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Phenotypic Diversity and Ploidy Level of Some Dioscorea Dumetorum Genotypes

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Abstract: *Dioscorea dumetorum (Kunth), Pax. has suffered research neglect in varietal identification and improvement. Understanding of the ploidy and phenotypic diversity is fundamental for the utilisation of the species in breeding programme. Twenty-three D. dumetorum genotypes were laid out in randomised complete block design with three replications in two years. Sixteen morphological traits were employed to characterize the genotypes. Ploidy levels were determined from chromosome counts and flow cytometric analysis of the 23 genotypes. Two main groups subsisted from the clustering analysis. Traits of discriminatory significance for the species were: tuber weights, tuber girth, leaf length, number of tubers per plant and tuber length. Genotypes in cluster II had higher tuber weight (1.23±0.10 kg), girth (21.42±0.91 cm) and leaf length (10.36±0.30 cm) compared with 1.07±0.12 kg, 19.72±1.04 cm and 10.08±0.34 cm observed in cluster I respectively. The 23 genotypes exhibited tetraploidy = 4x. The chromosome count and flow cytometry methods agreed. Average chromosome counts in TDd 3908 and TDd 3098 were 36.71±2.28 and 38.00±2.16 respectively. DNA content range from 33.16 AU (TDd 05-23) to 46.98 AU (TDd 04-146) with mean of 40.29±0.94 AU. Considerably high genetic diversity exist in the species however, some variability may still be hidden in larger population. Molecular assessment of larger population of this species is suggested for subsequent study*.
Keyword: *D. dumetorum, diversity, ploidy level, flow cytometry, cluster analysis*

I. Introduction

Yams; Dioscorea spp in the family Dioscoreacea and order Dioscoreale which has contributed immensely to the life and feeds of people in the sub-sahara Africa is widely distributed in the tropical and sub-tropical regions of the world. Out of the 644 species within the genus, only six have economic importance for food, medicine, and income in West Africa. Nigeria cultivates only four of the six species which are: D. cayenensis Lam., D. rotundata Pior., D. alata L., and D. dumetorum (Kunth), Pax. The former three are very well known and consumed, while the latter, D. dumetorum is least in production and utilization importance [1].

D. dumentorum, otherwise called clustered, trifoliate or bitter yam is indigenous to Eastern Nigeria [2]. Its tubers occur in clusters and edible flesh could be white or yellow. The bitter taste; which may be responsible for the under exploitation and utilisation is due to the presence of alkaloids, oxalates and saponin. Processing and cooking have been found to appreciably reduce the compounds [2], [3], [4]. The species is a highly delectable food to some sector of the populace, especially the diabetics. There are reports [5], [6], [7] that consumption of D. dumentorum lowers blood sugar and studies on its nutritive composition revealed the presence of the basic food nutrients (carbohydrate, protein, lipids and vitamin) within the reported and acceptable dietary value for roots and tubers. Aside comparing well with D. rotundata, D. alata and sweet potatoes (Ipomeoa batatas), [8] reported that the protein and phosphorus content in D. dumentorum is higher than in D. rotundata, D. alata and D. cayenensis.

Morphological, biochemical and molecular methods have been adopted to understand the diversity of some yam species: D. cayenensis-rotundata complex [9], [10]; and D. alata [11]. However owing to its poor utilization, studies to understand the inherent diversity in D. dumentorum have received little attention; hence, prospective breeding programme for its genetic improvement lags behind other species. Most Dioscorea species are polyploidy. Through Flow cytometry and chromosome counting, ploidy levels of yam germplasm collections have been determined [12]; [13] and [14]; [15]; [16]. Understanding the ploidy status at intra-specific level presents a guide to appropriate allocation of genotypes within the germplasm and enhancement of proper match of parents for hybridisation.

The knowledge of morphological diversity and ploidy status of genotypes within the D. dumentorum germplasm is very important for the initiation of a breeding programme for the improvement of this neglected species. Therefore, the objectives of the present study includes: to understand the genetic diversity of twenty-three accessions of D. dumentorum using phenotypic morphological data and to determine the ploidy status of the twenty-three genotypes.

II. Materials And Methods

Plant materials: Twenty three D. dumentorum genotypes from six tropical countries, maintained at the Genetic Resource Centre of the International Institute of Tropical Agriculture (IITA) were employed in the study (See Table 1). Yam sett; 250g of each genotype was planted in mounds (40-50 cm high) spaced 1m x 1m apart at the IITA field, Ibadan, Nigeria. The experiment was laid out in randomized complete block design (RCBD) of three replications with 15 plants per plot.

Morphological characterization : Sixteen morphological variables were collected using yam descriptors [17]. To identify the significance and discriminatory potentials of each trait, analysis of variance (ANOVA), stepwise discriminant approach and the maximum likelihood Chi-Squared test were applied on the continuous and discreet variables respectively [18]. Data matrix of the 23 genotypes and the identified most discriminatory traits were submitted for further analysis. Similarities among genotypes were assessed by Ward's minimum variance cluster analysis [19]. ANOVA, Stepwise Discriminant, Likelihood Ratio tests and cluster analysis were made using the GLM, STEPDISC, FREQ and CLUSTER procedures from the Statistical Analysis System, SAS-V9.2 [20].

Ploidy analysis

Chromosome count: Tuber minisetts (50g) were grown in carbonized rice husk filled polyethylene bags ($250cm^3$) in a green house. Actively growing root tips (5mm) were collected from 3 to 7 weeks old seedlings from two D. dumentorum accessions (TDd 3908 and TDd 3098) between 1300 and 1320 hours, and fixed at 4^OC for 24 hours in a mixture of 1:3 glacial acetic acid: absolute ethanol. Samples were stained in Aceto-carmine solution (1%) at room temperature for 48 hours, heated, cooled and the root cap (0.5 mm long of the root tip) removed. The meristematic portion next to the root cap was cut into drops of 45% glacial acetic acid on a glass slide, crushed with the tip of a mounting needle and pressed vertically between folds of blotting paper. Prepared slides were observed with a Leitz Diaplan light microscope. Chromosomes were counted from metaphase spreads at the 1000x magnification using an oil immersion. Average number of chromosomes was used as an estimate of ploidy level.

Preparation of nuclei for flow cytometry

Procedure outlined by [21] was adopted with slight modifications. Nuclei were isolated from young and healthy leaves of each of the 23 genotypes by chopping 30 to 50 mg leaves with a razor blade in some ice cold extraction buffer: 0.5ml Otto 1 buffer (0.1M citric acid monohydrate, 0.5% Tween 20), [22]. The homogenate was filtered through 30µm pore size nylon filter and incubated for 2-3 minutes at room temperature. Staining was carried out by adding 2ml of Otto II buffer (0.4M Na_2PO_4) supplemented with 4 µg/ml of DAPI (4-6 diamidino-2- phenylindole) and 1 µl/ml mercaptoethanol. Relative fluorescence intensity of stained nuclei was determined sing a Partec Ploidy analyzer (Partec GmbH, Germany). The distribution of florescence intensities which signifies the relative DNA content of samples are usually given as channel numbers (arbitrary units). A reference sample (a cultivar with known ploidy) was used for calibrating the ploidy scale. In this analysis the reference sample was a tetraploid Dioscorea rotundata, variety amula with 4x = 40 chromosomes. The ploidy analyzer was adjusted to channel 50 which is the G1 peak of nuclei isolated from the tetraploid standard. Samples for analysis were then run through the cytometer. The calibration was checked periodically and kept constant between runs to minimize variation. To estimate ploidy level, position of the G1 peak of histograms obtained for each sample was compared to that of the standard. Coefficient of variation (CV) was determined as the quotient of standard deviation of the peak and the mean peak of positions using Partec software package.

III. Results

Six of the 16 variables recorded were continuous and showed varying strength in distinguishing the 23 D. dumentorum accessions. Leaf length varied from 8.18 to 12.26 cm and the biggest tuber girth was about twice the girth of smaller tubers (Table 2). Agglomerative hierarchical clustering dendrogram showing relationship among the 23 yam accessions is shown in Figure 1. Clustering was based on similarities for some morphological traits among the 23 D. dumentorum accessions. Two main clusters each with two sub clusters were formed. Each cluster group shown on the dendrogram was characterized using all the variables from the study; however only quantitative traits with significant f-values and qualitative traits with significant Likelihood Chi square ratio of $P \leq 0.01$ and $P \leq 0.05$ were used to characterize the clusters (Table 3 and 4).

The two clusters formed were discriminated by tuber weight, tuber girth, leaf length, number of tubers per plant and tuber length. Cluster II had higher value for tuber weight, tuber girth and leaf length (Table 3).

Out of the ten qualitative traits, the most discriminative variables were quantity of spine on the stem, aerial bubil and tuber shape (Table 4). Accessions possessing more spines on the vine (88.88%) are grouped in

cluster II. The group also lack aerial bubils and possess round to irregular shaped tubers (Table 4). Accessions in cluster I have fewer spines on their vines and possess round to oval oblong tuber shapes. Out of the 23 accessions, 15 were males, 2 females, 1 have both male and female flowers (monoecious) while the rest 5 did not flower in the trials.

Chromosome count: The chromosomes at the mitotic stage of metaphase were small, dot like and clumped together (Plate 1). Chromosome counts at higher magnification revealed an average range of 36 - 40 chromosomes and hence are tetraploids (Table 7). The standard variation of counts ranged from 1.06 to 2.28.

Flow cytometry: All the accessions revealed tetraploid chromosome levels with the dominant peak of each sample approximately on channel 50 (Table 8). The relative DNA content in channel numbers/arbitrary units (AU) has a mean of 40.29 AU, and ranged from 33.16 AU to 46.98 AU. The CVs for the G1 peaks of plant nuclei varied between 5.1 and 9.8.

IV. Discussion

Clustering analysis resolved the D. dumentorum into two clusters. Variability was observed within the species which indicated morphological polymorphism from which breeders can make selections of suitable parents for further breeding work. The variability observed within most species in Dioscorea is associated with cross pollination and sexual recombination. Variability may also have arisen as a result of spontaneous mutation followed by long term selection and cultivation in various environments [11]. Although the D. dumentorum accessions were land races from different West African countries, they did not show grouping based on geographical location. Variation observed might thus be due to a mixture of planted clones and recombinant genotypes from natural sexual reproduction growing together on farmers' field. Furthermore, lack of relatedness in cluster formation to source of collection suggests that geographical diversity need not influence genetic diversity [23]; [24]. In yams, general appearances of the plant and the tuber are of great importance in identification of cultivars [9]. Both quantitative and qualitative traits morphologically differentiated the 23 accessions. The traits that best discriminate the D. dumentorum accessions were tuber girth, tuber weight, leaf length, number of tubers per plant, tuber length, spines on stem, aerial bubil and tuber shape. These traits may be useful as markers for classification and for genetic improvement of the crop [11].

Accessions without aerial bubils tend to produce tubers with larger girth and weight. They also had fewer spines on their stems. This suggest that assimilates was shared between aerial and underground tubers, subsequent selection for large tubers may be based on selecting accessions with no aerial bubils.

Sex ratios revealed higher proportion of male to female flowers in the species (2 females: 16 males). Higher ratio of male to female flowers (27:64) was also recorded in D. alata from Kerala [25] and (33:44) in D. cayenesis/D. rotundata complex from Benin Republic [9].

The present study revealed that ploidy level estimation by both conventional chromosome counting and flow cytometry were complementary. Earlier reports [26]; [27] and [12] admitted that chromosome counting in yams is difficult; however, flow cytometry offers a quick and robust method. Reported [12]; [14]; [15]; [28]; [29] levels of ploidy in Dioscorea species especially D. alata and D. cayenensis-rotundata complex includes: tetraploid (4x), hexaploids (6x) and octoploid (8x); [16] reported diploid and triploids in D. dumentorum. However, in the present study, the 23 D. dumentorum accessions were tetraploids. This is in agreement with the findings of [27], [12] and [29], that tetraploids were most frequent in the Dioscorea species. Preponderance of tetraploids over other ploidy levels was also observed in Cote d'lvoire [30]; [31], Cameroon [13] and Benin republic [32]. While tetraploids' occurrence seemed to be most frequent in Dioscorea, hexaploids and octoploids are evolutionary changes from the tetraploids. Moreover, ploidy level beyond octoploid is scarce in yam.

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Tables and Figures

Table 1: List and country of origin of the 23 D. dumentorum evaluated in the present study

S/N	Accession number	Local name	Country of origin
1	TDd 05-26	-	Cote d'Ivoire
2	TDd 3110	Agbota 457	Togo
3	TDd 05-5	-	-
4	TDd 05-10	-	-
5	TDd 3790	Peeba	Nigeria
6	TDd 3779	Amola	Nigeria
7	TDd 3100	Dokoute 33	Togo
8	TDd 04-146	-	-
9	TDd 05-16	-	-
10	TDd 3101	Alavanyo-wudidi	Ghana
11	TDd 05-24	-	-
12	TDd 3114	-	Gabon
13	TDd 3848	Leife	Benin

14	TDd 3093	Vote 333	Togo
15	TDd 05-3	-	-
16	TDd 05-6	-	-
17	TDd 05-23	-	-
18	TDd 4088	Dakoate	Togo
19	TDd 3829	Klimp- 36	-
20	TDd 3112	-	Nigeria
21	TDd 3095	Koute 281	Togo
22	TDd 3908	Akucha	Benin
23	TDd 3098	-	-

: Record not available

Table 2: Measures of Variability among the 23 accessions of D. dumentorum based on some quantitative characters

Character	Mean	Min	Max	S.D	CV (%)	P-Value
Leaf length	10.23	8.18	12.26	0.72	7.00	6.52***
Leaf width	6.77	5.69	8.37	0.55	8.13	6.00***
Tuber length	12.65	10.27	14.70	1.16	9.17	3.51**
Tuber girth	20.68	14.66	30.43	2.30	11.11	6.28***
Number of tubers per plant	1.42	1.00	2.10	0.23	15.95	6.75***
Tuber weight per plant	1.16	0.59	1.87	0.20	16.88	10.99***

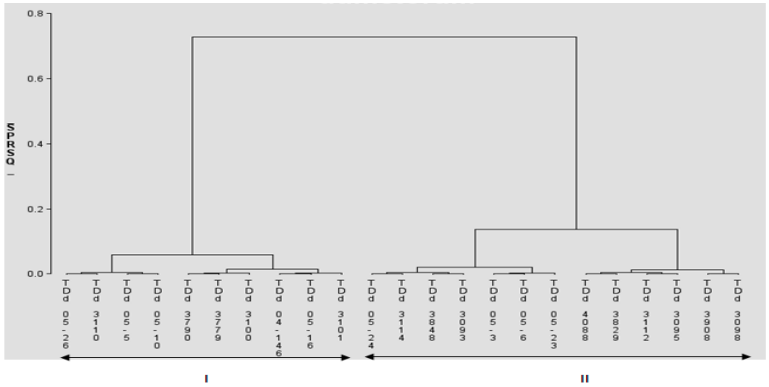

Figure 1: A dendrogram showing the groupings of the 23 accessions of D. dumentorum

Table 3: Intra-cluster variability of the 23 accessions of D. dumentorum based on some quantitative descriptors

Variables	CL I (Mean ± S.E)	CL II (Mean ± S.E)	Grand Mean
Tuber weight	1.07 ± 0.12	1.23 ± 0.10	1.16
Tuber girth	19.72 ± 1.04	21.42 ± 0.91	20.68
Leaf length	10.08 ± 0.34	10.36 ± 0.30	10.42
No. of tubers	1.47 ± 0.11	1.39 ± 0.10	1.42
Tuber length	12.79 ± 0.40	12.54 ± 0.35	12.65

Table 4: Percentage distribution of different significant qualitative characters within the cluster groups of 23 D. dumentorum accessions

Character	State	Clusters		
		1	2	Total
Spine on stem**	Few	9 (64.29%)	5 (35.71%)	14
	Many	1 (11.11%)	8 (88.88%)	9
Total (n)		10	13	23
Aerial bubil**	Absent	8 (38.10%)	13 (61.90%)	21
	Present	2 (100.00%)	0 (0.00%)	2
Total (n)		10	13	23
Tuber shape**	Round	7 (53.85%)	6 (46.15%)	13
	Oval	0 (0.00%)	1(100.00%)	1
	Oval-oblong	2 (100.00%)	0 (0.00%)	2
	Cylindrical	1 (100.00%)	0 (0.00%)	1
	Irregular	0 (0.00%)	6 (100.00%)	6
Total (n)		10	13	23
Leaf hairiness ns	Sparse	3 (33.33%)	5 (66.67%)	8
	Dense	7 (46.66%)	8 (53.33%)	15
	Total	10	13	23
Young leaf color ns	Pale green	4 (44.44%)	5 (55.55%)	9
	Dark green	7 (50.00%)	7 (50.00%)	14
	Total	11	12	23
Flowering/ Sex ns	Non-flowering	2 (40.00%)	3 (60.00%)	5

	Female	0 (0.00%)	2 (100.0%)	2
	Male	8 (53.33%)	7 (46.67%)	15
	Monoecius	0 (0.00%)	1 (100.0%)	1
	Total	10	13	23
Surface texture of	Smooth	6 (54.55%)	5 (45.45%)	11
	Rough	4 (33.33%)	8 (66.66%)	12
	Total	10	13	23
Leaf density ^{ns}	Low	2 (40.00%)	3 (60.00%)	5
	Intermediate	3 (50.00%)	3 (50.00%)	6
	High	5 (41.67%)	7 (58.33%)	12
	Total	10	13	23
Tuber flesh color ^{ns}	White	4 (40.00%)	6 (60.00%)	10
	Yellowish	1 (100.0%)	0 (0.00%)	1
	Creamish	5 (41.67%)	7 (58.33%)	12
	Total	10	13	23

n= Number of accessions by cluster.
**: P≤0.01 (Significance in the Likelihood Ratio Chi-Square test of association); $^{ns =}$ (not significant)

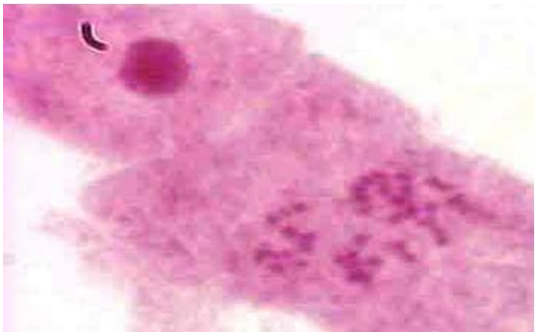

Plate 1: Cells at prophase and metaphase of D. dumentorum (chromosomes dot-like and clumped together) (400x)

Table 5: Chromosome counts in two accessions of D. dumentorum

Species	**No. of chromosome Counts (Replicates)**							**Results**			**Remark**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**Mean**	**Std. Dev**	**CV**	**Ploidy level**
D.dumentorum											
TDd 3908	36	35	34	39	35	40	38	36.71	2.28	6.21	4x
TDd 3098	39	35	36	37	40	41	38	38.00	2.16	5.68	4x

Table 6: Fluorescence intensity in arbitrary units (AU) and ploidy levels of the 23 D. dumentorum accessions

Accession ID	**Relative fluorescence intensity(AU)**	**CV (%)**	**Ploidy level**	**Comment**
TDd 05-26	44.12	6.9	4x	Tetraploid
TDd 3110	34.16	9.5	4x	Tetraploid*
TDd 05-5	44.28	8.4	4x	Tetraploid
TDd 05-10	44.12	8.5	4x	Tetraploid
TDd 3790	45.73	7.1	4x	Tetraploid
TDd 3779	35.47	9.1	4x	Tetraploid*
TDd 3100	34.16	9.5	4x	Tetraploid*
TDd 04-146	46.98	7.3	4x	Tetraploid
TDd 05-16	43.62	7.4	4x	Tetraploid
TDd 3101	33.38	6.7	4x	Tetraploid*
TDd 05-24	43.92	5.1	4x	Tetraploid
TDd 3114	44.87	8.3	4x	Tetraploid
TDd 3848	44.19	6.2	4x	Tetraploid
TDd 3093	38.79	6.9	4x	Tetraploid
TDd 05-3	35.65	7.0	4x	Tetraploid*
TDd 05-6	37.18	7.3	4x	Tetraploid*
TDd 05-23	33.16	9.8	4x	Tetraploid*
TDd 4088	43.73	7.4	4x	Tetraploid
TDd 3829	40.93	7.9	4x	Tetraploid
TDd 3112	41.78	7.7	4x	Tetraploid
TDd 3095	39.26	9.4	4x	Tetraploid
TDd 3908	38.99	9.4	4x	Tetraploid
TDd 3098	38.40	9.6	4x	Tetraploid

* may have lower number or smaller sized chromosomes