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Determination of ploidy among Yam (*Dioscorea* spp.) landraces in Kenya by flow cytometry

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Yam (*Dioscorea* spp.), a traditional crop in Kenya has not undergone improvement and little has been done to understand its genetic background. The taxonomy and phylogeny of the local landraces has not been fully studied. The main cultivated species is *Dioscorea minutiflora* Engl. Others found with low distribution are *Dioscorea alata* L., *Dioscorea bulbifera* L. and *Dioscorea odoratissima* Pax. Flow cytometry was used to estimate the ploidy level of 155 accessions of Kenyan yam including two checks, TDr.18544 a tetraploid and TDc.98136 an octoploid from International Institute of Tropical Agriculture (IITA), Nigeria. Also included in the study were *Dioscorea dumetorum* Pax, *Dioscorea asteriscus* Burkill and *Dioscorea schimperiana* Kunth which are yam wild relatives. Leaf samples were harvested from the field genebank and nuclei extracted using an extraction buffer (Partec GmbH, Munster Germany). Plant nuclei were isolated and stained with propidium iodide then analyzed in a flow cytometer. Seven ploidy levels of 3x (11.4%), 4x(37.5%), 5x(29.2%), 6x(14.6), 7x(3.1%); 8x(3.1%) and 10x(0.6%) were observed. Tetraploids (4x) formed the highest proportion followed by pentaploids (5x). The highest ploidy, decaploid, (10x), was found in *D. odoratissima* Pax, a conspecific form of *Dioscorea preahensilis* found under cultivation in two farms in Western Kenya. No diploids were observed in the study. Ploidy level was not associated with geographical habitat of the landraces while farmer-named varieties were not associated with ploidy levels. The findings generated new knowledge and form a basis for future yam research and improvement in the country. Further work is required to establish the phylogeny of Kenyan yam landraces.

Key words: Ploidy, yam, Dioscorea, flow cytometry, Kenyan.

INTRODUCTION

Yams are one of the oldest food plants known. They have been cultivated since 50,000 BC in Africa and Asia. In addition to these continents, yams also currently grow in the tropical and subtropical regions of North and South America (Burkill, 1960). *Dioscorea* genus is the only dioecious genus in the family *Dioscorea*ceae and comprises of about 600 species (Wilkin et al., 2005). Out of the 600 known species only a few are edible, these include but not limited to *Dioscorea alata* L., *Dioscorea rotundata Poir*, *Dioscorea bulbifera* L., *Dioscorea esculenta*, *Dioscorea trifida* L., *Dioscorea dumetorum Pax*., *Dioscorea opposite* Thunb. and *Dioscorea cayenensis* Lamb. Although, a very important crop for food and medicinal purposes (Poornima et al., 2007). *Dioscorea* has presented a challenge to systematists for many years due to its great morphological diversity, dioecy and small flowers (Wilkin et al., 2005). Yam is a neglected or underutilized crop in Kenya and is mainly

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distributed around the eastern parts of Mount Kenya and around the Aberdares. Smaller pockets are found in parts of the coast, the Rift Valley and western Kenya (Maundu et al., 1999). Kenya's yam diversity is represented by a number of species including *Dioscorea minutiflora Engl.*, *D. bulbifera L.*, and *D. dumetorum* Pax that are grown for food by mainly elderly farmers in the Eastern, Central, Western and Coastal regions of the country (Maundu et al., 1999). *D. dumetorum* was found in the coastal area though not in cultivation. The main species cultivated in the central region of the country is *D. minutiflora* which is known as a wild species in the main yam growing areas of West and central Africa.

D. *alata* L. has also been reported to be cultivated in parts of coastal and western regions (Muthamia et al., 2013). *D*. *bulbifera* has a smaller distribution in the central parts of the country. *Dioscorea odoratissima* Pax*,* has been found in cultivation in two farms in Western Kenya. This species has been described as conspecific form of *Dioscorea praehensilis* Benth*.* (Wilkin, 2001). Area under yams in Kenya increased from 882 ha in 2009 to 1,224 ha in 2010. Production increased to 8,035 tons in 2010 compared to 4,427 tons in 2009 (MoA, 2010). The demand for yams in urban towns and cities has been on the rise over the last few years. Yams are of both cultural and economic importance in the country where they have been used during ceremonies like youth initiation, payment of dowry and other rites (Mutegi et al., 2004; Mwirigi et al., 2009). Local farmers have been cultivating their own landraces for years; with little or no genetic improvement from the research and development sectors. These landraces manifest wide morphological characteristics and equally variable local names. Mwirigi et al. (2009) reported morphological differences among cultivated landraces in Kenya. There is limited research and literature of the crop in the areas of taxonomy, breeding and agronomy. These landraces are also facing genetic erosion due to many factors (Mutegi et al., 2004; Mwirigi et al., 2009).

An understanding of ploidy level among other research areas of the local landraces is useful before initiating yam breeding programmes. In Kenya, *D. minutiflora* Engl. is the major species grown and has a wide distribution and cultivar diversity. This species has been cultivated and selected by farmers over the years and appears also to have mixtures of other close wild relatives. The taxonomy of these cultivars is not well understood and requires further work (Paul Wilkin, personal communication). Yam is polyploid with ploidy levels of 2x (diploid), 3x (triploid) and higher ploidy levels. Segregation studies of microsatellite markers have revealed that the so called tetraploid species *D. rotundata* Poir. (2n = 40) is diploid with the basic chromosome number of x = 20 (Scarcelli et al., 2005). Gemma et al. (2009) have reported presence of diploids, triploids and tetraploids in *D. alata* microsatellite segregation analysis.

In their studies, the basic chromosome number of *D. alata* is x = 20. Several approaches have been used to determine the ploidy level in plants but in recent years since the emergence of flow cytometry traditional method have been abandoned. The small "dot-like" and often clumped yam chromosomes have complicated the use of conventional counting of chromosomes on microscope sides (Martin et al., 1966). Flow cytometry is an efficient and very successful method as is evident from the number of works to estimate the ploidy level and genome size of yam species (Hamon et al., 1992; Gamiette et al., 1999; Dansi et al., 2001a, 2001b; Egesi et al., 2002). Flow cytometry measures the content of nuclear DNA, based on the intensity of fluorescence produced by DNA- specific cytological stains. The ploidy level can be determined by comparing the samples with a known standard.

Knowledge of the amount of genetic material contained in the cell is informative and opens a vast array of applications from basic research to breeding (Dolezel et al., 2005). No previous ploidy studies have been conducted on the local landraces in the country and therefore this knowledge will go a long way in paving way for future research and hybridization programmes and understanding the phylogeny of yams in Kenya. Understanding the ploidy levels of the local yam landraces is essential for guiding future improvement and conservation programs of this crop.

MATERIALS AND METHODS

Plant materials

A total of 155 collections (Table 1) were collected from 17 districts in the country representing five administrative provinces where yams are grown (Figure 1). The species included were *D. cayenensis* Lam., *D. rotundata*, *D. odoratissima*, *D. asteriscus* Burkill, *D. dumetorum* Pax., *D. schimperiana* Kunth, *D. minutiflora* Engl., *D. alata* L. and *D. bulbifera* L. These were planted in a field genebank in Muguga, located at latitude 1° 13' 06'' S and longitude 36° 37'50'' E. Young and tender leaves were harvested from the field and placed in cool boxes before transporting them to the lab. Leaf samples of two accessions TDc. 98136 (*D. cayenensis* Lam.) and TDr.18544 (*D. rotundata*) which are known to be octoploid and tetraploid respectively were obtained from IITA, Nigeria. TDc.98136 was used as the internal control. The control serves as the internal standard since the ploidy level is known.

Nuclei isolation and fluorescent staining

To isolate and stain nuclei about 0.5 cm^2 of 3 to 4 week young fresh leaf of each yam accession were chopped together with a known octoploid standard, TDc 198136 from IITA, Nigeria using a double-edged razor blade in a 7 cm Petri dish containing 1000 µl of extraction buffer (Partec GmbH, Munster Germany). About one-third leaf weight of the internal standard was chopped and two-thirds leaf from the sample.

The suspension was incubated for 1 min and then filtered through 50 µm pore size. The filtrate was then centrifuged at 300 g for 5 min then resuspended in 200 µl of extraction buffer (Partec GmbH, Munster Germany), vortexed gently then incubated for 1 h at room temperature. The filtrate was finally stained with 600 µl of staining solution with 3.6 µl of propidium iodide solution and 3.6 µl of RNAse solution. After 1 h of incubation, the stained nuclei were analyzed using a flow cytometer (BD FACS Canto II). The linear log

Table 1. Ploidy levels of 155 yam landraces and wild relatives.

Accession	Local name	*Specie*	Ploidy level	Collection district	Region
1	Mbeu-nkuru 1	*D. minutiflora*	5x	Meru Central	Eastern
2	Karugwaci 1	*D. minutiflora*	3x	Meru Central	Eastern
3	Karugwaci 2	*D. minutiflora*	5x	Meru Central	Eastern
4	Ikambo	*D. minutiflora*	4x	Meru Central	Eastern
5	Karugwaci 3	*D. minutiflora*	5x	Meru Central	Eastern
6	Karugwaci	*D. minutiflora*	4x	Meru Central	Eastern
7	Mbeumburia	*D. minutiflora*	6x	Meru Central	Eastern
8	M'Ikinyori 1	*D. minutiflora*	4x	Meru Central	Eastern
9	Mbeu-Iguru 1	*D. minutiflora*	5x	Meru Central	Eastern
10	Aerial yam 1	*D. bulbifera*	5x	Meru Central	Eastern
11	Baribate	*D. minutiflora*	4x	Meru Central	Eastern
12	Ndingwa	*D. minutiflora*	4x	Meru Central	Eastern
13	Mtoikinyori 6	*D. minutiflora*	4x	Meru Central	Eastern
14	Mbeu-Iguru 2	*D. minutiflora*	3x	Meru Central	Eastern
15	Ntigania	*D. minutiflora*	5x	Meru Central	Eastern
16	Mbeu-nkuru 2	*D. minutiflora*	4x	Meru Central	Eastern
17	Nderema	*D. minutiflora*	4x	Meru Central	Eastern
18	Majara	*D. minutiflora*	6x	Meru Central	Eastern
19	Mueru	*D. minutiflora*	5x	Meru Central	Eastern
20	Ciotu	*D. minutiflora*	4x	Meru Central	Eastern
21	Ntoikinyori 9	*D. minutiflora*	4x	Meru Central	Eastern
22	M'Ikinyori 2	*D. minutiflora*	4x	Meru Central	Eastern
23	Aerial yam 2	*D. minutiflora*	8x	Meru Central	Eastern
24	Carungai	*D. bulbifera*	6x	Meru Central	Eastern
25	Ndianthi 1	*D. minutiflora*	4x	Embu	Eastern
26	Mundu-wakinyoni 1	*D. minutiflora*	3x	Embu	Eastern
27	Maribate 1	*D. minutiflora*	5x	Embu	Eastern
28	Tharangutu	*D. minutiflora*	4x	Embu	Eastern
29	Njuvi 1	*D. minutiflora*	5x	Embu	Eastern
30	Maribate 2	*D. minutiflora*	4x	Embu	Eastern
31	Mundu-wakinyoni 2	*D. minutiflora*	3x	Embu	Eastern
32	Nthiru	*D. minutiflora*	4x	Embu	Eastern
33	Ndianthi 2	*D. minutiflora*	5x	Embu	Eastern
34	Njuvi 2	*D. minutiflora*	6x	Embu	Eastern
35	Mundu-wakinyoni 3	*D. minutiflora*	4x	Embu	Eastern
36	Ndianthi 3	*D. minutiflora*	5x	Embu	Eastern
37	Ngwanjiru 1	*D. minutiflora*	5x	Embu	Eastern
39	Njuvi 3	*D. minutiflora*	3x	Embu	Eastern
40	Mundu-wakinyoni 4	*D. minutiflora*	5x	Embu	Eastern
41	Tharangutu	*D. minutiflora*	6x	Embu	Eastern
42	Kimeru	*D. minutiflora*	5x	Embu	Eastern
43	Ngwanjiru 2	*D. minutiflora*	5x	Embu	Eastern
44	Ndendera	*D. minutiflora*	4x	Embu	Eastern
45	Ndianthi 4	*D. minutiflora*	4x	Embu	Eastern
46	Mundu-wakinyoni 5	*D. minutiflora*	5x	Embu	Eastern
47	Theru	*D. minutiflora*	5x	Embu	Eastern
48	Icara B	*D. minutiflora*	4x	Embu	Eastern
49	Itarekia	*D. minutiflora*	4x	Embu	Eastern
50	Icara A	*D. minutiflora*	5x	Embu	Eastern
51	Nthonoya	*D. minutiflora*	4x	Embu	Eastern
52	Mundu-wakinyoni 5	*D. minutiflora*	3x	Embu	Eastern

Table 1. Contd.

53	Mundu-wakinyoni	*D. minutiflora*	6x	Embu	Eastern
54	Njuvi	*D. minutiflora*	5x	Embu	Eastern
55	Maribate	*D. minutiflora*	4x	Embu	Eastern
56	Njuvi	*D. minutiflora*	5x	Embu	Eastern
57	Tharangutu	*D. minutiflora*	3x	Embu	Eastern
59	Ncubi	*D. minutiflora*	4x	Igembe	Eastern
60	Mbeu-nkuru	*D. minutiflora*	4x	Igembe	Eastern
61	Thaana	*D. minutiflora*	6x	Igembe	Eastern
62	Mtoikinyoni/ Mwatuma	*D. minutiflora*	3x	Igembe	Eastern
63	Mtoikinyoni/ Mwakakianda	*D. minutiflora*	4x	Igembe	Eastern
64	Mtoikinyoni	*D. minutiflora*	3x	Igembe	Eastern
65	Nkwanjeru	*D. minutiflora*	5x	Igembe	Eastern
66	Mbonyongo	*D. minutiflora*	5x	Igembe	Eastern
67	Twambo	*D. minutiflora*	6x	Igembe	Eastern
68	Acumbi	*D. minutiflora*	5x	Igembe	Eastern
69	Karugwaci	*D. minutiflora*	5x	Igembe	Eastern
70	Mbeu-nkuru	*D. minutiflora*	4x	Igembe	Eastern
71	Ntwambo	*D. minutiflora*	4x	Igembe	Eastern
72	Naro	*D. minutiflora*	5x	Igembe	Eastern
73	Ntharieruri	*D. minutiflora*	4x	Igembe	Eastern
74	Nkwanyoni	*D. minutiflora*	4x	Igembe	Eastern
75	Mwiyosia	*D. minutiflora*	5x	Igembe	Eastern
76	Mbonyongwe	*D. minutiflora*	3x	Igembe	Eastern
77	Nkwamburi	*D. minutiflora*	5x	Igembe	Eastern
78	Kaaka	*D. minutiflora*	4x	Igembe	Eastern
79	Nthonia	*D. minutiflora*	5x	Igembe	Eastern
80	Nthania	*D. minutiflora*	3x	Igembe	Eastern
82	Ntoikinyori/ Mugandu	*D. minutiflora*	5x	Igembe	Eastern
83	Ngundu	*D. minutiflora*	6x	Igembe	Eastern
84	Ntharieruri	*D. minutiflora*	7x	Igembe	Eastern
86	Ntoroarure	*D. minutiflora*	5x	Igembe	Eastern
87	Mujochia/	*D. minutiflora*	4x	Igembe	Eastern
88	Tharierure	*D. minutiflora*	6x	Igembe	Eastern
89	Mbeu-nkuru	*D. minutiflora*	5x	Igembe	Eastern
90	Nkwamburi	*D. minutiflora*	5x	Igembe	Eastern
91	Mweu	*D. minutiflora*	4x	Igembe	Eastern
92	Mbithi	*D. minutiflora*	5x	Igembe	Eastern
93	Nduru	*D. minutiflora*	4x	Igembe	Eastern
94	M’thari	*D. minutiflora*	4x	Igembe	Eastern
95	Nereri	*D. minutiflora*	4x	Igembe	Eastern
96	Riari	*D. minutiflora*	4x	Tigania	Eastern
97	Mbeu-Mwera	*D. minutiflora*	6x	Tigania	Eastern
98	Rwera	*D. minutiflora*	4x	Tigania	Eastern
99	Ndenda	*D. minutiflora*	5x	Tigania	Eastern
100	Baicuru	*D. minutiflora*	5x	Imenti South	Eastern
102	Nakirima	*D. minutiflora*	4x	Imenti South	Eastern
103	Kiere	*D. minutiflora*	3x	Imenti South	Eastern
104	Nkerekere	*D. minutiflora*	4x	Imenti South	Eastern
105	Kamwere A	*D. minutiflora*	4x	Meru South	Eastern
106	Nkandau	*D. minutiflora*	5x	Meru South	Eastern
107	Ngondu	*D. minutiflora*	3x	Meru South	Eastern
108	Kirandi	*D. minutiflora*	6x	Meru South	Eastern
109	Njoka	*D. minutiflora*	4x	Meru South	Eastern

Table 1. Contd.

110	Ngoci	*D. minutiflora*	5x	Meru South	Eastern
111	Nkandau	*D. minutiflora*	4x	Meru South	Eastern
112	Ndianthi	*D. minutiflora*	5x	Nyeri North	Central
113	Njuhi	*D. minutiflora*	5x	Nyeri North	Central
114	Muchara	*D. minutiflora*	4x	Nyeri North	Central
115	Ngwanjiru	*D. minutiflora*	4x	Nyeri North	Central
116	Ndiandi	*D. minutiflora*	6x	Kirinyaga	Central
117	Mundu-wakinyoni	*D. minutiflora*	5x	Kirinyaga	Central
118	Muraru	*D. minutiflora*	4x	Kirinyaga	Central
119	Ngwanjiru	*D. minutiflora*	4x	Kirinyaga	Central
120	Ndiru	*D. minutiflora*	4x	Kirinyaga	Central
121	Njuhi	*D. minutiflora*	6x	Kirinyaga	Central
122	Kesse	*D. minutiflora*	5x	Taveta	Coast
123	Kyanchangao	*D. minutiflora*	6x	Taveta	Coast
124	Nduu	*D. bulbifera*	8x	Taveta	Coast
125	Kilukwa	*D. alata*	6x	Taveta	Coast
126	Kiye	*D. alata*	3x	Taveta	Coast
127	Mafore	*D. alata*	4x	Taveta	Coast
128	Kilikwa	*D. minutiflora*	4x	Taveta	Coast
129	Emodo	*D. alata*	5x	Teso	Western
130	Emodo	*D. odoratissima*	10x	Teso	Western
131	Emodo	*D. odoratissima*	8x	Teso	Western
132	Emodo	*D. odoratissima*	6x	Bungoma West	Western
133	Embama	*D. odoratissima*	5x	Hamisi	Western
134	Chihama	*D. minutiflora*	6x	Trans Nzoia West	Western
135	Gikuyu	*D. minutiflora*	3x	Trans Nzoia West	Western
136	Gikwa	*D. minutiflora*	4x	Trans Nzoia West	Western
141	Gikuyu	*D. minutiflora*	4x	Uasin Gishu	Rift Valley
142	Njiru	*D. minutiflora*	6x	Uasin Gishu	Rift Valley
143	Icoho	*D. minutiflora*	6x	Molo	Rift Valley
144	Ngiriri	*D. minutiflora*	4x	Molo	Rift Valley
149	Ndwananthi	*D. minutiflora*	4x	Igembe	Eastern
150	Ndenda	*D. minutiflora*	5x	Igembe	Eastern
151	Ikooro	*D. minutiflora*	4x	Igembe	Eastern
152	Nkwarwarene	*D. minutiflora*	4x	Igembe	Eastern
153	M'Maru	*D. minutiflora*	8x	Igembe	Eastern
154	M'Kinambati	*D. minutiflora*	4x	Igembe	Eastern
155	Ikooro	*D. minutiflora*	5x	Igembe	Eastern
156	Nareri/ Cianderi	*D. minutiflora*	3x	Igembe	Eastern
157	Mbura-mwitu	*D. minutiflora*	7x	Igembe	Eastern
158	Rweere	*D. minutiflora*	4x	Igembe	Eastern
159	Ndenda	*D. minutiflora*	3x	Igembe	Eastern
160	M'Maru	*D. minutiflora*	5x	Igembe	Eastern
162	Ngwa naro	*D. minutiflora*	4x	Kiambu	Central
164	Chihama	*D. odoratissima*	6x	Hamisi	Western
170	*D. dumetorum*	*D. dumetorum*	3x	Msambweni	Coast
172	*D. asteriscus*	*D. asteriscus*	6x	Malindi	Coast
176	*D. schimperiana*	*D. schimperiana*	7x	Trans Nzoia West	Western
177	*D. schimperiana*	*D. schimperiana*	7x	Trans Nzoia West	Western
TDc98136	*D. cayenensis*	*D. cayenensis*	8x	IITA	West Africa
TDr18544	*D. rotundata*	*D. rotundata*	4x	IITA	West Africa

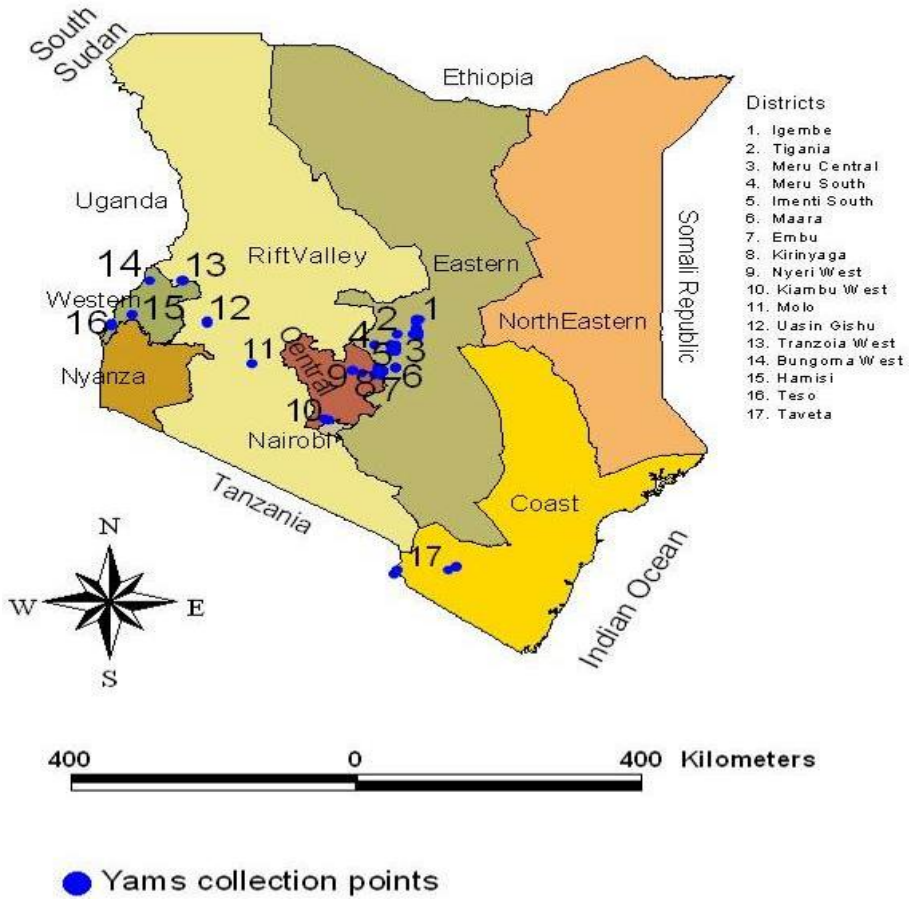

Figure 1. Yam distribution and collection areas in Kenya.

and peak fluorescence signals of the propidium-stained nuclei were collected along with forward and side scatter signals (SSC-A). Histograms of linear DNA fluorescence were analysed in FlowJo (v.7.6.5., Treestar Inc.). The instrument was adjusted such that the G1 peak of nuclei of the standard octoploid plant was set at channel 150. Wavelengths were captured as channels (PE-A) on the horizontal axis. This setting was checked from time to time during the entire analysis and kept constant to minimize deviations.

Peaks representing triploids were expected at channel 55, for the tetraploids at channel 75, hexaploids at channel 110 and octoploid at channel 150. Three measurements were made for each isolation and at least 500 nuclei for every sample examined.

Flow cytometry analysis

Ploidy level for each landrace was calculated by the formula: FI / FIR x (ploidy of internal reference), where Fluorescence of Internal Reference (F.I.R) is the index value of the internal reference. F.I is the mean position of the internal reference (Gamiete et al., 1999). Histograms showing fluorescence intensity frequency distributions for the internal standards were generated (Figures 2, 3, 4 and 5). Two peaks, G1 and G2, representing the two cell cycles were observed. G1 peak is reported in this study. Using curve fitting algorithms FlowJo deconvolutes the DNA histograms into three mathematical distributions, representing the populations of cells in each phase of the cell cycle. In this Jean-Dean-Fox model RMS represents root mean squared; % G1, G2 and S are the fraction of cells in the G1, G2 and S cell cycle phases respectively. G1 μ, G2 μ, G1 cv, G2 cv are the distribution stats of G1 and G2 peaks while %<G1, %> are the fraction of cells below G1 and above G2 (Fox, 1980).

RESULTS AND DISCUSSION

Seven ploidy levels were observed (Table 1). Eighteen accessions (11.4% of total 155 were triploid; 59 (37.5%) tetraploid; 46 (29.2%) pentaploid; 22 (14.6%) hexaploid; 4 (3.1%) heptaploid; 5 (3.1%) octoploid and 1 (0.6%) decaploid. Tetraploids formed the largest group followed by pentaploids and hexaploids. Among the four *D. alata* accessions Ac. 125 was 6x, Ac 126 3x, Ac 127 4x and Ac 129 5x. Among the *D. bulbifera* accessions Ac 10 was 5x, Ac 23 8x and Ac 124 8x, no tetraploid was observed in this species. Among the seven accessions identified as *D. odoratissima*, also found in cultivation in one part of the country, two accessions were tetraploid; three hexaploid, one pentaploid and one decaploid (10x). Among the wild species of *Dioscorea*, *D. asteriscus* was hexaploid; three D*. schimperiana* accessions were heptaploid and one accession of *D. dumetorum* was found to be a triploid. There were no diploids reported. No relationship was observed between ploidy and geographical regions / districts. Only one accession, Ac 153, among the accessions

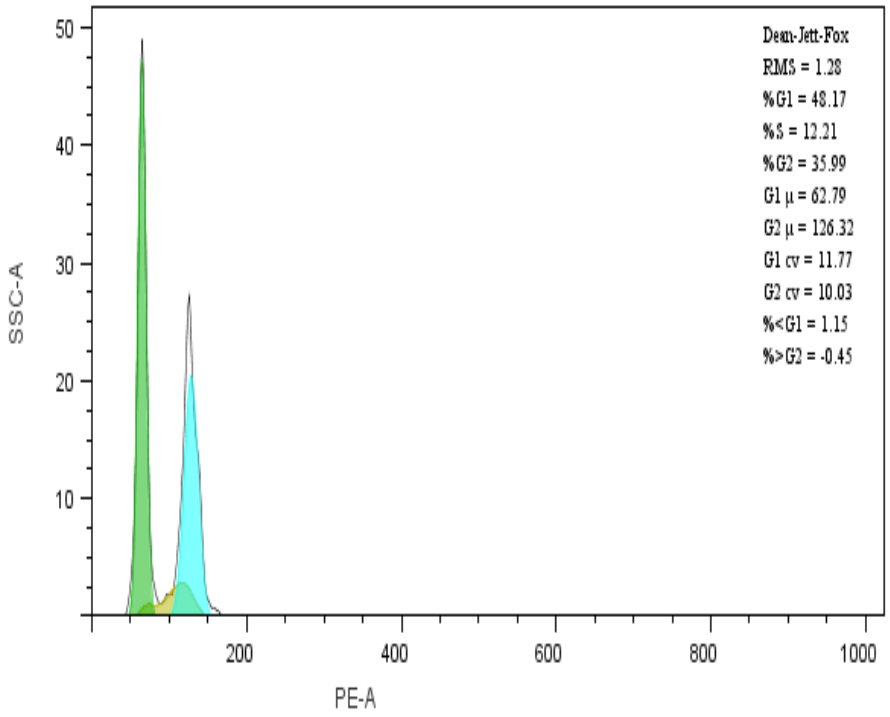

Figure 2. Histogram* showing a triploid (3x) accession. *Longer peak represents G1 cell cycle; Shorter peak represents G2 cell cycle.

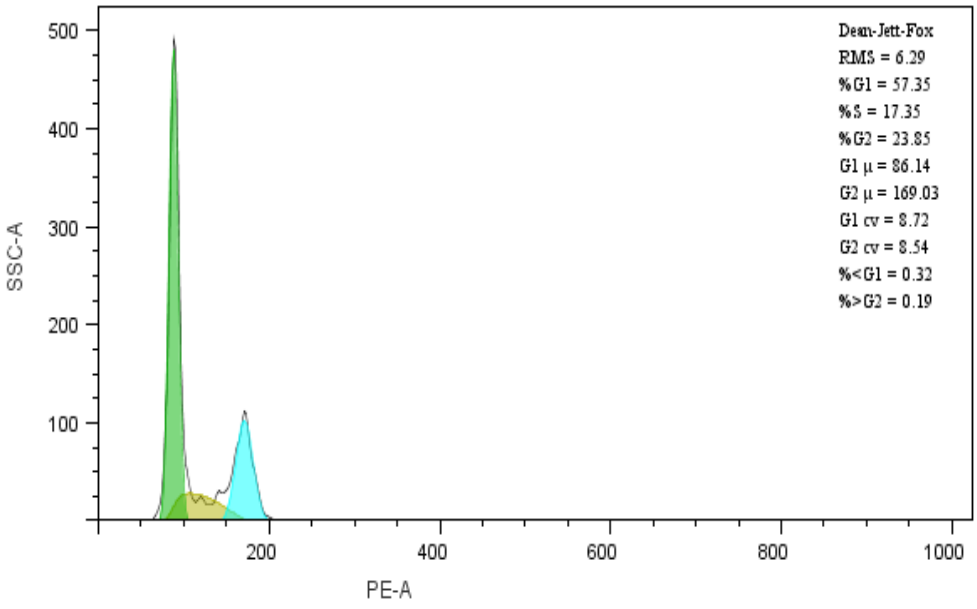

Figure 3. Histogram showing tetraploid (4x) accession.

in the major yam areas of the country was found to be octoploid. Accessions with similar morphological characters were found to manifest variation in ploidy levels. The two checks, TDr.18544 and TDc.98136 from IITA were tetraploid and octoploid respectively as indicated from the originating institution.

In a young plant, majority of cells are not in division and these cells are in the G1 stage. Their nuclear DNA content reflects the ploidy level of the plant. Cells in division pass from the G1 stage to G2 stage where they have a double DNA content (De Laat et al., 1987). Distribution of nuclei over the G1 channel represents the

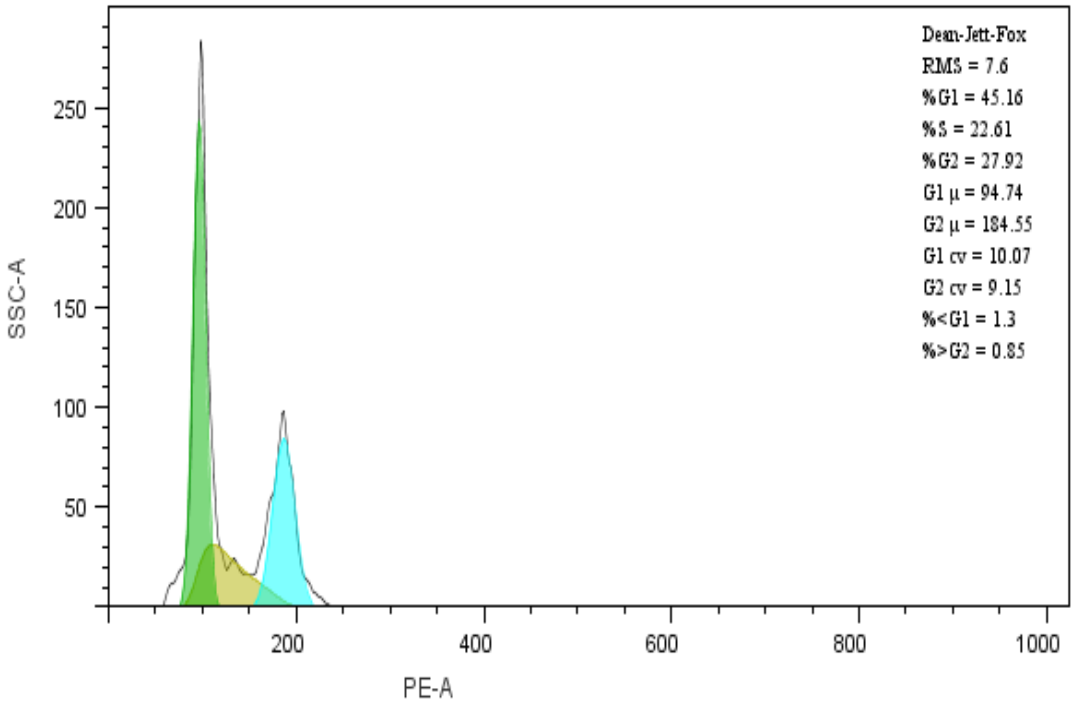

Figure 4. Histogram showing pentaploid (5x) accession.

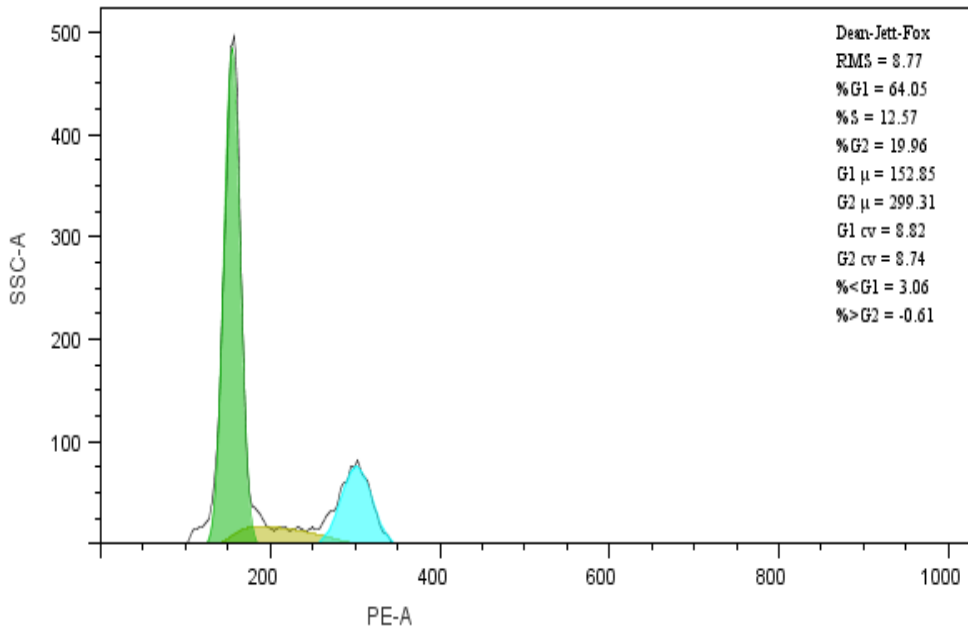

Figure 5. Histogram showing octoploid (8x) accession 23.

nuclear DNA content and hence ploidy level. Seven ploidy groups; 3x, 4x, 5x, 6x, 7x, 8x and 10x were identified in the study with differences in accessions in each ploidy group. Hamon et al. (1992) reported ploidy levels of 3x, 3.5x, 4.5x, 7x and 8.5x for *D. alata*. The lowest ploidy was 3x and highest 10x. Accession 130 also a domesticated species had the highest ploidy.

Different ploidy levels have been reported in other studies. Gamiete et al. (1999), Malapa et al. (2005) and Dansi et al. (2001) reported three ploidy levels 4x, 6x and 8x among cultivated yams in INRA collections in Guadeloupe and Cameroon. Sharma et al. (1956, 1957) found levels of ploidy to be 3x, 5x and 7x by chromosome count in *D. alata*. Recent segregation studies of microsatellite mar-

kers have shown that the so called tetraploid species *D. rotundata* is a diploid (Scarcelli et al., 2005). Obidiegwu et al. (2009) reported tetraploids, hexaploids, octoploids, and one mixoploid individual among 170 accessions of Guinea yams from West African countries. The accessions with 7x were all *D. schimperiana*, the 10x accession was *D. odoratissima*. The octoploid 8x group was comprised of one accession each of *D. odoratissima*, *D. bulbifera* and *D. cayenensis*, the standard used from West Africa. *D. dumetorum* was triploid as shown in previous studies. TDr 18544 from West Africa was a tetraploid, thus confirming its ploidy status. The two accessions of *D. alata* Ac 127 and Ac 129, used in the study were tetraploid, and hexaploid respectively.

Gemma et al. (2009) have proposed ploidy of 2x, 3x and 4x corresponding to diploid, triploid and tetraploid with the basic chromosome number of x = 20 for *D. alata*. Accessions 174 and Ac 164, both *D. odoratissima* were tetraploids. Ac 173 and Ac 132 also *D. odoratissima* had a ploidy of 5x. Ploidy of 7x was observed only in *D. schimperiana*, 8x and 10x were observed in accessions with *D. bulbifera*, *D. odoratissima* and the West African internal standard TDc.98136, respectively. There was no pattern to associate ploidy with geographical or regional source of the accessions.

Natural crossing between different groups may have occurred in history while the presence of 10x in the population supports hybridization process. The presence of heterogenous foliage among the cultivated cultivars supports crossing between different ploidy groups. There was no relationship between ploidy and geographical regions/districts, suggesting that ploidy is not influenced by location. Landraces with varying ploidy levels were found in the same locality. Landraces with similar names were also found to have varying ploidy levels. Subsequent mutations accompanied by fertilization may have played a role in evolution and polyploidization of yam in Kenya as has happened in other yam growing regions of the world. This study is the first attempt to estimate ploidy levels of yam in Kenya. The findings will form the basis for future research in addressing the mechanism of inheritance among the local landraces and further work on molecular characterization and taxonomic verification.

Conclusion

There were variable ploidy levels among the local yam landraces ranging from 3x, 4x, 5x, 6x, 7x, 8x and 10x. The highest proportion of ploidy was represented by tetraploids while no diploids were observed. There was no relationship between ploidy level and geographical regions. Similar landrace names were not associated with similar ploidy levels. There is need for further work on taxonomy and phylogeny of the local landraces.

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