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African Journal of Plant Science

Table of Content: Volume 9 Number 7, July 2015

ARTICLES

Adverse impact of Banana Xanthomonas Wilt on farmers' livelihoods in Eastern and Central Africa
Jackson Nkuba, William Tinzaara, Gertrude Night, Nicholas Niko, Wellington Jogo, Innocent Ndyetabula, Leornard Mukandala, Privat Ndayihazamaso, Celestin Niyongere, Svetlana Gaidashova, Ivan Rwomushana, Fina Opio and Eldad Karamura

Phytochemical evaluation of various parts of *Dracaena arborea* Link. and *Dracaena mannii* Bak.
Chinyere V. Ilodibia, Rachael U. Ugwu, C. U. Okeke, Ebele E. Akachukwu and Chinelo A. Ezeabara

Assessing the genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] accessions from Sudan using simple sequence repeat (SSR) markers
Ali Z. B., YAO K. N., Odeny D. A., Kyalo M., Skilton R. and Eltahir I. M.

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African Journal of Plant Science

Full Length Research Paper

Adverse impact of Banana Xanthomonas Wilt on farmers' livelihoods in Eastern and Central Africa

Jackson Nkuba[1]*, William Tinzaara[2], Gertrude Night[3], Nicholas Niko[4], Wellington Jogo[2], Innocent Ndyetabula[1], Leornard Mukandala[1], Privat Ndayihazamaso[4], Celestin Niyongere[4], Svetlana Gaidashova[3], Ivan Rwomushana[5], Fina Opio[5] and Eldad Karamura[2]

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Banana is a key crop in the livelihoods of many people in the Great Lakes region of East and Central Africa. For more than a decade now, the crop has been threatened by Banana Xanthomonas Wilt (BXW) which has spread throughout the region but at different rates. The disease attacks all banana cultivars and can cause up to 100% yield losses at farm level if effective control measures are not put in place. However, limited information on impact of BXW at regional level is available to guide interventions. Thus, this study assessed the impact of BXW on farmers' livelihoods in Kagera basin of Tanzania, Burundi and Rwanda. A total of 436 households (Tanzania 120, Burundi 208 and Rwanda 108) mostly from major banana-producing and BXW-affected districts were sampled and interviewed in a household survey. Thirty-three to seventy-five of the total banana mats per farm in the three countries were infected with BXW. Banana production losses caused by BXW were valued at US$ 10.2 million and US$ 2.95 million in Tanzania and Rwanda, respectively, banana sales by farmers dropped by 35% while bunch prices unpredictably doubled. Since banana is a key component of these farming communities, the banana production losses resulted in significant reduction in household food security and incomes. To cope with these challenges, most households are diversifying into other food crops such as maize, cassava and sweet potatoes. This poses a number of socio-economic and biological implications that require further investigation.

Key words: Banana Xanthomonas Wilt incidence, economic loss, food security.

INTRODUCTION

Banana is an important crop for the livelihoods of people in the Great Lakes region of East and Central Africa (ECA) including Burundi, Rwanda, eastern Democratic Republic of Congo (DR Congo), Uganda, western Kenya

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and north western Tanzania (Sharrock and Frison, 1999). In this region, annual per capita consumption of banana ranges from 400-600 kg, the highest in the world (Kalyebara et al., 2006). Currently, the livelihoods of banana farmers in these areas are threatened by Banana Xanthomonas Wilt (BXW) disease. The disease was first reported in the region in 2001 in Uganda and DR Congo (Tushemereirwe et al*.,* 2003), 2002 in Rwanda, 2005 in Tanzania and Kenya (Karamura et al*.,* 2005), and 2010 in Burundi (Niko et al*.,* 2011). Since the outbreak of the disease in ECA region, a number of efforts to elucidate both pathogenesis and epidemics have been undertaken in order to develop and fine tune management practices (Biruma et al., 2007; Karamura and Tinzaara, 2009).

All banana germplasm in ECA, including endemic highland cooking and brewing cultivars (AAA-EA), exotic brewing, dessert and roasting types (AB, AAA, AAB, ABB), and hybrids, are susceptible to the disease. Some ABB cultivars, for example, 'Pisang awak, are particularly susceptible to insect vector transmission and are believed to facilitate the rapid spread of the disease (Tushemereirwe et al., 2003). Some cultivars such as Dwarf Cavendish (AAA desert) which has persistent male bracts escape insect transmission. Unlike other diseases which cause gradually increasing losses over years, the impact of BXW is both extreme and rapid. The economic impact of BXW is due to death of the whole mat that would otherwise contribute to the ratoon plant production cycles. This disease has similarities to other bacterial wilts of banana, caused by *Ralstonia solanacearum* (Thwaites et al., 2000). Once these pathogens have become established, disease control is very difficult (Eden-Green, 2004).

In Uganda, at the height of epidemics (between 2001 and 2004), 33% of the total banana mats were infected with BXW in four heavily affected districts (Karamura et al., 2010). Total banana yield losses due to BXW infection were estimated at 10-17% per year during this period (Kalyebala et al., 2006). In DR Congo a study by Mwangi et al. (2006) estimated household income losses of about US$ 1500 per year. However, to the best of our knowledge, the few BXW socio-economic studies that have been done to date did not fully quantify the economic losses and BXW effects on household food security and incomes in the other EAC countries of Tanzania, Rwanda and Burundi which have been also greatly affected by BXW disease in banana production. Quantifying the economic losses due to BXW is critical for generating evidence to support policy advocacy for investment of resources by stakeholders (government, private sector, research and extension services) in managing the disease.

The overall objective of this study, therefore, was to assess the socio-economic impact of BXW disease on livelihoods of people of Burundi, Rwanda and Tanzania. Specifically, the study aimed at (i) assessing the spread of BXW disease, (ii) quantifying the economic loss due to BXW disease, and (iii) assessing the impact on household food security due to BXW disease in different banana farming communities.

METHODOLOGY

Household surveys between 2009 - 2011 was conducted in seven districts of Kagera region of Tanzania, sixteen provinces of Burundi and twelve districts of Rwanda for comparison between different communities. Selection of the sites was based on banana production potential, and incidence and intensity of BXW. A total of 120 households were purposely sampled in Kagera region, 208 households in Burundi and 108 households in Rwanda. A common structured household questionnaire was administered through face-to-face interviews. Data were collected on banana production constraints, number of banana plants (mats) affected by BXW, number of banana plants uprooted after being affected by BXW, banana production, consumption, banana sales before and after BXW attack, food security status and coping mechanism in relation to BXW and post-harvest products produced. Secondary data was obtained from local government offices in districts or provincial offices of each country. Data analysis was done using SPSS statistical software. Descriptive analysis of means, cross tabulations and variances were used to examine differences in various factors between BXW affected and unaffected farm households. The extrapolation method was used to compute the estimated banana economic losses resulting from BXW effect. Where applicable, a student's-test at 0.05 level of significance was performed to examine differences between variables such as before and after BXW outbreak, and between well and poorly managed banana farms.

RESULTS AND DISCUSSION

Ranking of BXW versus other banana production constraints

Farmers' ranking of banana production constraints in Burundi, Rwanda and Tanzania show that BXW disease was ranked first by most farmers (Figure 1). The importance of other banana constraints greatly varied between countries. Banana weevils (*Cosmopolites sordidus*), nematodes (including *Radopholus similis*, *Helicotylenchus multicinctus* and *Pratylenchus goodeyi*), Fusarium wilt (*Fusarium oxysporum cv cubence*) and Black Sigatoka (*Mycosphaerella fijiensis*) followed in that order for Tanzania.

In Rwanda, the second banana production constraint was Fusarium wilt followed by Black Sigatoka and Banana Bunchy Top Virus (BBTV) while in Burundi the second constraint was Fusarium wilt, BBTV, banana weevils and nematodes. The high ranking of BXW is probably due to the high yield losses the disease causes (up to 100 percent loss) if not controlled early enough (Karamura et al., 2010). This implies that for any banana intervention or investment, the control of BXW should be given first priority.

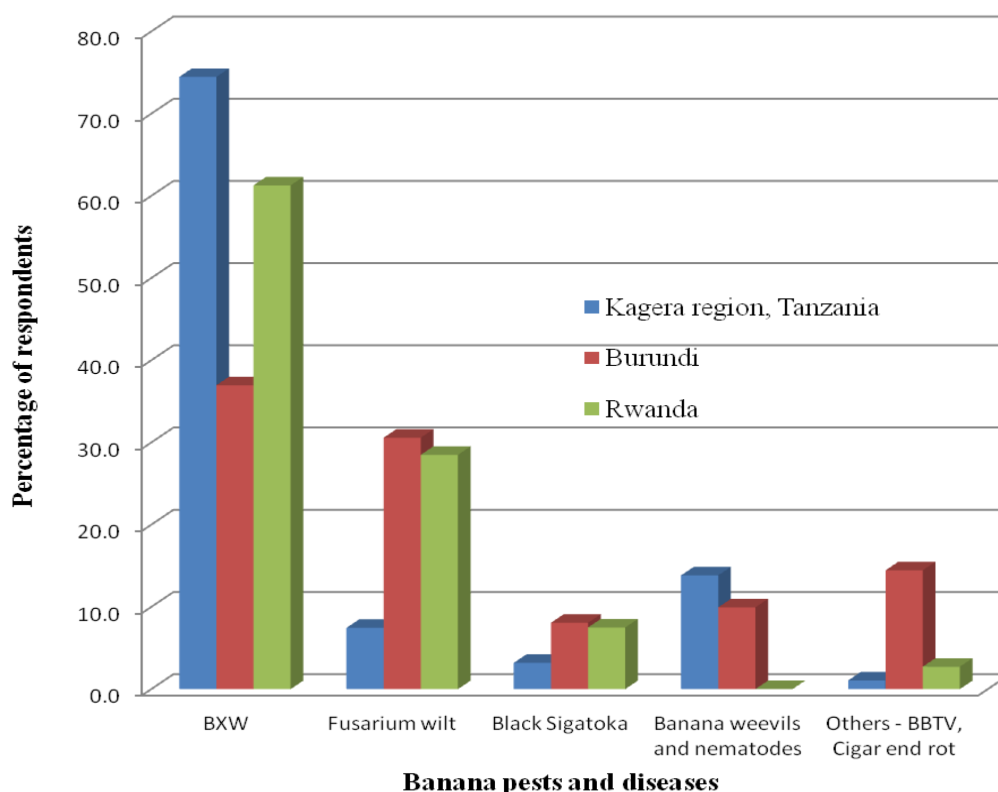

Figure 1. Farmers perceptions on importance of BXW compared with other banana pests and diseases.

BXW spread and banana management

The BXW spread was differently experienced between countries. In Kagera region of Tanzania, a total of 20,735 banana-growing households were reported by agricultural district departments to have farms infected with BXW disease in 2011. This was equivalent to 44% BXW incidence of the total villages found in that region (Table 1). About 30 banana mats per household were affected by BXW disease (Table 2). The highest number of affected farms was in Bukoba district (11,876) followed by Muleba district (6,035). However, Karagwe district had the highest percentage (73%) of villages affected by the disease. Biharamulo district was the only district that managed to control the disease after its outbreak in two wards in 2008.

BXW was first reported in Rwanda in 2005 (Reeder et al., 2007) in the district of Rubavu, Western Province, but farmers reported symptoms as having appeared in their fields in 2003. Percentage of banana fields with BXW was highest in Rutsiro (89%) and lowest in Gakenke, Kayonza and Ruhango (11%). The disease was not found in Kicukiro and in Ngoma. On average, the BXW incidence was highest in Rutsiro (35.9%) and lowest in Kayonza (1.1%). In Burundi, BXW disease was first confirmed in November 2010 in 11 of the 16 provinces of the country with a mean incidence of 25.5% of banana households. On average, 33% of the total banana mats per household were infected with BXW disease.

The incidence of BXW was slightly different between poorly and well managed fields. About 56 and 44% of poorly and well-managed farms, respectively were affected by BXW disease. However, the chances of BXW infection were not significantly different between well-managed and poorly-managed banana farms ($P < 0.05$). Likewise, the incidence of BXW was not significantly different between age categories of banana fields. About 80% of banana fields were more than 20 years old. This implies that farmers' knowledge on BXW spread mechanisms, symptoms and control options were almost the same between farmers with well and poorly managed fields as well as between farm age categories. Therefore, implementation of interventions to control the BXW disease should be equally imparted to all farmers with well and poor managed banana fields. The previous BXW awareness campaigns conducted through conventional extension approaches created good awareness to banana communities (Karamura and Tinzaara, 2009). In

Table 1. BXW incidences in Kagera region, June 2011.

District	Total number of villages	Villages affected with BXW (%)
Bukoba	92	68
Muleba	161	44
Missenyi	74	51
Karagwe	117	73
Biharamulo	74	0
Ngara	68	2
Total	586	44

Table 2. Number of banana mats affected by BXW disease per household in Kagera June 2011.

District	No. of mats affected by BXW	No. of households affected by BXW	Average number of banana mats affected by BXW per household
Bukoba	58,883	11,876	5
Muleba	67,200	6,035	11
Misenyi	10,920	650	17
Karagwe	460,086	2,134	217
Biharamulo	0	0	-
Ngara	17,776	80	222
Total	614,865	20,735	30

some cases, awareness on BXW management by farmers was high but adoption of control measures on affected farms was very low. For instance, in Tanzania and Rwanda where 92.5 and 60.8% of the farmers were knowledgeable on early de-budding of male flower by forked sticks, only 13 and 47% of them practiced the measures, respectively. This calls for strengthening jointly efforts and promotion of management technologies through participatory dissemination approaches accompanied by by-law enforcement of crop disease control by communities and local government authorities.

In Kagera, the proportion of BXW infected mats per household ranged from a single diseased mat to a whole banana field. The majority of farmers had proportion infection between 1 and 30% of their fields. Karagwe had the highest number of infected mats per household followed by Muleba, Bukoba, Ngara and then Missenyi. The average number of banana mats affected by BXW per household ranged from 5 to 222 with an overall average of 30 mats (Table 2). The difference could be attributed to differences in adoption rates of control measures among communities caused by differences in levels of awareness creation activities, importance of banana crop in the respective communities and level of by-law enforcement. Karagwe farmers were less aware of the disease symptoms, spread mechanisms and the recommended BXW control measures as compared to other districts. Farmers' knowledge on BXW management and awareness creation needs to be improved. The mechanisms of spread of BXW are mainly by insects such as bees, working tools, water runoff, grazing livestock and through planting effected planting materials (Karamura et al., 2005; Tushemereirwe et al., 2006). Therefore, based on the nature of the spread of the disease, community or participatory approaches should be emphasised in creating awareness of the disease and its management. Where creation of awareness on BXW and its control measures are done, then the application by farmers can be strengthened by the respective community organs or leadership.

Estimation of economic losses caused by BXW disease

Number of banana bunches harvested before and after BXW was significantly different in all countries ($P < 0.05$). Similarly, banana bunches per household sold and consumed before and after BXW were significantly different (Table 3). For instance, in Kagera region, there were enormous decline in banana products produced per month per household after BXW (Table 3). A decline was recorded in production of banana juice and beer by 62 and 64% after banana succumbed to BXW disease,

Table 3. Banana bunches harvested and products before and after BXW.

Products produced per household per month	Before BXW outbreak	After BXW outbreak	% Change (decline)
Banana juice (litres)	99.0*	37.2*	62
Banana beer (litres)	153.0*	54.7*	64
Number of bunches sold	9.4*	6.1*	35
Number of bunches consumed	11.0*	8.3*	25

*Significant at 0.05 level.

Table 4. Estimated banana economic loss caused by BXW in Kagera region in 2012.

District	No. of mats affected by BXW	Economic loss(TSh)	Economic loss(US$)
Bukoba	58,883	1,472,075,000	981,383
Muleba	67,200	1,680,000,000	1,120,000
Misenyi	10,920	273,000,000	182,000
Karagwe	460,086	11,502,150,000	7,668,100
Biharamulo	0	-	-
Ngara	17,776	444,400,000	296,267
Total	614,865	15,371,625,000	10,247,750

Assumption: One banana mat can produce 2.5 bunches per year and recovering period is 2 years.

respectively. Also, a dramatic decline in number of banana bunches sold and consumed per household was reduced by 35 and 25%, respectively (Table 3). In addition, banana price per bunch increased from an average of US$ 3.30 in 2007 before the BXW became a threat problem to US$ 4.80 during BXW peak in 2009, an equivalent increase of 46% of banana farm gate price.

BXW disease has negatively affected banana production and consequently the income accrued from banana sales dramatically dropped. It is estimated that a total banana economic loss of US$ 14.05 million was caused by BXW disease in the three countries of Tanzania, Rwanda and Burundi by 2012. The estimated monetary losses as a result of BXW infection in different districts of Kagera region are presented in Table 4. The total monetary loss due to BXW disease was about US$ 10.2 million (equivalent to 3 - 5% of the total banana value in Kagera region) excluding the recovery costs. Karagwe district had a huge loss accounting for over US$7.5 million. Similarly, at farm level in Burundi, harvest losses per household ranged from 65 to 99% in the BXW victim households in the three counties. Production losses of cooking banana which is a key to food security were 80%. Losses in beer and dessert types grown mainly for income ranged from 73 to 87%. In all the three countries, farmers cope with multiple strategies including increased cultivation of other crops such as maize, cassava and sweet potatoes and rearing animals.

In Rwanda, the BXW affected area of banana production in 2007 was estimated to be 433 ha of bananas with an economic loss of USD 638,675. In 2009, the estimated area affected by BXW was 2,000 hectares, that is, about five times more in period of two years which is equivalent to economic losses of US$ 2.95 million. Banana prices at farm gates dramatically increased by 33 to 100%. In Rwanda, price per bunch ranged from US$ 0.4 to US$ 4.00 before BXW, but they ranged from US$ 0.53 to US$ 8.00 during BXW peak period.

Banana fields in Burundi were affected by BXW much later as compared to the other two countries. In this country, it was estimated that on average, 33% of the total banana mats per household were infected by BXW since first reported in November 2010. In all areas, BXW rapidly reduced banana yields between 65 and 99%. The total banana economic loss caused by BXW outbreak in Burundi was estimated at US$ 0.9 million. About 8% of BXW affected households abandoned completely banana production.

Effect of BXW on household food security and dietary intake

Household food security was heavily affected by the outbreak of BXW in these countries. Usually, over 50% of the household diet comes from bananas (Table 5) in Kagera region. Likewise, banana contributes substantially to household diets in Rwanda with 32% of the households having banana contributing more than 50% to their diets (Table 5). The outbreak of BXW affected

Table 5. Contribution of bananas to household diet and coping mechanisms.

Criteria		Percentage	
		Tanzania (Kagera region)	Rwanda
(a) Contribution of banana to a household diet			
(0)	No contribution	8	10
(1)	less than 25%	14	32
(2)	between 26% and 50%	28	25
(3)	More than 50%	50	33
(b) Number of days in a week consume bananas either raw or cooked:			
(0)	We don't consume banana or plantain	8	-
(1)	< 2 days in a week	18	-
(2)	3-5 days in a week	53	-
(3)	Every day of the week	20	-
(c) Effect of outbreak of BXW to household diet:			
(0)	Not at all	30	50
(1)	Averagely	28	12
(2)	Affected very much	42	38

about 34 and 44% of the villages in Kagera in 2009 and 2011, respectively. About 70% of the interviewed farmers reported that there was a significant change in their dietary patterns due to the outbreak of BXW disease. Household food security was assessed as per measures developed by Webb et al. (2006) and Maxwell (1996).

About 42 and 38% of respondents reported that BXW affected household diet 'very much' in Tanzania and Rwanda, respectively (Table 5). The majority of households in Rwanda (46.1%) had two meals per day while 38.5% had three meals per day. One meal per day was reported by 14.4% of households and only 1% of households reported having four meals per day. For households having only one or two meals per day, the respondents reported either poor farm production (12%), lack of money (22%) or both reasons (66%). Lack of money has implications for access to food and also poor households are unable to access food not grown on their farm. The coping strategies of households whose banana farms were affected by BXW include eating substitute foods such as maize, root and tuber crops, and reduced number of meals and amount of food.

In the communities affected by BXW disease, the percentage of households who were not able to eat the food preferred in all months of the year increased from 14% before the BXW disease reference to 64% after affected by BXW. Thus, there was an increase in the occurrence of failing to get the food preferred by 50% for the households whose banana farms are affected by BXW (Table 6).

The results show that the percentage of households eating a smaller meal than desired increased from 16% before the BXW disease to 59% after banana farms were affected by BXW. The percentage of households who ate fewer meals in a day because there was not enough food increased from 20% before BXW to 50% after BXW infection with slight differences between countries (Table 6).

Farmers' coping mechanisms included consuming other foods such as maize and root and tuber crops (36%), reducing number and size of meals (10%) or both strategies (42%). Banana farmers reported increasing the cultivation of maize, root and tuber crops, and rearing of small ruminant animals. Although selling of household assets in order to buy food preferred was not recorded, in long run, households will be forced if BXW control measures are not adopted.

Conclusion and recommendations

People's livelihoods are at high risk due to outbreak of BXW disease. Up to June 2011, the BXW disease was still spreading in the region due to the fact that a number of farmers and their respective communities are not adhering to the BXW control measures. Banana yield losses caused by BXW disease was 3 - 5% of the total banana production up to 2012. BXW disease rapidly caused a decline in production of banana juice and beer by more than 62%. Amount of banana bunches sold and consumed per household dramatically declined by 35 and 25%, respectively. Banana prices at farm gate dramatically increased by 33 to 100% in all areas affected by BXW. This poses a big threat to the household food security, income and assets in long term if effective BXW control measures are not in place. Before the outbreak of BXW disease, about 50% of the household diet came from bananas. However, the outbreak of BXW disease

Table 6. Household food security status before, during and after affection of banana farms with BXW disease.

Criteria	Tanzania		Rwanda	
	Before BXW	BXW Peak	Before BXW	BXW Peak
1. Households not able to eat food preferred:				
(i) Percentage	36.7	64.2	14	56.3
Frequency of happening:				
(i) Rarely	47.7	24.6	71.4	35.4
(ii) Often	52.3	75.4	28.6	64.6
Reasons for household members not able to eat food preferred:				
(i) Lack of crop variety in farm	56.8	10.4		
(ii) Lack of enough farm produce	11.5	44.1		
(iii) Lack of money	9.0	2.6		
(iv) All the above	22.7	42.8		
2. Households eating a smaller meal than needed amount:				
(i) Percentage	15.8	47.5	20.0	58.9
(b) Frequency of happening:				
(i) Rarely	68.1	38.9	63.4	37.4
(ii) Often	31.9	61.1	36.4	62.8
3. Household members eating fewer meals in a day because there was no food:				
(i) Households eaten fewer meals	20.0	44.2	29.2	50.0
(b) Frequency of happening:				
(i) Rarely	41.5	26.5	56.0	23.8
(ii) Sometimes	12.5	13.1	8.0	16.0
(iii) Often	46.0	60.4	36.0	59.5

has affected the household dietary by 70%. As coping mechanisms, the food deficit caused by BXW effect is supplemented by other food crops such as maize, cassava and sweet potatoes, which has many implications in many socio-economic and biological aspects not investigated by this study. Therefore, sustainable joint efforts from all stakeholders are needed to rescue banana dependent families in the ECA region including by-law enforcement on BXW control measures by each community. In short-medium term, consumption of other foods as a coping strategy suggests that other crops should be promoted to mitigate the effects of BXW. However, clean banana planting material should also be availed to farmers who wish to replant banana after complete destruction of banana mats or entire fields.

Conflict of Interest

The authors did not declare any conflict of interest.

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Full Length Research Paper

Phytochemical evaluation of various parts of *Dracaena arborea* Link. and *Dracaena mannii* Bak.

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Phytochemical evaluation of leaves, stems and roots of *Dracaena arborea* (Link) and *Dracaena mannii* (Bak) present in southeastern Nigeria was carried out, to determine their taxonomical data with regards to their phytochemicals contents (flavonoid, saponin, tannin, cyanide, lectin, phytate and calcium oxalate) using standard methods. The results show varying quantities of the phytochemicals in the leaves, stems and roots of the two *Dracaena* species with some parts lacking some of the phytochemicals. The highest quantity of the phytochemicals was contained in the leaves of both species when compared to other parts respectively. The result also revealed no significant statistical difference in the phytochemistry of the two *Dracaena* species. The implication is that the two species are closely related and this justified their placement in the same genus *Dracaena* while the slight differences between them support their separation into different species. The result also indicated that the two species could be used in ethnomedicine for the treatment of diseases. In addition, these parts could be the possible sources of these phytochemicals.

Key words: *Dracaena arborea, Dracaena mannii*, phytochemicals.

INTRODUCTION

Dracaena consists of about 40 species (Waterhouse, 1987; Venter, 1996), and to Huxley (1992), it consists of 50 species. Sharma (1993) and Dutta (2003) described it as a genus of about 150 species. The genus was first described by Linnaeus in 1767. Some species of *Dracaena* include *Dracaena fragrans, D. surculosa, D. draco, D. marginata, D. arborea, D. goldiana, D.sanderina, D. deremensis, D. reflexa, D. mannii* etc. *Dracaenas* are either shrubs or trees and are divided into two broad groups based on their growth habits- tree *Dracaenas* and shrubby *Dracaenas*. Tree *Dracaena*s include *Dracaena americana* (Central American dragon tree), *D. draco* (Canary Islands draco tree), *D. marginata, Dracaena mannii* etc. while shrubby *Dracaenas* include *D. aletriformis, D. bicolor, D. cincta, D. concinna*, etc.(Waterhouse, 1987). *Dracaenas* are used as ornamentals, medicinal plants, in photo engraving, in research, as hedge plants, colourants, etc. In Europe and Canada, they are cultivated

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Figure 1. (a) *D. mannii*. **(b)** *D. arborea.*

and sold as ornamentals, (Huxley, 1992). *Dracaena arborea* Link. and *Dracaena mannii* Bak. which are commonly found in South eastern Nigeria are the points of interest in this research.

To classify plants, taxonomists make use of morphology, phylogeny, physiology, phytochemistry, anatomy, cytology, palynology etc. as taxonomic lines of evidence to determine their similarities and differences in order to group them into various taxa. Any data which show differences from species to species are of taxonomic significance and thus constitute part of the information or evidences which may be used by taxonomists (Stace, 1980). Phytochemicals are used in determining the relationship among taxa of different categories. Some of the major classes of the chemical evidence include flavonoids, alkaloids, amino acids, fatty acids, carotenoids, aromatic compounds etc (Sharma, 1993). Cronquist (1981) cited the following examples to indicate the use of chemistry in solving taxonomic problems: Caryophyllales produce betalain and not anthocynins; Polyoniales produce anthocynins and not betaains; Juglandales are aromatic plants while Fagales are non-aromatic; highly aromatic compounds are found in Lamiaceae; alkaloids are very common in Solanaceae; Sapindaceae have plenty of tannins.

Determination of these differences and similarities with regards to phytochemistry of the two species based on the outcome of the study were the objectives of this research.

MATERIALS AND METHODS

Sources of materials

Leaves, stems, fruits and roots, of *Dracaena* species were collected from Nsukka town (N06°.86. 43.5 and E07°.42.56.0) in Nsukka, Nsukka Local Government Area Enugu State, Nigeria.

The *Dracaena* species (Figure 1) were authenticated at Bio-diversity Development and conservation, Nsukka, where the voucher specimens were deposited.

Phytochemical procedure

Seven phytochemicals were examined. They included cyanide, phytate, lectin, alkaloids, flavonoids, saponins and calcium oxalate crystals.

Preparation of plant materials for phytochemicals analysis

Fresh leaves, stems and roots of *D. mannii* and *D. arborea* were washed and blended with an electric blender. 250 g of each of the ground samples were soaked in 200 ml of water for 24 h. They

were then filtered with cheese cloth. The extract (50% yields) were concentrated by means of rotary evaporator, and subjected to tests.

Qualitative test was conducted first to determine the presence or absence of these phytochemicals. This was done using standard procedure as describe by Harborne (1973).

Quantitative phytochemicals analysis

Similarly, quantitative test was carried out using standard procedures.

Determination of cyanide

1 ml of each of the sample extract was transferred into different test tubes; 4 ml alkaline picrate was added into each and allowed to stand for 5 min. Blank containing distilled water, and a standard were prepared and the absorbance read at 490 nM with spectro-photometer. This is in accordance with the method of Onwuka, (2005).

Determination of calcium oxalate

Following the method of Pearson (1978), 10 ml of each extract was transferred to100 ml flasks and 30 ml diethyl ether added into each flask. The pH of each filtrate was adjusted to 7.0 with NH_4OH. 0.1 M KMnO4 was titrated against each filtrate, noting the initial and final volumes of $KMnO_4$.

Determination of alkaloid

In accordance with the method of Harborne (1973), 20 ml of each of the extract was concentrated by heating over a water bath to one quarter of the original volume. NH_4OH solution was added drop wise until precipitation is completed and allowed to settle. The precipitates were collected and washed with dilute NH_4OH solution and then filtered. The residues were weighed and reported as the crude alkaloid.

Determination of saponin

According to the method outlined by Obadoni and Ochuko (2001), 10ml of each extract was transferred in 250 ml separator funnels and washed with 20 ml diethyl ether. Two layers were separated in each- the aqueous layer and the ether layer. The aqueous layers were recovered and the ether layers discarded. The purification process was repeated. 60 ml of n-butanol was added into the extracts and the extracts washed twice with 10ml of 5% aqueous NaCl. The remaining solutions were heated over a water bath. After evaporation, the samples were dried in already weighed beakers in an oven to constant weights. The final weights were obtained and the saponin calculated in percentages.

Determination of flavonoid

Following the procedure of Boham and Kocipa (1974), 100 ml 0f each extract was filtered using Whatman filter papers number A_1, B_1, C_1, A_2, B_2, C_2 and the filtrates were transferred into weighed crucibles and evaporated to dryness over a water bath. Each crucible was re-weighed and percentage flavonoid calculated.

Determination of lectin

In accordance with the method of Harborne (1973), to 1 ml of each extract diluents in a test tube, 1 ml of heparinized rabbit blood was added. A blank of red blood cells and normal saline was prepared and the extracts in the test tubes were allowed to stand for 4 h at room temperature. 1 ml of normal saline was added to all the tubes and allowed to stand for 10 min after which the absorbance was read at 620 nM using a spectrophotometer. The test tube containing only the blood cells and normal saline served as the blank. The lectin units were determined using the equation: Letin unit/mg= (b-a) x F; where b = absorbance of test sample solution; a= absorbance of blank; F= experimental factor given by F= (1/w x vf/va) D where w= weight of sample; vf= total volume of extract; va= volume of extract used in the assay; and D= dilution factor (if any).

Determination of phytate

Using the procedure according to the Oberlease (1973), 0.5 ml of the extracts each was pipetted into 4 test tubes fitted with ground glass stoppers. 1 ml of 0.2 g NH_4+Fe (111) sulphate. $12H_2O$ in 100 ml and 2 N HCl made up to 100 ml were added to each and fixed with clips. The tubes were heated in a boiling water bath for 30min. taking care that the tube remained well stoppered the first 5 min. They were cooled in ice water for 15 min and allowed to adjust to room temperature. The content of each tube was mixed and centrifuged for 30 min at 3000 r.p.m. 1 ml of each supernatant was transferred to 4 test tubes and 1.5 ml of solution 3 (10 g of 2,2-dipyridine + 10 ml thioglycollic acid in distilled water made up to 100ml) was added. The absorbance was read at 519 nM using spectrophotometer against the blank.

Statistical procedure

The results were analyzed using t-test and results were presented in mean±SD.

RESULTS

Table 1 shows the presence or absence of the seven phytochemicals in the parts tested.

Flavonoid, cyanide and saponin were present in all the parts tested in both plants. Calcium oxalate was lacking in the leaf and root of *D. mannii* and root of *D. arborea*. Alkaloid was lacking in the stem and root of *D. mannii* and in root of *D. arborea* while phytate was absent from the roots of both *Dracaenas* species. Lectin was absent from the leaves of *D. arborea* and from stem and root of *D. mannii.*

Table 2 shows varying quantities of the seven phytochemicals in the leaves, stems and roots of the two *Dracaena* species with some parts lacking the phyto-chemicals. *D. arborea* leaves contained the highest percentage of calcium oxalate crystals while *D. mannii* leaf contained the highest percentage of lectin, flavonoid and phytate. The root of *D. arborea* lacked calcium oxalate, alkaloid and phytate while the root of *D. mannii* lacked calcium oxalate, alkaloid, phytate as well as lectin. The leaf of *D. mannii* lacked calcium oxalate while that of *D. arborea* lacked lectin. Both *D. mannii* and *D. arborea* were low in cyanide content in all the parts tested. The

Table 1. Qualitative phytochemical content of the leaf, stem and roots *of D. arborea* and *D. mannii*

Phytochemicals	*D. arborea*			*D. mannii*		
	Leaf	Stem	Root	Stem	Leaf	Root
Calcium oxalate	+	+	-	+	-	-
Flavonoid	+	+	+	+	+	+
Saponin	+	+	+	+	+	+
Alkaloid	+	+	-	-	+	-
Lectin	-	+	+	-	+	-
Phytate	+	+	-	+	+	+
Cyanide	+	+	+	+	+	+

+, = presence; =, absence.

Table 2. Quantitative phytochemical content of the leaf, stem and roots of *D. arborea* and *D. mannii.*

Phytochemicals	*D. arborea*			*D. mannii*		
	Leaf	Stem	Root	Leaf	Stem	Root
Calcium oxalate	6.25±0.01	2.44+0.34	0.00 -0.00	0.00+ 0.00	2.50- 0.53	0.00 +0.04
Flavonoid	3.87±0.20	4.14±0.82	2.20±0.25	4.50± 0.32	2.00±0.25	1.10±0.15
Saponin	1.18±0.10	0.48±0.13	0.15±0.14	1.00± 0.31	1.00±0.32	0.50 ±0.37
Alkaloid	0.53±0.10	2.08±0.54	0.00±0.00	0.50±0.42	0.00±0.00	0.00 ±0.00
Lectin	0.00±0.00	10.02±0.20	0.18±0.26	170.00±0.25	0.00±0.00	0.00 ±0.00
Phytate	17.95±0.30	11.30±0.50	0.00±0.00	90.00±0.14	22.20±0.19	0.00±0.00
Cyanide	0.12±0.10	0.10±0.10	1.20±0.15	0.16 ± 0.66	0.10±0.25	1.50 ±0.27

same percentage of saponin was found in the leaf and stem of *D. mannii* and in the leaf of *D. arborea* while the leaf of *D. arborea* and the stem of *D. mannii* contained the same percentage of cyanide.

DISCUSSION

The results of the study showed varying quantities of the seven phytochemicals in the leaves, stems and roots of the two *Dracaena* species with some parts lacking some of the phytochemicals (Table 1*). D. arborea* leaf contained the highest percentage of calcium oxalate crystals (6.25± 0.01) while *D. mannii* leaf contained the highest percentage of lectin (170 0.25), flavonoid (4.50 0.32) and phytate (90 0.14), respectively (Table 1). The root of *D. arborea* lacked calcium oxalate, alkaloid, and phytate while the root of *D. mannii* lacked calcium oxalate, alkaloid, phytate as well as lectin. Both species were low in cyanide content in all the parts tested. The same percentage of saponin was found in the leaf and stem of *D. mannii* and in the leaf of *D. arborea* while the leaf of *D. arborea* and the stem of *D. mannii* contained the same percentage of cyanide.

None of the two species lacked entirely all the seven phyt ochemicals in all the parts tested this is in agreement with the result of Watson and Dallwitz (1992) in which they stated that *Dracaena*s are non cyanogenic and lack alkaloids. However, the leaves, stems and roots of both species tested positive for flavonoid and saponin which is in consonance with their report that *Dracaenas* contain flavonoid and saponin. Also, Pennisi and McConnel (2004) reported the presence of cuticular deposits of calcium oxalate crystals in 14 species of *Dracaena* they studied. This was also reported in this research in the leaf and stem of *D. arborea* and in the stem of *D. mannii*. These phytochemicals are known to have medicinal properties.

Flavonoids are phytochemical compounds which are widely distributed in all the vascular plants, (Harborne, 1973). They have antioxidant activity; some of the activity attributed to flavonoid includes anti-allergic, anti-cancer, anti-oxidant, anti-inflammatory and anti-viral. Flavonoid functions in defense against herbivores, management of diseases such as malaria, diabetes and hypertension, (Judd et al., 1999; Thompson, 1994).

Alkaloids are structurally diverse and are derived from different amino acids or mevalonic acids by various biosynthetic pathways (Robinson, 1981). They have an intensively bitter taste and many are extremely poisonous (Dutta, 2004). The amazing effect of these alkaloids on human has led to the development of pain-killer medication,

spiritual drugs and serious additions by those who are ignorant of the properties of the powerful chemical (Harborne et al., 1973). They are physiologically active in animals, usually even at very low concentrations, and many are widely used in medicine (for example cocaine, morphine, atropine, colchicines, quinine, and strychnine).

Saponins are glycosides with distinctive foaming characteristics (Judd et al., 1999). They are natural detergents found in many plants (Ajali, 2004). Saponins have both antibacterial and antifungal properties and are used extensively in cosmetics, such as lipsticks and shampoo.

Calcium oxalate is a phytochemical compound that forms niddle-shaped crystals. It is found in large quantity in the poisonous plant, dumb cane (*dieffenbachia*). It is also found in various species of Oxalis, Araceae and Agavaceae (Fabricant and Farnsworth, 2001). According to them, calcium oxalate is poisonous when ingested and even a small dose of it is enough to cause intense sensations of burning in the mouth and throat, swelling, and choking. In large doses, it causes severe digestive upset, breathing difficulties and if enough is consumed, convulsion, coma and death. Recovery from severe calcium oxalate poisoning is possible, but permanent liver and kidney damage may have occurred.

Phytate also known as phytic acid or inosytol hexaphosphate (IP6) is the storage form of phosphorus in many plant tissues. Phosphorus in this form is generally not bio-available to humans because it lacks the digestive enzyme, phytase required to separate phosphorus from the phytate molecule (Fabricant and Farnsworth, 2001). Recent studies have indicated that phytic acid may have some preventive effect in prostrate, breast, pancreatic and colon cancer (Fabricant and Farnsworth, 2001).

Lectins are glycoproteins of 60,000-000 MW that are known for their ability to agglutinate erythrocytes in vitro and are of therapeutic use against HIV-1 (Robinson, 1981). Jacalin, a plant lectin has been found to completely block human immuno deficiency virus type 1 in vitro infection of lymphoid cells. This activity of jacalin is attributed to its ability to specifically induce the proliferation of $CD4^+$ T lymphocytes in human. Lectins could also be potential use in cancer treatment strategies due to the fact that lectin present on the surface of tumour cell are capable of binding exogenous carbohydrate-containing molecules and internalize them by endocytosis (Robinson, 1981).

Cyanogenic glycosides are poisonous and have been implicated for the death of some domestic animals for example the ornamental *Euchalyptus* (Dubrovsky, 2005). Cyanogenic glycosides in plants yield free hydrocyanic acid otherwise known as prussic acid glycosides, when hydrolyzed by ß-glycosides or when other plant cell structure is disrupted or damaged, for example by freezing, chopping, or chewing. Microbial action in the rumen can further release free cyanide (Greenwood, 1989). Ruminants are more susceptible than monogastric animals, and cattle slightly more so than sheep (Dubrovsky, 2005).

Conclusion

The result shows that there is no significant difference in the phytochemistry of *D. mannii* and *D. arborea.* In other words, the two species are closely related and this justifies their placement in the same genus *Dracaena*. The slight differences existing between them also justify their separation into different species. Also, that, the two species could be used in ethnomedicine for the treatment of diseases such as malaria, diabetes, bacterial and fungal infections and even cancer.

Conflict of interest

The authors have declared that there is no conflict of interest.

Authors' contributions

This work was carried out in collaboration of all authors. Authors RUU and CVI designed the study, carried out the experiment and wrote the first draft of the manuscript. All authors managed the analyses of the study. Author CUO supervised the work. All authors read and approved the final manuscript.

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Full Length Research Paper

Assessing the genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] accessions from Sudan using simple sequence repeat (SSR) markers

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Genetic diversity and phylogenetic relationships among 252 cowpea (*Vigna unguiculata* (L.)Walp) accessions collected throughout the six geographical regions of Sudan were evaluated using simple sequence repeat (SSR) molecular markers. Eighteen (18) published primer pairs were selected based on their informativeness, out of which 16 primer pairs gave reproducible results among all of the cowpea accessions tested. A total of 129 alleles were detected from the 16 loci with an average of 8.1 alleles per locus. Heterozygosity values ranged from 0.01 to 0.13 with an average occurrence of 0.05 while the gene diversity ranged from 0.34 to 0.85 with an average of 0.60. The polymorphism information content (PIC) varied from 0.33 to 0.83 with an average of 0.56. Sudanese Cowpea germplasm clustered into three main groups with control germplasm obtained from the International Institute for Tropical Agriculture (IITA) showing distribution along two groups. This study confirms earlier suggestions that cowpea was first introduced into Sudan from West African countries into western Sudan (Kordofan and Darfur) regions. Accession TVU 8812-IITA Benin was found to be the most divergent cowpea accession within the individuals followed by accession HSD 5738 Sudan-Blue Nile and HSD 6782 Sudan-South Kordofan.

Key words: Simple sequence repeat, microsatellites, genetic diversity, cowpea.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a tropical grain legume widely distributed in sub-Saharan Africa, Asia, Central and South America as well as parts of southern Europe and the United States (Singh et al., 1997). Domestication of cowpea is presumed to have occurred in Africa given the exclusive presence of wild cowpea (Steele, 1976) although knowledge about the general region or regions of origin and number of domestication events within Africa is fragmented (Faris, 1965; Purseglove, 1968; Steele, 1976; Ng and Padulosi, 1988;

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Coulibaly et al., 2002). It is very important, widely adapted, and versatile grain legume of high nutritional value. Cowpea is mainly produced and consumed in Africa, where it provides a major low-cost dietary protein for millions of smallholder farmers and consumers, who cannot afford high protein foods, such as fish and meat. The s eed protein content is reported to range from 23 - 32% of seed weight (Nielson, 1993) and therefore is often referred to as a “poor man’s meat” (Diouf et al., 2005). In many parts of West Africa, cowpea hay is also critical as livestock feed, especially during the dry season (Wests et al., 1982).

Being a legume, cowpea is nitrogen-fixing (Sanginga, 2003) and fits perfectly in the traditional intercropping systems that are common in Africa, especially given its ability to tolerate shade. The total area under cowpea cultivation is more than 12.5 million hectares worldwide, with an annual production of around 4.5 million metric tons (Singh et al., 2002).

Cowpea remains one of the most important summer adapted food grain legumes grown under rained conditions in Sudan. Despite its importance in Sudan, the yields remain extremely low at an average of 0.26 tons/ha FAO STAT (2010). This production is mainly limited by a wide range of biotic and abiotic constraints.

Cowpea is believed to have been introduced into the western regions of Sudan from West Africa from where it spread to other regions. A national effort to conserve Sudanese cowpea collections resulted in the conservation of more than 250 accessions from different agro-ecological zones at the central gene bank in Wad Medani. These cowpea accessions have been morphologically characterized using the International descriptor list for cowpea published by International Board for Plant Genetic Resources (IBPGR). Traditional selection methods in cowpea depended mainly on the observed morphological variations even though morphological characteristics are easily influenced by the environment (Meglic et al., 1996). The genetic diversity information is extremely important, accurate assessment of genetic variability is important for the preservation and utilization of germplasm resources (Huaqiang et al., 2012).

There is an urgent need to undertake more detailed genetic characterization of cowpea germplasm in order to optimally exploit the resources for improved cowpea production in Sudan. Such analysis would also reveal the true origin of Sudanese cowpea germplasm and establish the extent at which the global cowpea collection at IITA would benefit the cowpea breeding programs in Sudan.

Simple sequence repeat (SSR) markers are one of the most frequently used markers in the genetic diversity analysis of cowpea (Li et al., 2001; Ogunkanmi et al., 2008; Lee et al., 2009; Asare et al., 2010; Badiane et al., 2012). The earliest cowpea SSR research is conducted by Li et al. (2001) and 27 SSR primers have been developed. Comparative studies in plants have shown that SSR markers, which are single locus markers with multiple alleles, provide an effective means for discriminating between genotypes (Powell et al., 1996; Li et al., 2001). This study assessed the genetic diversity of 245 Sudanese cowpea accessions alongside 22 global accessions obtained from IITA, Nigeria using SSR markers. The main objectives were to understand the extent of genetic variation and likely origin of Sudanese germplasm as well as create a mini-core collection based on.

MATERIALS AND METHODS

Plant materials

Seeds of 231 Cowpea (*V. unguiculata* L. Walp.) accessions obtained from Plant Genetic Resources Unit of the Agricultural Research Corporation of Sudan representing six different agro-ecological zones of Sudan that is, Northern, River Nile, South Kordofan, North Kordofan, Blue Nile and Bahr Eljabel State (Figure 1) in addition to 36 global cowpea accessions obtained from International Institute of Tropical Agriculture (IITA)-Ibadan-Nigeria (Table 1) were used in the present study. These materials (267 accessions) were planted in the greenhouse of Bioscience Eastern Central Africa BecA-ILRI Hub-Kenya for seedling establishment.15 accessions failed to germinate in the green house; a rest of 252 accessions was successfully grown and used.

DNA extraction

Young leaves sampling were taken eight days after sowing in 1.5 mL Eppendorf tube, frozen immediately in liquid nitrogen and stored in -80°C, then leaves samples were manually grinded using micropestle. Genomic DNA isolated from young seedlings leaves following ZR plant/seed DNA protocol. DNA quality and quantity check done using Nano-drop spectrophotometer and 1% Agarose gel electrophoresis stained with Gel red was used to run the gel. The DNA was normalized by adjusting its concentration to 25 ng μL^{-1} in an optical 96-well Reaction plates using sterile de-ionized water.

Microsatlite amplification

A total of 18 polymorphic SSR markers were used to screen 252 cowpea DNA samples (Table 2). The forward and reverse primers for each of the 18 SSR markers were labeled at the 5´ end of the oligonucleotide using fluorescent dyes to enable detection. PCR reaction were performed in 10 µL final volume in a mixture containing (Tag DNA polymerase1U, dNTPs 1 mM and Reaction buffer 1x) in bulk Polymerase chain reaction (PCR) premix, 5 mM reverse and forward primers, 2 µL DNA, 0.2 µL of 25 mM $MgCl_2$ and 7.2 µL of double distilled water. The optimal annealing temperature varied according to the T_m of the primer pairs and was determined using gradient PCR. For each amplification process, an initial denaturation of DNA at 95°C for 3min was followed by 30 cycles consisting of 30 sec at 94°C, 30 s at 50 to 60°C for annealing temperature (Table 2) 2 min at 72°C extensions a final extension of 15 min at 72°C was performed and the amplification products analyzed on 2% agarose gels in Tris Borate buffer stained with Gel red for visualization to establish polymorphism (Figure 2).

PCR analysis

PCR products of 4 primers with different dyes coloaded together in 96-well working plate vortexes and spined then a sub sample of 1.4

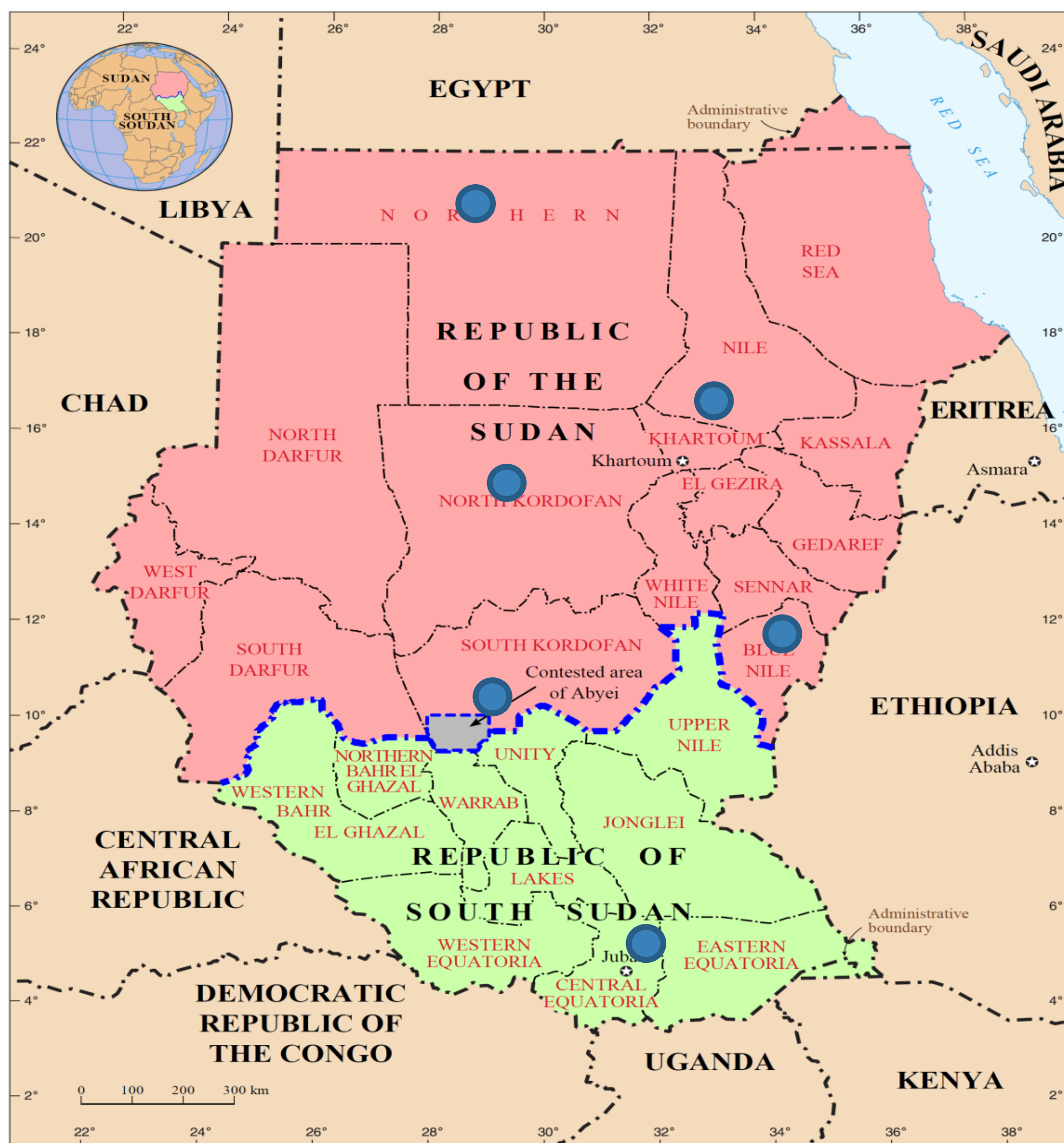

Figure 1. Different ecologica Zones where sample collected.

Table 1. List of cowpea accessions used; names, and origin.

S/N	Accession	Origin	S/N	Accession	Origin
1	HSD 10295	Sudan	127	HSD 5695	Sudan
2	HSD 10297	Sudan	128	HSD 5698	Sudan
3	HSD 10311	Sudan	129	HSD 5699	Sudan
4	HSD 10323	Sudan	130	HSD 5701	Sudan
5	HSD 10355	Sudan	131	HSD 5702	Sudan
6	HSD 10368	Sudan	132	HSD 5703	Sudan
7	HSD 10393	Sudan	133	HSD 4845	Sudan
8	HSD 10394	Sudan	134	HSD 4846	Sudan
9	HSD 10402	Sudan	135	HSD 4847	Sudan
10	HSD 10438	Sudan	136	HSD 4848	Sudan
11	HSD 10439	Sudan	137	HSD 4849	Sudan

Table 1. Contd.

S/N	Accession	Origin	S/N	Accession	Origin
12	HSD 10474	Sudan	138	HSD 4850	Sudan
13	HSD 10491	Sudan	139	HSD 4851	Sudan
14	HSD 10502	Sudan	140	HSD 4853	Sudan
15	HSD 10506	Sudan	141	HSD 4854	Sudan
16	HSD 10513	Sudan	142	HSD 4855	Sudan
17	HSD 10531	Sudan	143	HSD 4856	Sudan
18	HSD 10550	Sudan	144	HSD 4857	Sudan
19	HSD 10551	Sudan	145	HSD 4858	Sudan
20	HSD 10666	Sudan	146	HSD 4859	Sudan
21	HSD 10698	Sudan	147	HSD 4860	Sudan
22	HSD 10699	Sudan	148	HSD 4861	Sudan
23	HSD 1310	IITA	149	HSD 4862	Sudan
24	HSD 1311	IITA	150	HSD 4863	Sudan
25	HSD 1313	IITA	151	HSD 4864	Sudan
26	HSD 1314	IITA	152	HSD 4865	Sudan
27	HSD 2968	Sudan	153	HSD 5130	Sudan
28	HSD 2976	Sudan	154	HSD 5131	Sudan
29	HSD 2979	Sudan	155	HSD 5133	Sudan
30	HSD 2984	Sudan	156	HSD 5134	Sudan
31	HSD 3347	Sudan	157	HSD 5665	Sudan
32	HSD 3586	Sudan	158	HSD 5666	Sudan
33	HSD 3587	Sudan	159	HSD 5668	Sudan
34	HSD 3588	Sudan	160	HSD 5669	Sudan
35	HSD 3589	Sudan	161	HSD 5670	Sudan
36	HSD 3590	Sudan	162	HSD 5672	Sudan
37	HSD 3591	Sudan	163	HSD 5673	Sudan
38	HSD 3592	Sudan	164	HSD 5674	Sudan
39	HSD 3593	Sudan	165	HSD 5675	Sudan
40	HSD 3594	Sudan	166	HSD 5676	Sudan
41	HSD 3595	Sudan	167	HSD 5677	Sudan
42	HSD 3596	Sudan	168	HSD 5704	Sudan
43	HSD 3598	Sudan	169	HSD 5706	Sudan
44	HSD 3600	Sudan	170	HSD 5707	Sudan
45	HSD 3602	Sudan	171	HSD 5708	Sudan
46	HSD 4356	Sudan	172	HSD 5710	Sudan
47	HSD 4357	Sudan	173	HSD 5711	Sudan
48	HSD 4358	Sudan	174	HSD 5724	Sudan
49	HSD 4359	Sudan	175	HSD 5729	Sudan
50	HSD 4360	Sudan	176	HSD 5737	Sudan
51	HSD 4361	Sudan	177	HSD 5738	Sudan
52	HSD 4362	Sudan	178	HSD 5838	Sudan
53	HSD 4363	Sudan	179	HSD 5839	Sudan
54	HSD 4364	Sudan	180	HSD 5840	Sudan
55	HSD 2845	Sudan	181	HSD 5841	Sudan
56	HSD 2846	Sudan	182	HSD 5843	Sudan
57	HSD 2963	Sudan	183	HSD 5844	Sudan
58	HSD 2964	Sudan	184	HSD 5845	Sudan
59	HSD 2966	Sudan	185	HSD 5846	Sudan
60	HSD 2967	Sudan	186	HSD 5847	Sudan
61	HSD 4406	Sudan	187	HSD 5848	Sudan
62	HSD 4410	Sudan	188	HSD 5850	Sudan

Table 1. Contd.

S/N	Accession	Origin	S/N	Accession	Origin
63	HSD 4411	Sudan	189	HSD 5852	Sudan
64	HSD 4412	Sudan	190	HSD 5853	Sudan
65	HSD 4414	Sudan	191	HSD 5854	Sudan
66	HSD 4480	Sudan	192	HSD 5855	Sudan
67	HSD 6782	Sudan	193	HSD 5856	Sudan
68	HSD 4836	Sudan	194	HSD 5858	Sudan
69	HSD 4837	Sudan	195	HSD 5859	Sudan
70	HSD 4838	Sudan	196	HSD 5861	Sudan
71	HSD 4839	Sudan	197	HSD 5862	Sudan
72	HSD 4840	Sudan	198	HSD 5864	Sudan
73	HSD 4842	Sudan	199	HSD 5865	Sudan
74	HSD 4843	Sudan	200	HSD 5678	Sudan
75	HSD 4844	Sudan	201	HSD 5679	Sudan
76	HSD 4373	Sudan	202	HSD 5680	Sudan
77	HSD 4374	Sudan	203	HSD 5681	Sudan
78	HSD 4375	Sudan	204	HSD 5683	Sudan
79	HSD 4376	Sudan	205	HSD 5684	Sudan
80	HSD 4377	Sudan	206	HSD 5685	Sudan
81	HSD 4378	Sudan	207	HSD 5686	Sudan
82	HSD 4379	Sudan	208	HSD 5687	Sudan
83	HSD 4380	Sudan	209	HSD 5688	Sudan
84	HSD 4381	Sudan	210	HSD 5689	Sudan
85	HSD 4382	Sudan	211	HSD 5690	Sudan
86	HSD 4383	Sudan	212	HSD 4568	Sudan
87	HSD 4384	Sudan	213	HSD 6560	Sudan
88	HSD 4385	Sudan	214	HSD 6561	Sudan
89	HSD 4386	Sudan	215	HSD 6612	Sudan
90	HSD 4387	Sudan	216	HSD 6628	Sudan
91	HSD 4388	Sudan	217	HSD 6629	Sudan
92	HSD 4389	Sudan	218	HSD 6659	Sudan
93	HSD 0306	Sudan	219	HSD 6671	Sudan
94	HSD 0311	Sudan	220	HSD 6698	Sudan
95	HSD 0576	Sudan	221	HSD 6706	Sudan
96	HSD 0806	Sudan	222	HSD 6715	Sudan
97	HSD 0836	Sudan	223	HSD 6732	Sudan
98	HSD 10296	Sudan	224	HSD 6744	Sudan
99	HSD 4365	Sudan	225	HSD 6759	Sudan
100	HSD 4366	Sudan	226	HSD 6760	Sudan
101	HSD 4367	Sudan	227	HSD 4370	Sudan
102	HSD 4369	Sudan	228	HSD 1317	IITA
103	HSD 4371	Sudan	229	HSD 1320	IITA
104	HSD 4372	Sudan	230	TVU 8779	Benin
105	HSD 1315	IITA	231	TVU 8812	Benin
106	HSD 1316	IITA	232	TVU 8834	Benin
107	HSD 1318	IITA	233	TVU 10281	Benin
108	HSD 1319	IITA	234	TVU 10754	Benin
109	HSD 1321	IITA	235	TVU 8262	BorkinaFaso
110	HSD 1323	IITA	236	TVU 8516	BorkinaFaso
111	HSD 1324	IITA	237	TVU 9257	BorkinaFaso
112	HSD 2844	Sudan	238	TVU 10835	Cameron
113	HSD 4390	Sudan	239	TVU 8082	Cotedivoire

Table 1. Contd.

S/N	Accession	Origin	S/N	Accession	Origin
114	HSD 4391	Sudan	240	TVU 7325	Ghana
115	HSD 4392	Sudan	241	TVU 7614	Mali
116	HSD 4395	Sudan	242	TVU 7625	Mali
117	HSD 4396	Sudan	243	TVU 7647	Mali
118	HSD 5691	Sudan	244	TVU 10362	Mali
119	HSD 5694	Sudan	245	TVU 467	Mauritania
120	HSD 5696	Sudan	246	TVU 43	Nigeria
121	HSD 5697	Sudan	247	TVU 889	Nigeria
122	HSD 4368	Sudan	248	TVU 4669	Nigeria
123	HSD 1325	IITA	249	TVU 4783	Nigeria
124	HSD 4394	Sudan	250	TVU 6966	Nigeria
125	HSD 5692	Sudan	251	TVU 8923	Nigeria
126	HSD 5693	Sudan	252	HSD 5700	Sudan

Table 2. List of polymorphic primers.

SSR primer	Primer sequence	Annealing temperature range
VM30_F	5'**NED**_CTC TTT CGC GTT CCA CAC TT	59-62
VM35_F	5'**PET**_GGT CAA TAG AAT AAT GGA	48-53
VM37_F	5'**VIC**_TGT CCG CGT TCT ATA AAT	49-56
VM39_F	5'**NED**_GAT GGT TGT AAT GGG	48-53
VM40_F	5'**6FAM**_TAT TAC GAG AGG CTA TTT	48-52
VM51_F	5'**PET**_CAT TGC CAC TGG TTT CAC TTA	48-62
VM53_F	5'**VIC**_GAG TTC CGT TCG TTG TGA GTA GAG	48-62
VM54_F	5'**NED**_CAC ACA CAC ACA TAG ATA TAG	53-60
VM57_F	5'**6FAM**_GGA AGG GGT AGA GGA AAA GTG AA	57-62
VM70_F	5'**PET**_AAA ATC GGG GAA GGA AAC C	48-62
VM74_F	5'**NED**_CTG CTA CAC CTT CCA TCA TTC	48-62
VM94_F	**5'6FAM**_TCG AAC TTT GGC TTG AGG	**48-62**
Bmd17_F	5'**VIC**_GTT AGA TCC CGC CCA ATA GTC	48-62
SSR-6569_F	5'**PET**-GTTAACATCAGTCCCTTTCA	52-56
SSR-6573_F	5'**VIC**-TGTATGTAATGGAATCGTAA	48-54
SSR-6577_F	5'**6FAM**-GAACTTGATAGGATCCTAGA	57-62

ml from the mixture added to 8 ml Hi-Di (Formamide) and 500 LIZ mixture in 96 PCR plate then the reaction vortexes spines and loaded on PCR machine at 95°C as Denaturation temperature for 3 min the product fast cooled in ice for 10 min before analysis. Fragment analysis was performed on the ABI 3730 sequencer machine, and peaks were sized and the alleles classified using the Gene mapper software (Applied biosystems). The informativeness of each primer pairs was realized using the polymorphic information content (PIC) using the Power Marker software program. The genetic structure of the accessions was investigated by Analysis of Molecular Variance (AMOVA) (Input as Allelic Distance Matrix for F-Statistics using GenAlEx software program; whereas the principal coordinate analysis (PCoA) was performed to identify genetic variation patterns among the cowpea genotypes.

RESULTS

Polymorphism of SSRs in cowpea germplasm

A set of 18 primer pairs pre-selected by their ability to PCR amplify SSRs in cowpea germplasm were used to examine the genetic diversity and phylogenetic relationships among 252 cowpea accessions Sixteen of the primer pairs gave polymorphic DNA fragments following fragment analysis of PCR amplification products. One primer pair generated monomorphic allelic amplification profile across all cowpea genotypes tested, (Figure 3)

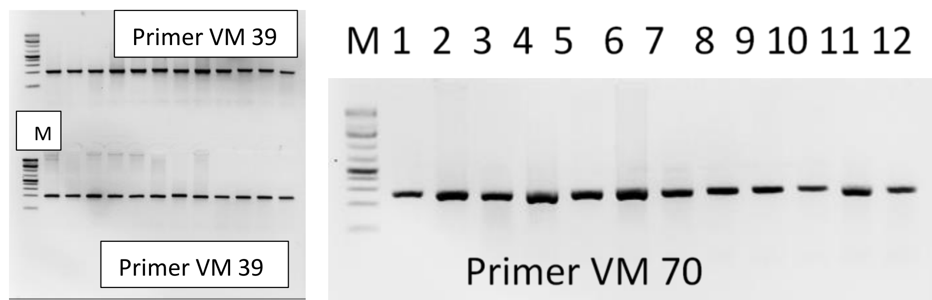

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Figure 2. PCR Ampification of cowpea genomic DNA from 252 accessions.

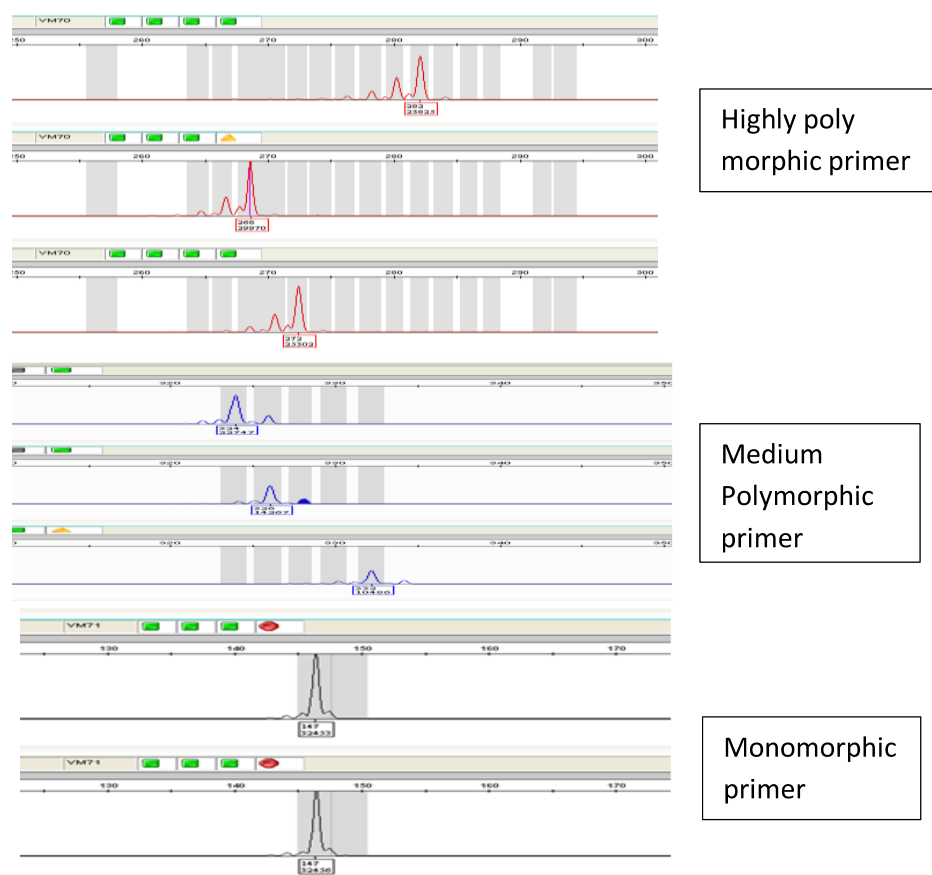

Figure 3. Capillary electrophorese product detection and band scoring.

and the other one showed an inconsistent fragment band therefore the two pairs were excluded. The informative (sixteen) SSRs were able to distinguish the whole accessions of the cowpea used in this study. A total of 129 alleles at 16 loci could be scored. The number of alleles detected per primer pairs varied from 2 to 17 with an average of 8.1 alleles. Polymorphic Information Content (PIC) ranging from 0.33 to 0.83 with a mean of 0.56 (Table 3).

Marker SSR 6569 detected the highest alleles number which was 17 while the lowest one 2 showed by BMD17.Two primers VM70 and SSR6577 detected 16 alleles each whereas four primers; VM30, VM 39, VM 51 and VM 94 had 8 alleles each (Table 3). Marker SSR6569 exhibited highest gene diversity with 0.85 while the least 0.34 was detected by Marker VM74. All primers studied were able to detect the levels of heterozygosity which was observed ranging from 0.01 to 0.13 with a

Table 3. Number of alleles, gene diversity, heterozygosity and polymorphism information content for the primers used in this study.

Marker	Major allele frequency	Allele no.	Gene diversity	Heterozygosity	PIC
VM30	0.61	8	0.60	0.08	0.57
VM 39	0.73	8	0.45	0.04	0.43
VM51	0.59	8	0.56	0.01	0.50
VM 53	0.60	7	0.56	0.05	0.50
VM57	0.63	5	0.54	0.05	0.49
VM70	0.36	16	0.82	0.07	0.80
VM 35	0.60	11	0.58	0.07	0.54
VM40	0.66	5	0.53	0.03	0.49
VM54	0.71	3	0.42	0.02	0.34
VM37	0.40	6	0.67	0.06	0.61
VM 94	0.39	8	0.75	0.03	0.71
BMD 17	0.46	2	0.60	0.13	0.51
SSR 6569	0.24	17	0.85	0.08	0.83
VM 74	0.80	6	0.34	0.02	0.33
SSR 6573	0.51	3	0.58	0.06	0.50
SSR 6577	0.35	16	0.82	0.05	0.80
Mean	0.54	8.1	0.60	0.05	0.56

Table 4. Private alleles, average observed (Ho), expected (He) Heterozygosis and percentage of polymorphic Loci among cowpea accessions studied.

Population	Number of private alleles	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Percentage of polymorphic loci (%)
Bahr Eljabel	2	0.000	0.339	81.25
Blue Nile	5	0.062	0.521	100.00
IITA	8	0.055	0.531	100.00
North Kordofan	5	0.057	0.550	100.00
Northern	2	0.163	0.507	93.75
River Nile	1	0.074	0.445	81.25
South Kordofan	10	0.046	0.526	100.00
Grand Mean	4.7	0.065	0.488	93.75
SE	0.107	0.009	0.020	3.34

mean of 0.05 (Table 3).

The highest number of private alleles was 10 observed by South Kordofan while the lowest was 1 revealed by River Nile Region, the average Observed (Ho) and Expected (He) Heterozygosity among Sudanese cowpea Accessions varied among the different Agro ecological Regions; The highest average of (Ho) 0.163 was recorded by Northern while the lowest 0.000 observed by Bahr Eljabel Region. Highest average of (He) was 0.550 detected by North Kordofan whereas the lowest 0.339 observed by Bahr Eljabel Region, four regions (Blue Nile, IITA, North Kordofan and South Kordofan) revealed 100% polymorphic loci (Table 4).

Phylogenetic analysis

Analysis of Molecular variances (AMOVA) Input as Allelic Distance Matrix for F-Statistics showed that the genetic variation of the total accessions among the geographical

Table 5. Summary of Analysis of Molecular Variance (AMOVA) for Sudanese cowpea Input as Allelic Distance Matrix for F-statistics analysis.

Source	Df	SS	MS	Est. Var.	Genetic variation (%)
Among Pops	6	193.840	32.307	0.388	8%
Among Individuals	245	2133.670	8.709	4.141	83%
Within Individuals	252	107.500	0.427	0.427	9%
Total	503	2435.010		4.956	100%

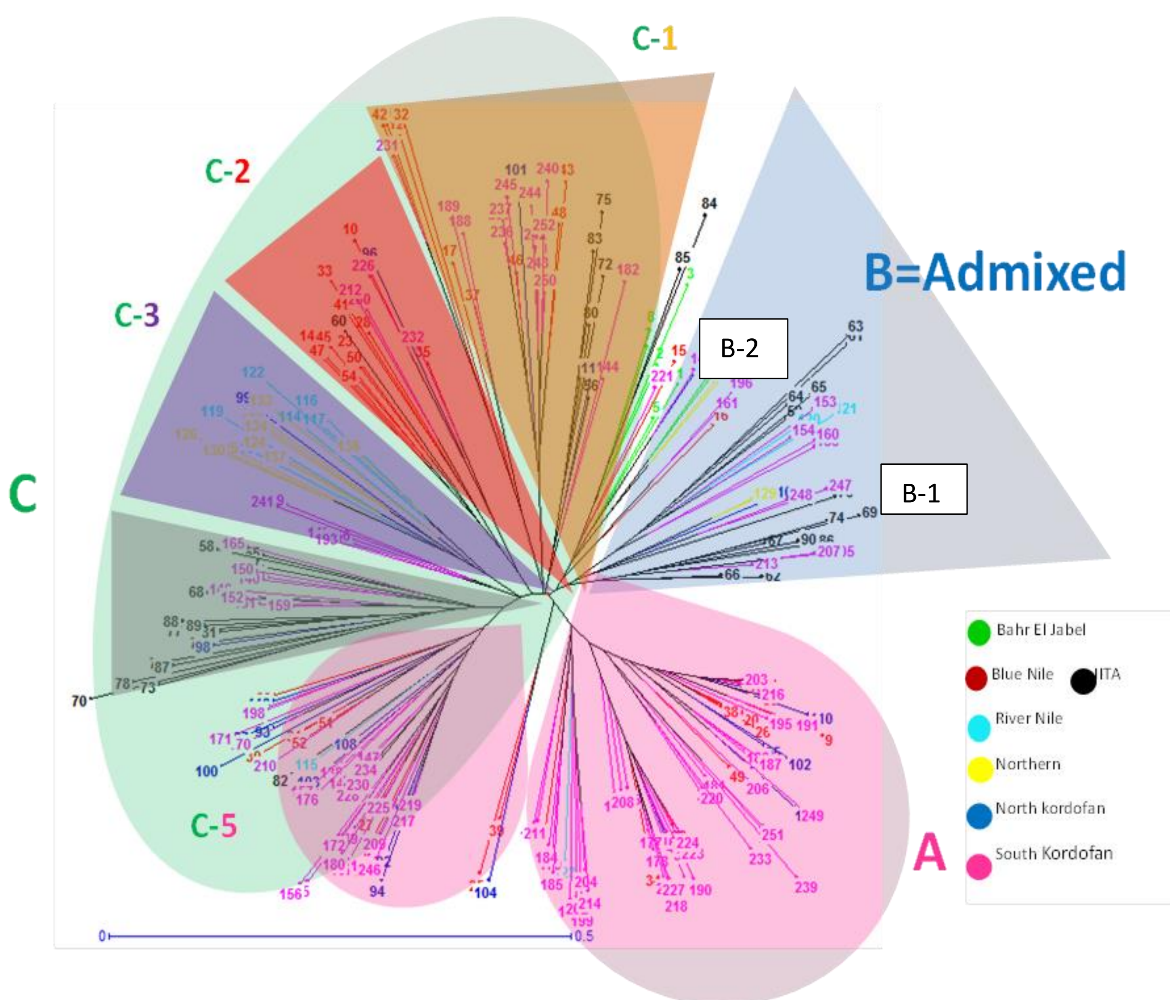

Figure 4. Phylogenetic tree among 252 Cowpea Accessions studied revealed by neighboring joining analysis.

regions was 8%, variation within individuals of sub-population was 9% while the variation among individuals of total population was 84% these results revealed that there was low differentiation among population studied with great diversity among individuals of Sudanese cowpea (Table 5).

Based on their molecular profiles resolved using informative SSRs, the 252 cowpea accessions used in this study clustered into three main groups, which they designated as groups A, B and C (Figure 4). Groups A and B were almost the same in size. Group B divided into two main sub-groups, sub group 1 and sub group 2, the main constituents of sub-group 1 are the out group IITA and South Kordofan germplasm, all Bahr Eljbel germplasm clustered together in sub-group 2 with some of South Kordofan (Figure 4). While group C which

Table 6. Pairwise Population Matrix of Nei Unbiased Genetic Distance among Population.

Agro-ecological zones	Bahr Eljabel	Blue Nile	IITA	North Kordofan	Northern	River Nile
Bahr Eljabel	0.000					
Blue Nile	0.264	0.000				
IITA	0.303	0.240	0.000			
North Kordofan	0.272	0.071	0.131	0.000		
Northern Sudan	0.303	0.170	0.190	0.115	0.000	
Rive Nile	0.261	0.247	0.267	0.194	0.087	0.000
South Kordofan	0.233	0.034	0.165	0.031	0.135	0.218

considered as a largest group comprised the different accessions from different Ecological zones and it contains the main accessions that are the most diverged in the collection that is, TVU 8812 IITA-Benin, followed by accession HSD 5738 –Sudan Blue Nile and HSD 6782-Sudan South Kordofan, Group C also divided into five sub groups where South and North Kordofan were clustered together, Northern and River Nile, South Kordofan and IITA, and South Kordofan and Blue Nile. (Figure 4).

Genetic distance

Generally, genetic distances among cowpea genotypes are low, reflecting the initial bottleneck during domestica-tion, and maintained by the inherent self-pollination mechanism in the crop (Asare et al., 2010). On the whole, the genetic difference observed among the different ecological zones was varied, the least GD 0.031 observed between North and South Kordofan while the greater distance 0.303 was observed by two pairs; IITA; Bahr Eljabel and Northern; Bahr Eljabel state. The lowest GD between Sudanese regions and the out-group was observed by North Kordofan 0.131 then South Kordofan with 0.165, this closely related Genetic Distance to the out group germplasm can confirm the earlier suggestion that cowpea crop introduced to Sudan from West African Countries to the Western part of Sudan (Darfur and Kordofan) and from there it spread to the rest of the county, never the less this result showed a light bar of possibility of other sources of Sudanese cowpea a part from West African countries this can be clear from phylogenetic tree where Group A is mostly dominated by South Kordofan while IITA cluster together with Sudanese germplasm in other two groups (Figure 4). Blue Nile germplasm was observed to be more closely related to South Kodofan with GD of 0.034 then to North Kordofan with 0.071 GD (Table 6).

Three pairs of accessions (HSD 5700 Blue Nile, HSD 4854 South Kordofan; HSD 5701 Blue Nile, HSD 4854 South Kordofan and HSD 4568 South Kordofan, HSD 6560 South Kordofan) possessed genetic distances 0.000, suggesting that the members of these pairs may in fact be either separately collected with different names in the same Region or gene flow between Regions.

DISCUSSION

Variability in SSR markers

The delineation of cowpea germplasm into groups of genetic relatedness will be valuable for guiding introgression efforts in breeding programs and for improving the efficiency of germplasm management (Bao-Lam Huynh et al., 2013).

In the present study, the 16 informative SSR primer pairs used to analyze the 252 cowpea germplasm from Sudan and IITA-Nigeria resulted in 2 to 17 alleles per primer pairs with an average of 8.1. This result is in agreement with Badiane et al. (2012), who found number of alleles in Senegal cowpea varied from 1 to 16. Fatokun et al. (2008) detected alleles ranging from 4 to 13 alleles among 48 wild cowpea lines collected from different Agro- ecological zones in Africa with an average of 7.5 alleles per primer. However, previous works in Ghana, Burkina Faso, Senegal and Nigeria have revealed detections of allels ranging from 1 to 6, 5 to 12, 1 to 9 and 2 to 5 respectively (Asare et al (2010), Sawadogo et al. (2010) Diouf and Hilu (2005) and Adetiloye et al. (2013)). These variations in numbers of alleles can be attributed to the types of primers used in each study and/or the rate of polymorphism of each primer pairs.

In this study the polymorphic information content (PIC) ranged from 0.33 to 0.83 with a mean of 0.56. Fatokun et al. (2008) observed PIC ranging from 0.29 to 0.87 with a mean of 0.68 among the 48 wild cowpea lines. However other cowpea Researchers (Li et al. (2001), Badiane et al. (2012) and Asare et al. (2010) reported PIC ranging from (0.02 to 0.73, 0.08 to 0.33 and 0.07 to 0.66) respectively. The informativeness of PIC value measured by Botstein et al. (1980) scale revealed that the mean PIC value ≥ 0.5 is highly informative, 0.25~0.50 reasonably

informative and < 0.25 is slightly informative, and Loci (Marker) with many alleles and a PIC value near 1 are most desirable (Botstein et al., 1980).

Gene diversity in this study was 0.60 on average ranging from 0.34 to 0.85. In Senegal cowpea gene diversity varied from 0.08 to 0.42 with mean of 0.28 (Badiane et al., 2012), whereas In Ghana cowpea germplasm gene diversity ranged from 0.12 to 0.68 with an average of 0.44 (Asare et al., 2010). The results of gene diversity reflect the proportion of polymorphic loci across the genome. Therfore according to the result of this study the markers used were highly polymorphic compared to those used by the Badiane et al. (2012), and Asare et al. (2010).

Heterozygosity in this study was observed ranging from 0.01 to 0.13 with a mean of 0.05, Asare et al. (2010) revealed Variation in heterozygosity among Ghanaian cowpea SSRs increasing from 0.01 to 0.84 with an average occurrence of 0.19. The low value of Heterozygosity agrees with previous series reported by several cowpea researchers who documented that cowpea in general has a narrow genetic base due to the result of a bottleneck induced by a single domestication event which involved in the origin of this crop, where the proportion of heterozygozity is likely to be low, and likely its inherent nature of self-pollination mechanism (Pasquet, 2000; Coulibaly et al., 2002; Ba et al., 2004).

Genetic relationship among population

Generally, genetic distances among cowpea genotypes are low, reflecting the initial bottleneck during domestication, which was maintained by the inherent self-pollination mechanism in the crop (Asare et al., 2010). In this study the shorter genetic distance of 0.031, which was found between North and South Kordofan states, might be due to gene flow resulting from the seed exchange practiced by farmers particularly within and between these two neighbouring states. The same phenomenon was observed in Northern and River Nile states, which are also neighbouring states in the northern region of the country, showing a shorter genetic distance of 0.087 mostly due to the same reasons.

The greatest genetic distance of 0.303, which was found among the accessions obtained from the IITA compared to the accessions collected from Bahr Eljabel state, as well as among accessions collected from Northern state compared to those collected from Bahr Eljabel state, can be attributed to the far distances between those non-neighbouring geographical regions, where the possibility of gene flow due to seed exchange is almost lacking. Accessions obtained from the IITA were representing materials that were originally collected from other countries in west Africa. Northern state is located in the far north of Sudan whereas Bahr Eljabel is within republic of South Sudan. On the other hand, the shorter genetic distance found between the IITA and North Kordofan accessions as well as IITA and South Kordofan accessions can interpreted by the earlier suggestion that cowpea crop was introduced to Sudan from West African Countries to the western part of Sudan (Kordofan and Darfur), from where it spread to the rest of the country. The closely genetic distance between Blue Nile state and North and South Kordofan states can be attributed also to seed and or grain exchange between farmers and inhabitants of these states for cultivation and food uses. Moreover, an ethnic relationship with similar cultural background exists between some groups of populations in these three states, which reflecting in similar nutritional and cultivation habits and practices within them.

Three pairs of accessions in this study were found to have genetic distances of 0.00, which indicate the common genetic make-up within each pair of accessions, though they were observed to have different farmers' variety names. The same variety with the same morphological characters can have different names following the locality or ethnic groups. Consequently, a better understanding of the genetic variation and strong sound footing system of classification of the cowpea collection in Sudan with molecular markers is urgent need.

Conclusions and recommendations

The current study suggested low level of genetic diversity among Sudanese Cowpea population with great diversity with individuals. Therefore broadening the genetic base of Sudanese cowpea population may be achieved through introgression of new alleles either from wild cowpea germplsam or by out breeding with more closely related species to Cowpea *V. triphylla* and *V. reticulate*.

A total of 11 markers (SSR 6569, VM 70, SSR 6577, VM 94, VM 37, VM 30, VM 35 BMD 17, VM 53, VM 51 and SSR 6573) were highly informative (0.5 <PIC<0.83) and can be used in future diversity studies as marker core set in cowpea; IITA germplasm grouped together with Sudanese germplasm revealing the similarity of Sudanese cowpea to West African countries. Sudanese Cowpea Core collection can be created from South Kordofan, River Nile and Bahr Eljabel germplasm.

Conflict of interest

The authors have not declared any conflict of interest.

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