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Ploidy level and nuclear DNA content of some accessions of water yam (*Dioscorea alata*) collected at Savè, a district of central Benin

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Summary

Ploidy level and nuclear DNA content of some accessions of water yam (*Dioscorea alata*) collected at Savè, a district of central Benin

The nuclear DNA content and the ploidy levels of 43 accessions of water yam (*Dioscorea alata*) collected at Savè (a district of central Benin) were determined by both conventional chromosome counts (from root tip cells) and flow cytometry. The great majority (42) of the accessions had 40 chromosomes and a nuclear DNA content varying from 1.0 pg to 1.26 pg (mean 2C value was 1.07 pg). One accession had 80 chromosomes with a DNA content of 2.12 pg. The ploidy levels (2X and 4X or 4X and 8X) of the analyzed individuals are discussed. These findings have important implications for yam breeding in relation to yam genetic resources.

Key words: chromosome, Benin, *Dioscorea alata*, DNA content, flow cytometry, ploidy, yam

Résumé

Niveau de ploïdie et teneur en ADN nucléaire de certaines accessions d'igname ailée (*Dioscorea alata*) collectées à Savè, district du Bénin central

La teneur en ADN nucléaire et les niveaux de ploïdie de 43 accessions d'igname ailée (*Dioscorea alata*) collectées à Savè (district du Bénin central) ont été déterminés à la fois par comptage conventionnel des chromosomes (de cellules de l'extrémité des racines) et par cytométrie en flux. La grande majorité (42) des accessions possède 40 chromosomes et la teneur en ADN nucléaire varie de 1,0 pg à 1,26 pg (la valeur 2C moyenne est 1,07 pg). Une accession possède 80 chromosomes et une teneur en ADN de 2,12 pg. Les niveaux de ploïdie (2X et 4X ou 4X et 8X) des exemplaires analysés sont discutés. Ces résultats ont des implications importantes pour les programmes de sélection de l'igname en relation avec les ressources génétiques de celle-ci.

Resumen

Nivel de ploidia y contenido de ADN nuclear en algunas accesiones de ñame de agua (*Dioscorea alata*) recogidas en Savè, un distrito de Benin central

Se determinaron el contenido de ADN nuclear y los niveles de ploidia de 43 accesiones de ñame de agua (*Dioscorea alata*) recogidos en Savè (un distrito de Benin central) tanto por recuento convencional de cromosomas (de células de los ápices radicales) como por la citometría de flujo. La gran mayoría (42) de las accesiones tenían 40 cromosomas y un contenido de ADN nuclear que variaba de 1,0 pg a 1,26 pg (el valor medio 2C era de 1,07 pg). Una accesión tenía 80 cromosomas con un contenido de ADN de 2,12 pg. Se consideraron los niveles de ploidia (2X y 4X o 4X y 8X) de los ejemplares analizados. Estos hallazgos tienen implicaciones importantes para el mejoramiento del ñame en relación con los recursos genéticos de esta especie.

Introduction

In Benin, as in many countries of West Africa, yams (*Dioscorea* spp.) play a unique role in the nutrition of the population, in the local economy, and also in social and religious festivities (Dansi et al. 1997; Orkwor et al. 1998). Four yam species or complexes of species (the *D. cayenensis/D. rotundata* complex, *D. alata*, *D. dumetorum* and *D. bulbifera*) are cultivated in Benin. Among these, the *D. cayenensis/D. rotundata* complex and *D. alata* are the most important. In terms of volume of production and utilization, *D. alata* (known as greater yam or water yam) is second to the *D. cayenensis/D. rotundata* complex also referred as to Guinea yam (Dansi et al. 1997). Various cultivars are known, which differ in their growth habit, shape and size of leaves, and shape, size and colour of the skin and flesh of the tuber. Unlike Guinea yam, which has been fairly well studied in Benin (Dansi et al. 2001a), water yam has not received the attention that it deserves with regard to its quantitative and qualitative improvement. Many genotypes are reported to be susceptible to pests and diseases (Degras 1986; Dansi et al. 1997; Orkwor et al. 1998), its traditional cultivars have never been collected and characterized, and the true diversity existing within this species still remains unknown.

To develop new elite genotypes with ecological adaptation and resistance to pests and diseases, plant breeders will need access to a wide range of diversity. Therefore, a better knowledge of the existing traditional cultivars held by farmers is necessary. For this reason, an important morphological, molecular and cytogenetic characterization programme focusing on the water yam's traditional landraces held by farmers was initiated in 2002 at the Genetics Laboratory of the Faculty of Science and Technology of the University of Abomey-Calavi, Benin. As *D. alata* is polyploid (Martin and Ortiz 1963, 1966; Baquar 1980; Essad 1984; Abraham and Nair 1991; Hamon et al. 1992; Abraham 1998; Gamiette et al. 1999; Egesi et al. 2002) knowledge of the ploidy level of the cultivars identified within this germplasm is important for breeding purposes.

Dioscorea is one of the most difficult genera for cytotaxonomic and cytogenetic studies (Essad 1984).

Determining ploidy levels in yam by counting chromosomes is tedious, difficult and time-consuming, because yam chromosomes are small, generally dot-like, and are often clumped together, complicating the counting process (Baquar 1980; Zoundjihékpon et al. 1990; Dansi et al. 2001a). To overcome these difficulties, flow cytometry has been recently used to determine ploidy levels in yams (Hamon et al. 1992; Gamiette et al. 1999; Dansi et al. 2001a, 2001b; Egesi et al. 2002). In ploidy analysis, flow cytometry assay has some important advantages over conventional chromosome 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, 1991b; McMurphy and Rayburn 1991; Dolezel 1997).

As part of the genetic characterization programme of Benin landraces of *Dioscorea alata*, a first germplasm collection mission was conducted in 2002 in central Benin (Savè district) to collect existing landraces. We report the ploidy levels and the nuclear DNA content of some of the accessions collected, as determined by both conventional chromosome counting and laser flow cytometry.

Materials and methods

Plant material

The material consisted of 43 accessions of water yam (*D. alata*) collected during December 2002 in central Benin (district of Savè) and maintained as a field collection and *in vitro* at the Faculty of Science and Technology, University of Abomey-Calavi, Benin.

Chromosome counting

Slides were prepared by the technique outlined by Zoundjihékpon et al. (1990) and Dansi et al. (2001a, 2001b). The root tips were obtained soon after sunrise (before 0700 hours) from plants grown in the field. Roots were pre-treated in a 2 mM 8-hydroxyquinoline solution for about 6 h on filter paper in a Petri dish to accumulate metaphase cells. The roots were fixed in 1:3 acetic alcohol 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 a magnification of 1000×. Because of difficulties in counting yam chromosomes reported by many authors (Baquar 1980; Zoundjihékpon et al. 1990; Dansi et al. 2001a), three accessions (DAL4b, DAL6c and DAL8a; see Table 1) were randomly chosen from the count to serve as check samples.

Preparation of nuclei and flow cytometry

A procedure described by Kamaté et al. (2001) was followed and *Pisum sativum* L. cv. Express long (2C=8.37 pg) was used as standard. To release the nuclei, a 50-mg sample of young healthy yam leaves (collected from the field) was mixed with 20 mg of *P. sativum* and chopped with a razor blade in 600 µl of buffer. The buffer was modified from that of Galbraith et al. (1983) by the addition of 10 mM sodium metabisulphite (added every 4 h), 0.1% triton X-100 and 1% polyvinylpyrrolidone (MW10000, Sigma). The homogenate was filtered through a 48-µm nylon mesh and supplemented with 10 mg mL^{-1} ribonuclease A (RNase A, Boehringer, Meylan, France) and 50 µg mL^{-1} ethidium bromide (Sigma). After 30 min of incubation, the samples were analyzed.

Flow cytometry measurements were performed with an EPICS V (Beckman-Coulter, Roissy, France) cytometer equipped with a 100-µm nozzle. At least 5000 nuclei were analyzed per sample. The total DNA content (2C defined as that contained by the G1 phase of the cell cycle) was calculated by multiplying the known DNA content of *P. sativum* by the quotient between the 2C peak positions of *Dioscorea* and *Pisum* in the histogram of fluorescence intensity. To do this, it is assumed that there is a linear correlation between the fluorescent signals from stained nuclei of the unknown specimen and the known internal standard, and the amount of DNA present.

Results

All the three randomly selected accessions (DAL4b, DAL6c and DAL8a) analyzed as check samples had 40 chromosomes. In accessions DAL4b and DAL8a some cells showed 41 and 42 chromosomes within the same root tips, while counts of about 43 were observed infrequently. In all the accessions analyzed, chromosomes were small, with some appearing dot-like and others appearing rod-shaped without any visible centromere region. In most of the cells, chromosomes were clumped together, hence complicating the counting process.

Among the 43 accessions investigated by flow cytometry (see Table 1), 42 had a relative nuclear DNA content (2C value) varying from 1.0 to 1.26 pg (1.07 pg on average) and one (DAL3b) had 2.12 pg.

All the three check samples investigated by chromosome counting showed a DNA content of about 1 pg.

Discussion

The cytology of *D. alata* has been reported by various workers, highlighting the occurrence of different levels of polyploidy (Sharma and De 1956; Martin and Ortiz 1963; Ramachandran 1968; Baquar 1980; Abraham and Nair 1991; Hamon et al. 1992; Abraham 1998; Gamiette et al. 1999; Egesi et al. 2002). According to these authors, individuals with 40 chromosomes are tetraploids. In this study, all the accessions having the same nuclear DNA content as the check samples were considered to have 40 chromosomes and therefore to

Table 1. Accession number, DNA content and ploidy level of some water yam accessions from Savè (central Benin)

No.	Accession number	DNA content	Ploidy
1	DAL2a	1.05	4X
2	DAL3a	1.03	4X
3	DAL3b	2.12	8X
4	DAL3c	1.06	4X
5	DAL3d	1.08	4X
6	DAL3e	1.04	4X
7	DAL4a	1.04	4X
8	DAL4b	1.02	4X
9	DAL4c	1.01	4X
10	DAL4d	1.03	4X
11	DAL5a	1.12	4X
12	DAL5b	1.07	4X
13	DAL5c	1.09	4X
14	DAL5d	1.09	4X
15	DAL6a	1.04	4X
16	DAL6b	1.02	4X
17	DAL6c	1.07	4X
18	DAL6d	1.03	4X
19	DAL6e	1.07	4X
20	DAL6f	1.04	4X
21	DAL7a	1.13	4X
22	DAL7b	1.09	4X
23	DAL7c	1.09	4X
24	DAL8a	1.08	4X
25	DAL8b	1.04	4X
26	DAL8c	1.05	4X
27	DAL8d	1.14	4X
28	DAL9a	1.05	4X
29	DAL9b	1.16	4X
30	DAL9c	1.18	4X
31	DAL9d	1.13	4X
32	DAL10a	1.26	4X
33	DAL10b	1.04	4X
34	DAL10c	1.06	4X
35	DAL10d	1.02	4X
36	DAL11a	1.17	4X
37	DAL11b	1.00	4X
38	DAL11c	1.11	4X
39	DAL11d	1.08	4X
40	DAL11e	1.05	4X
41	DAL11f	1.10	4X
42	DAL12a	1.03	4X
43	DAL12b	1.03	4X

be tetraploids. The only accession having a nuclear DNA content of 2.12 pg (DAL3b) was considered octoploid with 80 chromosomes.

Hexaploid individuals were not found among the sample analyzed. This does not, however, mean that they are absent in Benin, as they may be found elsewhere. As found in Benin (Dansi et al. 2001a), Cameroon (Dansi et al. 2001b) and Côte d'Ivoire (Zoundjihékpon et al. 1990; Hamon et al. 1992) with the *D. cayenensis/D. rotundata* complex, the tetraploids also seem to form the largest group of *D. alata* in Benin. This is in agreement with the findings of Essad (1984), who indicated that tetraploid individuals are the most frequent in the *Dioscorea* species.

In yam, the presence of extra chromosomes in the cells of some individuals as observed in this study is not rare and has already been 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).

Abraham and Nair (1991) reported normal meiosis with tetraploids (which showed 20 bivalents at diakinesis or metaphase I) and sterility with hexaploid and octoploid individuals. They also reported that plants with higher ploidy levels (6X and 8X) have characteristic morphological features such as thicker leaves and increased size of perianth and stigmatic lobes. These observations led them to consider the 2n=40 individuals as diploidized tetraploids and the 2n=80 plants as autopolyploid. The DNA content observed in this study of the plant having 80 chromosomes (2.12 pg) is double the average DNA content of those having 40 chromosomes (e.g. 1.07 pg), which is in agreement with their reasoning. Our team concur with Abraham and Nair's hypothesis and believe, as previously reported by Daïnou et al. (2002), that 2n=40 plants are diploids while those with 60 and 80 chromosomes are polyploid (triploids and tetraploids). The same situation exists with the *D. cayenensis/D. rotundata* complex (and with their wild relatives *D. abyssinica* and *D. praehensilis*), in which Zoundjihékpon (1990) reported that hexaploid and octoploid plants have larger pollen and somatic cells than those of tetraploid plants. *In situ* molecular hybridization is being conducted at CIRAD (Guadeloupe) to confirm or disprove this assumption.

The variation in the nuclear DNA content observed is either due to manipulation errors or is an actual phenomenon. In fact, such intraspecific variations in nuclear DNA content are frequent in plants and have already been reported in many plants, including maize (Laurie and Bennett 1985; Rayburn et al. 1989; Biradar and Rayburn 1993), rice (Martinez et al. 1994), soybean (Hammatt et al. 1991) and Guinea yam (Dansi et al. 2001a).

This investigation is the first report of cytogenetic work on water yam (*D. alata*) in Benin Republic. It is an important pre-breeding 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 modern cultivars.

Conclusion

Water yam (*D. alata*) is polyploid in Benin, as in Côte d'Ivoire, Nigeria and Cameroon. Individuals with 40 chromosomes are the most frequent. In yam breeding, an accurate knowledge of the ploidy level of the cultivars is required. Because of the difficulties in chromosome counting previously highlighted in yam, the use of flow cytometry, giving results in agreement with chromosome counts, offers the most suitable tool for this purpose.

The accessions 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 that will contribute to the alleviation of poverty in Benin.

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