Scientific Research and Essay Vol. 4 (5) pp. 448-452, May, 2009
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 © 2009 Academic Journals

Full length Research Paper

Estimation of the nuclear DNA content in some representative of genus *Dioscorea*

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Accepted 20 April, 2009

Nuclear DNA content of 54 accessions of 6 *Dioscorea* species collected from West African countries were determined using flow cytometry. Observed ploidy levels are diploid (2x), triploid (3x), tetraploid (4x), hexaploid (6x) and octoploid (8x). DNA index varied from 0.36 to 1.54 depending on the cultivar and considered specie. Estimated nuclear DNA sizes ranged from 0.702 ± 0.004 pg for G_1 nuclei of diploid *Dioscorea dumetorum* to 2.573 ± 0.020 pg for G_1 nuclei of octoploid *Dioscorea cayenensis*. Our result showed that there is a decrease in genome size with higher ploidy level. The study contributes a step towards a genome understanding of this group of plants.

Key words: *Dioscorea*, DNA content, flow cytometry, genome, ploidy level.

INTRODUCTION

Yams (*Dioscorea* spp.) are economically important starch staple in tropical and Sub tropical regions of the world, particularly West Africa, Asia, Far East, the Pacific and the Caribbean regions. These regions are considered main centres of yam domestication and diversity (Asiedu et al., 1997). The species of economic importance are *D. alata* L., *D. bulbifera* L., *D. cayenensis* Lam., *D. dumetorum* (Kunth) Pax., *D. rotundata* Poir and *D. esculenta* (Lour) Burk.

These specie play an important role in food and agricultural system diversification, broadens the food base and brings food security to 300 million people in the low income food-deficit countries (LIFDC's) of the tropics providing them with about 200 dietary calories daily (FA0, 1999). Obstacles militating basic conventional yam breeding in the *Dioscorea* genus can be summed up in the many constraints in the functioning of its sexual reproductive system.

From the flowering achievement to the establishment of seedling, limitations appear in; flowering expressions, sex availabity, gametogenesis, pollination, fecundation, embryogenesis and seed setting. These difficulties of the sexual recombination within yams suggest a complex speciation. Despite the few scanty literatures little is known about the phylogeny of most of these species and many taxonomic ambiguities that lead to cytological irregularities have so far remain unresolved. The C-value of an organism, i.e. the amount of DNA in the unreplicated nuclear genome (Swift, 1950), influences various cellular parameters, such as cell and nuclear volume and chromosome size, and developmental parameters like minimum generation time or duration of meiosis, among others. It is therefore not suprising that a correlation of genome size with breeding system has also been found (Price and Bachmann, 1976; Govindaraju and Cullis, 1991).

The development and application of molecular cytogenetics to yams will greatly improve the understanding of chromosome structure and karyotype variation within the species. The aim of this preliminary study is to increase

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the knowledge of nuclear DNA contents of six *Dioscorea* species using flow cytometry technique.

MATERIALS AND METHODS

Plant material

Fifty three (53) accessions representing six *Dioscorea* species collected from 10 countries of West and Central Africa (Table 1) were planted in 30 cm size pots filled with sterilized loamy soil and maintained in a screenhouse at the IITA (Ibadan, Nigeria).

Sample preparation for ploidy analyses

Healthy looking young leaves were collected from plants in the screen house, bagged, transported at cold temperature and kept in a refrigerator with a temperature of 4°C for a maximum period of 5 days until analysis. For each accession, nuclei were isolated and suspended following the method of Galbraith et al. (1983), by chopping approximately 50 mg of sample material with a sharp double-edged razor blade in a glass Petri dish containing 1 ml LB01 lysis buffer (5 mM TRIS, 2 mM Na_2EDTA, 0.5 mM spermine.4HCl, 80 mM KCl, 20 mM NaCl, 15 mM β-mercaptoethanol, 0.1% (v/v) TritonX-100, pH 7.5) (Doležel et al., 1989). The inclusion of β-mercaptoethanol in the buffer constitution was indispensable for coping with the negative effect of phenolic compounds released from the leaves upon chopping.

The nuclear suspension was filtered using an 80 µm nylon filter and stained with 50 mg mL^{-1} propidium iodide (PI, Fluka, Buchs, Switzerland). RNase (Fluka) at a concentration of 50 mg mL^{-1} was also added to avoid staining of double-stranded RNA by PI. After a 10 min incubation period on ice, samples were ready for FCM analyses. Due to its close but non-overlapping genome size, *Solanum lycopersicum* L. (2C = 1.96 pg DNA; Doležel et al. 1992) was chosen as reference standard.

Flow cytometric analyses

Samples were analysed using a Beckman Coulter EPICS-XL flow cytometer (Beckman Coulter®, Hialeah, FL, USA) equipped with an air cooled argon-ion laser regulated at 15 mW and operating at 488 nm. PI fluorescence was collected through a 645-nm dichroic long-pass filter and a 620-nm band-pass filter. Each day, before starting sample analysis, the instrument was checked for linearity with Flow-Check fluorospheres (Beckman-Coulter®). The results were acquired using the SYSTEM II software version 3.0 (Coulter Electronics). The amplifier system was adjusted so that the G_0/G_1 peak of nuclei isolated from diploid individuals (the lowest ploidy level detected in this study) appeared at channel 200 in a scale with 1,024 channels. These settings were kept constant throughout the whole experiment. For each sample, fluorescence data was acquired from at least 3,000 nuclei.

In initial experiments, ploidy level was determined by comparing the mean fluorescence intensity of nuclei from sample material with that of the reference standard, used internally. After a sample of each of the expected ploidy levels was obtained, either the reference standard was used externally or no standard was added during sample preparation. In the later situation, when deviations larger than 10.0% (from the assigned channel position) were obtained, the ploidy level was estimated by preparing a new sample with both test material and internal reference standard. Further-more, in some cases, samples of unknown ploidy level were co-chopped with control individuals of known ploidy level. Total DNA content was calculated as the sample peak mean, divided by the *S. lycopersicum* peak mean and multiplied with the DNA amount of the *S. lycopersicum* standard.

Sample 2C DNA content

= [(sampleG_1 peak mean)/ (*S. lycopersicum* G_1 peak mean)] x *S. lycopersicum* 2C DNA content (pg DNA).

RESULTS

The histograms that were obtained after analysis of stained nuclei of six *Dioscorea* species enabled precise determination of ploidy status, as illustrated in the provided histogram (Figure 1). Channel number values for diploid, triploid, tetraploid, hexaploid and octoploid accessions approximated a 1:1.5:2:3:4 ratio (Figure 1). The coefficient of variation values of the G_0/G_1 peaks were usually within the limit of 5% (Galbraith et al., 2002) as shown in Table 2. Table 1 showed that the five ploidy levels that were found in the studied population include: diploids (2x), triploid (3x), tetraploid (4x), hexaploid (6x) and octoploids (8x). Estimated nuclear DNA sizes ranged from 0.702 ± 0.004 pg for G_1 nuclei of diploid *D. dumetorum* to 2.573 ± 0.020 pg for G_1 nuclei of octoploid *D. cayenensis* (Table 1).

DISCUSSION

The estimated nuclear DNA range 2C = 0.702 - 2.573 pg in our study seem quite close with the result of Hamon et al. (1992) who estimated nuclear DNA content in *Dioscorea* in the range 2C = 0.88 - 2.88 pg. Our study suggests *Dioscorea* species could be considered taxa with a low size genome when compared to a known range of genome size in plants (Bennett and Leitch, 1997).

Eleven of the studied *D. alata* individuals were tetraploid while the remaining two were octoploid. The high number of tetraploids observed in this specie suggests that higher ploidy levels may have arisen from auto-polyploidization involving diploid individuals. The absence of hexaploid individuals supports the inference of a diploid auto-polyploidization origin. The nuclear DNA content (Table 1) estimation of *D. alata* individuals ranged 2C = 1.15 ± 0.008 pg while the octoploids were 2C =1.96 pg for the two individuals. Our result showed that there is a decrease in genome size with higher ploidy level as tetraploids in *D. alata* gave an estimated range of 2C = 1.15 ± 0.008 pg while the octoploid individuals were estimated to 2C =1.96 pg (Table 1).

Sharma and Sen (2002) considered that with polyploidization, chromosomes tend to diminish their size slightly, each one equally and hypothesized that this strengthening mechanism was a defence strategy against the increased possibility of mutations with polyploidy (an increase in nuclear DNA amount increases the probability of mutations). Interestingly the results of Chenuil et al. (1997) obtained in the *Barbus* (Cyprinidae) indicate that shortening of microsatellites and reduction of their num-

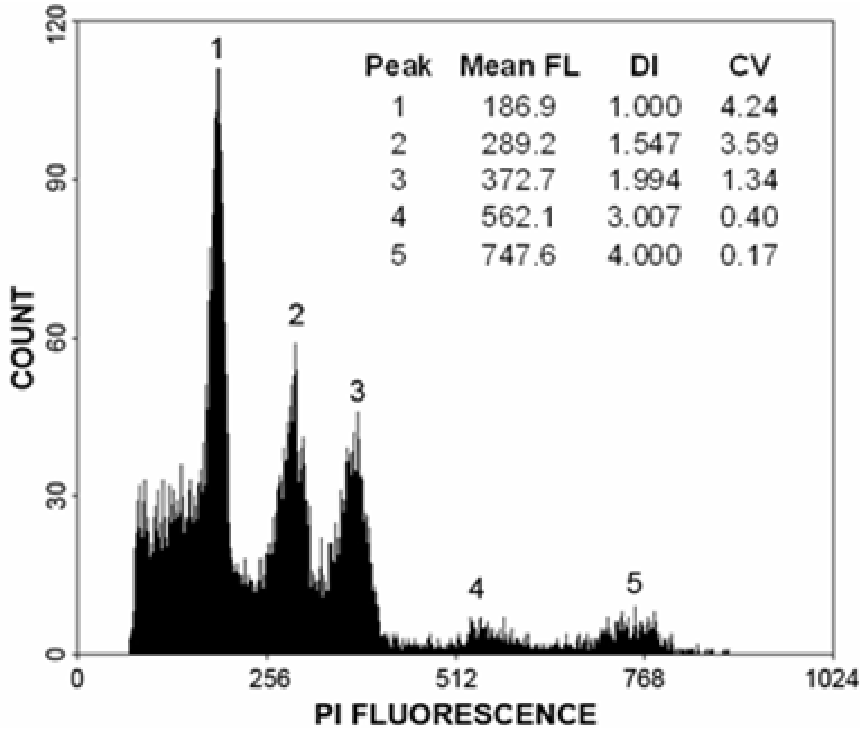

Figure 1. Histogram of relative fluorescence intensity of nuclei isolated simultaneously from five accessions of *Dioscorea* species with different ploidy levels: diploidy, triploidy tetraploidy, hexaploidy and octoploidy. Peaks 1, 2, 3, 4 and 5 correspond to nuclei at the G_0/G_1 phase. The mean channel number (Mean FL), DNA index (DI = mean channel number of sample / mean channel number of reference standard), and coefficient of variation (CV, %) value of each peak are also given.

Table 1. *Dioscorea* species alphabetically arranged with their genebank accession number, country of collection, ploidy level and nuclear DNA (2C) in pg (means ± standard error).

Species	Genebank accession number	Country of collection	Ploidy level	2C nuclear DNA content (pg) Mean ± S.E
	TDa 3202	Togo	4x	
D. alata	TDa 4142	Sierra Leone	4x	
	TDa 3231	Togo	4x	
	TDa 1066	Togo	4x	
	TDa 1210	Togo	4x	
	TDa 3898	Benin	4x	1.15 ± 0.008
	TDa 1178	Benin	4x	
	TDa 1060	Togo	4x	
	TDa 1103	Togo	4x	
	TDa 1250	Côte d' Ivoire	4x	1.96
	TDa 3215	Togo	4x	
	TDa 1108	Togo	8x	
	TDa 3920	Nigeria	8x	
D. bulbifera	TDb 2857	Equatorial Guinea	6x	
	TDb 3029	Togo	6x	2.40 ± 0.022
	TDb 3061	Togo	6x	

Table 1. Continued.

D. cayenensis	TDc 3704	Congo	6x	1.53 ± 0.110
	TDc 2811	Togo	6x	
	TDc 2798	Togo	6x	
	TDc 2794	Côte d' Ivoire	6x	
	TDc 3712	Congo	8x	
	TDc 2828	Ghana	8x	
	TDc 3807	Nigeria	8x	2.57 ± 0.020
	TDc 2815	Nigeria	8x	
D. dumetorum	TDd 3106	Togo	2x	0.70 ± 0.004
	TDd 3908	Benin	2x	
	TDd 3108	Ghana	2x	
	TDd 3935	Benin	2x	
	TDd 4118	Togo	2x	
	TDd 3907	Nigeria	3x	1.06
D. esculenta	TDe 4149	Guinea	4x	
	TDe 3039	Togo	4x	2.10 ± 0.230
	TDe 3036	Ghana	4x	
	TDe 3035	Côte d' Ivoire	4x	
D. rotundata	TDr 3331	Ghana	4x	
	TDr 1907	Ghana	4x	
	TDr 2246	Cote d Ivoire	4x	
	TDr 2766	Nigeria	4x	
	TDr 3683	Nigeria	4x	
	TDr 1529	Togo	4x	
	TDr 3964	Benin	4x	
	TDr 4065	Nigeria	4x	
	TDr 2611	Nigeria	4x	
	TDr 3983	Nigeria	4x	1.426 ± 0.008
	TDr 1787	Togo	4x	
	TDr 2159	Côte d' Ivoire	4x	
	TDr 2127	Nigeria	4x	
	TDr 1888	Côte d' Ivoire	4x	
	TDr 1946	Not Available	4x	
	TDr 2439	Nigeria	4x	
	TDr 1762	Togo	4x	
	TDr 1485	Togo	4x	
	TDr 3681	Nigeria	4x	

number could be one of molecular mechanisms of eliminating excessive DNA from organisms of higher ploidy levels.

D. cayenensis had four accessions each as hexaploid and octoploid. The absence of tetraploids in the studied accessions of *D. cayenensis* is in agreement with previous studies of Dansi et al. (2001a) who reported only hexaploid and octoploid individuals in their studied germplasm. *D. cayenensis* hexaploid individuals nuclear DNA content ranged 2C = 1.58 ± 0.110 pg while the octoploids gave an estimated range 2C = 2.57 ± 0.020 pg. A trend observed earlier in *D. alata* was noticed in *D. cayenensis* as our results showed that there is a decrease in genome size with higher ploidy level in *D. cayenensis.* This suggests a common phenomenon in the *Dioscorea* genus.

All *D. rotundata* accessions were tetraploid. This supports earlier studies of Gamiette et al. (1999) and Dansi et al. (2001a, b) who found out those tetraploids are more common in this specie. An estimated nuclear DNA content 2C = 1.42 ± 0.008 pg was observed for the tetraploid *D. rotundata.*

D. esculenta individuals analysed were tetraploid with an estimated nuclear DNA 2C = 2.10 ± 0.230 pg while *D. bulbifera* were all hexaploid with an estimated nuclear DNA 2C = 2.40 ± 0.022 pg. Five of *D. dumetorum* individuals measured were diploids while a single individual was triploid. *D. dumetorum* showed estimated nuclear DNA range 2C = 0.70 ± 0.004 pg for diploid (2x) indivi-

Table 2. A summary of ploidy level analysis with the DNA index (DI = mean channel number of sample / mean channel number of reference standard) and mean and range of the coefficient of variation CV (%) obtained.

Ploidy level	No. of individuals	DI	CV (%)	CV range (%)	
				Min.	Max.
Diploidy	5 (9.43 %)	0.36	3.90	1.42	5.29
Triploidy	1 (1.89 %)	1.54	3.59	-	-
Tetraploidy	34 (64.15 %)	0.59	4.32	1.12	5.39
Hexaploidy	7 (13.20%)	0.75	2.85	0.34	4.00
Octoploidy	6 (11.32 %)	1.30	2.15	0.12	4.63
Total	53				

duals and 2C = 1.06 pg for the single triploid (3x). The occurrence of a diploid cytotype of *D. dumetorum* suggests a diploid hypothesis in this specie. Although this result does not rule out a possibility of higher ploidy levels, it thus supports the hypothesis that polyploidi-zation may have been limited in *D. dumetorum.* A second hypothesis could be that the specie may have undergone a protracted processes of diploidization which may have involve changes that constitute decay of the original wholesale duplications of genomic characters such as DNA amount thus obscuring a polyploidy origin and state and restore a near diploid condition The triploid (3x) individual suggests that polyploidization by fusion of reduced (*n*) and unreduced (2*n*) gametes may be common phenomenon in *D. dumetorum.* The occurrence of triploid (3x) individual in our study confirms the work of Sharma and De (1956).

Flow cytometry is shown here to be a useful tool to indicate the ploidy and genome size estimate of six *Dioscorea* species. Each has a certain amount of nuclear DNA, which is a systematic value. Although it does not permit absolute and definitive conclusions, it contributes a step towards a genome understanding of this group of plants.

ACKNOWLEDGEMENTS

This study would not have been possible without the support of the Kirkhouse trust (UK), through the travel grant to the first author. The authors are grateful to FCT for providing the grants of João Loureiro (FCT/BPD-/36601 /2007) and Eleazar Rodriguez (FCT/BD/27467/- 2006). The technical assistance of Hammed Adeola and Olatunji Aina of the Central Biotechnology Laboratory (CBL), International Institute of Tropical Agriculture (IITA), Ibadan is acknowledged.

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