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Use RAPD markers to determine the extent of diversity in cocoyam genotypes in the Eastern region of Ghana

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Experiments conducted in Ghana show that cocoyam exists as mixtures of clones in farmers farms. This work aimed to use RAPD markers to determine the extent of diversity in cocoyam genotypes collected from farms at different locations in the Eastern region of Ghana. The study also investigated whether the genotypes have different adaptation to different farming systems (intercropping with plantain and sole cropping) and tillage methods (mounds and flat). The genotypes were grouped into two main clusters at 0.65 similarity coefficient of variation with accessions Pameng Red 3 and Pramkese 2 being the most diverse. The genotypes began separating at 85% similarity index into three discrete groups. Group I, (Pameng 1, Dwenase 2 and 3) did not separate at 100% similarity index. The other two groups consisted of (Pameng 2, Gyampomani 1, Gyampomani 2, Dwenase 1) and (Pramkesse 1 and Gyampomani 3). The analysis of variance of the growth parameters of the genotypes under the tillage and farming systems revealed significant differences. Generally, genotypes in group II grew better under the farming systems and tillage practices studied whiles Pramkesse 2, which did not cluster with any other genotypes in its major cluster, grew poorly under the two farming systems.

Key words: Cocoyam, intercropping, solecropping, mounds, flat, RAPDS.

INTRODUCTION

In Ghana, farmers can identify at least three varieties of cocoyam on the basis of cormel skin colour as follows; mankani-pa, with red skin colour, mankani-fitaa, with white skin colour, mankani-serwaa, with pale skin colour (Karikari, 1971).

Cocoyam contributes significantly to the national food baskets. The FAO estimated that Ghana produced 1,063 tonnes of cocoyam representing about 18% of total world's production (Onwueme and Sinha, 1991). Today the demand for cocoyams has increased both in Ghana and other parts of the world. In Ghana, the high demand is brought about by the establishment of agro-processing companies which use cocoyam as raw material, and other exporters who export chopped cocoyam leaves to Europe.

In spite its importance as a staple food in many countries,

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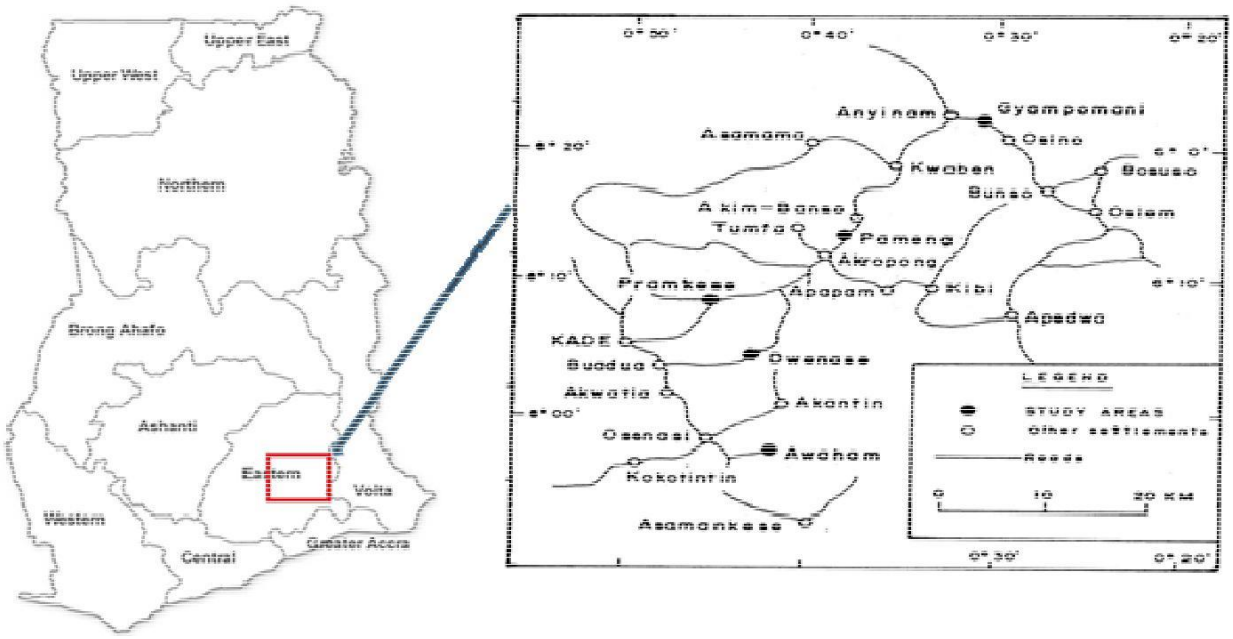

Figure 1. A map showing location of the study areas.

cocoyam has received very little research attention (Goenaga and Hepperly, 1990), and is regarded as an under-exploited and insufficiently studied crop (Nguyen and Nguyen, 1987; Giacometti and León, 1994; Watanabe, 2002).

Previous studies using microsett derived plants from 16 genotypes by Osei and Mintah (2002) indicated that differences exist in growth and yield of different cocoyam genotypes indicating that cocoyam exists as mixtures of clones in farmers' farms.

Hence, there exists a need to assess the extent of genetic diversity to determine these differences.

DNA based markers have become methods of choice in genetic diversity studies, as they analyse variation at DNA level. This excludes all environmental influences and time specificity, since analysis can be performed at any growth stage using any plant part and requires only small amounts of material (Mueller and Wolfenbarger, 1999; Rao, 2004).

Tillage method is considered one of the major factors for increasing the yield of cocoyam on a tuber yield per unit area basis (Ennin et al., 2009). Soils are tilled to create a soil environment favourable for plant growth and development. In general, root and tuber crops do not produce satisfactory yields on compacted or shallow soils (Ennin et al., 2009).

Driven by land economy, most peasant cocoyam farmers in Ghana practice intercropping by utilizing the space under the tree crop canopy for the cultivation of the cocoyam. For this reason, most of the cocoyams are grown under canopies of crops such as cocoa, oil palm and plantains. However few studies have been done to ascertain the agronomic and physiological implications of such intercropping to determine if some cocoyam genotypes are more sensitive to intercropping than others and, if so, could this be a guide in choosing genotypes or cultivars to grow under conditions of low light intensity.

The purpose of this study was:

1) To determine the extent of diversity in cocoyam genotypes collected from different locations in the Eastern region of Ghana.
2) To determine whether the genotypes have different adaptation to different farming systems (intercropping with plantain and sole cropping) and tillage methods (mounds and flat).

MATERIALS AND METHODS

Diversity studies and experimental material

The experiment was carried out at the Biotechnology Centre, College of Agriculture and Consumer Sciences, University of Ghana. Eleven cocoyam genotypes from five towns in the Eastern region of Ghana were used for the experiment. The towns were; Dweanase, Pramkesse, Gyampomani, Awaham and Pameng (Figure 1). The genotypes for the study were labelled as; Pameng 1 and 2, Dwenase 1, 2 and 3, Pramkese 1 and 2, Gyampomani 1, 2 and 3 and Pameng Red 3.

DNA extraction

The young or tender leaves of each genotype were harvested, kept on ice and taken to the laboratory for total DNA extraction. Total DNA was extracted from the leaf tissues using the GenElute™

Table 1. RAPDs (Operon F-series 10-mer) primers.

Primer	Sequence (5'-3')
OPF-08	GGGATATCGG
OPF-09	CCAAGTCTTC
OPF-13	GGCTGCAGAA
OPF-16	GGAGTCTGG
OPF-19	CCTCTAGACC
OPF-20	GGTCTAGAGG
OPF-10	GGAAGCTTGG
RAPD-DCA:OPF-08	GGGATA

Table 2. PCR programme conditions for the DNA amplification.

Programme	Number of cycles	Steps	Temperature (°C)	Hold time (s)
Denaturation	1	1	95	360
Denaturation	45	1(denature)	95	30
Denaturation	45	2(anneal)	35	30
	45	3(extension)	72	60
		4(final extension)	72	600
		Hold step	4	~ ∞

Plant Genomic DNA Miniprep Kit and stored in a freezer at -20°C for subsequent use.

DNA amplification

A modified protocol was used for DNA amplification, using eight selected RAPD primers (Williams et al., 1990). The (full) list of the eight selected RAPD primers and their respective sequences is presented in Table 1. The amplification mixture contained 1.5 uL PCR buffer, 1uL $MgCl_2$, 0.5 uL dNTP, 2µl primer, 0.5 uL *Taq* polymerase, and 1.5 uL template DNA in sterile de-ionized water. Conditions for the DNA amplification were as stated in Table 2.

Gel electrophoresis and PCR products

The resulting amplicons (amplification products) were taken through gel electrophoresis using 2% agarose gel (molecular biology grade) prepared using 1X TAE (Tris-Acetate EDTA) buffer and stained with ethidium bromide. 7 µl of amplicons were loaded into the wells generated in the agarose gel and run alongside 10 µl of standard molecular weight DNA markers at a constant voltage of 60 V for 21/2 h for all reactions. The products were visualized under UV light in 2% agarose gel stained with ethidium bromide.

Scoring and data analysis

The resulting bands after electrophoresis were scored as binary data with the help of Microsoft Office Excel® indicating the presence of bands as 1 and the absence of bands as 0. A generalized dendrogram was then drawn from the scored bands for analysis using GenStat® computer software, 9th edition.

Evaluation of genotypes under different tillage and farming systems

Experimental site and source of planting materials

The experiment was conducted at the University of Ghana Agricultural Research Centre, Kade in the Eastern Region. Microsett-derived planting materials of the cocoyam genotypes collected from five towns in the Eastern Region of Ghana were used for the study. Ten of the 11 genotypes used for the diversity study were used in the field evaluation. Pameng Red 3 was excluded from the field evaluation because it was collected late.

Field establishment and experimental design

Three months old split-corm derived suckers of local plantain cultivar were planted at a spacing of 3 m x 3 m for the plantain cocoyam intercrop system.

One month after planting the plantain suckers, two months - old microsett-derived planting materials of the different cocoyam genotypes were transplanted at a spacing of 1 m x 1 m in the sole and intercrop systems. The cocoyams were planted 0.5 m away from the plantains.

The experimental design was a split, split plot with the farming systems (sole and intercropping) as the main plot, the tillage system (planting on flat and mounds) as the subplot, and the genotypes as the sub sub plots. Each treatment was replicated three times.

Cultural practices

Compound fertilizer (NPK, 15-15-15) was applied at a rate of 100 g and 200 g per plant to cocoyam and plantain respectively. Watering and weeding were done whenever necessary.

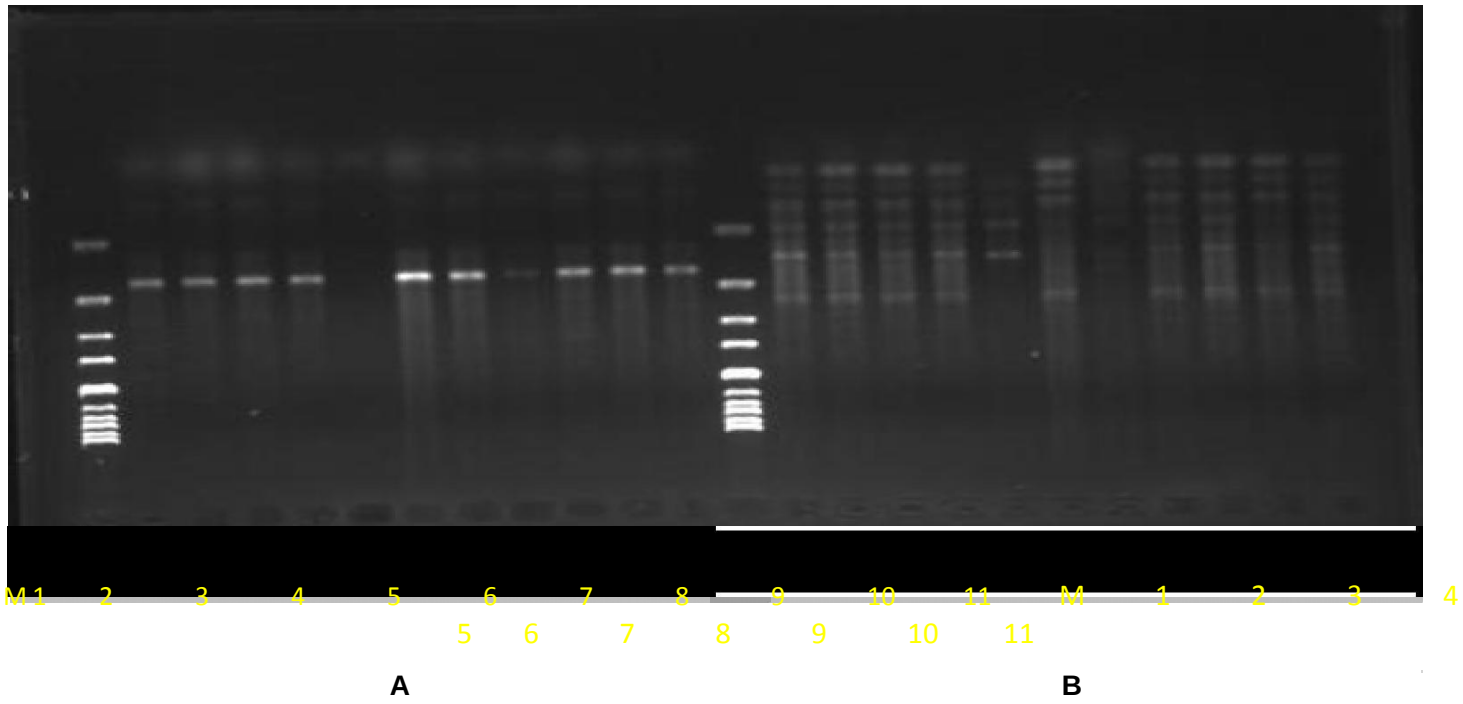

Figure 2. Cocoyam DNA Fingerprints: Amplified DNA bands of eleven cocoyam genotypes obtained after agarose gel electrophoresis of PCR products using primers (A) OPF-19 and (B) OPF-10. *1 = Pramkese 2; 2 = Dwenase 2; 3 = Dwenase 3; 4 = Pameng 1; 5 = Pameng red 3; 6 = Pameng 2; 7 = Pramkese 1; 8 = Gyampomani 3; 9 = Gyampomani 2; 10 = Dwenase 1; 11 = Gyampomani 1.*

Data collection (Growth parameters)

Growth parameters were measured once a month on ten plants per genotype. Data were collected from all ten plants located in the rows of each plot. The parameters evaluated were; plant height, number of leaves, plant girth , yield and leaf area. Genstat Discovery Edition 4 was used for the data analysis.

RESULTS

Genetic diversity of eleven cocoyam genotypes

Figure 2 shows the bands of Amplified DNA obtained after agarose gel electrophoresis of PCR products using primers OPF-19 and OPF-10 and total DNA from the eleven cocoyam genotypes. Similar results were obtained with other primers used in the study, except primer DCA-OPF-08 which produced no amplification products.

Cluster analysis

The cluster analysis based on RAPDs from seven primers (Table 2) grouped the genotypes into 2 major clusters. Major cluster 1 contained only Pameng Red 3 at 65% similarity index while major cluster 2 comprised all the other genotypes (Pramkesse 1 and 2, Pameng 1 and 2, Gyampomani 1,2 and 3, Dwenase 1,2 and 3). However major cluster 2 further separated into 2 sub clusters. Sub cluster 1 consisted of only Pramkesse 2 which is named group IV throughout the write up. Sub cluster 2 contained Pameng 1 and 2, Gyampomani 1, 2 and 3, Dwenase 1, 2 and 3. The genotypes in sub cluster 2) began separating at 85% similarity index into three discrete groups. One such group contained (Pameng 1, Dwenase 2 and 3,) which did not separate at 100% similarity index and is named as group I in the write up. The other two groups consisted of Pameng 2, Gyampomani 1 and 2, Dwenase 1 also named group II and Pramkesse 1 and Gyampomani 3 in the other (Group III) (Figure 3).

Effect of two farming systems and two tillage practices on the growth parameters of ten cocoyam genotypes

The results of the growth measurements of the different cocoyam genotypes indicated moderate levels of variability among the genotypes, and also due to their interactions with farming systems and tillage practices.

There were significant ($P<0.05$) differences in all the growth measurements due to genotypes, farming systems, tillage and the interactions except for the interactions between farming systems and tillage for plant height and girth.

Averagely genotypes in cluster II had superior growth than genotypes in clusters I and III in most of the growth parameters measured. Pramkesse 2 in cluster IV grew poorly for almost all parameters measured when compared with the other genotypes.

Generally most genotypes grew better under intercropping than solecropping for most of the parameters

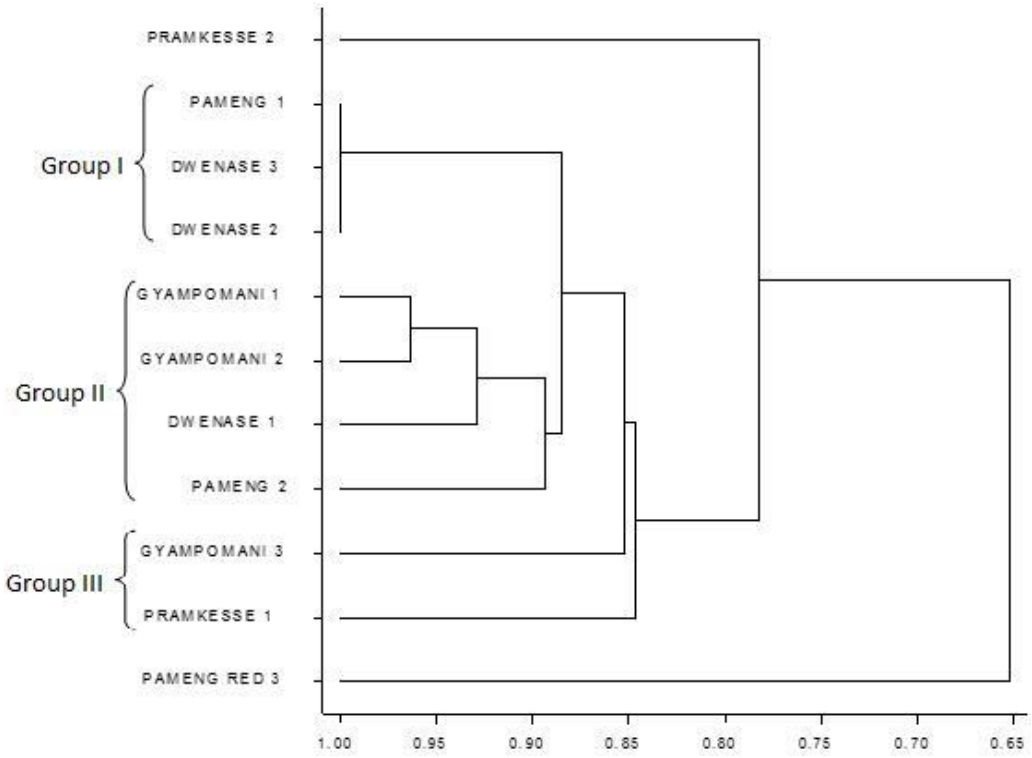

Figure 3. UPMGA cluster analysis for eleven cocoyam accessions: The neighbor-joining analyses revealed close genetic similarities between the cocoyam accessions.

Table 3. Effect of two farming systems (intercrop and sole cropping) and two tillage practices (mounds and flat) on the mean cormel fresh weights (kg/ha) of ten cocoyam genotypes at harvest.

Group No.	Genotype	Intercropping		Sole cropping		Genotypic means
		Flat	Mound	Flat	Mound	
I	PAM 1	6845	11135	5982	6330	7573
	DWEN 3	7120	8670	7035	5155	6995
	DWEN 2	12680	6090	4770	9280	8205
Mean		**8881.7**	**8631.7**	**5929**	**6921.7**	**7591**
II	GYAM 1	7600	8690	9322	7002	8153.5
	GYAM 2	8605	2535	3700	5725	5141.3
	PAM 2	2480	9185	11650	11600	8728.8
	DWEN 1	13500	6040	6728	6080	8087
Mean		**8046.3**	**6612.5**	**7850**	**7601.8**	**7527.7**
III	GYAM 3	4565	5485	5952	6248	5562.5
	PRAM 1	3822	4150	4655	4765	4348
Mean		**4193.5**	**4817.5**	**5303.5**	**5506.5**	**4955.3**
IV	PRAM 2	6690	220	4828	3500	3809.5

s.e.d. of interactions=1902.3 s.e.d. of genotypes=986.4.

measured. Genotypes in cluster II grew highest in plant height, girth, number of leaves, leaf area and cormel fresh weights under intercropping. Genotypes in cluster I followed next in superior growth after genotypes in

cluster II except for number of cormels in which genotypes in cluster I yielded more cormels than genotypes in cluster II on solecropping. However Pramkesse 2 which did not closely cluster with any genotype was more adapted to sole cropping than intercropping for most of the parameters measured. Significant differences were not obtained for interactions between the two farming systems, two tillage practices and farming systems and tillage practices for the mean fresh weight of cormels per hectare after analysis. However, there were significant differences between the ten genotypes as well as interactions between the genotypes, farming systems and tillage practices. The average genotypes in groups I and II produced higher fresh cormel weights per hectare than those in groups III and IV (Table 3). Fresh weights of cormels were higher under intercropping (8881.7 kg, 8631.7 kg) on the flat and on mounds for genotypes in group I than under sole cropping (5929 kg, 6921.7 kg) per hectare respectively. However genotypes in group II produced the highest fresh cormel weights per hectare under sole cropping than under intercropping. Pramkesse 2 in group IV yielded poorly in cormel fresh weights under intercropping particularly on mounds (Table 3). The cormel yield of Prankesse 2 was however better on the flat than on the mound per hectare.

Genotypes (B,C,F,G,H,I) recorded higher cormel fresh weights per plant under intercropping than on sole cropping. However, higher number of cormels per plant were generally recorded in sole cropping than in intercropping (Figure 4).

The genotypes in addition grew better on mounds than on the flat for most parameters with the exception of fresh weight of cormels per plant in which only four (A,D,G,J) out of the ten genotypes grew better on mounds than on flat land.

DISCUSSION

Genetic diversity assessment of eleven cocoyam genotypes collected from five towns in the Eastern region of Ghana

RAPD analysis

Seven out of the eight RAPD primers used in the PCR reactions produced amplification with the DNA of the eleven genotypes collected. Primer DCA- OPF-08 (sequence) did not produce amplification with the DNA of any of the cocoyam genotypes used in this study. This is probably due to the fact that the primer sequence (5'GGGATA3') has no homology with the cocoyam genome or it might be due to manufacturing error.

It is significant to note that the genotypes did not cluster according to their distinct towns of collection. This implies that there has been a significant flow of cocoyam germplasm between the five towns in the Eastern region where the genotypes were collected. The genotypes Pameng 1, Dwenase 2 and 3 were similar at 100% similarity index indicating that these genotypes are probably duplicates grown at different locations. They could have originated in localities different from where they were collected. This suggests that cocoyam genotypes may have been transported between localities as a result of the normal farmer to farmer exchange of planting materials. This exchange of genetic material may have been enhanced by the closeness of the five towns to each other. The clustering of the eleven genotypes into different groups may be due to genetic divergence of cocoyams over the two hundred years since its introduction to Ghana, and to re-introductions or occasional hybridization between clones and thus the crop exists as mixtures of clones in farmers' field. The result of this study is a useful guide in selecting cocoyam germplasm for breeding and conservation. Pameng Red 3 which was the most diverse among all the accessions may have some distinct agronomic characters. It therefore requires further evaluation in the field.

The genetic diversity of cocoyam observed in this work is in agreement with Offei et al. (2004) who used 10 random primers to study the genetic diversity and structure of seventy cocoyam accessions collected in the Eastern and Volta regions of Ghana. The 70 accessions did not cluster into their distinct geographical regions suggesting that there may have been movement of germplasm across the two regions.

Effect of two farming systems (intercropping and sole cropping) on growth components of ten cocoyam genotypes

Most genotypes which grew well under intercropping for plant height, girth, number of leaves and leaf area could be attributed to moisture conservation under intercropping since the plantains provided an amount of shade to the cocoyam therefore reducing the amount of evaporation. This observation agrees with the findings of Goenaga and Chardon (1993) that cocoyam requires moisture throughout its growing season (9 to 12 months).

The high litter fall from both the cocoyam and plantain plants and the activities of soil organisms as a result of the cool environment under the system, maintained soil fertility and this all led to most of the genotypes under intercropping growing superiorly than corresponding genotypes under sole cropping for most of the parameters measured. This observation is also in agreement with Karikari (1971) and Giacometti and León (1994) that cocoyam responds well to organic and chemical fertilization. In fertile soils the crop develops healthy leaves and produces higher yields. Schaffer and O'Hair (1987) also reported that leaves of cocoyam grown under moderate shade appear to be more

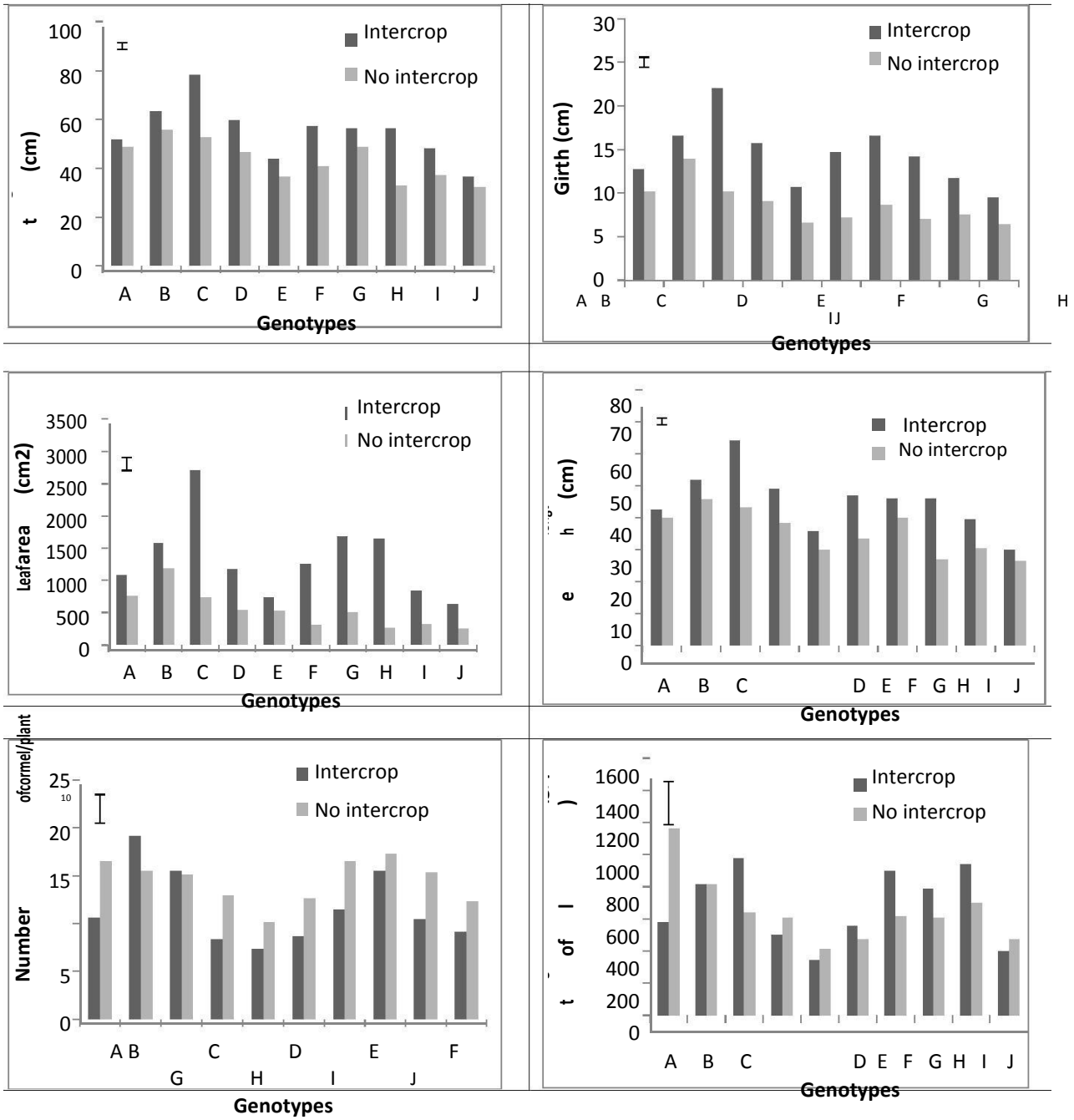

Figure 4. Effect of farming systems (intercrop and no intercrop) on growth and yield of cocoyam genotypes *A-Pameng 2, B-Gyampomani 1, C-Dwenase 1, D-Gyampomani 3, E-Pramkesse 2, F-Gyampomani 2, G-Pameng 1, H-Dwenase 3, I-Dwenase 2, J-Pramkesse 1.*

photosynthetically efficient than leaves grown in full sun. Therefore planting cocoyam as an understory crop in mixed cropping systems may maximize their photosynthetic efficiency. However for number of cormels per plant and cormel fresh weight per hectare of genotypes in group III, most genotypes were more adapted to full exposure or sole cropping than intercropping and this might be due to the different genetic compositions of the genotypes and also competitions between the two intercrops. This indicates that the same farming systems cannot be used for all cocoyam genotypes for optimum growth. This also agrees with the findings of Onwueme and Charles (1994), that yield of cocoyam varies from place to place, depending on the cultivation methods and the environmental conditions.

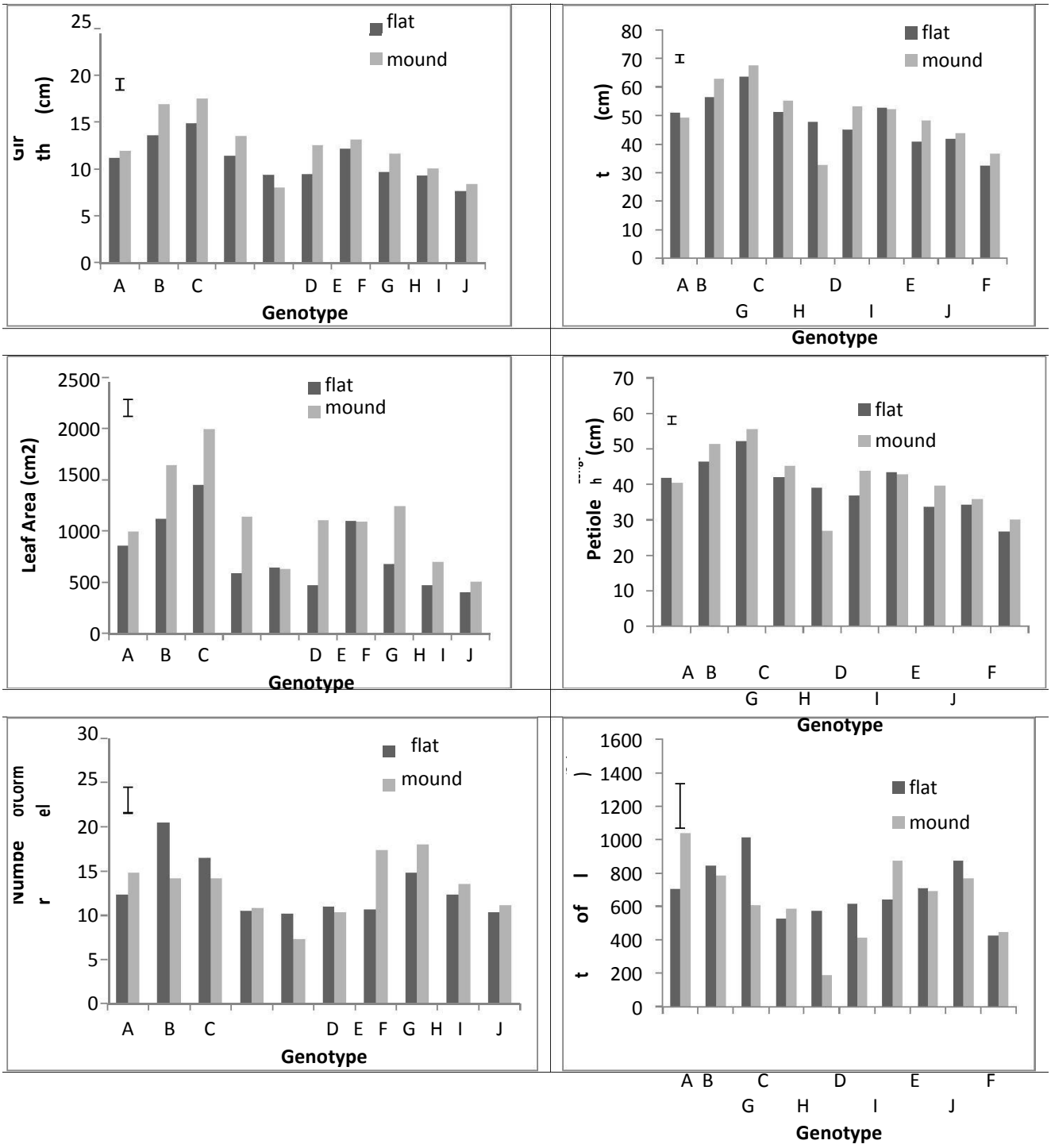

Figure 5. Effect of tillage methods (flat and mound) on growth and yield of cocoyam genotypes. *A-Pameng 2, B-Gyampomani 1, C-Dwenase 1, D-Gyampomani 3, E-Pramkesse 2, F-Gyampomani 2, G-Pameng 1, H-Dwenase 3, I-Dwenase 2, J-Pramkesse 1.*

Effect of two tillage practices (planting on mounds and flat) on growth components of ten cocoyam genotypes

Growing cocoyam on mounds resulted in increased growth in all the growth components measured compared to growing on flat land (Figure 5).

These results may probably be due to the loose nature of soils associated with mounding which enhanced infiltration of water and air, easy penetration of roots and also improved soil water management. This results is comparable to that found by Adekiya et al. (2009) who compared five tillage methods and their effects on growth and yield of cocoyam in the forest savannah transition zone of South West Nigeria and found out that manual mounding produced satisfactory results in mounding.

Conclusions

The seven RAPD markers used in the experiment suggested moderate to low levels of genetic variation (0.65 to 1.00 genetic similarity) among the eleven cocoyam genotypes sampled from the five towns in the Eastern region of Ghana. Clustering was not based on agro-ecological zones but rather dependent on inherent genetic variability. This means cocoyam accessions may have been transported between localities at random as a result of the normal farmer to farmer diffusion of planting materials. This may have been enhanced by the closeness of the five towns to each other.

The cocoyam genotypes showed genotypic differences for most of the growth parameters studied. Genotypes in groups I and II were generally high yielding and were morphologically superior to the other genotypes in the different groups. Genotypes in group III recorded moderate yield and morphological values. However the distantly related genotype Pramkese 2 in group IV in general recorded moderate to low values for most of the parameters that were observed. This suggests that the yield potential of cocoyam genotypes may be deduced from their morphology.

The cocoyam genotypes also showed different adaptations to the two farming systems and two tillage practices. For instance the distantly related genotype, Pramkesse 2 was more adapted to sole cropping and on flat than intercropping on mounds for most of the parameters measured.

This means that to produce optimum yields, different cultural practices maybe required for different cocoyam genotypes.

Conflict of Interest

The authors have not declared any conflict of interest.

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