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Genetic structure of seventy cocoyam (*Xanthosoma sagittifolium*, Linn, Schott) accessions in Ghana based on RAPD

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Seventy cocoyam accessions collected from the eastern and Volta regions of Ghana were studied using RAPD technique. Ten primers were used to study the genetic diversity and structure of the experimental material and a total of 120 different bands were detected. Levels of polymorphic fragments detected by the ten primers ranged from 69.2% to 100%. In the accessions from the eastern region, the mean observed and effective number of alleles per individual per population and mean Nei's gene diversity were 1.99 ± 0.01, 1.53 ± 0.12 and 0.312 ± 0.05, respectively. Mean observed and effective number of alleles per individual per population and Nei's gene diversity were 1.76 ± 0.17, 1.44 ± 0.16 and 0.264 ± 0.08, respectively for the accessions from the Volta region. Considering the entire collections, the mean observed and effective number of alleles per individual per population and Nei's gene diversity for primers were 1.99 ± 0.02, 1.54 ± 0.12 and 0.319 ± 0.05, respectively. In terms of Nei's F-statistics in the subdivided populations for all primers, the overall gene diversity (Ht) ranged from 0.230 to 0.396 with a mean of $0.313 + 0.06$, within sample gene diversity (Hs) ranged from 0.214 to 0.372 with a mean of $0.286 + 0.06$, gene differentiation (Gst) ranged between 0.005 and 0.178 with a mean of 0.086. Geneflow estimate ranged between 2.309 and 99.500 with a mean of 5.314. The accessions grouped into three main clusters. Accession BD96/183 was the most diverse and may be incorporated into cocoyam breeding programs. The 70 accessions did not cluster into their distinct geographical regions suggesting that there may have been movement of germplasm across the two regions.

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Cocoyam (*Xanthosoma sagittifolium*) originated from tropical America (Coursey 1968). The crop was introduced into west Africa in 1940 (Purseglove 1972). In Ghana, cocoyam is cultivated throughout the forest belt as a shade crop for young cocoa seedlings. Cocoyam plays an important role in poverty alleviation and food security. The cormels are used for human consumption while the corms serve as planting material. The corms supply easily digestible starch and are known to contain substantial amounts of protein, vitamin C, thiamine, riboflavine and niacin (Cobley and Steele 1976: Maduewesi and Oyinke 1980). The leaves are important source of proteins and vitamins. Cocoyam accessions have been characterized based on morphological and isozyme markers (Karikari 1973; Doku 1983; Aguegia et al. 1994). Genetic improvement of cocoyam is limited by a poor knowledge of genetic diversity within the species.

RAPDs are useful genetic markers and offer breeders the opportunity for improving the efficiency of selection in crop improvement (Heun and Helentjaris 1993). The randomly amplified polymorphic DNA (RAPD) technique is popular among the various DNA marker assisted techniques currently available (Williams et al. 1990), because of its speed, low cost and necessity of only minute amounts of plant material. It is less restricting than the restriction fragment length polymorphism (RFLP) technique (no hybridization and no use of radioisotope). RAPDs have been employed for cultivar identification and also for plant genome analysis including population genetics (Hu and Quiros 1991; Koller et al. 1993; Asemato 1996; Isabel et al. 1995), systematics and phylogeny (Adams and Demeke 1993). Though it is not always reproducible, RAPD detects a large number of markers (Kazan and Muchlbaue 1993). The RAPD approach can expedite gene tagging, genome mapping, genetic fingerprinting, assessing genetic variation and identifying hybrids (Mayer et al. 1997).

RAPD markers have been extremely useful to assess patterns of genetic diversity in a number of plant species (Bussell 1996; Fritsch and Rieseberg 1996; Ouborg et al. 1999). Despite important limitations due to their dominant nature, RAPD markers can be used to assess fixation indexes and population genetic parameters when appropriate statistical analysis is used (Huff et al. 1993; Lynch and Milligan 1994; Stewart and Excoffier 1996). Palacios and

González-Candelas (1997) have illustrated following modifications proposed by Stewart and Excoffier (1996), the estimation of these basic population genetic parameters based on RAPD profile similarity. The RAPD technique is, therefore, a powerful tool which can contribute to the improvement of cocoyam through evaluation of accessions and the detection of gene flow (Marmey et al. 1994).

Genetic improvement of a crop requires knowledge of genetic diversity within its species. In Ghana, genetic improvement in cocoyam has been slow due to lack of knowledge on genetic diversity in the crop. The purpose of the present study was to study the genetic diversity and population structure of cocoyam accessions using RAPD markers with a view to generating the necessary information for breeding programs in cocoyam.

MATERIAL AND METHODS

Seventy cocoyam accessions were used in the study, collected from the Plant Genetic Resources Center, Bunso. Eighteen of the accessions were collections from the Volta region of Ghana while fifty-two were accessions from the eastern region of Ghana (Table 1).

RAPD analysis

Fifty primers synthesized by Operon Technologies were screened and 10 of them, OPAM-03, OPAM-04, OPAM-05, OPB-01, OPB-19, OPC-05, OPC-17, OPD-01, OPE-09 and OPJ-01, which were polymorphic, and also produced reproducible profiles, were used for DNA amplification.

DNA extraction

DNA was extracted from one gram of young cassava leaves harvested on ice using the method by Doyle and Doyle (1990) with some modifications. Leaf tissues were ground in liquid nitrogen using mortar and pestle. The fine powder was then transferred to eppendorf tubes (about half full) and 500 µl of prewarmed (60°C) CTAB extraction buffer was added, mixed and incubated at 65°C for 1 h. One extraction with a phenol/chloroform was performed and DNA precipitated by addition of equal volumes of cold isopropanol and NaOAc. The pellets were washed with 70% ethanol and allowed to dry at room temperature. It was then dissolved in 1 × TE buffer (10 mM Tris (pH 8.0); 0.1 mM EDTA) and treated with RNase (5 µg μl^{-1}) at 37°C for 30 minutes. The DNA was extracted with phenol/chloroform and precipitated as described above. It was then washed in cold 70% ethanol and allowed to dry at room temperature and then dissolved in 100 µl of 1 × TE overnight at 4°C. The DNA concentration was determined by comparison with lambda DNA standards on agarose gels stained with ethidium bromide (0.5 µg μl^{-1}).

Table 1. *Cocoyam germplasm selected for RAPD analysis.*

Accession	Region	Accession	Region
1. AGA 97/162	Eastern	36. TA 97/056	Volta
2. AGA 97/161	Eastern	37. TA 97/037	Volta
3. AGA 97/206	Eastern	38. TA 97/018	Volta
4. AGA 97/029	Eastern	39. TA 97/017	Volta
5. RAX 93/008	Eastern	40. RAX 93/010	Volta
6. RAX 93/006	Eastern	41. RAX 93/009	Volta
7. RAX 93/005	Eastern	42. TA 97/140	Volta
8. RAX 93/004	Eastern	43. TA 97/137	Volta
9. RAX 93/002	Eastern	44. TA 97/090	Volta
10. RAX 93/001	Eastern	45. TA 97/083	Volta
11. SCJ 98/009	Eastern	46. TA 97/074	Volta
12. SCJ 98/007	Eastern	47. TA 97/068	Volta
13. SCJ 98/006	Eastern	48. SCJ 98/002	Volta
14. SCJ 98/006	Eastern	49. TA 97/021	Volta
15. SCJ 98/005	Eastern	50. TA 97/163	Volta
16. SCJ 98/003	Eastern	51. TA 97/160	Volta
17. SCJ 98/015	Eastern	52. TA 97/54	Volta
18. SCJ 98/014	Eastern	53. TA 97146	Volta
19. SCJ 98/013	Eastern	54. BD 96/085	Eastern
20. SCJ 98/012	Eastern	55. BD 96/079	Eastern
21. SCJ 98/011	Eastern	56. BD 96/078	Eastern
22. SCJ 98/010	Eastern	57. BD 96/077	Eastern
23. SCJ 98/023	Eastern	58. BD 96/123	Eastern
24. SCJ 98/022	Eastern	59. BD 96/118	Eastern
25. SCJ 98/021	Eastern	60. BD 96/111	Eastern
26. SCJ 98/020	Eastern	61. BD 96/102	Eastern
27. SCJ 98/019	Eastern	62. BD 96/093	Eastern
28. SCJ 98/106	Eastern	63. BD 96/092	Eastern
29. SCJ 98/094	Eastern	64. BD 96/163	Eastern
30. SCJ 98/024	Eastern	65. BD 96/158	Eastern
31. BD 96/070	Eastern	66. BD 96/145	Eastern
32. BD 96/064	Eastern	67. BD 96/135	Eastern
33. BD 96/059	Eastern	68. BD 96/126	Eastern
34. BD 96/059	Eastern	69. BD 96/124	Eastern
35. BD 96/091	Eastern	70. BD 96/183	Eastern

PCR amplification

PCR amplification was carried out using the ten primers mentioned above. Amplification was performed in a total volume of 30 µl PCR reaction mixture containing 10 mM Tris-HCl pH 8.0, 10 mM KCl pH 8.3, 3.5mM $MgCl_2$, 0.17 mM of dNTPs, 0.5 µM primer, 25 ng genomic DNA and 1.6 U Taq polymerase Stoffel fragment (Pharmacia). PCR was carried out in a Progene thermo-cycler with heated lid to reduce evaporation. The cycling programme involved an initial denaturing at 94°C for 1 min followed by 45 cycles at 94°C for 30 s, 37°C for 30 s, 72°C for 1 min and then a final 72°C for 5 min extension. The RAPD-PCR products were electrophoresed in 2%

agarose (Sigma, St. Louis, MO) gels in TAE buffer (Tris-acetate) at 40 volts for 5 h. The agarose was stained with ethidium bromide, visualised under UV and photographed using a Polaroid MP4 camera. A 100 bp DNA marker from Gibco BRL (New York, USA) was used as a standard.

Statistical analysis

Population genetics analysis. — Unambiguous RAPD bands were scored manually as present (1) or absent (0) from the gels. A data matrix of individual × marker containing the band scoring information was transformed to allele frequencies under the assumption that each amplified band corresponds to a different RAPD locus. The data set was then used to calculate observed and effective number of alleles per individual per population, genetic diversity estimates, Gst (Nei 1973) and geneflow (Nm) using the POPGENE (version 1.31) Microsoft Window-based software (Yeh and Boyle 1997). The observed number of alleles is the number of alleles with non-zero frequency and effective allele number estimates the reciprocal of homozygosity (Hartl and Clark 1997). The total number of alleles for either observed or effective was divided by number of individual accessions and scored as the means.

Phenetic analysis. — For the phenetic analysis, only polymorphic bands were included in the binary data set and similarities were calculated using Jaccard's coefficient (Jaccard 1908): $S_{ij} = a/(a+b+c)$ where S_{ij} is the similarity between two individuals I and j, a is the number of bands present in both I and j, b the number present in I but absent in j and c the number present in j but absent in i. Cluster analyses using the UPGMA (unweighted pair-group method with arithmethic averages; Sokal and Michener 1958) were carried out on the similarity matrices using the software Genstat 5 statistical package (Genstat 5 Committee 2000).

RESULTS AND DISCUSSION

RAPD profile

A total of 120 bands (Table 2) from the ten primers were scored as reliable and consistent and included in the analysis. There was variation in the number of amplified fragments generated by the primers from the cocoyam accessions. One hundred and twelve of the amplified fragments were polymorphic.

Genetic variation in the eastern region accessions. — A summary of genetic variation statistics for all loci is shown in Table 3. Observed number of alleles per individual per population ranged from 1.95 to 2.00 with a mean of 1.99 ± 0.01, while effective number of allele per individual per population ranged from 1.30 (OPAM-05) to 1.68 (OPC-17) with a mean of 1.53 ± 0.12. Nei's diversity ranged from 0.22 (OPAM-05) to 0.38 (OPC-17) with a mean of 0.31 ± 0.05.

Table 2. *Ten oligonucleotide primers used for RAPD analysis of the cocoyam germplasm showing the amplified (A) and polymorphic (P) fragments and percent polymorphism (PP).*

Primer	Nucleotide sequence	A	P	PP (%)
OPAM-03	CTTCCCTGTG	9	8	88.9
OPAM-04	GAGGGACCTC	12	11	91.7
OPAM-05	GGGCTATGCC	8	7	87.5
OPB-01	GTTTCGCTCC	9	7	77.8
OPB-19	ACCCCCGAAG	16	16	100.0
OPC-05	GATGACCGCC	10	9	90.0
OPC-17	TTCCCCCCAG	11	9	69.2
OPD-01	ACCGCGAAGG	16	16	100.0
OPE-09	CTTCACCCGA	17	17	100.0
OPJ-01	CCCGGCATAA	12	12	100.0

Genetic variation in the Volta region accessions. — A summary of genetic variation the Volta region accessions is presented in Table 3. The number of alleles per individual per population ranged from 1.47 (OPD-01) to 2.00 (OPC-17, OPD-01) with a mean of 1.76 ± 0.17 and effective number of alleles per individual per population ranged from 1.30 (OPC-05) to 1.69 (OPD-01) with a mean of 1.44 ± 0.16. Nei's diversity ranged from 0.10 (OPAM-04) to 0.40 (OPD-01) with a mean of 0.26 ± 0.08.

Genetic variation in all accessions. — A summary statistics for all seventy accessions is presented in Table 3. The observed number of alleles per individual ranged from 1.94 (OPE-09) to 2.00 with a mean of 1.99 ± 0.02. Effective number of alleles per individual ranged from 1.33 (OPAM-05) to 1.68 (OPC-07) with a mean of 1.54 ± 0.13. Nei's diversity ranged from 0.24 (OPAM) to 0.38 (OPC-17, OPD-01) with a mean of 0.32 ± 0.05.

Nei's analysis of gene diversity in the subdivided populations

Nei's genetic diversity in the subdivided populations of the accessions is presented in Table 4. The total genetic diversity (Ht) varied between 0.230 and 0.396 with a mean of 0.313 ± 0.06. Within sample genetic diversity (Hs) ranged between 0.214 to 0.375 with a mean of 0.286 ± 0.06. Gene differentiation (Gst) ranged from 0.01 (OPC-17) to 0.18 (OPE-09) with a mean of 0.086. Geneflow estimate ranged from 2.309 (OPE-09) to 99.500 (OPC-17) with a mean of 5.314.

Table 3. *Observed (O) and effective (E) number of alleles and Nei's genetic diversity for accessions. (a: the eastern region; b: the Volta region; c: combined accessions).*

Primer	Material	O	E	Nei's diversity
OPAM-03	a	2.00	1.60	0.347
	b	1.67	1.43	0.299
	c	2.00	1.63	0.363
OPAM-04	a	2.00	1.52	0.326
	b	1.89	1.17	0.102
	c	2.00	1.43	0.277
OPAM-05	a	2.00	1.30	0.219
	b	1.89	1.40	0.254
	c	2.00	1.33	0.236
OPB-01	a	2.00	1.40	0.251
	b	1.67	1.48	0.269
	c	2.00	1.48	0.287
OPB-19	a	2.00	1.53	0.314
	b	1.75	1.40	0.248
	c	2.00	1.54	0.323
OPC-05	a	2.00	1.42	0.255
	b	1.70	1.30	0.209
	c	2.00	1.42	0.256
OPC-17	a	2.00	1.67	0.381
	b	2.00	1.67	0.369
	c	2.00	1.68	0.380
OPD-01	a	2.00	1.57	0.348
	b	2.00	1.69	0.396
	c	2.00	1.65	0.380
OPE-09	a	1.95	1.63	0.338
	b	1.47	1.35	0.205
	c	1.94	1.63	0.349
OPJ-01	a	2.00	1.61	0.343
	b	1.83	1.51	0.287
	c	2.00	1.60	0.339
Mean	a	1.99 ± 0.01	1.53 ± 0.12	0.312 ± 0.05
	b	1.76 ± 0.17	1.44 ± 0.16	0.264 ± 0.08
	c	1.99 ± 0.02	1.54 ± 0.12	0.319 ± 0.05

Phenetic RAPD analysis

Jaccard's similarity coefficient values ranged for the comparison of the individual accessions from 0.15 to 0.84. The phenogram produced by UPGMA of the Jaccard similarity matrix from the pooled data of the 10 primers from the 70 samples is shown in Fig. 1. Three major clusters were identified from the phenogram. The first major cluster which was a single accession, BD 96/183 collected from Awaman Junction in the Volta region was relatively distant from the other accessions. It separated from the two other major clusters at the 25% level of similarity.

The second major cluster was a tight cluster and comprised 12 accessions (BD 96/091, BD 96/123, BD 96/118, BD 96/111, BD 96/102, BD 96/092, BD 96/163, BD 96/158, BD 96/145, BD 96/135, BD 96/126, BD 96/124) collected from 11 different localities but in the Volta region (Table 1). The third major cluster was subdivided into three subclusters at the 60% level of similarity. Subcluster 1 comprised eighteen accessions three of which (BD 96/079, BD 96/078, BD 96/077) were from the eastern region. Subcluster 2 was made up of 19 accessions all of which were from the eastern region (Table 1 and Fig. 1). The third subcluster comprised 20 accessions. Sixteen were collected from the eastern region and the remaining four (TA97/056, TA97/037, TA97/018, and TA97/017) from the Volta region.

The results showed that the number of RAPD markers generated per primer varied as a result of primer sequence and individual genotype. This outcome is consistent with studies in other crops (Williams et al. 1990). In Ghana, cocoyam accessions show different morphological forms such as corm and cormel colour, cormel size, number of cormels per plant, taste and number of leaves per plant. Though no attempt has been made to correlate

Table 4. *Nei's F-statistics in the subdivided populations for all primers.*

Primer	Over all gene diversity (Ht)	Within sample gene diversity (Hs)	Gene differentiation (Gst)	Gene flow (Nm)
OPAM-03	0.353	0.303	0.142	3.02
OPAM-04	0.230	0.214	0.077	6.64
OPAM-05	0.246	0.236	0.041	11.70
OPB-01	0.299	0.260	0.130	3.346
OPB-19	0.314	0.281	0.105	4.262
OPC-05	0.252	0.228	0.095	4.763
OPC-17	0.377	0.375	0.005	99.500
OPD-01	0.396	0.372	0.013	3.796
OPE-09	0.331	0.272	0.178	2.309
OPJ-01	0.329	0.315	0.043	11.128
Mean	0.313 ± 0.06	0.286 ± 0.06	0.086	5.314

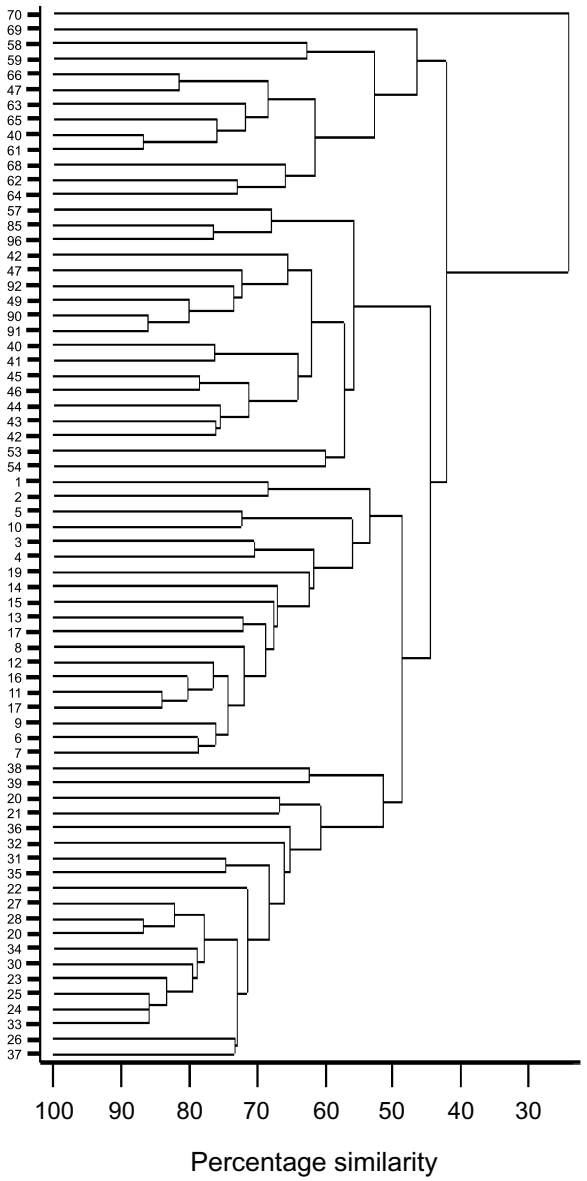

Fig. 1. Phenogram using UPGMA analysis showing relationships based on diversity of RAPDs among 70 accessions of cocoyam.

morphological differences with RAPDs polymorphisms, these morphological differences could reflect level of RAPD marker polymorphism, number of amplified fragments per primer and differences in marker frequencies in the 70 cocoyam accessions. The experimental total genetic diversity Ht of 0.313 ± 0.06 is also high and indicates that present day cocoyam might have developed from seeds as a result of open-pollination. The high diversity might have been maintained from generation to generation over the years. Mean coefficient of genetic differentiation of $Gst = 0.086$ indicates about 9 per cent total genetic variation between accessions from eastern and Volta regions Therefore, the largest amount of genetic variation (0.914) resided within the groups. RAPD analysis can be used to evaluate geneflow between species (Arnold et al. 1991; Quiros et al. 1991). An experimental geneflow estimate of 5.314 shows there has been a significant geneflow of cocoyam germplasm between accessions from the eastern and Volta regions, thus the seventy accessions did not cluster into their distinct geographical regions (Fig. 1). These accessions might have originated in localities different to those assigned based on their collection points. This suggests that 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 two regions to each other. Cocoyam is a vegetatively propagated crop and, therefore, unlikely to generate variation within a short span of time. The results of the current study will be useful in selecting germplasm for breeding and conservation. Accession BD96/183 which was the most diverse among all the accessions may have some distinct characteristics and can be incorporated in breeding programs in cocoyam in the country. The conservation of vegetatively propagated crops is logistically difficult and expensive. The clustering of the accessions could be a guide in selecting materials for preservation under limited storage facilities as exit in the Ghana.

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