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Full Length Research Paper

Characterization of rain-fed maize-based vegetable production systems in Northern Ghana

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The study was conducted in Northern Ghana comprising of the Northern, Upper East and Upper West regions. The objective of the study was to analyse maize-based rain-fed vegetable production systems with a view to explore their potential to contribute to food and income security for rural households. A combination of qualitative and quantitative data collection methods were employed during the study. Questionnaires were employed to collect quantitative data, through semi-structured interviews on socio-cultural and economic dimensions of vegetable production including household characteristics, consumer perspectives and the economics of production and consumption of vegetables. The targeted respondents were households and consumers. Rain-fed cereal-based vegetable production is a key component of the traditional farming system in Northern Ghana and served as the main source of vegetables for rural households. The results showed that all households in the study area integrated vegetables with maize mainly for home consumption. Only a few households cultivated vegetables with the sole purpose of income.

Key words: Agriculture, rain-fed, household, vegetable, maize-based, Ghana, farming system.

INTRODUCTION

According to the World Bank (2008), more than two-thirds of the poor in Sub-Saharan (SSA) live in rural areas and derive a living mainly by producing rain fed crops, livestock, trees and other agricultural activities. There is little doubt that, overall, agricultural growth contributes to economic growth and poverty reduction. However, the form in which this growth takes place is important in determining the effectiveness of its development impact. It is important, especially for developing countries, to

identify specific opportunities in the agricultural sector and tailor investment in sectors that will ensure the greatest impact on food security and poverty alleviation. (World Bank, 2001). One way this can be achieved is a systemic view of specific sub-sectors within the agricultural sector. This allows in-depth analysis of the complex contextual dynamics characterizing agricultural systems in order to examine, critically, the inherent constraints and opportunities that enable informed

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decisions.

According to the Ghana Living Standards Survey (2010), an estimated 1.2 million people, representing 5% of Ghana's population, are food insecure. Fifty-nine percent of these are in Northern Ghana (34% in Upper West region, 15% in the Upper East and 10% in the Northern Region), while an estimated 40% people are vulnerable of becoming food insecure in the rural areas of Northern Ghana. Nutrition security is critical to sustainable food security. However, inadequate access to minerals and vitamins remain a challenge, especially in the global south. The role of traditional African vegetables in local diets of indigenous populations in Africa has been recognized (Guarino, 1997; Mirghani and Mohammed, 1997). The importance of vegetables in local diets extends beyond providing the food needs of local populations. Vegetables are recognized sources of essential nutrients required for health growth and a healthy life (Udosen, 1995; Food and Agricultural Organization (FAO), 1988; Fube and Djonga, 1987). However, evidence exists to the effect that the consumption of fruits and vegetables in Africa, generally, falls below the recommended 400 g/day (Keatinge et al., 2011). This raises much concern not only about the nature of food consumption but legitimate questions must also be raised about the ability of farming systems in developing countries to provide the micronutrient requirements of large sections of the population that depend on farming for a living. Farming systems analysis offers an appropriate mechanism for detailed analysis, with a systemic view of specific sub-sectors of the agricultural systems, in this case, rain-fed traditional African Vegetables in Northern Ghana.

According to the FAO (2001), a farming system is defined as "a population of individual farming systems that have broadly similar resource bases, enterprise patterns, household activities and constraints, and for which similar development strategies and interventions would be appropriate". Following the FAO (1997) our analysis is approached from the perspective of the farm household, mainly as a resource manager and a beneficiary of the farm system. As a resource manager, the farm household provides direction and purpose for the entire farming system by defining the objectives of the farming system (Shaner et al., 1982). On the other hand, the farm household's decision is depended upon the broad contextual realities within which it operates and is influenced by both external and internal factors. Thus, a particular household's decision within the farming system is based on the broad social, economic, cultural and institutional boundaries within which it operates. The farm household therefore, provides an appropriate framework within which to analyze the dynamics within the farming systems.

As the world faces unprecedented food security challenges, there is the need to assess the constraints faced by farmers and to identify the opportunities

available to them in order to ensure a more realistic approach to addressing the situation through appropriate policies and interventions (Barrett, 2013). Maize-based farming systems offer enormous opportunity for economic growth and poverty reduction among poor farmers in Africa.

In an analysis of various farming systems and their potential to contribute to poverty reduction, Garrity et al. (2012) singled out maize based systems as offering the greatest potential pathway out of poverty. This, according to the authors, is due to the fact that an estimated 91 million farmers derive livelihood from maize based systems against the background that the incidence of poverty is high in maize based systems. In addition, small holder farmers, who coincidentally, are resource-poor, constitute an estimated 90% of the farmer population in maize based systems in Sub-Saharan Africa.

In Ghana, maize production has increased, both in area and output, quite significantly over the recent years, mainly under smallholder production. According to statistics from the Ministry of Food and Agriculture (2011), maize constitutes 55% of total grain output in Ghana. Almost invariably, rural farmers integrate traditional African vegetables in their maize fields, most often, to cater for household food needs and, in some cases, for cash income. Thus, rain-fed maize and associated systems have great potential to contribute to poverty reduction if the inherent opportunities are harnessed creatively to boost cash income and food for the rural poor. This is against the background that globally, the rate of expansion of irrigation infrastructure has not been as expected and, in fact, there are reasons to believe that irrigated land, as a proportion of total agricultural land is on the decline (Valipour, 2015).

In Africa, the value of irrigation-equipped area as a percentage of total agricultural area is only 5.8% (Valipour, 2014). In Ghana, irrigation agriculture accounts for only 1% of agricultural area (Ghana Irrigation Development Authority, 2012). Thus, large sections of the population still depend on rain-fed agriculture. Such a systemic analysis is important in understanding the contextual dynamics between water and other sectors relevant for food security such as water management, population, food, the environment and the long term interactions among them and the resulting impact on future food security (Khan et al., 2009, in Valipour, 2015).

MATERIALS AND METHODS

The study area

Figure 1 represents the study area. Northern Ghana is situated between 8° to 11° N latitude and 0° to 3° W longitude. Administratively, it comprises of the Upper West Region (UWR), Upper East Region (UER) and Northern Region (NR). The area falls within the dry land Savannah zone occupying an estimated 40% of the country (Gyasi, 1995). The rainfall pattern is mono-modal. The rainy season permits a growing season of 150 to 160 days in the

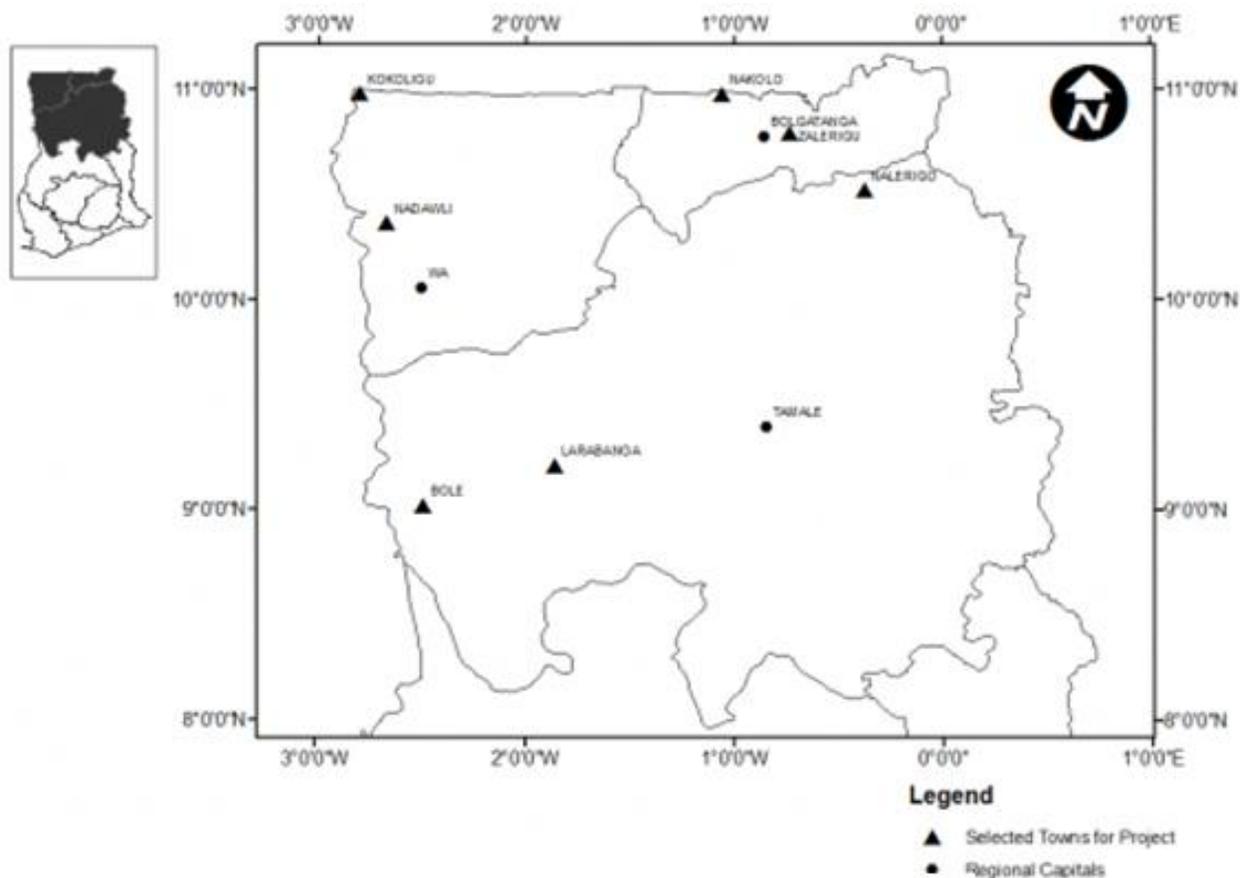


Figure 1. Map of Northern Ghana.

Upper East Region and 180 to 200 days in the two other regions. Mean total annual rainfall varies from 1,000 mm in the Upper East Region to 1,200 in the south eastern part of the Northern Region. The rainfall shows wide variations from year to year, both as regards the amount and the time when it occurs.

Food shortages are a common feature of the dry season (Destombes, 1999). According to the 2010 population and housing census the 3 northern regions together account for 17.3% of the total population of Ghana. Northern region accounts for the largest share (10.2%), followed by Upper East region (4.3%) and the Upper West region (2.8%). The Northern Region, despite being the largest, in terms of land mass, is the least populated among the 3 administrative regions of northern Ghana with a population density of 35 persons per sq. km. The Upper East region has the highest population density of 118 persons/sq. km while the Upper West region has a population density of 38 persons/sq. km.

Sampling and data collection

A combination of qualitative and quantitative data collection methods were employed during the study. Questionnaires were employed to collect quantitative data, through semi-structured interviews on socio-cultural and economic dimensions of vegetable production including household characteristics, consumer perspectives and the economics of production and consumption of vegetables. The targeted respondents were households and consumers. Two focus group discussions (FGD) with households

engaged in vegetable production, disaggregated on the basis of gender, were conducted per community. The FGD served to solicit further information, to validate issues arising out of the analysis of the questionnaires and to explore the qualitative basis of the results obtained.

Key informant interviews of key stakeholders including notable farmers, traders, transporters and relevant institutions such as the ministry of agriculture, farmers associations and non-governmental organizations (NGOs) were conducted to understand current dynamics regarding the production, consumption and marketing of vegetables under maize based production systems. The research team visited selected farms and interacted directly with farmers while observing production practices and other field operations in particular cropping patterns, cultural practices and inputs use.

RESULTS AND DISCUSSION

Type of vegetable cultivated

The most common vegetable cultivated in Northern Ghana are as indicated in Figure 2. At least, 60% of all vegetables are cultivated by farm households: pepper (100%), Okro (96%), tomato (91%), Alefu (84%), Ayoyo (73%) and cowpea leaves (62%). Among the cultivated vegetables, leafy vegetables constitute 62.4%. Although

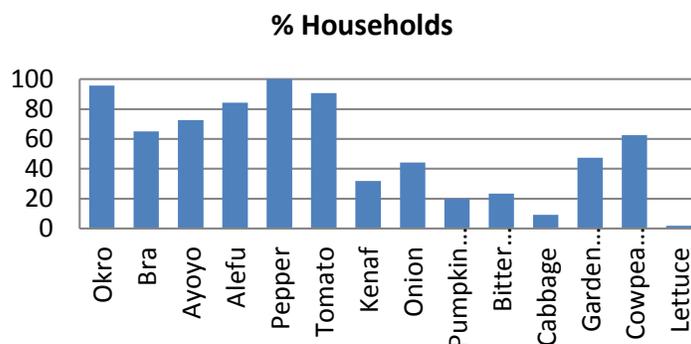


Figure 2. Common types of vegetables cultivated. Source: Field Survey (2012).

Table 1. Share of individual vegetable production.

Type of vegetable	Northern Ghana	Coastal Zone	Forest Zone
Garden Eggs	12	26	62
Leafy Vegetables	21	6	72
Okro	42	12	46
Onion	33	24	43
Pepper	20	18	62
Tomato	24	26	51
Other vegetables	12	11	78
Total	24	17	59

Source: Ghana Living Standards Survey (2008).

vegetables constitute a significant proportion of crops cultivated by households collectively, the wide range of vegetables cultivated makes the significance of any individual vegetable negligible in terms of its contribution to household income. Eggplant, tomato and pepper are, however, cultivated in substantial quantities usually as sole crops for commercial purposes in all three regions of northern Ghana. Thus, commercial viability appears to be a strong driver for scale-up in vegetable cultivation.

Importance of vegetable production among households

Northern Ghana ranks second as regards the production of major vegetables consumed locally in Ghana. Overall, Northern Ghana accounted for about 24% of all vegetables harvested in Ghana in 2007 (Ghana Living Standards Survey (GLSS), 2008). It is important to acknowledge that, with the exception of onion and tomato, the rest of the crops, as indicated in Table 1, were produced under rain-fed agriculture, most of them, integrated with major cereal crops like maize, millet and sorghum.

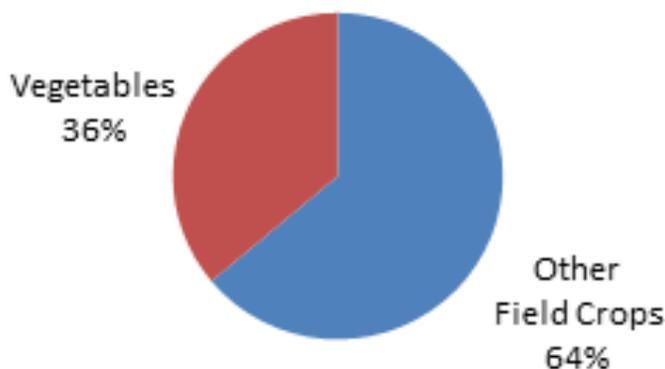
From the rankings in Table 2, it is obvious that rain-fed vegetable production is not among the most important economic activity in farm households considered from the amount of time the household invests in it. In most cases, it is women, who incidentally, have little time at their disposal during the farming season, who engage actively in vegetable production. Overall, only 15% of respondents ranked vegetable production as the most important economic activity.

In the Upper East region, however, vegetable production is quite important compared to the other regions with 24% of households in that region ranking it as the most important economic activity. This is not surprising since the practice is common among farmers, especially, during the dry season. The area is well noted for tomato, onion and water melon production during the dry season.

The existence of large irrigation schemes like the Tono, Vea and Bugri irrigation schemes has boosted vegetable production significantly in the Upper East region. Vegetable production ranked the least among households in the Upper West region. In the Upper East and Upper West regions vegetables are mostly cultivated as border crops while in the Northern region they are cultivated mostly as sole crops on small parcels of plots

Table 1. Ranking of vegetable production among other farming activities among households.

Rank	Northern (%)	Upper East (%)	Upper West (%)	Overall (%)
1	15	24	5	15
2	26	9	18	18
3	44	52	55	50
4	15	15	21	17

**Figure 3.** Share of vegetables among field crops. Source: Field Survey (2013).

for women by their husbands.

Share of vegetables among field crops

Vegetable production among rural farmers is quite significant when compared against all other field crops in terms of the different types of crops that are cultivated. It is a normal cultivation practice for farmers to inter-crop arable crops with vegetables. Every household cultivates, at least, 3 different types of vegetable either as sole crop or mixed cropped with other field crops. As indicated in Figure 3, vegetables constitute 36 % of all field crops. Since, they are cultivated mainly for household consumption, a wide variety of vegetables are cultivated but in small quantities to supply the household food requirements.

The following vegetables constitute at least, 60% of all vegetables cultivated by farm households: pepper (100%), Okro (96%), tomato (91%), Amaranthus (84%), Corchorus (73%) and cowpea leave (62%). Among the cultivated vegetables, leafy vegetables constitute 62.4%. Although vegetables constitute a significant proportion of crops cultivated by households collectively, the wide range of vegetables cultivated makes the significance of any individual vegetable negligible in terms of its contribution to household income. Eggplant, tomato and pepper are, however, cultivated in substantial quantities usually as sole crops for commercial purposes in all three regions of northern Ghana.

Production objectives

As indicated in Figure 4, most households undertake crop production for the dual purposes of cash and food. Crop production for the sole purpose of cash is very limited and accounts for only 5% of all households interviewed. Rain-fed vegetable production is secondary to other field crops and is cultivated mainly to supplement household income and for food.

During the rainy season most vegetable fields are converted for the production of cereal crops. Most leafy vegetables are cultivated for consumption while others like tomato, pepper and eggplant are cultivated mainly for cash. In the Upper East region relatively more households indicated engaging in vegetable production for the sole purpose of income. This response is not surprising since the region is noted for large scale commercial production of vegetables notably, tomato, onion and pepper under irrigation conditions during the dry season.

Gender

Women mostly undertake rain-fed vegetable cultivation while men concentrate on the cultivation of maize. Women usually integrate leafy vegetable in the household maize fields. In some cases, small plots of vegetable are cultivated independent of the family farm holding by women for the sole purpose of supplementing

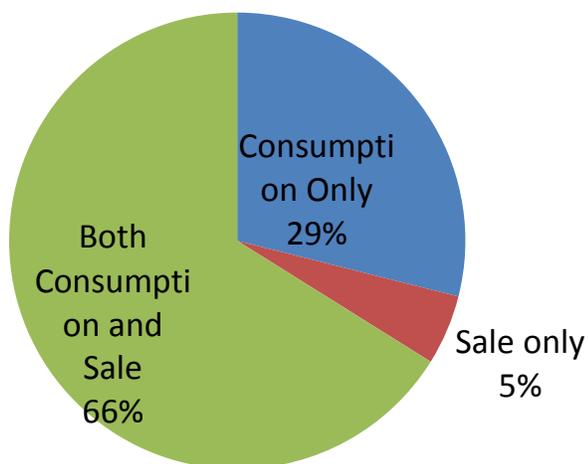


Figure 4. Production Objective. Source: Field Survey (2013).

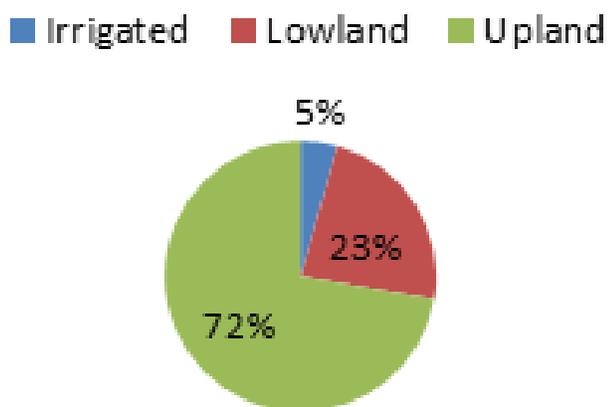


Figure 5. Land type. Source: Field survey (2013).

their income. Most of the men indicate low prices of vegetables as their main reason for their decision not to cultivate vegetables during the rainy season.

Land type and tenure

Most farmlands in the study area are located upland (72%). Irrigated land constitutes only 5% of farmlands while lowlands constitute 23% (Figure 5). However, there is a demonstrated preference for farmers to cultivate vegetables in lowlands due to better access to water (Figure 5). Land is typically owned outright by members of a household as indicated in Figure 6. Land is traditionally owned by households and held in trust for the members by the household head. As members come of age they are apportioned sections of the land to cultivate to feed their families and to provide for other needs. The consequence of this arrangement is that the size of land

Plot Ownership

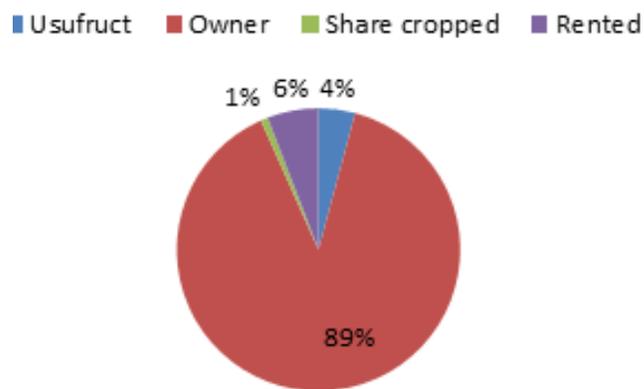


Figure 6. Land ownership. Source: Field Survey (2013).

available for individual members within households progressively reduces in size, diminishing usufructory rights generally. This has resulted in the increasing phenomenon of renting land for cultivation and share cropping arrangement by farmers and land owners previously unknown in Northern Ghana. The household head is the plot manager responsible for all decisions regarding the farm. However, the household head may not necessarily participate actively in farm operations. Such is the case of ageing household heads whose children have come of age. Usually, the eldest son in the household takes over active management of the plot from the household head.

Change in vegetable area under vegetable cultivation

The area dedicated to vegetable production has remained largely unchanged over the past 5 years (Table 3). Sixty-nine percent of all households indicated that their vegetable area has not changed. 21 and 10% indicated that the household’s farm area dedicated to vegetable production has increased and decreased, respectively. The main push factor for area expansion for vegetable production is access to profitable markets, as most households are able to obtain sufficient quantities of vegetables for household consumption. The trend is similar in all the regions under study. The northern region recorded the highest expansion of vegetable area and the most likely reason is the proximity of most of the study communities in that region to major urban markets, especially Yendi and Tamale.

Technical and institutional support

For smallholder farmers, producer organizations are essential to achieve competitiveness. Elsewhere, they

Table 3. Change in vegetable area over the past 5 years.

Parameter	Northern	Upper East	Upper West	Overall
No Change (%)	68	71	77	69
Increased (%)	28	18	10	21
Decreased (%)	4	11	13	10

Source: Field Survey (2013).

Table 4. Indicators of technical/institutional support.

Indicator	Northern (%)	Upper East (%)	Upper West (%)	Overall (%)
Membership of Farmer groups	49	43	33	42
Functions of farmer groups				
To assist each other on our farms	13	16	30	19
Information Sharing	25	24	28	25
Access to input	0	18	10	9
Access to Credit	5	8	3	5
Market Access	5	9	0	10
Frequency of meetings	3.0	3.0	2.0	3.0
Training in vegetable production	42	62	14	39
Number trainings in vegetable production	3	4	2	3
Access to material explaining vegetable production	11	21	0	11

Source: Field survey (2013).

have been known to have provided a great deal of empowerment to small holder farmers in the context of institutional constraints. Northern Ghana has a strong presence of NGOs that have shown substantial support to smallholder farmers in the formation and strengthening of farmer groups. However, as indicated in Table 4, these efforts have not achieved much as most of the farmer groups formed over the years have been difficult to sustain. In total, only 42% of households reported having members belonging to a farmer group. The two most important reasons for the formation of farmer organizations is access to information (25%) and self-help (19%). Other reasons include market access (10%), access to inputs (9%) and access to credit (5%) (Table 5).

Existing farmer groups are, however, quite active meeting, on the average, 3 times annually. Support to vegetable farmers by the Ministry of Agriculture and NGOs, generally, has been concentrated mainly on dry season irrigated vegetable farmers. Overall, 39% of all households indicated that some of the members had ever received formal training in vegetable production. In the Upper East region, however, significant numbers of farmers (62%) had received some form of training in vegetable production. This is largely accounted for by irrigated vegetable farmers on large irrigation schemes like the Tono, Veve and Bugri Irrigation schemes. The

situation in the Northern region is also similar to that in the Upper East region as the selected communities included some areas with irrigation schemes. Farmers in the Upper West region have received the least number of training in vegetable production accounting for only 14%. This is not surprising as irrigation agriculture is not very popular in the region. Very few farmers have access to learning material on vegetable production. Much as most vegetable farmers cannot read, as already discussed previously, the lack of access to learning material on vegetable production constitutes a major constraint since most farmers are unable to put into practice most of what they have been taught.

Resource endowment

Land

Vegetable producing households are typically smallholders. The average size of land cultivated by a household in the study area in 2012 is 0.4 to 1.5 ha. Each household cultivates several plots each year with some households cultivating up to 5 separate plots annually. The average number of years households have cultivated their current plots is between 13 and 18 years

Table 5. Key characteristics of vegetable plots.

Characteristic	Northern	Upper East	Upper West	Overall
No. of plots	3	4	4	5
Vegetable area (2011)	0.7	1.1	1.1	1.0
Upland	45%	54%	80%	60%
Lowland	55%	46%	20%	40%
Source of Irrigation water				
Dam/Dugouts	2.5	55	13	36
Well	2.5	2.5	10	5
Borehole	0	2.5	2.5	2
Stream	0	2.5	10	3

Source: Field Survey (2013).

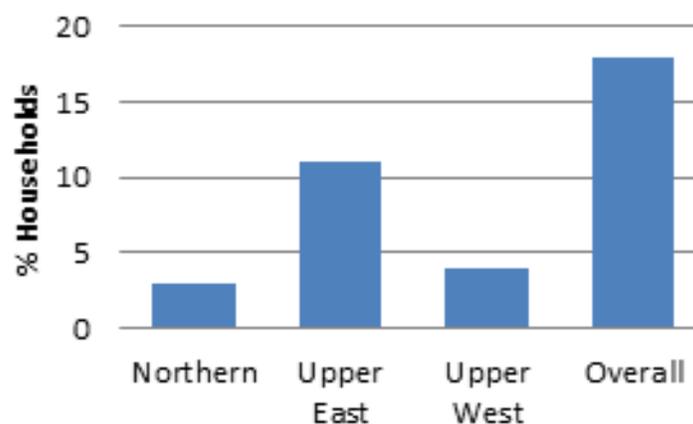


Figure 7. Household ownership of agricultural equipment. Source: Field Survey (2012).

indicating a high degree of intensification. Most of the land is located upland.

As would be expected irrigated vegetable production is not significant as field crops are mainly cultivated during the rainy season. Generally, irrigation infrastructure is very limited and therefore, irrigate vegetable production even during the dry season is not very significant and mostly limited to high value vegetables like tomato and onion. Leafy vegetables are, however, mostly cultivated during the rainy season.

Dry season vegetable production is, however, common in the Upper East region as the area is relatively more endowed with irrigation facilities than the other regions. The main source of water for irrigating vegetable fields is dam and dugouts, Table 5.

Agricultural equipment

The common agricultural equipment owned by households includes hoes and cutlasses as would be

expected of smallholders. Only 18% of all households own major agricultural equipment other the hoe and cutlass, such as tractor, donkey, knapsack and water pumps (Figure 7). Although most households do not own farm equipment, this is not a constraint to agricultural production as most households have access to farm equipment either by hiring or borrowing them. Over 95% of all households indicated that they can afford to hire equipment. Timely access to equipment, however, constitutes a critical challenge. In spite of the fact that government has put in place a policy over the past years to import tractors on a consistent basis, this is still not adequate (Table 6).

Transport

Bicycles, motorbikes and cars can be of great assistance to farmers in their farm activities in providing much needed means of transport. As indicated in Table 7, most households own bicycles. Although fewer households

Table 6. Access to agricultural equipment.

Parameter	Northern (%)	Upper East (%)	Upper West (%)	Overall (%)
Access	73	95	90	87
Type of Access				
Hired	92	90	94	91
Borrowed	8	10	6	9

Source: Field Survey (2012).

Table 7. Household ownership of means of transport.

Asset	Northern	Upper East	Upper West	Total
Bicycle (%)	98	70	88	86
Motorbike (%)	28	45	15	29
Car/truck (%)	0	13	0	4

Source: Field Survey (2012).

Table 8. Sources of information for vegetable farmers.

Source	Northern	Upper East	Upper West	Overall
FBOs (%)	37	41	23	34
Community extension agent (%)	33	56	29	40
Radio (%)	47	53	65	57
Extension officer (%)	76	58	53	59
Other farmers (%)	6	30	30	26
NGO (%)	18	85	85	63

Source: Field Survey (2012).

own motorbikes, the situation represents a significant increase over the previous years. This is mostly due to access to cheaper Chinese made motorbikes. Quite a significant number of households in the Upper East region own motorbikes. This is not surprising because commercial vegetable production of high value vegetable crops like onion and tomato is more common in the region.

Access to inputs and services

Agricultural information

Table 8 describes the most common sources of information for farmers in Northern Ghana. Farmers have several sources of agricultural information. The common sources of information for farmers include Farmer based organizations (FBOs), community extension officers, radio, extension officers, other farmers and NGOs. Although the northern region has a very high access to extension officers, the region ranked lowest in all other

sources of information.

Overall, 59% of all households have access to extension services on a regular basis. This poses a major constraint to vegetable production as information received is too general and not focused on vegetable production specifically. Seventy-one percent of all vegetable farmers indicated that they did not access specific information on vegetable production. NGOs are playing a very important role in passing information to farmers in the Upper East and Upper West Regions.

Most farmers complain of lack of access to technical information on vegetable production, especially, as regards disease and pest attacks. Some vegetable varieties notably tomato has been abandoned due disease and pest attacks.

Farm inputs

There is a very high rate of access to key inputs as indicated in Table 9. Compost is the least accessible input. Farmers have been trained and have technical

Table 9. Access to inputs and Services by farmers

Access to inputs and Services	Northern	Upper East	Upper West	Overall
Fertilizer	98	88	95	93
Herbicide	100	65	69	80
Manure	68	83	64	71
Tractor	95	98	92	95
Compost	10	40	18	23

Source: Field Survey (2012).

Table 10. Access to credit.

Did you receive any agricultural credit?	Northern	Upper East	Upper West	Overall
Yes (%)	10	22	20	18
No (%)	90	78	80	82

Source: Field Survey, 2012.

Table 11. Reasons accounting for farmers' inability to access credit.

Parameter	Northern	Upper East	Upper West	Overall
No need for credit (%)	7	22	16	14
Too expensive (%)	0	3	3	2
Not available locally (%)	57	13	31	31
Difficult to obtain (%)	37	63	50	53

expertise in compost preparation. However, the poor access to compost is mainly due to the labour intensive nature of its preparation. Despite the high rate of access by farmers to other inputs timely access, especially, fertilizer and tractor services have resulted in losses to farmers on an annual basis. Access to fertilizer has improved significantly with the introduction of government subsidy program. However, the timeliness of supply to farmers still poses a significant challenge to farmers.

Finance

Access to credit is a major constraint to vegetable farmers in the northern Ghana. Over 80% of households in the study area do not have access to financial credit. Table 10 indicates reasons why farmers are unable to obtain loans to finance farming activities.

Overall, 53% of farmers are unable to obtain loans because they perceive loans to be difficult to access. Thirty-one percent attribute their inability to access financial credit to the lack of financial services within their locality while 2% is of the opinion that interest rates are too high. Fourteen percent of respondents see no need to acquire additional financial support from financial

institutions (Table 11). Majority (71%) of farmers who were able to contract loans indicated that the loans they acquired were not sufficient for their farm needs. Interest rates ranged from 10 to 50% and loans were contracted for periods between 2 months and 2 years.

Key constraints

Poor access to markets

Poor access to markets constitutes the single most important constraint to effective commercialization of rain-fed vegetable production in northern Ghana. Physical access is a major difficulty as most farms are situated in rural settings and far away from urban markets.

Consequently, farmers face difficulty transporting their produce in substantial quantities to major market centers and therefore, rely extensively on intermediaries to sell their produce. Another dimension of poor market access by vegetable farmers is that these intermediaries often have strict control over entry into major markets. Thus, even when farmers have been able to transport their produce to such markets they are still constrained to sell

their produce through intermediaries who would often offer low prices to farmers.

Poor producer price

Poor producer price constitutes an important constraint to the effective commercialization of vegetable production. This is due to a combination of factors. Farmers, generally, have no direct access to urban markets either due to difficulty in transporting their produce or the intimidating presence of intermediaries. Consequently, most vegetable producers sell their produce at farm gates where prices are very low. Moreover, owing to the perishable nature of most of the vegetables and lack of appropriate storage facilities, farmers are compelled to sell their vegetables immediately after harvest in order to avoid huge post-harvest losses. This practice often results in a glut driving down prices significantly.

Poor access to improved varieties

Most farmers still cultivate traditional varieties (landraces) of vegetables. Apart from the fact that these local varieties are low yielding, they attract low prices in the market. A major challenge among farmers that has resulted in this situation is the inability of farmers to produce and keep seed. Thus, farmers have seen a gradual erosion of some varieties over the years.

Poor access to technical support

Access to extension services by farmers is generally poor due to high farmer-extension officer ratio. Moreover, technical support for vegetable production, in particular, is poor especially for rain-fed vegetable production.

Conclusion

Vegetable cultivation under cereal-based farming systems is traditionally a component of the farming system in Northern Ghana and secondary to cereal production. Majority of the vegetables cultivated under rain-fed maize-based production systems are traditional African leafy vegetables. Rain-fed leafy vegetables are, traditionally, women's crop as men would rather concentrate on the cultivation of cereals during the rainy season. In view of the limited access to irrigation for vegetable production, rain-fed vegetable production offers enormous potential to contribute to improved nutrition among rural households. However, for this potential to be achieved there is the need for improved processing and storage so as to ensure access during the off season.

Conflict of Interest

The authors have not declared any conflict of interests.

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Full Length Research Paper

Availability and morphological characteristics of endophytic fungi held in different methods of preservation

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Maintenance of microorganisms in mycology is of fundamental importance for retrospective and prospective studies that focus on their biology, etiology and epidemiology. The aim of this study was to evaluate the viability, contamination and morphological changes of endophytic fungi maintained under different preservation methods. We evaluated five preservation methods; constant sub-culturing, preservation under mineral oil, under distilled water, preservation at -20°C and storage at -70°C. Every 50 days, we evaluated the viability, purity and macro-micro morphology of the cultures. The main results are as follows: constant sub-culturing and preservation under distilled water have allowed the viability of all strains during the study period. Preservation in mineral oil resulted in the contamination of the strain *Aspergillus* F45 and micro-morphological modification of the *Fusarium* LU5 culture after 100 days of preservation. Preservation at -70°C caused macro-morphological changes in *Fusarium* LU6 after 100 days. Under the experimental conditions and the limited period of study (150 days) it was demonstrated that conservation under distilled water was the most appropriate form of preservation of endophytic microorganisms.

Key words: Endophytes, preservation methods, morphological characteristics.

INTRODUCTION

In fungi preservation, methodologies are used which aim to maintain cell viability and the metabolic properties of

these organisms for long periods. This activity is essential for retrospective and prospective studies that focus

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on the biology, etiology, epidemiology and production of substances which are of biotechnological interest (Ryan et al., 2000). The main preservation methods evaluated can be divided into "simple and cheap", such as constant sub-culturing, storage under oil (Kobayashi 1984; Smith and Onions, 1994), under water (Burdall and Dorworth, 1994; Smith and Onions, 1994), in soil (Smith and Onions, 1994) and in silica gel (Elliot, 1975; Smith and Onions, 1994), or they may be "complex and expensive", such as lyophilization (Kolkowski and Smith, 1995; Tan, 1997) and cryopreserved in liquid nitrogen (Smith, 1998).

The microorganisms called endophytes coexist inside of higher plants without causing any apparent signs of disease (Petrini, 1991). Currently these microorganisms came to be regarded as an important part of biodiversity. The endophytic mycoflora presents a distribution that differs according to its host. The distribution of endophytic mycoflora differs with the host. The specificity of the endophytes ranges from generalist to highly specific to the host and the environment. These endophytes protect their hosts from infectious agents and adverse conditions by secreting bioactive secondary metabolites (Azevedo et al., 2000; Strobel, 2003). Studies on the potential of endophytes, especially those related to the discovery of new substances, appear relevant both for use in the biological control of pests and diseases and for application in the chemical/biochemical or pharmaceutical industries (Stierle et al., 1995; Azevedo 1998; Azevedo et al., 2000; Stamford et al., 2001, 2002; Suto et al., 2002; Strobel, 2003).

The Laboratory Micobateriologia, CPCS-INPA, has been conducting bioprospecting of substances produced by endophytes which are useful for the treatment and diagnosis of tuberculosis. Some strains of industrial interest have been obtained and need to be preserved. However, the literature is very limited as to the influence of different methods of preservation on morphology and the production of secondary metabolites in fungi of this group.

The aim of this work was to evaluate the feasibility and morphological characteristics of endophytic fungi maintained in different preservation methods and specifically to: a) assess the continued viability and purity of the cultures; b) evaluate the influence of these conservation methods on the micro and macro morphological characteristics of the cultures.

MATERIALS AND METHODS

Microorganisms

Five endophytic fungi belonging to the collection of the National Institute for Amazon Research were used in this study. Four of them were isolated from *Caesalpinia ferrea* Martius (*Fusarium* LU5, *Fusarium* LU6, *Fusarium* LU11, *Aspergillus* F45) and these had been preserved under mineral oil. The fifth strain was isolated from *Himatanthus sucuba* (*Fusarium* H42) and had been preserved in distilled water.

Preservation techniques

Five conventional preservation techniques were evaluated in triplicate:

1) Constant sub-culturing: This technique was performed as described by Lacaz et al. (1991). Every 15 days, a fragment of viable culture was transferred to a new tube containing Potato Dextrose Agar (PDA) which was incubated at 25°C.

2) Preservation under mineral oil: The technique was performed as described by Braz et al. (2009). Glass vials (20 ml) were filled with 2 ml of PDA culture medium and then inoculated with the microorganism. After 7 days, the culture media was covered with 10 ml of mineral oil (autoclaved for two consecutive days). The vials were capped with a rubber stopper and an aluminium seal and stored at 25°C.

3) Preservation under distilled water: The technique was carried out as described by Diogo et al. (2005). Glass vials (20 ml) were filled with 10 ml of distilled water (autoclaved for two consecutive days). Five small fragments (25 mm²) taken from a 7 day culture and transferred to these vials. The vials were closed with a rubber stopper and an aluminium seal and stored at room temperature (28°C).

4) Preservation at -20°C: The technique was carried out as described by Girão et al. (2004). Glass vials (20 ml) containing 8 ml of distilled water, 0.5 ml of dimethyl sulfoxide DMSO (cryoprotectant) and 1 ml of glycerol (cryoprotectant) were autoclaved for two consecutive days. Small fragments (25 mm²) taken from a 7 day culture were transferred to these vials. The vials were closed with a rubber stopper and an aluminium seal and stored at -20°C.

5) Preservation at -70°C: The technique was performed as developed in the Laboratory of Mycobacteriology, CPCS-INPA. In 0.4 ml of distilled water, 0.025 ml of dimethyl sulfoxide DMSO (cryoprotectant), 0.050 ml of glycerol (cryoprotectant) and 10 mg of polypropylenespheres (0.5 mm diameter, with a central hole) were placed into 1.5 ml microtubes. Small fragments (25 mm²) taken from a 7 day culture were transferred to these vials. The vials were closed with a rubber stopper and an aluminium seal and stored at -70°C.

Culture assessments

Each 50 days, during a 150 days period, the preservation techniques were evaluated. The preserved mycelia were transferred to Petri dishes containing PDA medium. Information about the viability, purity, macro-morphology and micro-morphology of the obtained cultures were assessed (Lacaz et al., 2002).

RESULTS

The endophytic isolates selected for the present study belonged to the class of Ascomycetes, specifically from the genera *Aspergillus*, *Fusarium* and *Penicillium*. The first morphological characterization was carried out in the "day zero" and this was used as a standard for evaluating the changes in viability, purity, micromorphology and macro-morphology over 150 days of the experiment. Table 1 show the frequency (%) of cultures that presented viability or that presented contamination, during the 50, 100 and 150 days of the experiment.

The strains subjected to preservations methods must

Table 1. Evaluation of the cultures that presented viability or that presented contamination during the 50, 100 and 150 days of the experiment.

Preservation technique	Viability (n1 /n2)			Contamination (n3/n2)		
	50 days	100 days	150 days	50 days	100 days	150 days
Sub-culturing	5/5	5/5	5/5	-	-	-
Distilled water	5/5	5/5	5/5	-	-	-
Mineral oil	4/5	4/5	4/5	1/5	1/5	1/5
-20°C	4/5	4/5	4/5	-	-	-
-70°C	4/5	4/5	4/5	-	-	-

n1: viable cultures, n2 total cultures, n3 contaminated cultures.

be able to withstand extreme temperatures and environments. In this study the culture of the isolate *Fusarium* L6 presented no growth after the submersion in oil such as *Fusarium* LU11 that did not grow after freezing (-20 and -70°C) (Table 1). The preservation under mineral oil resulted in the fungi contamination of the culture of *Aspergillus* F45 (-20 and -70°C).

In addition, the preservation techniques of constant sub-culturing and mineral oil caused modifications only in the macro-morphology of *Fusarium* LU6 after 100 days of preservation, in both cases this strain lost the ability to produce a red pigment.

DISCUSSION

Regarding the "Constant sub-culturing" preservation, in the experimental conditions all the strains have remained viable. However, previous studies shows that, in the long-term, the loss of viability usually occurs (Roy et al., 2014). Other disadvantages of this method are: a) spent with culture media; b) culture contamination; c) changes in morphology; and d) strains lose the ability to produce secondary substances (Nakasone et al., 2004).

The methods of preservation under mineral oil and at low temperatures (-20 to -70°C) are described in literature as able to maintain the viability of fungal cultures for longer periods, more than 10 years (Braz et al., 2009). However, the strains subjected to preservation must be able to with stand the extreme physical conditions. In the present study the culture of the strains *Fusarium* LU6 and *Fusarium* LU11 became unfeasible after submersion in oil and freezing, respectively. Smith and Onions (1983) reported on the effectiveness of the method of preservation in mineral oil, they assessed preserved cultures and 47 of the 58 have remained viable after 32 years.

Although viability was maintained for all samples tested in this study, the preservation technique for constant sub-culturing causes changes in the macromorphology of a sample. As the mineral oil technique showed the worst results with lower viability, changes in the macro-morphology and contamination. These changes in fungi

macromorphology when preserved for long or short periods have been described in the literature, especially for techniques of constant sub-culturing (Rodrigues et al., 1992; Freitas et al., 2011). Interestingly, described by Roberts et al. (1992) demonstrated that changes in macromorphology such as lost characteristics when kept in constant sub-culturing were recovered after being preserved in distilled water, which is not common.

Universities and research institutes need preservation methods that do not occupy too much space and are inexpensive. The use of low temperatures (-20 and -70°C) with cryoprotectants, primarily glycerol, prevents the formation of ice crystals during freezing, reducing cellular damage caused by their formation (Mata and Pérez-Merlo, 2003). However, even being employed with protective substances, structures in some microorganisms do not support the physical conditions, it happened in the present study and in previous ones (Figueiredo and Pimentel, 1975).

The preservation technique of distilled water has been widely used for maintenance of microorganisms despite having been overcome, in terms of maintenance of viability and genetic changes by lyophilization technique. Studies show that this low cost technique can maintain fungi stored for periods of seven (Burdshall and Dorworth, 1994) to 11 years (Neufeld and Oliveira, 2008) with more than 90% viability and no morphological changes, thus demonstrating the effectiveness of this conservation method. Among the techniques tested in this study, the method of preservation in distilled water was more effective for endophytic fungi tested. There is a need for further studies with a longer preservation and compared to over current methods such as lyophilisation and cryopreservation in liquid nitrogen, and a greater number of samples in order to establish the best preservation criteria for the group of tested microorganisms. The differences in the nature of the microorganism related to the variation in retention time and stress suffered during the reactivation prevent the determination of standard procedure conservation applicable to all microorganisms. This demonstrates the importance of this study for this group of fungi, thus contributing to a better understanding of the conservation methods.

Conflict of Interests

The authors have not declared any conflict of interests.

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