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PLOIDY LEVEL OF THE CULTIVATED YAMS (*DIOSCOREA CAYENENSIS/D. ROTUNDATA* COMPLEX) FROM BENIN REPUBLIC AS DETERMINED BY CHROMOSOME COUNTING AND FLOW CYTOMETRY

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ABSTRACT

The ploidy levels of ninety Guinea yam (*Dioscorea cayenensis/D. rotundata* complex) cultivars identified within Benin were determined by both chromosome counting from root tip cells and flow cytometry. Three different ploidy levels (4x, 6x, 8x) were detected among the samples. Eighty cultivars were tetraploid, five were hexaploid, three were octoploid and two cultivars, 'Tam-Sam' and 'Youbé' were mixoploid with both 4x and 8x ploidy levels. Chromosome counts were in agreement with data from flow cytometry that provides an easier assay for ploidy analysis. Flow cytometry was found to be a reliable tool for rapid determination of ploidy level in yam.

Key Words: Chromosome, Benin Republic, *Dioscorea cayenensis/Dioscorea rotundata* complex, flow cytometry, mixoploid, ploidy, yam

RÉSUMÉ

Les niveaux de ploïdie de 90 variétés d'ignames cultivées du complexe *Dioscorea cayenensis/D. rotundata* du Bénin sont déterminés par dénombrement chromosomique et par cytométrie en flux. Trois différents niveaux de ploïdie (4x, 6x, 8x) sont mis en évidence. Quatre-vingt variétés sont tétraploïdes, cinq sont hexaploïdes, trois sont octoploïdes et deux (Tam-Sam et Youbè) sont mixoploïdes avec des cellules tétraploïdes (4x) et octoploïdes (8x). La cytométrie en flux a donné des résultats qui corroborent ceux du dénombrement chromosomique et apparaît, chez l'igname, comme une méthode rapide et efficace pour la détermination du niveau de ploïdie.

Mots Clés: Chromosome, République du Bénin, complexe *Dioscorea cayenensis/Dioscorea rotundata*, cytométrie en flux, myxoploïde, ploïdie, igname

INTRODUCTION

Tropical root and tuber crops occupy a pre-eminent position as food crops, next only to cereals and grain legumes, and they also form the subsidiary staple of over 20% of world population (Okwor, 1998). Among the tropical tuber crops, Guinea yam (*Dioscorea cayenensis/Dioscorea rotundata* complex) is one of the most important, especially in the so-called 'yam belt' of West Africa (Orkwor *et al.*, 1998).

In spite of its economic importance, Guinea

yam has not received the attention that it deserves with regard to its quantitative and qualitative improvement. Consequently, many genotypes are reported to be susceptible to pests and diseases (Degras, 1986; Dansi et *al.*, 1997, Orkwor *et al.*, 1998). For developing new elite genotypes for ecological adaptation and resistance to pests and diseases, plant breeders will need access to a wide range of diversity. Therefore, better knowledge of the existing traditional cultivars held by farmers is a pre-requisite.

In order to assess the diversity within this species complex (*D. cayenensis /D. rotundata)* in the Republic of Benin, systems of classification and identification based on morphological, isozymic and RAPD markers were recently used (Dansi *et al.*, 1998, 1999, 2000a, b).

D. cayenensis /D. rotundata complex being polyploid, the knowledge of the ploidy level of the cultivars identified within this germplasm is important. Determining ploidy levels in yam by counting chromosomes is tedious, difficult and time consuming. Yam chromosomes are small, generally dot-like and most often clumped together complicating the counting (Baquar 1980; Zoundjihékpon *et al.*, 1990). To overcome these difficulties, flow cytometry has been recently used to determine ploidy levels in yams (Hamon *et al.*, 1992; Gamiette *et al.*, 1999). In ploidy analysis, flow cytometry assay has some important advantages over conventional chromosomes counting. The method is non-destructive (one sample can be prepared from a few milligrams of leaf tissue), exceptionally rapid, sensitive and convenient, does not require dividing cells, and can detect both mixoploidy and aneuploidy (Galbraith *et al.*, 1983; De Laat *et al.*, 1987; Arumuganathan and Earle, 1991a, b; McMurply and Rayburn, 1991; Dolezel, 1997).

The objective of this study was to determine the ploidy level of the different cultivars identified within the *D. cayenensis /D. rotundata* complex of Benin Republic using both chromosome counting and flow cytometry.

MATERIAL AND METHODS

Plant materials. The material consisted of 90 yam cultivars of the *D. cayenensis/D. rotundata* complex of Benin Republic (Tables 1 and 2). These cultivars represent the ninety morphotypes identified within the Guinea yam germplasm collected in 1996 and 1997 in different localities of Benin (Dansi et *al.*, 1997; 1998; 1999). They are maintained as a field collection at the International Institute of Tropical Agriculture near Cotonou.

Of the ninety cultivars selected, 26 were analysed by chromosome counting (Table 1), 60 by flow cytometry (Table 2) and 4 by both chromosome counting and flow cytometry (Table 2). The 30 cultivars analysed by chromosome counting (Table1) were selected in such a way that the 26 cultivar groups identified within the Benin Republic' Guinea yam germplasm were represented.

For the purpose of the analysis, plants were cultured *in vitro* (nodal cutting culture) on the basal MS medium (Murashige and Skoog, 1962), supplemented with 2% sucrose, 0.05 mg/l kinetin, 20 mg/l cysteine and 0.7% agar, pH= 5.6 at 25°/ 22° C day/night. The media were sterilised at 121° C for 15 minutes and cooled before use. Lighting was provided by cool white fluorescent lamps (18 h photoperiod and 4000 lux light intensity).

Chromosome counting. Slides were prepared by the technique outlined by Zoundjihékpon *et al.*, (1990). The root tips were obtained soon after sunrise (before 7 a. m.) from plants grown in sawdust in a greenhouse. Roots were pre-treated in a 2mM 8-hydroxyquinoline solution for about 6 h on filter paper in a petri dish to accumulate metaphase cells. The roots were fixed in acetic – alcohol (1/3) for 48 h, followed by hydrolysis in 5N HCl for 45 min and 1N HCl for 10 min. Root tips were stained in Feulgen reagent in darkness for at least 2 h and slides were prepared by squashing in 45 % acetic acid. Prepared slides were stored at -20°C. After removing the cover slip, the slides were soaked in absolute ethanol for 1 h, air-dried for 15 min, stained in a Giemsa solution in phosphate buffer, pH=6.7, rinsed in distilled water for 2 min and dried again before mounting. The chromosomes were counted in 3-5 cells per slide and in 5-10 root tips per cultivar using a light microscope at magnification of 1000x.

Preparation of nuclei for flow cytometry. A procedure modified from Otto *et al.* (1981) and

Otto (1990) was used to prepare and stain the cell nuclei. To release nuclei, young and healthy plant leaves were cut into pieces in a glass petri dish. Following addition of 1 ml of ice-cold extraction buffer (0.1M citric acid containing 0.5% Tween 20), the leaf pieces were finely chopped with a sharp razor blade. The homogenate was filtered through a 45 µm nylon gauze filter to remove cell debris. Two ml of staining buffer containing 0.4 M sodium hydrogen phosphate and 4 µg/ml DAPI (4'-6-diamidino-2-phenylin dole) was added to the suspension of nuclei and samples were analysed immediately.

Flow cytometry. Flow cytometry measurements were performed with Partec Ploidy Analyser (Partec Gmbh, Germany) equipped with a 100W high-pressure mercury lamp. Instrument gain was adjusted so that the G1 peak of nuclei isolated from a control tetraploid plant (cultivar Kponan with a chromosome number of 40) was set at channel 50. This calibration was checked periodically to minimise variation due to runs and kept constant during the analysis of samples prepared from plants of unknown ploidy. Peaks representing G1 nuclei were then expected at channels 75 and 100 for hexaploid and octoploid ploidy levels respectively. Two measurements were made for each isolation and at least 2000 nuclei were examined each time. To estimate ploidy level, the position of the G1 peak on the histogram obtained for each of the individuals was compared to that of the cultivar 'Kponan'. A software package (Partec) was used for the calculation of CV-values.

RESULTS

Chromosome counts. Among the thirty cultivars investigated, two (Baridjo and Makpawa) were hexaploid and showed 60 chromosomes, one (Agangan) was octoploid with 80 chromosomes and the others were tetraploid with 40 chromosomes (Table 1). In cultivar 'Makpawa', some cells showed 62 and 63 chromosomes within the same root tips while counts of about 65 were observed infrequently. One or two extra chromosomes were also observed in some cells of the tetraploid cultivars 'Antawororou', 'Djikpiri', 'Guiena' and 'Issou agatou'.

In all the cultivars analysed, chromosomes were small with some appearing dot-like and others rod-shaped without any visible centromere region. In most of the cells, chromosomes appeared clumped together hence complicating counting.

Flow cytometry. After DAPI staining, isolated nuclei irradiated with UV radiation from young leaf tissue emitted fluorescence which was measured by the flow cytometer. For each sample, analysis of the relative fluorescence intensity of the isolated nuclei yielded a histogram showing a dominant peak corresponding to nuclei in the G1 phase of the cell cycle and a minor peak corresponding to G2 nuclei. The amount of debris in the samples was negligible. The dominant G1 peaks made ploidy estimation easy.

Three ploidy levels, tetraploid (4X), hexaploid (6X) and octoploid (8X) were observed among the sixty-four cultivars analysed (Table 2). Fifty-three cultivars were tetraploid (Fig. 1a), five were hexaploid (Fig. 1b) and three were octoploid (Fig.1c). Two cultivars Youbè and Tam-Sam were mixoploid (Fig. 1d). The relative nuclear DNA content in arbitrary units (AU), expressed as channel numbers varied from 43,7 AU to 52,2 AU for the tetraploids, from 65,4 AU to 76,9 AU for the hexaploids and from 85.5 AU to 100,3 AU for the octoploids (Table 2). The coefficient of variation (CV), determined as the quotient of standard deviation of the peak and the mean peak position (channel number), was 3.4 to 4.7% (mean = 3.8) for the G1 peaks of plant nuclei (Table 2). The small CVs reflected narrowness of the peaks and indicated good reliability of measurements. Clear separation of peaks was even obtained when a sample was prepared from a mixture of leaves from tetraploid, hexaploid and octoploid individuals (Fig. 1e).

DISCUSSION

Dioscorea is one of the most difficult genera for cytotaxonomic and cytogenetic studies (Essad, 1984). Many authors already reported the difficulties encountered in chromosome counting in cultivars of *D. cayenensis/D. rotundata* complex (Miège 1952, 1954; Baquar, 1980; Zoundjihékpon *et al.*, 1990) and even other *Dioscorea* species (Suessenguth, 1921; Ramachandran, 1968;

Baquar, 1980; Araki *et al.*, 1983; Essad, 1984, Gamiette *et al.*, 1999). In yam, the presence of extra chromosomes in the cells of some individuals as observed in this study is not rare and has been already reported by Miège (1954), Baquar (1980), Zoundjihékpon *et al.*, (1990) and Gamiette *et al.* (1999). The extra chromosomes are reported to be B chromosomes or satellites which in yam are sometimes as large as the chromosomes themselves (Essad, 1984).

Off the 90 cultivars analysed (Tables 1 and 2), eighty were tetraploid, three were octoploid, five were hexaploid and two were mixoploid. When considering the cultivar groups, twenty-three out of the twenty-six investigated appeared tetraploid while one (Alakissa) was octoploid and two (Baridjo and Makpawa) were hexaploid. As found in Côte d'Ivoire (Zoundjihékpon *et al.*, 1990; Hamon *et al.*, 1992) and Cameroon (Dansi *et al.*, 2000c), the tetraploids form the largest group in Benin Republic (Fig. 2). This is in agreement with the findings of Essad (1984) which indicated that tetraploid individuals are the most frequent in the *Dioscorea* species.

The diverse chromosome counts reported by Miège (1954), Sharma and De (1956), Martin and Ortiz (1963), Baquar (1980) and Essad (1984) in the *D. cayenensis /D. rotundata* complex, indicated the existence of two chromosome base numbers, X= 9 and X=10. Only the chromosome base number X=10 was observed in the Benin Guinea yam cultivars. Similar results have already been reported for yams in Côte d'Ivoire and Cameroon (Zoundjihékpon *et al.*, 1990).

DAPI is an AT base pair binding fluorescent dye, and is not suitable for DNA content estimation in plants (Michaelson *et al.*, 1991; Dolezel *et al.*, 1992; Godelle *et al.*, 1993; Dolezel *et al.*, 1998).

TABLE 1. Results of chromosomes counts and ploidy level of 30 Guinea yam (*Disocorea cayenensis / D. rotundata* complex) cultivars of Benin Republic

Cultivars	Cultivar groups	Chromosome number	Interpretation (*Ploidy level*)
Agangan	ALAKISSA	80	8X
Agogo	AGOGO	40	4X
Ahimon	AHIMON	40	4X
Antawororou	ANTAWORO ROU	40	4X
Banioure oloukobi	BANIOURE	40	4X
Baridjo	BARIDJO	60	6X
Boki	NONFORWOU	40	4X
Brizi	KOKOROGBANOU	40	4X
Dikpiri	DIKPIRI	40	4X
Douba yéssirou	DOUBA YESSIROU	40	4X
Gbèra	SOUSSOU	40	4X
Gnalabo	GNALABO	40	4X
Gnidou	GNIDOU	40	4X
Guiéna	SOUSSOU	40	4X
Hounbonon	TABANE	40	4X
Iberegnense	TAM-SAM	40	4X
Issou agatou	SOUSSOU	40	4X
Kangni	KRATCHI	40	4X
Kinkerekou	KOKOROGBANOU	40	4X
Kokouma	MOROKOROU	40	4X
Kpanhoura	KPANHOURA	40	4X
Kponan	KPONAN	40	4X
Makpawa	MAKPAWA	60	6X
Noualaye	NOUALAYE	40	4X
Oroutanai	MONDJI	40	4X
Orou yinsingué	SOUSSOU	40	4X
Ourtchoua	OURTCHOUA	40	4X
Terkokonou	TERKOKONOU	40	4X
Tognibo	TOGNIBO	40	4X
Wolouchahabim	PORCHEHBIM	40	4X

TABLE 2. Ploidy level of 64 Guinea yam (*Disocorea cayenensis / D. rotundata* complex) cultivars of Benin Republic as revealed by flow cytometry

Cultivars	Cultivar group	DNA amount (AU)	CV (%)	nterpretation *(Ploidy level)*
Agangan**	ALAKISSA	95,67	3,5	8X
Akpazin	KOKOROGBANOU	44,75	3,6	4X
Alakissa	ALAKISSA	100,37	3,4	8X
Ala n'kojèwoué	MONDJI	49,81	4,7	4X
Androki	SOUSSOU	48,52	4,5	4X
Ankpoloman	DOUMA YESSIROU	52,29	4,2	4X
Assaboné	GNALABO	43,71	4,8	4X
Baniakpa	KOKOROGBANOU	50,63	3,4	4X
Banioure bagarou	BANIOURE	51,51	4,7	4X
Banioure Montogué	BANIOURE	50,49	4,3	4X
Baridjo**	BARIDJO	76,98	4,1	6X
Bonakpo	KOKOROGBANOU	52,29	4,5	4X
Danwari	MONDJI	50,37	3,7	4X
Déba	KOKOROGBANOU	50,02	4,6	4X
Djatouba	NONFORWOU	49,99	3,8	4X
Djikpiri	ANTAWOROROU	51,67	3,9	4X
Djiladja	MONDJI	47,48	4,3	4X
Doundoua	ALAKISSA	85,53	4,5	8X
Effourou	MONDJI	48,79	3,6	4X
Fêni	AHIMON	46,99	3,8	4X
Gnawounkoko	AGOGO	52,18	3,9	4X
Gnifokpado	MONDJI	50,71	4,6	4X
Guirissa	SOUSSOU	51,46	4,8	4X
Ihdonou	TABANE	48,98	4,7	4X
Kagourou	TABANE	49,69	4,3	4X
Kee	AHIMON	51,02	4,5	4X
Kokoné	KOKOROGBANOU	50,05	3,9	4X
Kologo	KOKOROGBANOU	50,64	3,5	4X
Kouragouroko	OURTCHOUA	50,50	3,8	4X
Kpirou kpika	KOKOROGBANOU	49,87	3,4	4X
Kratchi	KRATCHI	48,75	3,7	4X
Laboko	KPOUNA	48,39	3,9	4X
Marétassou	GNALABO	51,79	4,4	4X
Monji	MONDJI	50,65	4,3	4X
Nonforwou	NONFORWOU	49,28	4,1	4X
Nindouin	MONDJI	49,56	3,9	4X
Morokorou	MOROKOROU	51,19	3,8	4X
Ofêgui	BARIDJO	75,27	4,8	6X
Omoya	KOKOROGBANOU	49,98	4,3	4X
Ossoukpana	KOKOROGBANOU	50,41	4,1	4X
Ouwonpèotina	BARIDJO	65,45	4,2	6X
Piédjè	MONDJI	50,76	4,3	4X
Porchèhhim	PORCHEHBIM	48,28	4,7	4X
Singou	KOKOROGBANOU	49,47	4,4	4X
Soagona	AGOGO	52,03	3,8	4X
Sobasson	OURTCHOUA	50,18	3,4	4X
Sogodo	MAKPAWA	74,49	3,9	6X
Soussouka	SOUSSOU	49,10	4,7	4X
Soussounin	SOUSSOU	50,72	4,5	4X
Soussou souanbou	SOUSSOU	50,37	3,7	4X
Tabané	TABANE	48,59	3,6	4X
Tam-Sam**	TAM SAM	49,97 / 99,93	4,2	4X/8X
Terlounto	GNALABO	44,47	4,1	4X
Tognibo**	TOGNIBO	48,68	3,7	4X
Walassi	BANIOURE	49,97	3,5	4X

TABLE 2. Contd.

Cultivars	Cultivar group	DNA amount (AU)	CV (%)	nterpretation *(Ploidy level)*
Wamai	SOUSSOU	50,19	3,8	4X
Wossou	AGOGO	51,94	4,2	4X
Yahou	SOUSSOU	50,14	4,4	4X
Yaka	TABANE	49,76	4,1	4X
Yakarango	KOKOROGBANOU	50,31	4,3	4X
Yoblè	MONDJI	50,67	3,8	4X
Youbè	MONDJI	46,80 / 93,83	4,2	4X/8X
Youèyouèdota	KOKOROGBANOU	50,39	4,6	4X
Yoroutassou	NONFORWOU	52,18	4,7	4X

C.V.: coefficient of variation. Cultivars analysed with the two methods are marked with asterisk

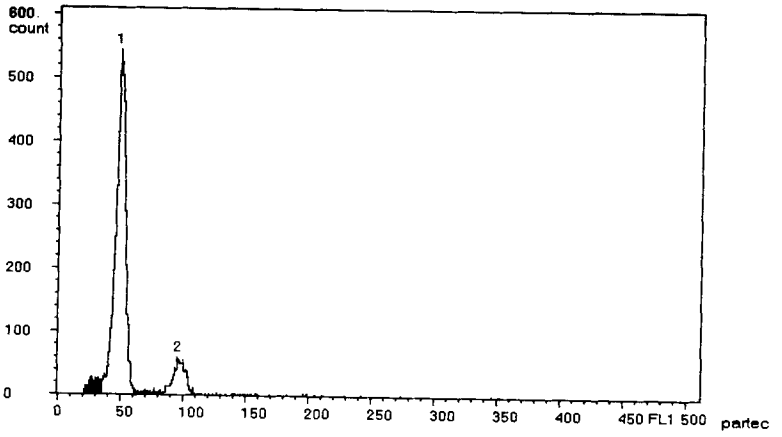

Figure 1a. Histogram of a tetraploid (cultivar Androki).

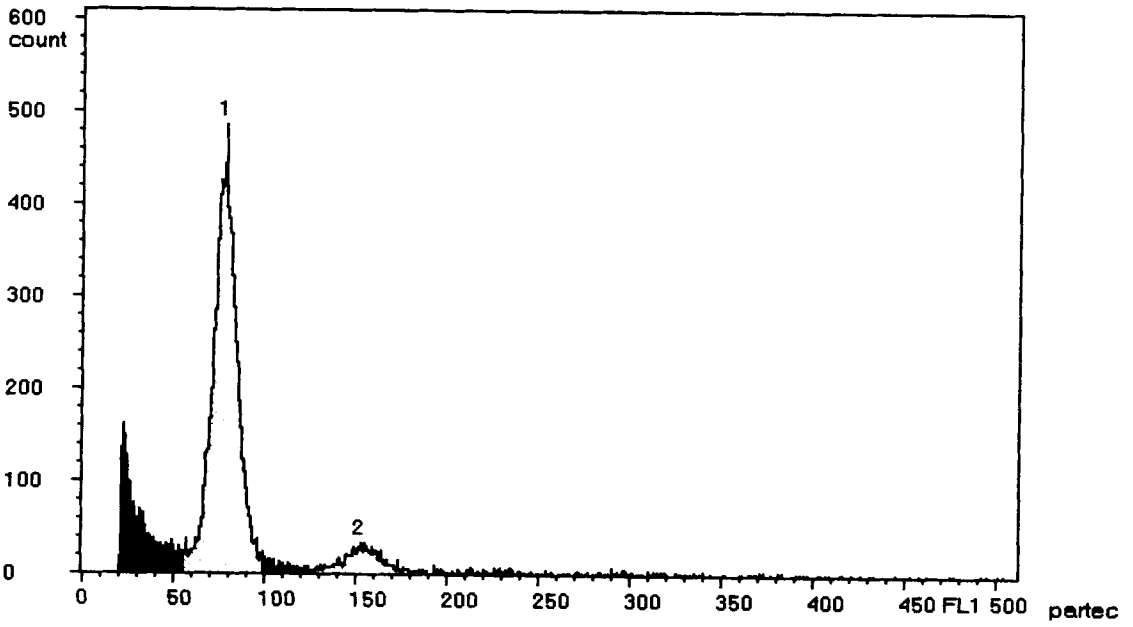

Figure 1b. Histogram of a hexaploid (cultivar Baridjo).

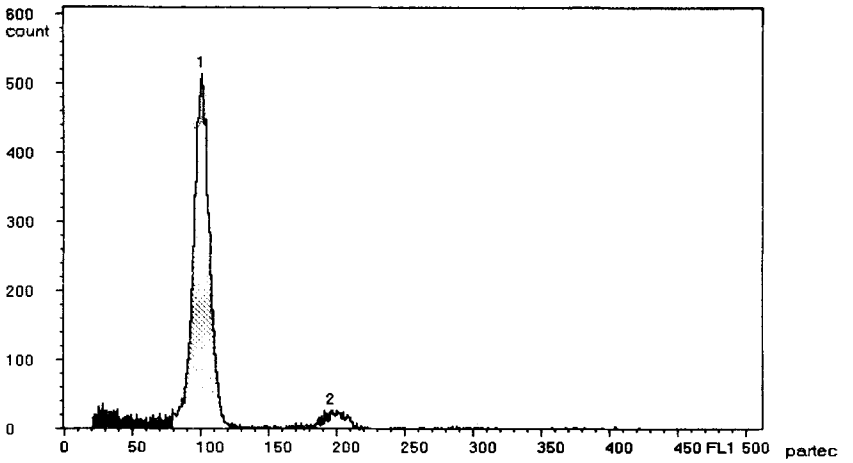

Figure 1c. Histogram of an octoploid (cultivar Alakissa).

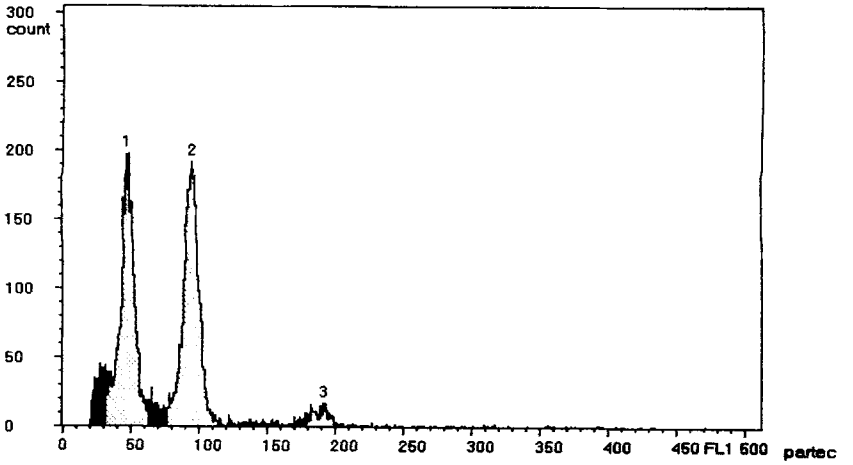

Figure 1d. Histogram of a mixoploid (cultivar Youbè).

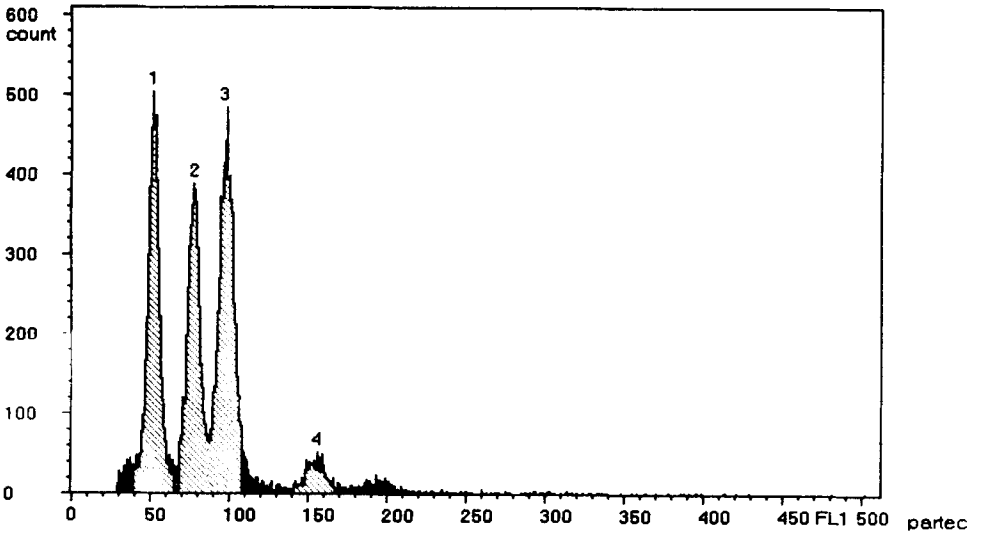

Figure 1e. Histogram obtained with a heterogeneous sample containing nuclei of tetraploid (cultivar Yakarango), hexaploid (cultivar Ofègui) and octoploid (cultivar Alakissa). The G2 peak of the tetraploid is masked by the G1 peak of the octoploid cultivar.

The Partec ploidy analyser used in this study only had filters for DAPI, so it was not possible to analyse the nuclear DNA content variation (in absolute units) within the yams of Benin. However, variations in the relative fluorescence intensity (Channel number) recorded in each of the classes of ploidy level may be an indication of a probable variation in the nuclear DNA content (in absolute unit) in the samples. Such intraspecific variation of nuclear DNA content are frequent in plants and have been already reported in many plants such as maize (Laurie and Bennett, 1985; Rayburn *et al.*, 1989; Biradar and Rayburn, 1993), rice (Martinez *et al.*, 1994), and soybean (Hammatt *et al.*, 1991). Analysis of the nuclear DNA content variation in the Benin yams may be important because it could be correlated with the flowering capacity and some agronomic traits of the plants as shown in potato by Valkonen *et al.* (1994).

In this study, no correlation was found between ploidy level and geographic origin of the yam cultivars in the Republic of Benin. This is contrary to the study of Miège (1954) who described local geographic centres of origin for the tetraploids, the hexaploids and the octoploids in Côte d'Ivoire.

Some authors (Chevalier, 1936, 1946; Burkill, 1960; Miège, 1968) treat *D. cayenensis* and *D. rotundata* as distinct species while others (Hamon, 1987; Ramser *et al.*, 1997; Dansi *et al.*, 1999, 2000a) consider the two as different compartments of the same species. In the present study, a correlation was found between subspecies or compartments and ploidy levels. Hence, all the cultivars (Agangan', 'Alakissa', Baridjo', Doundoua', 'Makpawa', Ofegui', 'Ouwon-peotina', 'Sogodo') belonging to the subspecies *D. cayenensis* are either hexaploids or octoploids while those of *D. rotundata* are all tetraploids.

Because the plant material used in the flow cytometry analysis were cultured *in vitro*, chromosome counts are needed to confirm the mixoploid status of the two cultivars 'Youbè' and 'Tam-Sam' in which two populations (4X and 8X) of cells were detected. In fact, such results may be the consequence of endomitosis that has occurred in some cells during the growth *in vitro*. The presence of 40 chromosomes, in all the cells investigated in the cultivar 'Iberegnense' that is morphologically identical to 'Tam-Sam' supports the above point of view.

This investigation is the first report of cytogenetic work on the cultivated yams belonging to *D. cayenensis/D. rotundata* of Benin Republic. It is an important prebreeding examination of the cultivars which will direct the yam breeding programme both towards the choice of initial material and towards the breeding methods that will supply the modern cultivars.

CONCLUSION

Guinea yams (*D. cayenensis/D. rotundata* complex) in the Republic of Benin, like in Côte d'Ivoire, Nigeria and Cameroon, are also polyploid with tetraploid, hexaploid and octoploid individuals. Among these, tetraploids individuals are the most frequent. In yam breeding programme, an accurate knowledge of the ploidy level of the cultivars is required. Because of the difficulties in chromosome counting previously highlighted, the use of flow cytometry which gave result in agreement of chromosome counts offers the most suitable tool for this purpose.

The ninety different cultivars for which ploidy levels were determined will be evaluated, multiplied and used for either breeding or other genetic investigations. This will help create new elite genotypes for improved yam productivity in Benin and other parts of West Africa.

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