same environment (Figure 3a). Nevertheless, when the gene copy number was compared between different environments, the quantity was higher in the forest, for both rhizospheric and bulk soils (without statistical differences between them), that figure out a great bacterial diversity than in the agricultural soil. It was also possible to observe that, in the agricultural soil, the gene copy number was higher in the rhizospheric soil than in the bulk soil (without statistical differences).

The molecular analysis of the *phz*F gene allowed us to observe the gene frequency in the studied environment. When the *phz*F gene copy number was compared in rhizospheric soils obtained from the forest and agricultural area, a higher number of copies, 4.82×10^8 copies/g of soil, in the agricultural area could be observed, as the number of copies in the forest area was 3.11×10^8 copies/g of soil. In the bulk soil samples, there was no statistical difference by the Duncan test at 5% probability for the same parameter analyzed.

The results showed that soil samples collected in the forest area, after cultivation with caupi bean, had 1.26 times more the *phz*F gene copy number than the bulk soil (2.46×10^8 copies/g of soil), with no significant statistical difference. In agricultural soil samples, the *phz*F gene copy number was also higher in rhizospheric environment, 4.82×10^8 copies/g of soil, than in bulk soil, 2.75×10^8 copies/g of soil (Figure 3b).

Comparison of bacterial communities in rhizospheric and bulk soils

The T-RFLP analysis was performed to access the bacterial community structure colonizing the rhizospheric soil cultivated with caupi bean, and to compare to the bulk soil, collected in the forest and agricultural area, based on separated T-RFs for the bacterial 16S rRNA marker among ADE soils.

The results of T-RFLP analysis showed a clear distinction in the bacterial community structure between the soil collected in the forest and agricultural areas, as also, soil samples that were influenced by the caupi bean roots and control, and bulk soil. The data exhibited joining at 55% similarity, mainly high among biological replicates (Figure 4).

The results presented global R values above 0.83 to rhizospheric and bulk soil samples, in the agricultural environment, and 0.704 in the forest environment for rhizospheric and bulk soil samples, being statistically significant at $p<0.05$ level by the ANOSIM test. The analysis of ordered MDS clearly showed difference of bacterial communities between soil samples. This structural difference could be related to different characteristics of each soil, and mainly to the influence of caupi bean roots.

It was also possible to observe, from the profiles generated by the T-RFPL analysis, the differences in the bacterial community structures in rhizospheric soil cultivated with caupi bean and bulk soils, likewise among the areas where the soils were collected (forest and agricultural), which make us to infer that the environment influenced in this composition. In the soil of forest environment, it was observed that the caupi bean rhizosphere was more homogeneous than in the bulk soil (Figure 5), and it was also more gathering samples were noted.

Furthermore, the RDA analysis results of T-RFLP profiles showed that these bacterial communities also differed in the structure, meaning that there was integration of the replies

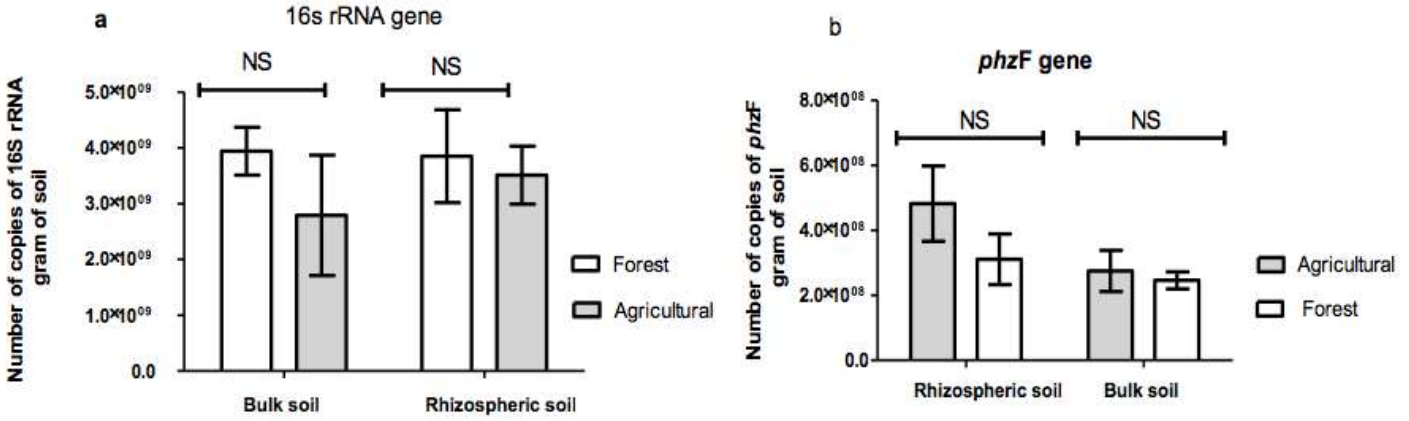

Figure 3. Mean of gene copy number of the bacterial 16S rRNA: (a) and number of phzF gene copy (b) determined by the quantitative PCR. Samples refer to ADE rhizospheric soil cultivated with caupi bean in two environment (forest and agricultural).

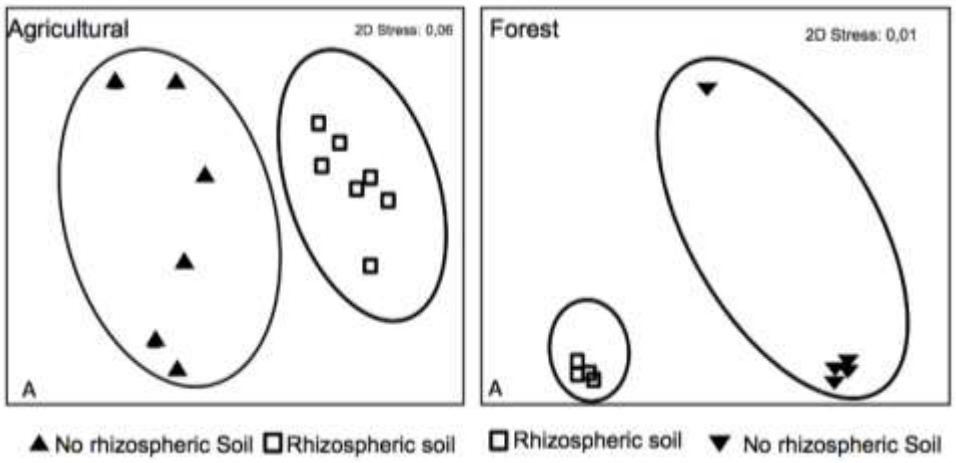

Figure 4. Multidimensional scale anelusis (MDS) based on T-RFs seperated by the 16S rRNA among areas and soil samples.

relating to both types of soils (rhizospheric and bulk soils) of each area. Anyway, there were no elements associated to the data variability, as those were responsible for 40% in the agricultural area and 44% in the forest as shown in Figure 6. The results also showed a distinct correlation of the chemical parameters with the bacterial communities among samples of rhizospheric and bulk soils.

DISCUSSION

Concerning the soil chemical analysis, it is important to reinforce that the ADE composition of stable pyrogenic organic matter allied to elevated contents of Phosphorus, Calcium, Magnesium and Carbon, which constitute a unique micro ecosystem, make it highly fertile (Glasser, 2007; Madari et al., 2006; Falcão et al., 2010; Lehmann et al., 2003). The pH of the forest soil was slightly elevated as compared to the soil in agricultural area, possibly explained by the burning of forest vegetation carried out by ancient communities as a practice to prepare the soil for new cultivation, which promotes the permutation exchange of cations from the ashes to the soil, causing pH acidification as described by Cenciani et al. (2009).

The molecular analysis of bacterial and phzF gene diversity in rhizospheric and bulk soils shows a great potential as reservoirs of new antibiotics. Distinct bacterial species colonizing soil communities, and also associated with plants roots produce natural antibiotics as a way to suppress pathogens attack. Gouda et al. (2018) emphasize the importance of plant growth promoting rhizobacteria as the best option for the plant

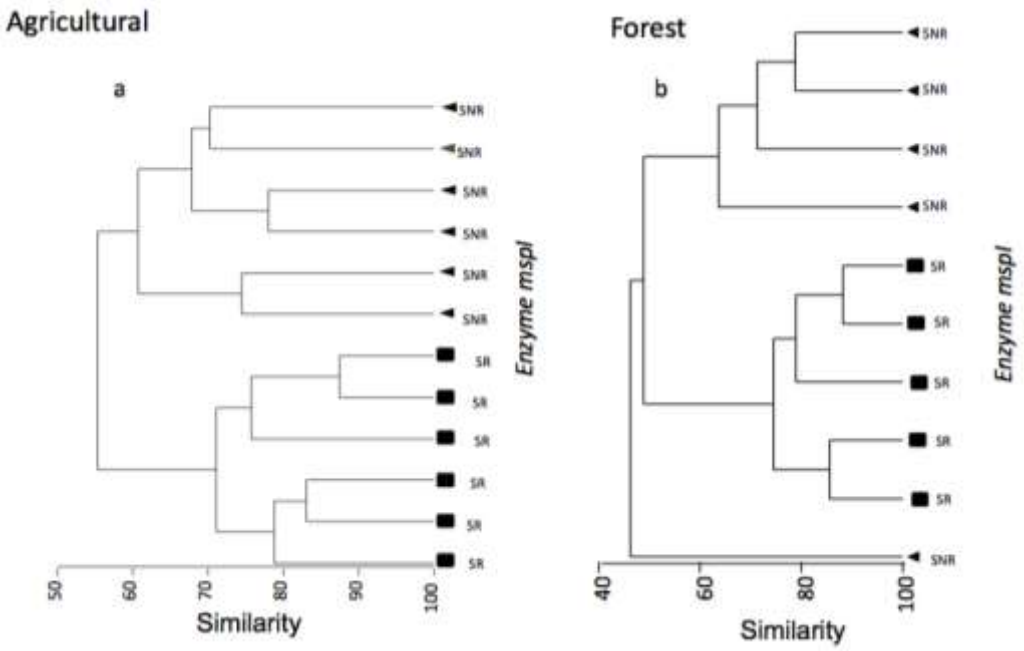

Figure 5. Similarity join between rhizopheric (SR) and bulk soil (SNR) collected in the forest (a) and agricultural environment (b). Obtained samples were grouped with algorithm, through the *Mspl* enzyme segmentation performed by CLUSTER analysis in Primer 6 software (Plymouth Marine Laboratory, PrimerE, United Kingdom).

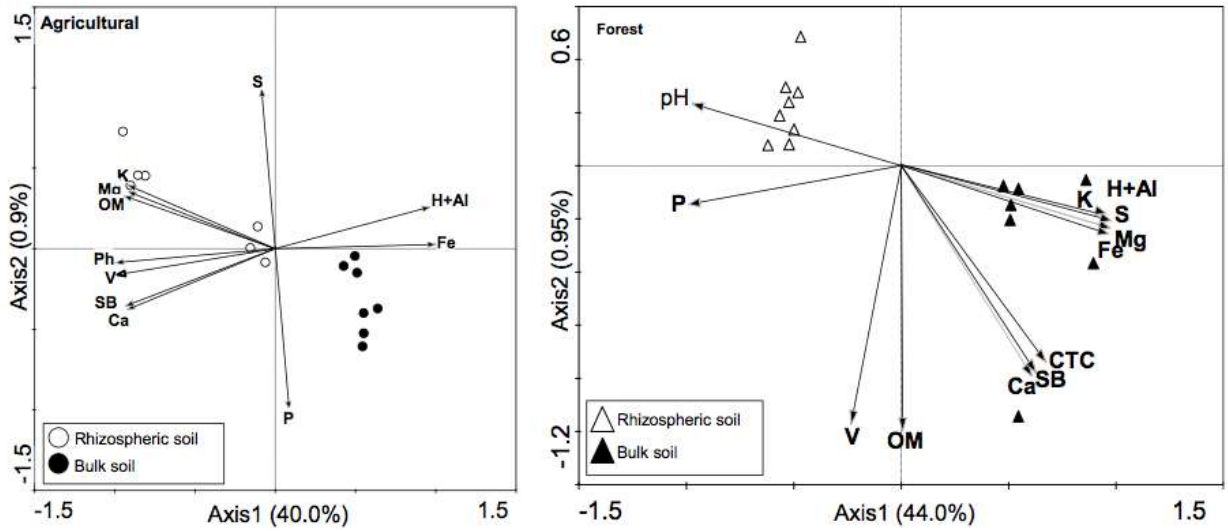

Figure 6. Redundancy analysis (RDA) of bacterial communities determined by the T-RFLP technique of chemical characteristics in the agricultural and forest soil.

and soil health instead of chemical fertilizers and pesticides. Rashid and Chung (2017) discuss plant hormone signaling regulation and biosynthesis by rhizobacteria and rhizofungi likewise the jasmonic acid, ethylene and salicylic acid pathways which trigger protection mechanisms against pathogens and insects.

The quantitative PCR analysis aimed at obtaining the total gene copy number of the bacterial 16S rRNA and the *phz*F gene in rhizospheric and bulk soil samples was precise concerning the gene copy quantification, allowing us to compare the frequency among soil samples in both forest and agricultural environments. The results suggest

that chemical, physical and biologic environmental conditions of the caupi bean rhizosphere could favor the incorporation of specific groups of bacterial organisms as mentioned elsewhere (Hinsinger et al., 2009). Undetected statistical difference concerning the elevated abundance of bacterial organisms in the rhizospheric and bulk soils seems to be related to the physicochemical stability and plant diversity in the ADE environment. The biological, chemical and physical conditions of the rhizosphere environment stimulate the plants to release organic ions to the environment, favoring the colonization or not of specific groups of prokaryotic organisms. Also, in the rhizosphere environment, the temperature and nutritional stress could directly affect its composition around the roots (Raiesi et al., 2015; Compant et al., 2005; Moreira and Siqueira, 2006). These findings focus on studies towards these groups of diversified bacterial organisms colonizing the plant rhizosphere microenvironment, considering the possibility of isolating and characterizing bacterial species through methods dependent or not on culture, manly to produce secondary metabolites as antibiotics.

The data obtained here suggest that the caupi bean rhizosphere microenvironment aggregates specific groups of bacterial organisms that harbor and/or express the *phz*F gene. After studying the evolution and dispersion of the *phz*F gene in rhizospheric soil samples, it corroborated the diversity of phenazine-producing bacterial organisms as previously discussed by Mavrodi et al*.* (2010), furthermore, implying that most of them are plant associated microorganisms and also that the *Pseudomonas* genus keeps mechanisms of gene conservation, while the *Burkholderia* and *Pectobacterium* genus operate mechanisms of horizontal gene transfer. The intra-genotypic diversity in a group of microorganisms that share the same antagonistic characteristics provides a largely unexplored resource to improve the biological control of plant pathogens (Raaijmakers et al., 2001; Weller et al., 2002).

The *phz*F gene diversity in the natural environment permits specific comprehension and regulation of these processes by some groups of bacteria that are relevant for the production of natural biocompounds *in vitro*. The results of this study add improvements to isolate and characterize phenazine-bacterial producers in soil systems, likewise in the plants rhizosphere. Besides, some groups of prokaryotes, colonizing the plant rhizosphere, produce phenazine when stimulated by phytopathogens attack.

It was observed that the caupi beans roots considerably influenced the bacterial community structure. It is also considered that the general characteristics of each area, forest and agricultural, could have shaped the bacterial community profile. Studies carried out by Lima et al*.* (2014), with ADE rhizospheric soil samples and controls, demonstrated in ordered MDS analysis, taking in account T-RFLP data that the rhizosphere of 2 leguminous species shaped the bacterial community structure which summed to soil properties.

The interaction among plants and bacteria is coordinated by intrinsic metabolic processes in both organisms, in a mutual recognition processes (Dini-Andreote and van Elsas, 2013). Furthermore, bacteria respond distinctly to the compounds secreted by plants roots (Melo and Azevedo, 2008), dictated by the soils' properties, which strongly influence the bacterial community structure (Jesus et al*.,* 2009).

The characterization of bacterial communities in rhizospheric environments could contribute to future studies related to the soil quality and usefulness of these microorganisms in the environment as nitrogen biological fixation, bioremediation, production of enzymes involved in plastic degradation, disease control in plants and pathogen suppression in the soil (Bettiol et al., 2005). Similar studies with soils from the Amazon region showed that bacterial communities were influenced by the land use and chemical characteristics of the vegetation metabolites (Jesus et al., 2009; Navarrete et al., 2010; Lima et al., 2014).

Studies of the functional bacterial communities in the ADE indicated that the land use interfered on the amount of bacterial organisms and, the soil high fertility is associated to the microbial diversity detected when compared with other Amazonian soils (Brossi et al., 2014). In the present results, there was clustering of these bacterial communities, even though without significant differences. The historic events in the studied areas suggest a positive influence in the direct maintenance of the bacterial communities structure.

Conclusion

The 16S rRNA and *phz*F genes were detected in the caupi bean rhizospheric soil applying the quantitative PCR assay, demonstrating that the gene copy number of *phz*F was elevated in rhizospheric soils in the forest and agricultural environment. The bacterial 16S rRNA copy gene number was elevated in bulk soil collected in the forest environment, emphasizing the bacterial diversity in relation to the agricultural environment. The bacterial community profile showed divergence according to plant type and roots influence.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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Full Length Research Paper

Hot water immersion disinfests enset (*Ensete ventricosum*) suckers from the enset root mealybug *Cataenococcus ensete* Williams and Matile-Ferrero

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The enset root mealybug, *Cataenococcus ensete* Williams and Matile-Ferrero (Homoptera: Pseudococcidae) is a pest of national significance attacking enset in south and south-western Ethiopia. Adults and nymphs of several overlapping generations feed on the crops' underground corms and roots, making them hard to reach and thus control. The main means of pest spread is through infested planting material. The objective of this study was to evaluate the effect of a hot water treatment of enset suckers against enset root mealybugs. Small, medium and large-sized infested enset suckers were each exposed to water temperatures of 21, 55, 75 and 95°C for periods of 10, 30, 60 and 300 s. Complete mealybug mortality was obtained for 60 s at 55°C and at 10 and 30 s exposure times at 75 and 95°C without affecting the performance of enset suckers of all size groups. Considering the ease of using boiling water for small-scale enset farmers, immersing suckers for at least 10 and up to 30 s at 95°C is advocated in order to eliminate all enset root mealybugs from enset suckers. The immersion of enset suckers in boiling water for 10 to 30 s can be easily demonstrated, with a much higher envisaged adoption rate by farmers.

Key words: Enset corm, enset roots, Ethiopia, integrated pest management (IPM), mortality, pseudostem, survival.

INTRODUCTION

Enset (*Ensete ventricosum* (Welw.) Chessman) is mainly cultivated across south and south-western Ethiopia on a total land area estimated to be around 300,000 ha (CSA, 2011). It is a multipurpose crop which is used as a source of food, feed, fibre, construction material and often also for medicinal purposes (Shigeta, 1996). It is estimated that more than 20 million Ethiopians, belonging to over 45 ethnic groups inhabiting the highlands of southern, south-western and central Ethiopia depend on enset as a major or co-major staple food (Brandt et al., 1997; CSA, 2004).

Numerous biotic and abiotic constraints affect enset cultivation. Important abiotic constrains are drought especially during the latter part of the 6 to 7 month dry season and frost at high (>3000 masl) elevation sites

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mainly during December and January. Among the biotic constraints, Xanthomonas wilt of enset has been affecting enset cultivation since several decades and is considered the most important biotic constraint (Castellani, 1939; Yirgou and Bradbury, 1968; Welde-Michael et al., 2008; Blomme et al., 2017).

The enset root mealybug (*Cataenococcus ensete* Williams and Matile-Ferrero) is a major destructive insect pest of enset in southern, south-western and central Ethiopia having been first reported at Wonago (Tsedeke, 1988; Addis et al., 2008). The pest has not been reported on banana in Ethiopia (Addis, 2005, personal communication). Enset root mealybugs have an elongate-oval body covered with bright white wax secretions on the dorsal and lateral sides. Although the insect has been present in various parts of the enset growing belt, it has only become a serious threat to enset production since around 2000 (Addis, 2005). Although empirical yield loss data are not available, more than 30% of the enset farms visited in southern and south-western Ethiopia were infested with enset root mealybugs (Addis et al., 2008). Similarly, Kefelegn et al. (2014) reported that 26% of sampled farms were infested with enset root mealybugs and enset root mealybug counts per plant were respectively 64, 51 and 76 in the Dilla Zuria, Gedeb and Wonago districts of southern Ethiopia. The insect attacks enset of all age groups, but based on field observations and farmer feedback, 2-4 year old plants were more frequently found to be infested with this pest (Kefelegn et al., 2014).

In addition, Mulualem and Walle (2014) and Kefelegn et al. (2014) reported variation in susceptibility or tolerance of enset landraces to the enset root mealybug. Enset root mealybug symptoms include slow plant growth, a lack of vigor and eventual plant death, especially under moisture-stress. Kefelegn et al. (2014) reported that corm and root damage was highest during the dry and hot period of the year. These authors also reported that the majority of farmers can only recognize enset root mealybug symptoms once severe plant damage occurs. Infested plants often display retarded growth with dried out outer leaves, but with a green central shoot. Enset plants attacked by root mealybugs have a significantly lower number of roots as compared to healthy plants. As a result, mealybug-damaged enset plants are more easily uprooted. The mealybugs are mainly spread across farms, villages and regions through infested planting materials (Addis et al., 2008). Enset corms used for the production of new suckers may be infested with enset root mealybugs. The mealybugs often remain unnoticed by farmers and are consequently distributed to new regions and farms through exchange or sale in the market of infested planting materials (Bizuayehu, 2002). The production of mealybug-free clean planting materials is the key control measure used to manage enset root mealybugs. Transplanting contaminated planting material also facilitates mealybug spread (Azerefegne et al., 2009). Kefelegn et al. (2014) assessed farmer's indigenous knowledge on how to manage/control enset root mealybugs. The authors reported that farmers practice clean seedling selection, use of farmyard manure, increasing soil moisture, uprooting the infested plants and burning the hole, removing alternative hosts, control of ants and variety selection.

A number of techniques have been developed to protect or disinfest banana suckers (*Musa* planting materials) from pests and pathogens (Speijer, 1999). Insecticides can be used to control root mealybugs. However, the high cost of insecticides and their often unavailability to resource poor subsistence farmers, residues on the corms and the possible development of resistance justify the search for new strategies. Moreover, enset is often intercropped with coffee and the application of insecticides is against the norm of organic coffee production. A combination of hot water treatment with insecticides is often used for quarantine security work to eliminate pests such as mealybugs, aphids, thrips, soft scales and ants (Hara et al., 1995; Hu et al., 1996). Exposure of weevil [larvae] and nematode-infested banana and plantain suckers to hot or boiling water for varying time durations has been used to obtain clean planting materials (Colbran, 1967; Gold et al., 1998; Speijer et al., 1999; Hauser, 2000, 2007; Tenkouano et al., 2006). Immersion of pared banana or plantain suckers in boiling water for at least 20 s and up to 30 s has been advised for the control of nematodes and weevil larvae (Coyne et al., 2010). Enset root mealybugs (*C. ensete* Williams and Matile-Ferrero) are currently not a pest on banana in Ethiopia and other countries in Africa (Blomme et al., 2017, personal communication). Another root mealybug *Geococcus* spp. has been reported in Kerala, India on banana. Various control options (e.g. soil ameliorants, botanicals, chemical insecticides and fungal bio-agents) were screened against this banana root mealybug (Smitha and Maicykutty, 2010). However, a hot/boiling water treatment for the control of *Geococcus* spp. on banana has not yet been reported in literature. In Ethiopia, the optimum hot water temperature and immersion duration for disinfesting enset suckers from root mealybugs without jeopardizing the growth and vigour of enset suckers has not yet been assessed. Therefore, this study was carried out in Ethiopia to evaluate the effectiveness of hot water immersion at different temperature levels and exposure durations for the control of enset root mealybugs. In case multiple effective options/combinations are obtained, ease of adoption by farmers will guide optimum temperature by immersion duration combination selection.

MATERIALS AND METHODS

Collection of infested enset plants and assessment of plant size and infestation level

Two to three-year old enset plants of the landrace 'Genticha' highly infested with enset root mealybugs were uprooted and collected from numerous farms in Aleta Wondo in the Sidama Zone, and Yirga

Cheffe and Kocherre in the Gedeo Zone, southern Ethiopia. A total of over 600 plants were collected. The aboveground part of the plants was cut at a height of 20 cm above the corm. In addition, dried up pseudostem leaf sheaths were trimmed off. All the cord roots were gently cut at their junction point with the corm using a knife and all decayed plant debris was removed from the corms together with the soil. Only infested plants/corms were retained. Subsequently, the plants were categorized into three size classes: small (a pseudostem circumference at soil level of <15 cm), medium (15-30 cm) and large (31-45 cm). A total of 160 infested suckers were randomly selected from each size class, totalling 480 suckers for the overall experiment. The average number of adult enset root mealybugs in each size class was estimated by counting the adult mealybugs on 40 selected enset plants from each size class (that is, 25% of corms). Suckers were moved from one heap to another heap and each 4th sucker was used for mealybug counts. On average, 58, 72 and 116 adult mealybugs/plant were found on the small, medium and large enset corm classes, respectively.

Thermal water treatments

Eighty litres of tap water were heated to 55, 75 and 95°C (that is, boiling water; 95°C as a result of the higher elevation at Hawassa, that is, 1665 m above sea level) on a wood-fired stove in a 100 L metal barrel. A volume of 20 L was left for the displacement of water while immersing 10 enset plants of the same size class at a time. The required temperature was inspected with a thermometer immersed in the water. When the water reached the required temperature ten enset suckers of each size class kept in a locally-made woven-leaf basket were entirely immersed in the hot water for each of the exposure durations of 10, 30, 60 and 300 s. Immersion of the three classes of enset plants (10 plants per treatment) in water at room temperature (21°C) was included as a control. After the thermal treatments, the suckers were planted in plastic pots of 25 cm diameter and 20 cm in height, having drainage holes in the bottom and filled with 8 kg of soil free from mealybug infestation. The soil was collected from the same site as the enset suckers and kept in sealed plastic bags for more than a month before transferring to pots in order to avoid any infestation from the soil. The insects cannot survive for more than three weeks in the absence of a host (Addis et al., 2008). The potted suckers were maintained in a semi-controlled greenhouse for 120 days and watered as required/when the soil was no longer moist. The screenhouse protected the plants from adverse weather conditions (e.g. heavy rain and related flooding) and from nocturnal animals which often eat corm parts such as porcupines and mole rats.

Data collection and analysis

Four months after planting the treated enset suckers, plant above and below ground growth traits were assessed. The enset plants were assessed visually and plants with fresh and green leaf laminas, pseudostem, corm and roots were considered as having survived the hot water treatment. For these surviving plants, plant height was measured from the ground (soil surface) to the lowest part of the petiole of the last emerging leaf (Tsegaye and Struik, 2003). Plants were subsequently dug up and the number of cord roots of each surviving plant in each treatment was recorded by counting all healthy-looking and functional cord roots. In addition, data were collected on the number of adult mealybugs present. All cord roots and dead corm tissues were carefully lifted up/removed in order to count each and every mealybug.

The overall effects of water immersion temperature and exposure duration were categorized into two effectiveness classes: treatments which completely disinfested enset from the root mealybug but with no death of enset suckers were categorized as “Effective”, while those treatments for which root mealybugs survived or caused any death of enset suckers as “Not effective”. The data were analysed as a two-factor experiment (temperature and exposure durations) in a completely randomised design per enset class size using Minitab (Minitab Inc., 2010). Treatment means were separated using Tukey’s honestly significant difference test (HSD) ($p< 0.05$). Percent whole plant dry weight gain was calculated per plant size class, by comparing the total dry weight of the 21°C treatment (control) with the other treatment and exposure duration combinations.

RESULTS

Effectiveness of hot water immersion to disinfest enset suckers from root mealybugs

The hot water treatment had varied effects on the survival of enset plants (Table 1). The survival of small, medium and large-sized enset suckers was not affected when immersed in water at 21°C for all exposure durations. Similarly, exposing the three size classes to all temperature treatments for up to 30 s did not affect plant survival. Reductions in plant survival started to appear at 75 and 95°C with exposure periods starting from 60 s. The 55°C hot water treatment did not affect the survival of the large sized enset plants, but it decreased the survival of both small and medium sized plants by 10% when exposed for 300 s (Table 1).

The 75°C thermal treatment reduced survival of the small sized plants to 70 and 10% for 60 and 300 s exposures, respectively, and survival of medium sized enset plants to 90 and 70%, respectively (Table 1). The survival of the large sized plants was not affected by a 300 s exposure. The boiling water treatment severely affected the survival of young enset plants. Only 60% of the small sized plants tolerated 95°C treatment for 60 s, while all of them died when exposed for 300 s. For the medium sized enset, 80 and 20% survived the 95°C treatment for durations of 60 and 300 s, respectively. The survival of the large sized enset was not affected at 95°C for durations up to 30 s, while 10 and 20% of plants died when exposed for 60 and 300 s, respectively.

Effect of hot/boiling water immersion on the growth of small-sized suckers

The small-sized enset suckers achieved significantly greater plant height, leaf dry weight and pseudostem dry weight for the hot water immersion treatment combinations of 55°C for 60 s, 75°C for 10 and 30 s, and 95°C for 10 and 30 s (Table 2). On the other hand, the temperature levels of 75 and 95°C for 300 s resulted in a significantly lower performance of the above-ground plant parts. Similar observations were also made for the below ground plant traits. The small enset suckers immersed in water at 21°C had an average total plant dry weight of 53 g/plant at 120 days post treatment, while it was around or > 100 g/plant for the combinations of 55°C for 60 s, 75°C

Table 1. Effectiveness of hot water immersion to disinfest enset suckers from enset root mealybugs. (The first number in parenthesis denotes the number of surviving mealybugs, while the second number after the comma represents the percentage of surviving suckers). Ten plants were assessed for each treatment combination.

Water temperature(°C)	Exposure time(s)	Size of enset sucker		
		Small	Medium	Large
21	10	NE*(89,100)	NE (97,100)	NE (152,100)
	30	NE (93,100)	NE (103,100)	NE (128,100
	60	NE (85,100)	NE (99,100)	NE (134,100)
	300	NE (88,100)	NE (112,100)	NE (169,100)
55	10	NE (9,100)	NE (14,100)	NE (24,100)
	30	NE (7,100)	NE (6,100)	NE (9,100)
	60	E (0,100)	E (0,100)	E (0,100)
	300	NE (0,90)	NE (0,90)	E (0,100)
75	10	E (0,100)	E (0,100)	E (0,100)
	30	E (0,100)	E (0,100)	E (0,100)
	60	NE (0,70)	NE (0,90)	E (0,100)
	300	NE (0,10)	NE (0,70)	E (0,100)
95	10	E (0,100)	E (0,100)	E (0,100)
	30	E (0,100)	E (0,100)	E (0,100)
	60	NE (0,60)	NE (0,80)	NE (0,90)
	300	NE (0,0)	NE (0,20)	NE (0,80)

*E=Effective (no root mealybugs and 100% survival of enset suckers), NE=Not effective (surviving root mealybugs or less than 100% survival of enset suckers).

for 10 and 30 s and 95°C for 10 and 30 s (Table 2). Weight gain of small enset suckers (compared to the 21C treatment) increased by 100, 90, and 86% for the treatments of 55°C for 60 s, 75°C for 10 s and 95°C for 10 s, respectively (Table 2).

Effect of hot/boiling water immersion on the growth of medium-sized suckers

Medium-sized enset suckers grew significantly better when treated at 55°C for 60 and 300 s and at 75 and 95°C for both 10 and 30 s (Table 3). As with the small-sized suckers, combinations of 75 and 95°C for 300 s significantly reduced all the above-ground performance parameters. On the other hand, the enset plants treated with water at 21°C (control) for all exposure periods had intermediate performance levels. They were only better than the treatments with the highest temperatures and longest exposure durations (75 and 95°C at 60 and 300 s), which caused very poor growth or mortality of the enset plants. Similar observations were made for the below ground plant growth traits (Table 3). The treated medium-sized suckers also had heavier corms as compared to the control groups treated with water at 21°C (except for the 75 and 95°C with 300 s exposure duration combinations). These corms attained up to around 70 g/plant and about 150 g/plant total dry weight, respectively (Table 3). Higher whole plant weight gains were recorded for the medium-sized suckers with treatments of 55°C for 60 s (70%), 75°C for 10 and 30 s (77%), and 95°C for 10 and 30 s (76 and 78%) (Table 3).

Effect of hot/boiling water immersion on the growth of large-sized suckers

Similar trends in temperature effects were observed on the above- and below ground plant growth traits of the large-sized enset suckers (Table 4). The large-sized plants grew significantly better when treated at 55°C for 60 and 300 s and at 75 and 95°C for 10 and 30 s (Table 4). As with the small and medium-sized suckers, combinations of 75 and 95°C for 300 s significantly reduced all the above-ground growth traits (Table 4). Similar observations were made as for the medium-sized suckers when comparing the control suckers with the treated ones. The large-sized enset suckers achieved about a 57-58% total plant dry weight gain when treated at 75 and 95°C hot water for 10 and 30 s (Table 4).

DISCUSSION

This study revealed that enset suckers could tolerate higher temperatures (75 and 95°C) for only shorter

Table 2. Effect of hot water treatment on above and below ground plant traits for small-sized enset suckers infested with enset root mealybugs at 120 days post treatment. 10 plants were assessed per water temperature level and immersion time.

Water temperature (°C)	Exposure time (seconds)	Plant height (cm)	Leaf DW# (g)	Pseudostem DW (g)	N° of cord roots	Corm DW (g)	Root DW (g)	Total plant DW (g)	Whole plant dry weight gain compared to the 21°C treatment (%)
21	10	23.3^{b}*	12.1^{a}	13.5^{a}	6.3^{a}	22.2^{a}	5.4^{a}	53.2^{d}e	
	30	23.2^{b}	12.0^{a}	13.6^{a}	6.4^{a}	22.1^{a}	5.4^{a}	53.1^{d}e	
	60	23.3^{b}	12.1^{a}	13.5^{a}	6.2^{a}	22.2^{a}	5.4^{a}	53.2^{d}e	
	300	23.4^{b}	12.2^{a}	13.6^{a}	6.3^{a}	22.2^{a}	5.3^{a}	53.3^{d}e	
55	10	24.2^{b}	13.5^{a}	20.4^{c}	8.8^{a}	31.9^{c}	6.4^{a}	72.2^{abcd}	35.7
	30	25.7^{ab}	16.0^{b}	24.7^{c}	10.9^{a}	35.0^{c}	7.2^{a}	82.9^{abcd}	55.8
	60	29.3^{ab}	22.1^{b}	31.7^{b}	13.1^{b}	44.6^{b}	8.2^{a}	106.6^{a}	100.4
	300	26.8^{ab}	18.1^{b}	23.1^{b}	11.5^{b}	32.3^{c}	6.5^{a}	80.0^{abcd}	50.4
75	10	38.8^{a}	20.0^{b}	28.60	12.8^{b}	44.8^{b}	7.8^{a}	101.2^{ab}	90.2
	30	39.1^{a}	19.4^{b}	27.9^{b}	12.2^{b}	43.4^{b}	7.7^{a}	98.4^{abc}	85.0
	60	22.9^{b}	15.2^{b}	17.9^{b}	8.6^{a}	24.2^{d}	4.8^{a}	62.1^{bcd}	16.7
	300	2.7^{c}	2.2^{c}	3.1^{d}	1.2^{c}	3.6^{e}	0.7^{b}	9.6^{ef}	-82.0
95	10	38.9^{a}	19.8^{b}	27.7^{b}	12.4^{b}	43.6^{b}	7.7^{a}	98.8^{abc}	85.7
	30	38.4^{a}	19.2^{b}	27.2^{b}	12.6^{b}	43.9^{b}	7.6^{a}	97.9^{abc}	84.0
	60	21.4^{b}	11.4^{a}	16.8^{b}	7.7^{a}	22.7^{d}	4.5^{a}	55.4^{cd}	4.1
	300	0.0^{c}	0.0^{c}	0.0^{d}	0.0^{c}	0.0^{e}	0.0^{b}	0.0^{f}	-100
Fpr		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	

#: DW: dry weight

*: means followed by the same letter are not significantly different according to Tukey's HSD test ($p<0.05$).

durations (<30 s) irrespective of their sizes. However, the larger sized suckers tolerated higher temperatures and exposure durations better than the smaller ones. Immersion of the enset suckers in tap water at a temperature of 21°C did not kill the mealybugs on suckers of all sizes (Table 1). However, few mealybugs survived on suckers treated at 55°C for 10 and 30 s. On the other hand, the 75 and 95°C hot water treatments killed all the mealybugs within 10 s.

Insects feeding on plants vary in their ability to survive thermal treatments. Water temperatures of 49°C and above have been shown to kill external insect pests in less than 10 minutes (Sharp, 1994). Researchers in Hawaii found that a 10 min immersion of cut flowers in 49°C achieved 100% mortality of all stages of *Pseudulacaspis cockerli* (Cooley) and *Coccus viridis* (Green) (Hara et al., 1997). They also found that a 12 min immersion in 49°C water eliminated 95% of ants, aphids, and mealybugs on red ginger flowers, *Alpinia purpurata* (Vielli). In another study, a hot water immersion treatment of 20 min at 49°C was effective in killing mealybugs and all other arthropods found externally on limes, or under the calyx of the fruit, and there were no surviving insects or mites (Gould and McGurie, 2000). For the long tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozetti), an estimated 19 minutes was required to reach 99% mortality on persimmons (*Diospyros kaki* L.) dipped in 49°C hot water (Lester et al., 1995). Researchers in New Zealand found that two-spotted spider-mites were more resistant to heat and required 40 min at 48°C to reach 99% mortality (Lester et al., 1995). In this study, the enset root mealybug was not able to survive a 55°C water treatment for one minute, while at 75 and 95°C complete mortality of mealybugs was obtained within 10 s. This study showed that certain combinations of water temperature and exposure duration can completely kill the insect pest without causing any damage to the plant. Hot water temperatures at 55°C for one minute, and at 75 and 95°C for 10 or 30 s can be used on small, medium and large-sized enset suckers without causing any plant damage. Large-sized plants could also be treated for 60 s at 75°C.

Hot water immersion treatments at 55°C for 60 s, 75°C for 10 to 30 s, and 95°C for 10 to 30 s on small, medium and large-sized suckers were effective. In addition, the water treatments at 55°C for 300 s, and 60 and 300 s at 75°C were also effective. The remaining combinations of

Table 3. Effect of hot water treatment on above and below ground plant traits for medium-sized enset suckers infested with enset root mealybugs at 120 days post treatment. 10 plants were assessed per water temperature level and immersion time.

Water temperature (°C)	Exposure time (seconds)	Plant height (cm)	Leaf DW[#] (g)	Pseudo stem DW (g)	N° of cord roots	Corm DW (g)	Root DW (g)	Total plant DW (g)	Whole plant dry weight gain compared to the 21°C treatment (%)
21	10	26.9[a]*	14.1[a]	22.7[c]	8.3[a]	44.3[a]	7.5[a]	88.6[c]	
	30	27.0[a]	14.1[a]	22.7[c]	8.5[a]	44.1[a]	7.4[a]	88.3[c]	
	60	27.1[a]	14.1[a]	22.6[b]	8.6[a]	44.6[a]	7.4[a]	88.7[c]	
	300	27.2[a]	14.1[a]	22.2[c]	8.4[a]	44.8[a]	7.3[a]	88.4[c]	
55	10	31.6[c]	15.4[c]	33.2[b]	12.3[c]	57.5[b]	9.5[b]	115.6[abc]	30.6
	30	35.7[c]	19.5[c]	36.2[b]	13.5[c]	61.5[b]	10.3[b]	127.5[abc]	44.1
	60	43.4[b]	21.6[c]	47.2[c]	16.2[b]	69.8[b]	11.5[b]	150.1[ab]	69.6
	300	43.5[b]	19.9[c]	35.9[d]	16.0[b]	63.9[b]	10.4[b]	130.1[abc]	47.0
75	10	43.8[b]	24.2[b]	48.8[b]	16.7[b]	72.0[d]	11.6[b]	156.6[a]	76.9
	30	43.6[b]	24.4[b]	49.4[b]	16.4[b]	71.1[d]	11.5[b]	156.4[a]	76.7
	60	32.4[c]	20.8[c]	37.3[c]	13.7[c]	53.5[b]	9.6[b]	121.2[abc]	36.9
	300	14.2[d]	15.5[d]	25.7[d]	6.4[d]	28.6[c]	5.4[d]	75.2[cd]	-15.0
95	10	43.7[b]	23.8[b]	49.1[b]	16.4[b]	71.7[d]	11.5[b]	156.1[a]	76.4
	30	43.9[b]	24.9[b]	48.5[b]	16.7[b]	72.8[d]	11.6[b]	157.8[a]	78.3
	60	25.8[f]	17.6[d]	30.6[e]	11.5[c]	36.8[c]	7.0[c]	92.0[bc]	4.0
	300	4.7[e]	4.2[e]	8.0[f]	2.1[e]	13.0[c]	2.0[e]	27.2[d]	-69.3
Fpr		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	

#: DW: dry weight

*: means followed by the same letter are not significantly different according to Tukey's HSD test ($p<0.05$).

temperature and exposure periods were either not enough to kill all the mealybugs or killed some proportion of the enset plants. The lower growth of the control plants (21°C water immersion) might have been caused by the presence of mealybugs which were not affected by the water temperature.

Conclusion

This study showed that the immersion of enset corms of all sizes in water at 95°C for at least 10 and up to 30 s prior to planting could be used for the elimination of all enset root mealybugs. In addition, these boiling water treatments significantly enhanced whole plant weight gain of the small, medium and large-sized enset suckers by as much as 100, 78 and 58% as a result of eliminating enset root mealybugs and probably plant-parasitic nematodes and other pathogens. The water treatments at 55 and 75°C are difficult to apply by small-scale subsistence farmers who do not have thermometers and may not have knowledge of temperature measurements. The immersion of enset suckers in boiling water for at least 10 and up to 30 s can be easily demonstrated, with a much higher envisaged adoption rate by farmers. This method could be applied by farmers when adult mealybugs are observed on corms and roots during transplanting and during preparation of corms for macro-propagation in order to obtain mealybug free suckers or planting material. It can also be applied by private seed businesses/entrepreneurs and at development project macro-propagation sites during corm preparation and before sale/distribution of suckers to farmers. Additional detailed socio-economic studies including a cost-benefit analysis and adoption research would be crucial to support out-scaling efforts. In addition, it is envisaged that immersion of banana suckers in boiling water for at least 10 and up to 30 s, e.g. in Kerala, India where the root mealybug *Geococcus* spp. is present on banana, could be highly effective and would warrant further research/confirmation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Table 4. Effect of hot water treatment on above and below ground plant traits for large-sized enset suckers infested with enset root mealybugs at 120 days post treatment. 10 plants were assessed per water temperature level and immersion time.

Water temperature (°C)	Exposure time (second)	Plant height (cm)	Leaf DW# (g)	Pseudo stem DW (g)	N° of cord roots	Corm DW (g)	Root DW (g)	Total plant DW (g)	Whole dry plant weight gain compared to the 21°C treatment (%)
21	10	35.3^{a}*	19.7^{a}	44.8^{a}	11.4^{a}	77.9^{a}	11.8^{a}	154.2^{de}	
	30	35.4^{a}	19.7^{a}	44.8^{a}	11.8^{a}	77.9^{a}	11.9^{a}	154.4^{de}	
	60	35.4^{a}	19.8^{a}	44.8^{a}	11.2^{a}	77.9^{a}	11.9^{a}	154.3^{de}	
	300	35.5^{a}	19.8^{a}	44.9^{a}	11.3^{a}	77.6^{a}	11.8^{a}	154.1^{de}	
55	10	36.4^{a}	27.5^{ba}	50.1^{c}	15.5^{a}	82.2^{a}	13.2^{a}	173.0^{abcde}	12.2
	30	43.2^{c}	30.5^{b}	61.4^{c}	16.2^{a}	84.5^{a}	14.9^{a}	191.3^{abcde}	24.0
	60	52.4^{b}	33.1^{b}	74.5^{b}	18.3^{b}	104.7^{b}	16.9^{b}	229.2^{abcd}	48.6
	300	52.7^{b}	31.7^{b}	72.1^{b}	18.2^{b}	101.3^{b}	16.7^{b}	221.8^{abcd}	43.8
75	10	52.6^{b}	38.0^{b}	79.7^{b}	19.0^{b}	109.0^{b}	17.4^{b}	244.1^{a}	58.2
	30	53.4^{b}	37.7^{b}	77.6^{b}	18.4^{b}	109.1^{b}	17.3^{b}	241.7^{ab}	56.7
	60	45.8^{a}	32.4^{b}	68.4^{c}	16.8^{b}	94.5^{bc}	15.7^{bc}	211.0^{abcd}	36.8
	300	27.1^{c}	29.5^{bc}	43.2^{d}	11.5^{c}	71.5^{bc}	11.7^{bc}	155.9^{cde}	1.1
95	10	52.9^{b}	38.2^{b}	77.3^{b}	18.1^{b}	108.7^{b}	17.2^{b}	241.4^{abc}	56.5
	30	51.8^{b}	38.8^{b}	78.2^{b}	18.4^{b}	108.1^{b}	17.7^{b}	242.8^{a}	57.4
	60	35.3^{a}	24.5^{c}	43.1^{d}	12.4^{c}	76.4^{c}	12.4^{c}	156.4^{bcde}	1.4
	300	25.8^{c}	18.2^{c}	40.3^{d}	9.0^{c}	55.9^{c}	9.2^{c}	123.6^{e}	-19.9
Fpr		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	

#: DW: dry weight
*: means followed by the same letter are not significantly different according to Tukey's HSD test ($p<0.05$).

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Combining ability of selected maize (*Zea mays* L.) inbred lines for major diseases, grain yield and selected agronomic traits evaluated at Melko, South West Oromia region, Ethiopia

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The genetic potentials of resistance to turcicum leaf blight (TLB), grey leaf spot (GLS), and common rust (CR) disease, grain yield and selected agronomic trait were studied in 45 F1 hybrids from a half diallel following Griffing's Model 1, Method 4. The 45 hybrids excluding parents were evaluated in 5 × 9 alpha lattice designs with three replications. The study was carried out at Jimma Agricultural Research Center during 2015 cropping season with the objective to evaluate combining ability for turcicum leaf blight, grey leaf spot, common rust disease, grain yield and selected agronomic trait in maize inbred lines. For analysis of variance, days to 50% anthesis, days to 50% silking, turcicum leaf blight, grey leaf spot, common rust disease severity index, days to maturity and grain yield data were collected. Mean square due to general combining ability (GCA) was highly significant (P ≤ 0.01) for all traits, while specific combining ability (SCA) mean square was non-significant except for grain yield, days to 50% tasseling, days to 50% of silking, and days to maturity. This study showed the importance of additive types of gene action in controlling the inheritance of the traits. Among ten inbred lines, L7 and L10 were best combiners for GLS tolerance with the highest negative value of GCA effects and inbred line L7 was the best combiner for TLB and CR tolerance with the highest negative value of GCA effect. Inbred lines L2 and L9 were best combiner for grain yield with the highest positive GCA effect value. Therefore, maize breeding program can engage in hybridization and synthetic variety formation based on the information of inbred lines with high negative value GCA effect for diseases tolerant and high GCA for grain yield.

Key words: Combining ability, general combining ability (GCA), inbred lines, specific combining ability (SCA).

INTRODUCTION

Maize (*Zea mays*) is the third most important cereal crop in the world after rice and wheat in production and it is stable food crop in Ethiopia. It is believed to have originated in Mexico and to have been introduced to Ethiopia in the 1600s to 1700s (McCann, 2005). It is cultivated in a wider range of environments than wheat

and rice, because of its greater adaptability (Koutsika-Sotiriou, 1999).

In Ethiopia, maize grows under a wide range of environmental conditions between 500 and 2400 m above sea level. The mid-altitude sub-humid agro-ecology is the most important maize producing environment in Ethiopia (Kebede et al., 1993). Therefore, maize production in Ethiopia is constrained by a number of abiotic and biotic stress factors, including pests and diseases (northern leaf blight, gray leaf spot, maize streak virus, rust and downy mildew) (Dagne et al., 2004).

Turcicum leaf blight (TLB), caused by the fungi *Exserohilum turcicum* is one of the widely distributed and economically very important diseases of maize production in the country. The infection appears during both off- and main-seasons, but it is more serious during the main-season in constantly wet and humid areas (Mosisa et al., 2012). According to Tewabech et al. (2012), the northern corn leaf blight (*E. turcicum*) has been reported to cause the highest grain yield loss of 50 and 16.4% loss of 1000 kernel weight on the susceptible cultivar.

The other leaf diseases including grey leaf spot (*Cercospora zeae-maydis* Tehon & Daniels) and common leaf rust (*Puccinia sorghi* Schr.) are also the most important infectious diseases of maize in the country. The disease incidence ranges from 95 to 100% in areas with constant moisture and high humidity and the yield loss can reach up to 70% (Tewabech et al., 2012).

To use inbred lines in hybrid breeding program, evaluation of inbred lines based on per se performance such as yield, disease tolerance and other character, and test cross performance, is the most common. For that reason, combining ability tests must be employed to choose individual inbred lines with potential for hybrid performance (Stoskopf et al., 1999). The diallel cross design proposed by Griffing (1956) is the first approach to testing combining ability, to know the type of gene action from the parent lines.

Diallel cross analysis also yields information on GCA and SCA of inbred lines. Combining ability analysis is one of the powerful tools in identifying the best combiners that may be used in crosses either to exploit heterosis or to accumulate productive genes. It also helps to understand the genetic architecture of various characters that enable the breeder to design effective breeding programs for future improvement of the existing materials (Sprague and Tatum, 1942). The combining ability could be used to provide information in the selection of elite inbred lines in order to establish the type of gene action, which controls the grey leaf spot resistance (Legesse et al., 2009).

Selection of inbred lines for hybrid breeding program has crucial role to produce hybrids which might perform better than the latest released commercial variety. Therefore, this study was under take to estimate general and specific combining ability in crosses of selected inbred lines and to identify inbred lines with better combining ability for grain yield and disease resistant traits for further use in breeding program.

MATERIALS AND METHODS

Experimental site

The experiment was carried out in the main cropping season of 2015 at Jimma Agricultural Research Centre (JARC) located at Melko. It is located in south western part of Oromia Region, 358 km from Addis Ababa and 12 km from Jimma Town. The center is located at 7°40' N' latitude and 36° E longitude at an altitude of 1753 m.a.s.l. The climate of the area is characterized as sub-humid with mean monthly maximum and minimum temperature of 26.3C and 11.6°C, respectively (IAR, 1997).

Experimental materials and management

Ten selected inbred lines (Table 1) were crossed in a half diallel following Griffing's Model 1, Method 4 (Griffing, 1956) and the resulting 45 F_1 hybrids (excluding parents) were evaluated in a 5 × 9 alpha lattice design (Patterson and Williams, 1976) in three replications. Each treatment was planted in two rows of 5.1 m length with spacing of 0.75 m between rows and 0.30 m between plants within the rows. The inbred lines used for crossing were selected in terms of resistance to major diseases (Grey Leaf Spot, Turccum Leaf Blight and Commun Rust). List of inbred lines is shown in Table 1.

Data collected

Ten plants were selected randomly in each plot and were labeled. These plants were measured individually and the mean value was recorded for the plot. The severity of major diseases such as grey leaf spot (GLS), TLB and common leaf rust (CR) was recorded on the whole plot using a 1 to 5 scale where 1=no symptoms, 2=moderate lesion below leaves subtending the ear, 3=heavy lesion development on and below the leaf subtending the ear with a few lesions above it, 4=severe lesion development on all but uppermost leaves may have few lesions and 5=all leaves dead. After the diseases were recorded; the severity of the disease was estimated using severity index formula (Wheeler, 1969):

$$\text{DSI } \% = \frac{\textit{The sum of all disease rate plants multiplied under each scale}}{\textit{Total number of scored plants X Maximum disease scale}} X100$$

Grain yield was determined as weight of the total shelled grain after adjusting grain moisture to 12.5% and then converted to ton per hectare. Days to 50% anthesis was recorded as the number of days from planting to the day when 50% of the plant in a plot started

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Table 1. List of parent inbred lines used in this study.

Lines code (Number)	Pedigree
1	[LZ-956343/LZ956003]-B-1-1-2-B-B/124-b(113)-3-1-1
2	Gibe1-91-1-1-1-1
3	CML444
4	DE78-Z-126-3-2-2-1-1-1(g)
5	30H83-3-5-1-1-1-1-1
6	CLM197
7	ILOO'E1-9-1-1-1-1-1
8	SZNYA99F2-7-2-1-1
9	30H83-7-1-5-1-1-1-1
10	SC-715-56-2-1-2-1-1

pollen shading. Days to 50% silking was recorded as the number of days from planting to the day when 50% of the plants in the plot had their silks emerged 2 to 3 cm above the sheath.

Data analysis

The statistical analysis of the data was carried out using SAS computer software version 9.2 (SAS, 2008) software. The plot based mean values for grain yield and other agronomic traits were subjected to analysis of variance (ANOVA) as described in Gomez and Gomez (1984). Analysis of combining ability was carried out as described in Griffing (1956). Significances of general combining ability and specific combining ability effects of the hybrids were determined by t-test using standard errors of GCA and SCA effect. The significance of GCA and SCA effects were tested by dividing the corresponding GCA and SCA effect values by their respective standard error and comparing the obtained t value with tabular t-value at error degree of freedom (Hailegebrial et al., 2015).

RESULTS AND DISCUSSION

Analysis of variance (ANOVA)

ANOVA showed significant difference among genotypes for all character. It showed highly significant ($P \leq 0.01$) and significant ($P \leq 0.05$) mean squares due to GCA for all traits, while mean square due to SCA was non-significant except for days to tessiling, days to silking, maturity date and grain yield traits (Table 2). This suggests that significant difference exists among the materials with respect to combining ability and thus both additive and non-additive gene actions were important for the expression of the traits.

Mean squares due to GCA and SCA were highly significant ($P \leq 0.01$) for grain yield. This indicated that the importance of both additive and non-additive types of gene action in controlling this trait. But the ratio of GCA/SCA was greater than unity for the trait, indicating that this trait is pre-dominantly controlled by additive type of gene action. Similarly Wende (2013) and Tessema et al. (2014) reported more importance of additive gene action than non-additive gene action for grain yield in maize. Mean square due to GCA showed highly significant difference ($P \leq 0.01$) for grey leaf spot, turcicum leaf blight and common rust, whereas SCA revealed non-significant difference (Table 2). This showed that as the resistant genes controlled by additive types of gene action for Grey leaf spot, turcicum leaf blight, and common rust diseases in this study. Cumulative gene action plays an important role in developing grey leaf spot, turcicum leaf blight and common rust tolerant variety. Similarly, an experiment conducted by Legesse et al. (2009) showed non-significant SCA mean square for northern leaf blight (NLB) diseases in combining ability and heterotic grouping of highland transition maize inbred lines. But in other study, Dagne et al. (2008) and Nzuve et al. (2013) reported that the GLS resistant gene was controlled by both additive and non-additive types of gene action.

Mean squares due to GCA and SCA were highly significant ($P \leq 0.01$) for days to 50% anthesis, days to 50% silking and days to maturity (Table 2), indicating that days to 50% anthesis and days to 50% silking were governed by both additive and no-additive types of gene action. But additive type of gene action had preponderance to control days to 50% anthesis and days to 50% silking traits, since GCA/SCA ration was greater than unity. Study conducted by Abdel-Moneam et al. (2009), Shushay (2014) and Habtamu (2015) also indicated the importance of additive and non-additive gene action for this trait. In contrast to this, Melkamu et al. (2013a) and Alam et al. (2008) reported the predominance of non-additive types of gene action for this trait in maize combining ability studies that were done in inbred lines.

General combining ability effect

The estimates of GCA effects of the parents for different characters are shown in Table 3. A wide range of variability for GCA effects was observed among the parents for disease resistance and in other traits.

Table 2. Analysis of variance for TLB, GLS, CR, grain yield and selected agronomic traits of inbred lines by half diallel method, 2015.

SV	Df	Mean squares						
		GY (t/ha)	GLS (1-5)	TLB (1-5)	CR (1-5)	AD (day)	SD (day)	MD (day)
Cross	44	3.8**	95.5*	123**	76.4*	13.9**	12.6**	9.8**
GCA	9	8**	233.2**	196.6**	111.7*	71.3**	64.1**	29.2**
SCA	35	2.4*	71.7	117	71.4	3.5**	3.7**	7**
GCA/SCA		3.3	3.3	1.7	1.6	20.4	17.3	4.2
CV (%)		14.9	21.2	18.5	22.9	1.7	1.5	1.2
Error	90	1.3	60.7	60.3	52.4	1.7	1.5	3.7

*,**Significant and highly significant at 0.05 and 0.01, respectively. AD: Days to anthesis (day), CR: common rust (1-5 scale), Df: degree of freedom, GCA: general combining ability, GLS: grey leaf spot (1-5 scale), GY: grain yield per hector (ton/h), MD: maturity date (day), TLB: turcicum leaf blight (1-5scale), SCA: specific combining ability, SD: days to silking (day), SV: source of variation.

Table 3. Analysis of general combining ability effects for the ten inbred lines for GLS, TLB, CR, grain yield and selected agronomic traits, 2015.

Trait Lines	GY (t/ha)	GLS (1-5)	TLB (1-5)	CR (1-5)	AD (day)	SD (day)	MD (day)
L1	-0.4	-2.3	4.1*	2.3	-1.4**	-0.9**	0.3
L 2	0.8**	2	-3	-1	1.1**	1.1**	0.3
L 3	0.1	4.6**	4.4*	3.6*	-2.1**	-2**	-1**
L 4	-0.2	-0.2	-0.4	1.3	-3.1**	-3.2**	-1.4**
L 5	-1**	-1.3	-1.5	-0.1	0.7*	0.4	2.1**
L 6	-0.6*	3.8*	2.3	1.6	1.0**	1**	0.8*
L 7	0.5*	-3.5*	-4.3*	-3.3*	-0.2	-0.3	-0.9*
L 8	-0.4	-0.3	0.5	-1.3	1.3**	1.3**	-0.1
L 9	0.7**	-2.5	-1.6	-0.8	0.2	0.6*	-1**
L 10	0.5*	-4.8**	-0.5	-2.3	2.5**	2**	0.9*
SE	0.2	1.5	1.7	1.4	0.3	0.2	0.4

*,**Significant and highly significant at 0.05 and 0.01, respectively. AD: Days to anthesis (day), CR: common rust, GLS: grey leaf spot, GY: grain yield per hectar (ton/ha), MD: maturity date (day), SD: days to silking (day), TLB: turcicum leaf blight.

General combining ability effect varied among lines in all the disease severity percentage; this means that there is a genetic variation between the lines. Usually, lines with negative and significant GCA effect values are good general combiner for disease tolerant variety development; whereas lines with positive and significant GCA effects values are poor general combiner. Inbred lines L7 and L10 showed negative and significant GCA effects for grey leaf spot, while only line L7showed negative and significant GCA effects for turcicum leaf blight and common rust disease as well. This indicates that these lines 7 and 10 have potential for tolerance to grey leaf spot disease and diseases useful in breeding program to develop resistant variety. Lines L3 and L6, for example, exhibited positive and significant GCA effects for grey leaf spot, turcicum leaf blight and common rust traits and line 1 for grey leaf spot; therefore, they might contribute diseases susceptible alleles in the synthesis of new varieties. The results of this study are in agreement with the findings from Dagne et al. (2008), Legesse et al. (2009) and Girma et al. (2015).

Four inbred lines (L2, L7, L9 and L10) were found to be the best general combiners for grain yield as these lines had showed significant and positive GCA effects (Table 3). Inbred lines with positive and significant GCA effects are desirable parents for hybrid development as well as for developing synthetic varieties as they may contribute favorable alleles in the synthesis of new varieties. Inbred lines L5 and L6 had negative and significant GCA effects indicating that these inbred lines were poor combiners for grain yield. Lines with positive and significant GCA effects have potential to form high yielding cross combinations with different number of lines. The results of this study are also in agreement with the findings of Dagne et al. (2007) and Tessema et al. (2014). Similarly, Legesse et al. (2009) and Amiruzzaman et al. (2010) reported negative and positive significant GCA effects for grain yield, respectively.

Among the ten inbred lines, eight exhibited significant GCA effects for days to anthesis, but only three lines (L1, L3 and L4) revealed negative and significant GCA effects which is important in developing early flowering hybrids in

areas having short growing period. The five lines L2, L5, L6, L8 and L10 showed positive and significant GCA effects, which are undesirable for this trait (Table 3). Only three lines (L1, L3 and L4) revealed negative and significant GCA effects, whereas five lines (L2, L6, L8, L9 and L10) showed positive and significant GCA effects (Table 3) for days to 50% silkings trait. Inbred lines with negative and significant GCA effects are best general combiner for days to 50% silkings trait in breeding program as they are used to develop early maturing variety in low moisture areas and the reverse is true for areas receiving rainfall for longer periods.

Inbred lines with positive and significant GCA effects for days to silking had the tendency to increase late maturity, indicating that they could be used in breeding program to develop late mature varieties in long rain season receiving area. Similarly, negative and positive GCA effects were also reported by Dagne et al. (2008) and Girma et al. (2015). Lines L3, L4, L7 and L9 revealed negative and significant GCA effects; this indicated that they are the best general combiners for this trait in breeding program to develop early mature varieties. Nowadays, parent with negative and significant GCA effect for days to anthesis and days to silking are desirable in maize breeding, for areas with early termination of rain in Ethiopia to develop early mature varieties. Inbred lines L5, L6 and L10 displayed positive and significant GCA effects for days to maturity which are not desirable for drought stress areas. Similar results were reported by Habtamu (2015) for days to maturity.

Specific combining ability (SCA) effect

ANOVA for specific combining ability effects was carried out for traits which revealed significant mean squares due to specific combining ability. The estimated SCA effects of the crosses for grain yield and selected agronomic characters are shown in Table 4. Among the forty five crosses, only five hybrids (L3 × L7, L5 × L6, L6 × L7, L8 × L9, and L9 × L10) revealed significant SCA effects for grain yield (Table 4). Among forty five crosses, only the hybrid L6 × L7 exhibited positive and significant SCA effect for grain yield; this indicated that this cross has good specific combinations for grain yield. On the other hand, most of the hybrids showed positive SCA effects for grain yield. Such cross combination could be effectively exploited in maize hybrid breeding program, since there are dominance or epistasis gene effects. On other hand, L3 × L7, L5 × L6, L8 × L9, and L9 × L10 exhibited negative and significant SCA effects for grain yield which is an undesirable feature for this trait, as these crosses revealed reduced grain yield performance. The result of this study is in conformity with the findings of Dagne et al. (2008) and Hailegebrial et al. (2015), who also indicated positive and negative SCA effects for grain yield.

Among the forty five crosses, eight hybrids showed significant SCA effects for days to 50% anthesis (Table 4). Hybrids L1 × L3, L1 × L9, L2 × L10, L4 × L10, and L7 × L8 showed negative and significant SCA effects for the character desirable for drought stress area. Hybrid L7 × L8 (-2.6 days) showed maximum negative and significant SCA effect indicating that this combination increases early maturity of maize in breeding program. Hybrids L2 × L7, L3 × L8 and L7 × L10 exhibited positive and significant SCA effects which could be used in late mature maize variety development breeding program especially in long rain season area, because the highest positive SCA effect hybrid L7 × L10 for days to 50% anthesis exhibited also the highest grain yield (9.2 t/ha).

The maximum SCA effect is 2.2 days for days to 50% silking (L3 × L8), whereas the earliest SCA effect is -2.6 days for days to 50% silking of L7 × L8. Hybrids L1 × L3, L1 × L9, L2 × L10, L3 × L4 and L7 × L8 revealed negative and significant SCA effects for days to 50% silking, whereas L1 × L2, L1 × L8, L3 × L8 and L3 × L10 exhibited positive and significant SCA effects (Table 4). Negative and significant SCA effect for days to 50% silking is desirable in breeding programs to develop early mature maize variety, while positive and significant SCA effect for days to 50% silking is desirable to develop late mature variety. The results of this study are inconsistent with finding of Legesse et al. (2009). In contrast to this, Melkamu et al. (2013b) reported that none of the hybrids showed significant SCA effects for days to silking, since all the tested hybrids had similar days to 50% silking; this is because there is no more SCA effect variation within crosses for days to 50% silking.

Among forty five crosses, only eight hybrids revealed significant SCA effects for days to maturity (Table 4). Hybrids L1 × L2, L1 × L10, L5 × L6 and L5 × L10 exhibited negative and significant SCA effects, whereas hybrids L1 × L6, L2 × L10, L3 × L4 and L6 × L10 showed positive and significant SCA effects. The SCA effects revealed by the earliest hybrid cross (L1 × L2) for days to maturity showed only a seven-day difference as compared to the latest hybrid cross (L1 × L6). Even if crosses with negative and significant SCA effects was desirable at area receive short rain season, but most of the crosses, positive and significant SCA effects revealed the highest grain yield than crosses with negative and significant SCA effect hybrid in this study. This indicates that for long rain season, areas late mature maize variety is preferable. Hybrid with medium and long maturity time appeared resistant to the three common maize disease studied. Similarly, Dagne et al. (2008) reported positive and negative SCA effect for days to maturity in combining ability of maize inbred lines for grain yield and reaction to grey leaf spot disease. On the other hand, Girma et al. (2015) reported that crosses with negative and significant SCA effects for days to maturity can be exploited in hybrid breeding program in maize research for reduced maturity dates, while crosses with positive and significant

Table 4. Analysis of specific combining ability effects for grain yield and other traits, 2015.

Crosses	GY (t/ha)	AD (day)	SD (day)	MD (day)
L1×L 2	0.1	1	1.4*	-2.4*
L 1×L 3	-0.1	-1.7*	-2.2**	-1
L 1×L 4	-0.4	1.2	1.2	-0.8
L 1×L 5	0.4	-0.6	-1.1	0.8
L 1×L 6	1	0.4	0.4	4.2**
L 1×L 7	-0.8	-0.06	-0.3	1
L 1×L 8	0.5	1	1.4*	-1.3
L 1×L 9	-0.03	-1.5*	-1.8*	1.3
L 1×L 10	-0.4	-0.1	1.1	-1.9*
L 2×L 3	0.07	-0.3	-0.6	0.7
L 2×L 4	-0.08	-0.02	-0.1	-0.9
L 2×L 5	-0.3	-0.02	0.3	0.2
L 2×L 6	-0.5	0.9	0.1	-0.4
L 2×L 7	-0.4	1.5*	1.1	-1.5
L 2×L 8	0.7	-0.4	-0.6	1.7
L 2×L 9	0.3	-0.2	-0.2	0.3
L 2×L 10	0.2	-2.4**	-1.3*	2.4**
L 3×L 4	0.7	-0.8	-1.6*	2.1*
L 3×L 5	0.5	-0.2	-0.3	0.5
L 3×L 6	-0.1	-0.5	-0.1	-1.8
L 3×L 7	-2.1*	0.7	0.9	-0.1
L 3×L 8	0.5	1.8*	2.2**	-0.6
L 3×L 9	0.5	0.3	0.3	0.9
L 3×L 10	0.1	0.6	1.5*	-0.6
L 4×L 5	-0.5	0.5	0.6	0.2
L 4×L 6	-0.02	-0.7	-1	-0.8
L 4×L 7	0.6	-0.6	0.7	-0.1
L 4×L 8	-0.5	0.8	0.6	-0.2
L 4×L 9	0.3	1.2	0.4	-0.3
L 4×L 10	0.07	-1.4*	-1	0.5
L 5×L 6	-2.8**	0.7	0.5	-1.9*
L 5×L 7	0.4	-0.3	0.5	0.7
L 5×L 8	0.7	0.2	-0.01	0.9
L 5×L 9	0.9	0.3	0.8	0.8
L 5×L10	1.1	-0.6	-1	-2.1*
L 6×L 7	1.6*	-1	-0.2	-0.9
L 6×L 8	0.9	-1	-0.6	1.3
L 6×L 9	0.1	-0.1	0.2	-1.8
L 6×L 10	-0.02	1	0.8	2*
L 7×L 8	-0.8	-2.6**	-2.6**	-1.1
L 7×L 9	0.9	0.1	0.3	1.2
L 7×L10	0.7	2.2**	-0.2	1
L 8×L 9	-2.1*	-0.4	-0.1	-0.7
L 8×L 10	-0.2	0.7	-0.2	0.1
L 9×L 10	-1.3*	0.4	-0.2	-1.6
SE	0.6	0.7	0.6	0.9

*,**Significant and highly significant at 0.05 and 0.01, respectively. AD: Days to anthesis (day), GY: grain yield (ton/ha), MD: maturity date, SD: days to silking (day), SE: standard error.

SCA effect for days to maturity are undesirable as these crosses showed a tendency to increase maturity date.

Conclusion

In this study, inheritance of grey leaf spot, turcicum leaf blight and common rust maize disease were controlled by additive type of gene action. This suggests the possibility of breeding through recurrent selection and backcrosses to incorporate candidate genes into susceptible parents. Of the ten inbred lines, only L7 and L10 showed negative and significant GCA effects for GLS disease, whereas only L7 manifested negative and significant GCA effect for TLB and CR disease, which is a desirable character to develop disease tolerant variety. This means that these parents had potential to cross with many other inbred lines to develop disease tolerant hybrids in breeding program.

Lines L2, L7, L9 and L10 had positive and significant GCA effects for grain yield, which mean that these parents could be used to improved grain yield in breeding programs. Among the F1 crosses, only L6 × L7showed positive and significant SCA effects for grain yield indicating its potential for use in future breeding program to develop hybrid variety. Most of the crosses also revealed positive SCA effects for grain yield; therefore, they might be used in hybrid variety development program. Generally, the results of this study could be exploited to develop disease tolerant and high yield varieties of maize particularly adapted to middle-altitude agro-ecology areas. The cross combinations with good performance in terms of disease resistant and grain yield were recommended for further evaluation in multi-location experiments to confirm their tolerance to diseases, yield superiority and stability.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Diallel analysis in *Citrullus mucosospermus* (Fursa) for fruit traits

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For the estimation of genetic parameters (such as heterosis effect, potence ratio, combining ability and reciprocal effects) of five fruit traits, in order to suggest breeding strategies in *Citrullus mucosospermus* (Fursa), a field trial was carried out at Kononfla city in Western Côte d'Ivoire using 4x4 full diallel. All 12 F_1 hybrids and their parents were planted in a randomized complete block design with three replications. A positive heterosis effect relative to mid-parental values was observed to hybrids having one parent *Bebu* for characters' fruit weight, fruit diameter and fruit volume. Variances due to general (GCA) and specific (SCA) combining abilities were significant (P<0.001) for all studied traits, indicating the involvement of both additive and non-additive gene actions in the inheritance of these traits. Non-additive gene effects were predominant for all studied traits. *Bebu* was the best general combiner for all traits except fruit number which the best combiners were three morphotypes of *Wss*. For NF, Wss1×Wss2 and Wss2×Wss3 recorded the highest positive SCA values. Crosses involving *Bebu* as one parent, presented the best SCA values for other traits.

Key words: *Bebu*, *Wlêwlê* small seeds, heterosis, potence ratio, combining ability.

INTRODUCTION

In Côte d'Ivoire, the term "pistachio'' is used to designate the oleaginous cucurbit species including *Citrullus mucosospermus* (Fursa) (Zoro Bi et al., 2003). This species belongs to *Citrullus* genus of *cucurbitaceae* family and is native to West Africa where it was domesticated (Chomicki and Renner, 2015). *C. mucosospermus* or egusi watermelon is specially cultivated for its oleaginous seeds which have nutritive, therapeutic, social and economic values (Adetutu et al., 2015; Kumawat et al., 2017). For example, dried slightly toasted and ground seeds are used as soup thickener. The "egusi" seeds are reported to be rich in nutrient such as carbohydrates (10.45 to 26.30%), proteins (21.78 to 30.42%) and lipids (41.78 to 56.08%) (Marie et al., 2015). Further, the seeds are good sources of amino acids, vitamins (B1 and B2) (Abrefa et al., 2002) and minerals

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like sulfur, calcium, manganese, potassium and zinc (Acar et al., 2012; Manika et al., 2015; Marie et al., 2015). Edible oil extracted from *C. mucosospermus* seeds can also be used in cosmetology and pharmaceutic industries (Manika et al., 2015; Erhirhie and Ekene, 2013).

Despite their economic, cultural, therapeutic and nutritive importance, along with their good adaptation to extremely divergent agro-ecosystems, *C. mucosospermus* cultivation is neglected. This crop is underutilized (also called orphan crops) and lacks attention from research and development. Consequently, there is lack of data about genetics and breeding of this important crop in many African countries particularly, in Côte d'Ivoire (Adjoumani et al., 2016a, b). In addition, lack of high yielding traditional cultivars (Koffi et al., 2009) along with a lack of local high yielding hybrids constitute one of the major problems of *C. mucosospermus* cultivation in Côte d'Ivoire. The productivity of this crop should be increased by improving the genetic architecture through hybridization between different elite cultivars or through selection of high yielding cultivars. Estimation of heterosis, potence ratio, gene actions, combining ability and reciprocal effects of yield and its components should be greatly emphasized for the improvement of this crop. One of the several biometrical techniques available to plant breeders to achieve above-mentioned information in the crop species is diallel analysis (Seyyed-Nazari et al., 2016). Diallel crosses reportedly provided early information on the genetic behavior of these attributes in the first generation (Farshadfar et al., 2011, 2014). That is why they have been widely used in plant breeding programs to obtain information on the behavior of a group of parents *per se* and their hybrid combinations (Queiroz et al., 2017). They represent the best strategy for determining the general (GCA) and specific (SCA) combining abilities which help to identify the best parents and hybrid combinations and, provide sufficient genetic data about character inheritances (Farshadfar et al., 2011, 2014; Iqbal et al., 2015; Farshadfar and Amiri, 2015; Chukwu et al., 2016; Golabadi et al., 2017). In fact, the significance of the GCA reflects additive gene actions while the significance of SCA reflects the non-additive genetic effects which indicates relevant non-allelic interactions (Hossein et al., 2014; Fasahat et al.,2016; Seyyed-Nazari et al., 2016; Golabadi et al., 2017; Queiroz et al.,2017; Santos et al., 2017). If both GCA and SCA values are not significant, epistatic gene effects may play a remarkable role in determining these characters (Farshadfar and Amiri, 2015; Fasahat et al., 2016). However, no data concerning combining ability of *C. mucosospermus* local cultivars are available in the literature. The study of reciprocal effet as well as extra chromosomal inheritance or maternal effects is important in breeding programs which allows the determination of parents to be used as donors or recipients of pollen (Bahari et al., 2012).

The objectives of this work were to study through a complete diallel cross analysis, (i) the level of heterosis over the mid- and better parent, (ii) the type of gene actions, (iii) GCA and SCA effects of parents and crosses respectively and (iv) reciprocal effects in order to suggest breeding strategies for *C. mucosospermus.*

MATERIALS AND METHODS

Experimental site

The study was carried out in West-Center of Côte d'Ivoire precisely at Kononfla city located in Sinfra department which is in the Marahoue District. Kononfla is located at latitude 6° 37′ 18″ N, longitudes 5° 54′ 37″ W and altitude 243 m above sea level. Average temperature is between 25 and 30 °C and rainfall varies from 1500 to 2000 mm per annual. Soil type in the area is of ferralitic form.

Plant material

Plant material comprised four accessions of *C. mucosospermus* which were collected from four regions (North, Center, West-Center and East) of Côte d'Ivoire (Table 1). The choice of the four genotypes is based on a wide range of morphological diversity in the fruit and seed characteristics that prominently distinguish one line from another.

Experimental design, inbred line and F_1 hybrid creations

The purity of four parent accessions (*Bebu, Wss1, Wss2 and Wss3*) was achieved previously after four generations of self-pollination before crosses in order to obtain different F_1 families. Then, seeds of four parental lines were sowed during the period from January to April 2016 for crossing. At anthesis, hybridization was made among the four inbred lines according to a full diallel mating design to produce 12 F_1 hybrids along with their four inbred lines. Field evaluation of the parents and their 12 F_1's hybrids was conducted from May to August 2016 in a randomized complete block design with three replications. The experimental field area was 2067 m² (53 m × 39 m) and divided into three blocks. Each block comprised 16 plots with a space of 1 m between plots and the area of one plot was 32 m² (8 m × 4 m). Only one F_1 family or parental inbred line was sowed on 3 rows distant each other from 2 m within a plot. A total of 3 seeds of each family were planted per hole with an intra-row spacing of 2 m. Two weeks after sowing, seedlings were separated and the most vigorous were chosen per hole. The experimental field was regularly weeded during the vegetative growth stage.

Fruit traits measurements

Data collection was made on 10 plants sampled at random in each plot giving a total of 30 plants per genotype. Three fruits per plant were randomly selected in parental and F_1 generations to evaluate five agronomic traits that are:

***Fruit maturity period*:** determined by counting days between sowing and the fruit harvest characterized by total plant drying,
***Fruit number per plant*:** determined by counting total fruits per plant,

Table 1. Description of parental C. mucosospermus accessions used for full diallel cross.

Accession names	Origin of collect	Code of accessions	Characteristics
Bebu	Korhogo (North)	B	Big fruit with big seeds
Wlêwlê small seeds 1	Béoumi (Center)	Wss1	Small white fruit with small seeds
Wlêwlê small seeds 2	Gohitafla (West-Center)	Wss2	Small green fruit with small seeds
Wlêwlê small seeds 3	Tanda (East)	Wss3	Medium green fruit with small seeds

***Fruit weight per plant*:** Each fruit was weighed with an electronic balance (Kein HD5K5; sensibility 5 g). Fruit weight per plant corresponds to average of 3 fruits weight per plant,
***Fruit diameter per plant*:** diametric lateral opposite board after fruit median section was measured with digital caliper (Stainless Hardened). Fruit diameter per plant corresponds to average of 3 fruits diameter per plant,
***Fruit volume per plant*:** Fruit of *C. mucosospermus* cultivars are supposed spherical and their volume are evaluated like spherical volume as follow : $VFr = 4/3\pi r^3$, where r = DFr/2).

Statistical analyses and genetic parameter estimates

The data was subjected to statistical analyses using R version 3.3.1 (R Development Core Team 2011) Software. Analysis of variance (ANOVA) and the post ANOVA test. (Turkey test) was performed according to Turkey' HSD at 0.05 level of probability to test differences among treatment means. Heterosis percentage according to the mid-parent (MPH) and better parents (BPH), for the different studied characters were calculated using the formula proposed by Mather and Jinks (1971) as follows:

$$\mathbf{MPH} = \frac{(\mathbf{F1 - MP})\times\mathbf{100}}{\mathbf{MP}}$$

$$\mathbf{BPH} = \frac{(\mathbf{F1 - BP})\times\mathbf{100}}{\mathbf{BP}}$$

where F_1 = mean value of the particular hybrid, MP = (P1 + P2)/2 = mean value of the two parents for that hybrid, and BP = better parent mean value for that hybrid.

The t-test was manifested to determine whether F_1 hybrid means were statistically different from mid parent and better parent means. Potence ratio was calculated according to Smith (1952) to determine the degree of dominance as follows:

$$\mathbf{P} = \frac{(\mathbf{F1 - MP})}{\mathbf{0.5}\times(\mathbf{P2 - P1})}$$

where P = relative potence of gene set, F_1 = first generation mean, P1 = the mean of lower parent, P2 = the mean of higher parent, and MP = mid-parents value = (P1 + P2)/2. Complete dominance was indicated when P = ±1, while partial dominance is indicated when "P" value varies between −1 and +1 except the value zero, which indicates absence of dominance. Over-dominance was considered when potence ratio exceeds 1. The positive and negative signs indicate the direction of dominance of either parent.

The analysis of variance of GCA, SCA and reciprocal effects and their respective effect estimates were carried out according to Griffing's (1956) model 1 method 1. The statistical model for the mean value of a cross (i × j) in Griffing's analysis is:

$$Y_{ij} = m + g_i + g_j + s_{ij} + r_{ij} + 1/b\sum e_{ijkl}$$

where Y_{ij} = mean of $(i\times j)^{th}$ genotype over replications k (k = 1, 2,. . ., b), m = general population mean, g_i and g_j = general combining ability effects of i^{th} and j^{th} parent, respectively, s_{ij} = specific combining ability effect for the cross involving i^{th} and j^{th} parent, r_{ij} = reciprocal effect involving the reciprocal crosses between the i^{th} and j^{th} parents and $1/b\sum e_{ijkl}$ = mean error effect.

The significance of the estimates of variance due to GCA, SCA and reciprocal effects was tested using F-values at threshold probabilities of 1 and 5%, while significance of estimates of GCA, SCA and reciprocal effects was tested using their respective standard errors. The estimates of genetic components were obtained based on the expectations of the mean squares, Zeinanloo et al. (2009):

Component due to GCA; σ^2 GCA = (MS_{GCA} - MSerror) /6n
Component due to SCA; σ^2 SCA = MS_{SCA} - MSerror

where MS_{GCA} = variance due to GCA, MS_{SCA} = variance due to SCA, MSerror = mean error, and n = number of replications.

RESULTS

Agronomic performances of F_1 hybrids and their inbred lines

Table 2 shows the mean values of four *C. mucosospermus* inbred lines and their F_1 hybrids for fruit characters. Mean values of parental lines showed that the cultivar *Bebu* (B) and accessions of cultivar *Wlêwlê small seeds* (*Wss*) have very contrasted characters (Figure 1). *Bebu*'s fruit reached a short maturity period thus it was precocious and possessed big fruits with a low fruit numbers per plant. In opposition, accessions of *Wss* had small fruits with higher fruit numbers and it was late because it possessed a long fruit maturity period. However, among *Wss* accessions, *Wss3* presented big fruit with a long period of their maturity while *Wss1* and *Wss2* differed only by fruit color.

Results about F_1 hybrids illustrate that most of them produced average values that tend to be either than their respective mid-parental values or exceed the better-parental values. Thus, for characters' fruit weight (FW), fruit diameter (FD) and fruit volume (FV), crosses involving cultivar *Bebu* as one of parents, obtained mean values which were equal or superior to the better-parental values while for the same traits, other crosses recorded average values more or less equal to their respective mid-parental values. The same crosses (without *Bebu* as one parent) obtained mean equal to their parental mean values for fruit number (NF) and fruit maturity period

Table 2. Means ± standard deviation of four inbred lines and their F_1 hybrids for the studied fruit traits.

Genotypes	FMp (das)	NF	FW (g)	FD (mm)	FV (mm^3)
B	83.77 ±1.7 [d]	2.93 ± 0.87 [e]	910.44 ± 242.52 [d]	122.63 ± 10.55 [bc]	986683.4 ± 246596.14 [bcd]
Wss1	98.33 ± 4.01 [b]	6.23 ± 1.81 [abc]	574.89 ± 101.33 h	104.34±5.89 [f]	600499.5 ± 102484.35 [ef]
Wss2	98.50 ± 4.18 [b]	6.27 ± 1.95 [abc]	615.89 ± 138.77 [g]h	106.25±8.23 [ef]	639101.7 ± 145291.80 [f]
Wss3	102.50 ± 5.04 [ab]	6.50 ± 1.89 [ab]	728.55 ± 150.83 [e]	109.67±7.54 [de]	700236.2 ± 142036.46 [e]
B × Wss1	91.27 ± 3.67 [c]	4.13 ± 1.04 [de]	929.56 ± 218.97 [d]	120.86±9.26 [bc]	940260.3 ± 207389.74 [d]
Wss1 × B	91.47 ± 4.07 [c]	4.27 ± 1.39 [de]	991.56 ± 182.60 [c]	122.30±6.67 [c]	966365.7 ± 159288.65 [cd]
B × Wss2	90.63± 4.29 [c]	4.10 ± 1.09 [de]	1092.11 ± 212.21 [a]	127.35±7.96 [a]	1094127± 206775.62 [a]
Wss2 × B	90.83 ± 4.60 [c]	4.63 ± 1.67 [d]	1050.78 ± 174.81 [ab]	126.09±5.86 [ab]	1056282.3 ± 147136.80 [ab]
B × Wss3	93.53 ± 5.53 [c]	4.60 ± 1.40 [d]	1006.44 ± 150.92 [bc]	124.99±6.73 [ab]	1031283.1 ± 166786.40 [abc]
Wss3 × B	93.87 ± 5.92 [c]	4.83 ±1.62 [cd]	1010.44 ± 155.77 [bc]	125.87±8.08 [ab]	1056181.8 ± 177508.46 [ab]
Wss1 × Wss2	99.67 ± 3.92 [ab]	6.60 ± 1.99 [ab]	654.78 ± 131.07 [fg]	107.88±6.96 [def]	665518.7 ± 125721.43 [ef]
Wss2 × Wss1	99.83 ± 4.45 [ab]	7.30 ± 2.26 [a]	648.33 ± 171.41[fg]	106.33±9.43 [def]	644139.2 ± 168207.46 [ef]
Wss1 × Wss3	102.00 ± 5.96 [ab]	5.07 ± 2.43 [bcd]	679.44 ± 145.33 [f]	110.30±7.90 [d]	713433.5 ± 155849.68 [e]
Wss3 × Wss1	101.33 ± 6.29 [ab]	6.83 ± 2.05 [a]	690.26 ± 161.50 [ef]	110.10±7.44 [de]	708472.0 ± 148178.12 [e]
Wss2 × Wss3	102.83 ± 6.39 [a]	6.87 ± 1.89 [a]	679.78 ± 148.47 [f]	110.27±7.37 [d]	711439.2 ± 148236.90 [e]
Wss3 × Wss2	103.00± 5.19 [a]	7.27 ± 2.35 [a]	643.83 ± 113.28 [fg]	106.63±6.76 [def]	642380.3 ± 124939.76 [ef]
F	42.5	17.128	108.04	111.7	113.64
P	< 2.2 10^{-16}	< 2.2 10^{-16}	<2.2 10^{-16}	< 2.2 10^{-16}	< 2.2 10^{-16}

Means within a column followed by the same letter are not significantly different (P = 0.05).B = Cultivar *Bebu*; Wss1 = *Wlêwlê small seeds* 1; Wss2 = *Wlêwlê small seeds* 2; Wss3 = *Wlêwlê small seeds* 3; FMp = Fruit maturity period; NF = Number of fruits per plant; FW = Fruit weight; FD = Fruit diameter; FV = Fruit volume; das= days after sowing.

Figure 1. Fruits of four parental lines showing their differences. ***Wss1*****:** *Wlêwlê small seeds* 1 ; ***Wss2*****:** *Wlêwlê small seeds* 2 ; ***Wss3*****:** *Wlêwlê small seeds* 3; **B:** cultivar *Bebu.*

(FMp), while for the same traits, crosses which used *Bebu* as one parent, recorded average values noticeably equal to their respective mid-parental values.

Heterosis and potence ratio estimations in F1 hybrids for five fruit traits

Estimates of heterosis (%) of F1 hybrids over mid and better parents along with potence ratio values for some fruit traits are presented in Table 3.

Fruit maturity period

Percentages of heterosis of F_1 hybrids according to mid-parent were not significant and were positive for majority of crosses. Hybrids of all crosses involving *Bebu* as one of the parents, recorded the highest significant values of negative heterosis relative to the best parental values, indicating the precocity of these hybrids in comparison with better parent. Potence ratio values were comprised between -1 and 1 without 0 for all crosses except Wss1 × Wss2 and Wss2 × Wss3 which exhibited higher values

Table 3. Heterosis percentages (according mid-parent (MPH) and better parent (BPH)) and potence ratios (P) values for the studied fruit traits.

Genotypes	FMp (das)			NF			FW (g)			FD (mm)			FV (mm^3)		
	MPH	BPH	P	MPH	BPH	P	MPH	BPH	P	MPH	BPH	P	MPH	BPH	P
B × Wss1	0.24 ns	-7.19*	0.03	-9.82ns	-33.69ns	-0.27	25.16*	2.10ns	1.11	6.49ns	-1.45ns	0.81	18.48ns	-4.70ns	0.76
Wss1 × B	0.46ns	-6.98*	0.06	-6.91ns	-31.55ns	-0.19	33.51**	8.91ns	1.48	7.77*	-0.27ns	0.96	21.77*	-2.06ns	0.89
B × Wss2	-0.55ns	-7.99*	-0.07	-10.87ns	-34.57ns	-0.30	43.10**	19.95ns	2.23	11.29**	3.85ns	1.58	34.60**	10.89ns	1.62
Wss2 × B	-0.33 ns	-7.78*	-0.04	0.72ns	-55.68ns	0.02	37.69**	15.41ns	1.95	10.18**	2.82ns	1.42	29.94**	7.05ns	1.40
B × Wss3	0.43ns	-8.75**	0.04	-2.47ns	-29.23ns	-0.07	22.81*	10.54ns	2.06	7.61*	1.93ns	1.36	22.27*	4.52ns	1.31
Wss3 × B	0.79ns	-8.42**	0.08	2.47ns	-25.64ns	0.07	23.30*	10.98ns	2.10	8.37*	2.64ns	1.50	25.22*	7.04ns	1.49
Wss1 × Wss2	1.27ns	1.18ns	15.00	5.60ns	5.32ns	21.00	9.97ns	6.31ns	2.90	2.46ns	1.54ns	2.72	7.38ns	4.13ns	2.37
Wss2 × Wss1	1.44ns	1.35ns	17.00	16.80ns	16.49ns	63.00	8.89ns	5.27ns	2.58	0.98ns	0.08ns	1.09	3.93ns	0.79ns	1.26
Wss1 × Wss3	1.58ns	-0.49ns	0.76	-20.42ns	-22.05ns	-9.75	4.25 ns	-6.74ns	0.36	3.08ns	0.58ns	1.24	9.70ns	1.88ns	1.26
Wss3 × Wss1	0.91ns	-1.14ns	0.44	7.33ns	5.13ns	3.50	5.91 ns	-5.26ns	0.50	2.90ns	0.40ns	1.16	8.93ns	1.18ns	1.17
Wss2 × Wss3	2.32ns	0.33 ns	1.17	7.57ns	5.64ns	4.14	1.12ns	-6.70ns	0.13	2.14ns	0.55ns	1.35	6.24ns	1.60ns	1.37
Wss3 × Wss2	2.49ns	0.49 ns	1.25	13.84ns	11.79ns	7.57	-4.22ns	-11.63ns	-0.50	-1.23ns	-2.77ns	-0.78	-4.07ns	-8.26 ns	-0.89

ns: no significant *and **: significant at 5% and at 1% respectively. B = Cultivar *Bebu*; Wss1 = *Wlêwlê small seeds* 1; Wss2 = *Wlêwlê small seeds* 2; Wss3 = *Wlêwlê small seeds* 3; FMp = Fruit maturity period; NF = Number of fruits per plant; FW = Fruit weight; FD = Fruit diameter; FV = Fruit volume.

than 1.

Number of fruits per plant

Estimates of heterosis (%) of F_1 hybrids over mid and better parents were not significant for all hybrids and exhibited a range of heterosis (%) from -20.42 to 16.80% and from -55.48 to 16.49 % over the mid and higher parent, respectively. Crosses using *Bebu* as one parent recorded potence ratio comprised between -1 and 1 while in the other crosses, potence ratio values were superior to 1. However, Wss1 × Wss3 exhibited the only negative value of potence ratio (-9.75).

Fruit weight

Only hybrids of crosses having *Bebu* as one parent recorded a significant positive heterosis according to mid-parent (Figure 2). Percentages of heterosis according to the best parent were not significant for all hybrids. In the majority of cases, potence ratio were over 1.

Fruit diameter

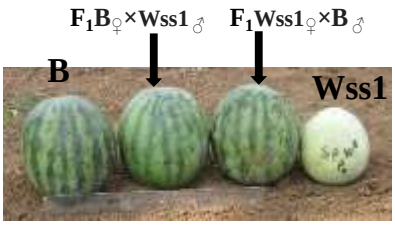
All hybrids with one parent *Bebu*, exhibited significant positive heterosis over mid-parent (Figure 2) except hybrids from B × Wss1 which obtained non-significant value of heterosis according to mid-parent like other hybrids. Concerning heterosis according to the best parent, values recorded were not significant and were positive for all hybrids except hybrids resulting from B ×Wss1, Wss1×B and Wss3 × Wss2. The same hybrids have obtained potence ratio values comprised between -1 and 1 while the other hybrids had values superior to 1.

Fruit volume

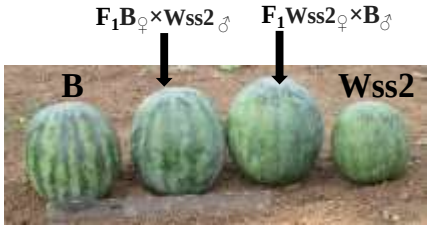
Crosses involving *Bebu* as one of the parents, exhibited significant positive heterosis over mid-parent (Figure 2) except cross B×Wss1 which recorded non-significant value of heterosis according to mid-parent as well as other hybrids. For heterosis according to the best parent, values obtained were not significant and were positive for all hybrids except hybrids resulting from B ×Wss1, Wss1×B and Wss3× Wss2. These hybrids had potence ratio values comprised between -1 and 1 while the other hybrids recorded values superior to 1.

General and specific combining abilities for five fruit traits in pistachio cultivars

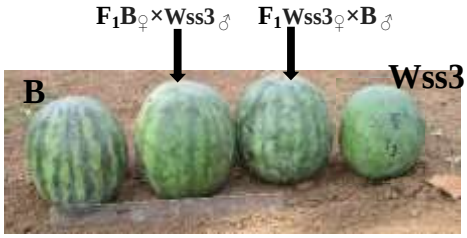
Analysis of variances for combining ability



2a: Fruits of *Bebu* (**B**), of hybrids (**F_1**) and of *Wlêwlê small seeds* 1 (**Wss1***)*



2b: Fruits of *Bebu* (**B**), of hybrids (**F_1**) and *Wlêwlê small seeds 2* (**Wss2***)*



2c: Fruits of *Bebu* (**B**), of hybrids (**F_1**) and *Wlêwlê small seeds* 3 (**Wss3)**

Figure 2. F1 hybrids and their respective parents showing heterosis in crosses involving parental line Bebu as one of parents.

Table 4. Analysis of variances for general and specific combining abilities (GCA and SCA), reciprocal, maternal and non-maternal effects and variance components.

Sources of variation	Df	FMp	NF	FW	FD	FV
GCA	3	163.03**	7.86**	120318.05**	295.56**	135497.88**
SCA	6	6105.20**	21.40**	381786.08**	8293.85**	400030.63**
REC	6	0.06ns	0.34ns	584.90ns	1.68ns	665.37ns
MAT	3	0.05ns	0.53ns	188.20ns	0.86ns	226.39ns
NMAT	6	0.07ns	0.16ns	981.61ns	1.25ns	1104.3 ns
error	30	0.78	0.11	827.23	1.78	809.89
σ² GCA		9.01	0.43	6638.38	16.32	7482.67
σ² SCA		6104.42	21.30	380958.86	8292.07	399220.74
GCA/SCA		0.00	0.02	0.02	0.00	0.02

ns: no significant, *and **: significant at 5% and at 1% respectively. FMp = Fruit maturity period; NF = Number of fruits per plant, FW = Fruit weight; FD = Fruit diameter; FV = Fruit volume. REC = reciprocal effect; MAT = maternal effect; NMAT = non –maternal effect; σ²GCA= Component due to GCA mean squares; σ²SCA= Component due to SCA mean squares; GCA/SCA= ratio σ²GCA/ σ²SCA.

revealed that the mean squares (MS) of GCA and SCA were highly significant ($p < 0.001$) for all studied traits suggesting the presence of both additive and non-additive gene actions in the inheritance of these traits. The magnitude of specific combining ability variance was higher than general combining ability variance for all studied trait indicating that non-additive gene actions play an important role in the expression of these studied traits. The very low values of GCA/SCA ratio for these traits further substantiated this finding. The mean squares of reciprocal, maternal and non-maternal effects were not significant for all characters studied, indicating that these characters were under strict nuclear control (Table 4).

The estimates of general combining ability (GCA) and specific combining ability (SCA) effects for studied traits are presented in Table 5. Concerning GCA effects, results revealed that *Bebu* have recorded the highest significant positive values of GCA for fruit weight (FW), fruit diameter (FD) and fruit volume (FV). The same inbred line showed the highest significant negative values of GCA for number of fruit (NF) and fruit maturity period (FMp).

A negative value of GCA for FMp indicates earliness in fruit maturity while a positive value translates lateness in the fruit maturity. Thus, *Bebu* appeared as the best general combiner for FW, FD, FV and FMp while *Wlêwlê small seed* accessions were the best general combiners for NF since they exhibited highest positive values of GCA for this trait. Results about specific combining ability (SCA) effect showed that B × Wss2 cross had the highest negative estimated value of SCA for FMp indicating hybrids from this cross were precocious. Wss1×Wss2 followed by Wss2×Wss3 exhibited the highest positive values of SCA for NF. For characters FW, FD and FV, crosses using *Bebu* as one parent presented significant positive values of SCA with the highest values recorded by B×Wss2.

DISCUSSION

A 4 x 4 diallel cross design is used for agronomic performance study in inbred lines and their F_1 hybrids through effects of heterosis, potence ratio, general and

Table 5. Estimates of GCA and SCA in four inbred lines and their hybrids for five fruit traits.

Genotypes	FMp	NF	FW	FD	FV
AGC of parents					
B	-6.57**	-1.47**	181.03**	8.97**	192.46**
Wss1	1.32*	0.31**	-88.73**	-4.31**	-92.38**
Wss2	1.51*	0.64**	-56.52**	-2.99**	-60.76**
Wss3	3.74*	0.53**	-35.78**	-1.68**	-39.32**
SE_{ij}	0.27	0.10	8.81	0.41	8.71
ASC of crosses					
B × Wss1	0.16ns	-0.16ns	61.56**	1.80*	30.96ns
B × Wss3	0.07ns	0.13ns	56.50**	3.02**	68.32**
B × Wss2	-0.67ns	-0.32ns	140.24**	5.62**	121.24**
Wss1 × Wss2	0.46ns	0.48**	-9.89ns	-0.71ns	-14.31ns
Wss1 × Wss3	0.15ns	-0.41*	2.67ns	1.07ns	20.37ns
Wss2 × Wss3	1.21**	0.37*	-52.59**	-2.00**	-45.28**
SE_{ij}	0.49	0.18	16.08	0.75	15.91

ns: no significant *and **: significant at 5% and at 1% respectively. B = Cultivar *Bebu*; Wss1 = *Wlêwlê small seeds* 1; Wss2 = *Wlêwlê small seeds* 2; Wss3 = *Wlêwlê small seeds* 3; FMp = Fruit maturity period; NF = Number of fruits per plant; FW = Fruit weight; FD = Fruit diameter; FV= Fruit volume. SEij = standard error.

specific combining ability and reciprocal effects. Concerning agronomic performances, the results showed that cultivar *Bebu* and accessions of *Wlêwlê small seeds* (Wss) have very contrasted traits. The results also revealed that accessions of Wss cultivar were distinct one from other, indicating existence of variability between them. Our finding come to substantiate those of Zoro Bi et al. (2006), Adjoumani et al. (2012) and Gbotto et al. (2016).

The comparison of parental agronomic performance with these of their F_1 hybrids revealed that some hybrids presented mean values equal or superior to those of the best parent for some traits, occurring heterosis effects over mid- and the best parent. The significance of heterotic effects showed that non-additive genetic type of gene action affects such traits (Wannows et al., 2015). Indeed, according to Yousfi (2011), hybrids vigor is based on the complementation of parental gametic contribution by favorable dominant genes and therefore, the expression of heterosis effect could be resulted from non-additive gene action. Also, for explaining these hybrid vigor, Moll and Stuber (1974) listed three possible causes of hypothesis, partial to complete dominance, over dominance and epistasis. The results of heterosis in one crop suggested that hybrid vigor is available for the commercial production of this crop and selection of desirable hybrids among the crosses having heterotic and heterobeltiotic effects in other characters is the best way to improve the yield of this crop (Wannows et al., 2015). However, it is worth noting that a positive heterosis effect for FMp translating the lateness is not desirable. On the contrary, a negative heterosis effect for this trait indicates the earliness in the fruit maturity and therefore is desirable. Some hybrids had recorded mean values more or less equal or inferior to mid parental values for some traits justifying the low or very low heterosis effects observed. This fact could be explained by additive gene effects in the inheritance of these traits. In fact, Mather and Jinks (1971) reported that, under additive gene action, the F_1 mean is expected to be midway between the means of their parental combination. According to Jung and Lelly (1985), absence of heterosis or its low value in barley probably reflected a large additive gene action. Potence ratio values were strictly superior to zero in some crosses or were strictly inferior to zero in other crosses. These values of potence ratio reflected various degrees of gene dominance or recessiveness. Thus, in some F_1 crosses, potence ratio values were comprised between 0 and 1 or were superior to 1 for some trait suggesting that these traits were respectively under partial or over dominance gene actions control. On the contrary, traits having potence ratio values comprised between -1 and 0 or inferior to -1, indicated the presence of partial or over recessiveness gene action respectively in the inheritance of these characters. Similar results had been reported by El-Tahawey et al. (2015), Kamer et al. (2015) and Wannows et al. (2015). These various degrees of dominance are in agreement with hypothesis of partial or over dominance proposed by Moll and Stuber (1974) for explaining the hybrid vigor. In fact, hybrids which manifested positive heterosis effect over mid-parent have recorded potence ratio values comprised between 0 and 1 or superior to 1 while hybrids with a negative heterosis effect have obtained values of potence ratio comprised between – 1 and 0 or inferior to -1 (Kamer et al., 2015; Wannows et al., 2015).

Results concerning combining ability showed that

analysis of variances due to general and specific combining ability were highly significant for all studied traits suggesting the presence of both additive and non-additive gene actions in the expression of these traits. No studies were done about general and specific combining ability in *C. mucosospermus*. However, the works carried out on its sister *C. lanatus* and on another *cucurbitaceae* species have also emphasized the importance of both additive and non-additive gene action in the inheritance of many traits (Feyzian et al., 2009; Gvozdanović-Varga et al., 2011; Bahari et al., 2012; El-Tahawey et al., 2015: Golabadi et al., 2015; Ogbu et al., 2016; Santos et al., 2017). The presence of both additive and non-additive gene actions in the expression of these traits suggests the use of reciprocal recurrent selection or Bi-parental mating for improving these traits (Jatoth et al., 2014). The magnitude of specific combining ability variance was higher than general combining ability variance for all studied traits bringing about the lower values of AGC/ASC ratio for all traits indicating that non-additive gene effects play an important role in the expression of these traits. Similar results had been reported by Hemalatha et al. (2014), Pandey et al. (2014) and Iqbal et al. (2015) who had found lower values of GCA variance compared with SCA variances which caused lower values of ratio AGC/ASC respectively in maize (*Zea mays* L.), Pigeonpea [*Cajanus cajan* (L.) Millsp.] and dry beans (*Phaseolus vulgaris* L.). This result confirms these obtained with potence ration values. The predominance of non-additive gene action in the expression of investigated characters suggests the possibility of the hybrid vigor exploitation (Hasanuzzaman et al., 2012; Pandey et al., 2014, Poodineh and Rad, 2015; Santos et al., 2017) or the possibility to postpone the selection to later generations for improving genetically these traits (Kiran et al., 2012).

The parental lines comparison in GCA term, showed that cultivar *Bebu* was the best general combiner for all studied traits except number of fruits per plant which the best general combiner were the three accessions of *Wss*. These four inbred lines could be used in *C. mucosospermus* breeding programs. In fact, the highest GCA effects shown by genotypes for a trait indicate that these genotypes contain more genes with additive effects (Nataša et al., 2014) and have high potential for generating superior offspring. This underlies that this trait is heritable (Ogbu et al., 2016). Consequently, these genotypes could be a good parent for this trait in a breeding program designed to improve that trait (Zeinanloo et al., 2009; Nataša et al., 2014). In addition, high significant values of GCA for one trait reveals selection and hybridization methods would result in interesting genetic improvement for this trait thanks to desirable genes accumulation of two parents in the targeted genotype (Golabadi et al., 2015). However, lines with higher GCA effects can be used more effectively in the development of synthetic variety (Hemalatha et al., 2014).

Results concerning SCA effect showed that crosses with high SCA effects involved at least one parent with high general combining ability for all characters. Significant SCA effects for one trait, obtained in crosses which were involving one parent with high GCA effect (high x low), suggested the involvement of additive x dominance gene interaction in expression of this trait (Nataša et al., 2014). According to Banerjee and Kole (2009), the diversity in parental GCA-effects plays an important role for the production of hybrids F_1 with significant positive SCA effect. Crosses with significant SCA effects could be advanced further for the isolation of transgressive segregants in order to develop good inbred lines (Hemalatha et al., 2014; El-Tahawey et al., 2015).

Conclusion

Overall, parent lines exhibited significant genetic variations for investigated traits in this study. A positive heterosis effect relative to mid-parental values was observed to hybrids having one parent *Bebu* for characters' fruit weight, fruit diameter and fruit volume, while a negative heterosis effect relative to the best parental values was recorded to same hybrids for fruit maturity period. Non-additive gene actions involved in the inheritance of all studied traits, suggests the exploitation of heterosis effect or the postponement of selection to later generations for improvement of these traits. *Bebu* appeared the best general combiner for FMp, FW, FD, and FV while *Wss* accessions were for NF suggested that these four parents can be incorporated into *C. mucosospermus* breeding programs. Crosses Wss1×Wss2 and Wss2×Wss3 showed significant SCA effects for NF while crosses using *Bebu* as one parent presented significant positive values of SCA for FW, FD and FV. B×Wss2 had recorded a negative value of SCA for FMp. All these crosses could be used for developing high yielding genotypes.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

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ABBREVIATIONS

GCA, general combining ability; **SCA,** Specific combining

ability; **B,** Cultivar *Bebu;* **das,** Days after sowing; **Wss,** *Wlêwlê* small seeds; **FM,** Fruit maturity period; **NF,** Number of fruit per plant; **FW,** Fruit weight; **FD,** Fruit diameter; **FV,** Fruit volume.

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