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Investigation and confirmation of chromosome numbers and raphide morphology of Six yams genotypes

Norman Tongoona and E. S Shan

Njala Agricultural Research Center (NARC), PMB 540, Freetown, Sierra Leone.

Abstract

The ability to count chromosomes is among the valuable tools used by plant breeders and cytogeneticists. This exercise is difficult with yams (*DIOSCOREA* spp.) since the chromosomes are small, dot-like, frequently having only a few cell division visible in a single root tip. The aim of this study was to investigate and confirm the chromosome numbers and raphide morphology of six yam genotypes which included two genotypes of *DIOSCOREA ALATA* (ER 07/030 and ER 07/036) and four genotypes of *D. ROTUNDATA* (NR 07/060, NR 07/071, SR 07/072 and TDr 95/18544) using the root tip method. A total of ten minisetts per genotype each weighing 20 g were grown in quarter liter pots containing vermiculite medium. Root tips were prepared using the acetocarmine staining technique. Genotype ER 07/030 had 20 chromosomes, while ER 07/036, NR 07/060, NR 07/071 and SR 07/072 had 40 chromosomes, and TDr 95/18544 had 60 chromosomes. These findings were in agreement with the ploidy results obtained by flow cytometry (FCM), which indicated that FCM was a reliable technique for rapid determination of ploidy level in yams. Results of the raphide study in *D. ALATA* showed the type III morphotype in ER 07/036 with six to eight sided crystals and symetrical pointed ends. The remaining genotypes exhibited no visible presence of raphides. Findings obtained in this study would provide guidance in a yam improvement programme both in terms of selection of initial breeding material and choice of breeding methods. The development of flowering genotypes and the exploration of chromosome size and content form part of future research.

Keywords: *Dioscorea*, chromosome number, raphide morphology, genotypes.

INTRODUCTION

Tropical root and tuber crops are a subsidiary staple to over 20% of the world's population occupying an important position after cereals and grain legumes (Orkwor et al., 1998). Yams consist of more than 600 species, but only six species including *Dioscorea cayenensis* (yellow yam), *D. alata* (water yam), *D. rotundata* (white yam), and *D. bulbifera* (aerial yam) are important staple crops consumed in West Africa (Mwenye et al., 2010). *Dioscorea hispidata* (Asiatic bitter yam), locally called Ubi gadong, is also an economically wild yam species providing food for millions of people in the tropics and subtropics (Nashrivah et al., 2010). In Sierra Leone, yam is considered as the third most important root and tuber crop after *Manihot esculenta* (cassava) and *Ipomoea batatas* (sweet potato). It plays key roles as famine reserve- and cash- crop for poor resource farmers (Norman, 2010).

Despite its economic importance, yam has not been accorded the keen attention of researchers in many areas in Africa, especially in Sierra Leone. In order to develop new elite genotypes for ecological adaptation and reasonable tolerance to local pests and diseases, plant breeders require wide genetic diversity (Dansi et al., 2000b). Among the many constraints limiting

conventional breeding of *Dioscorea* spp., ranging from flowering to seedling development, are: flowering expression, pollen viability or egg receptivity, gametogenesis, pollination, fecundation, embryogenesis and seed set. These constraints encountered in sexual recombination of yams are due to the complex speciation in the crop (Obidiegwu et al., 2009). Although scanty information on yam phylogeny exists, many taxonomic ambiguities associated with cytological irregularities still remain unresolved. In addition, various cellular parameters including cell and nuclear volume and chromosome size, and developmental parameters such as minimum generation time or duration of meiosis, among others, are influenced by the C-value of an organism (Dolezel and Bartos, 2005). Therefore, genome size normally determines the breeding system. Genome size lacks a precise definition; it has either been used to describe the DNA amount in G_1 phase nucleus or unreplicated haploid chromosome set (n). This problem is exacerbated in polyploids, where genome size is used to describe the haploid (n) and monoploid (x) chromosome set(s) (Greilhuber et al., 2005).

Feulgen densitometry, image cytometry, and flow cytometry (FCM) are among the cytometric techniques which have played a significant role in plant taxonomy, biosystematics, and ecology in determining chromosomal and ploidy level data (Suda et al., 2006). The merits of FCM lie in its simplicity and speed, the small amount of tissue sample required the use of various types of plant tissues: leaves, stems, roots, sepals, petals and seeds in FCM assays. This provides the possibility of extensively exploring rare and endangered plant species with no risk of population destruction (Sgorbati et al., 2004). Through FCM, ploidy level at various spatial scales, interactions among cytotypes, and evolutionary processes in diploid-polyploid sympatric populations can also be reliably assessed (Baack, 2004; Husband and Sabara, 2004). Moreover, FCM holds great potential in reshaping former taxonomic concepts and facilitating robust classification based on cytotype characteristics (Rosenbaumova et al., 2004). Thus, the application of molecular cytogenetics to the species of *Dioscorea* under study will greatly improve an understanding of chromosome structure and karyotype variation within the species (Suda et al., 2006).

Chromosome observation is necessary to clarify the structure, function, organisation and evolution of yam genomes. However, the determination of ploidy level in yam somatic cells by chromosome counting is limited by the polyploid nature of the crop, dot-like nature of chromosomes and small volume of mitotic cells. These characteristics hinder the preparation of a distinct and well-spread chromosomes visible in a single focal plane (Staudt, 1989). A simple, rapid and reliable procedure is needed to determine the chromosome number of meristematic regions of yam root tips (Dansi et al., 2001). Furthermore, an understanding of the ploidy and chromosome status in plants generated from anther, ovary and callus cultures, or cell fusion for the identification of haploids, heterokaryons or doubled haploid genotypes is imperative in augmenting plant breeding efforts to develop new genotypes.

Raphides are calcium oxalate crystals which comprise of five basic morphological types such as needle-shaped raphides, rectangular or pencil-shaped styolids, mace-head-shaped aggregates called druses, block-shaped aggregates called crystal sand and prisms of different shapes (Horner and Wagner, 1995). Beside root and tuber, calcium oxalate crystals have also been noted in various tissues or organs within plants including leaves, stems and seeds, performing functions such as calcium storage, defense mechanism against predators by tearing soft tissues of the throat or esophagus of predators and provide structural strength (Nakata, 2003; Franceschi and Nakata, 2005). The varying character morphology and physiological functions are possibly under genetic control, and thus, may have taxonomic potential for botanists and archaeologists (Horner and Wagner, 1995).

Morphological characterization of 52 germplasm from Sierra Leone indicated that 43 of the genotypes belonged to *D. alata*, while seven belonged to *D. rotundata* and two *D. bulbifera* (Norman, 2010). Determination of ploidy level of these accessions also indicated that 45 genotypes were tetraploids, three diploids, two hexaploids, and one genotype each found to be triploid and pentaploid. The aim of this study was to investigate the number of chromosomes and type of raphide present in two *Dioscorea* species using conventional chromosome counting technique.

MATERIALS AND METHODS

Plant materials and growth conditions

An experiment to investigate and confirm the ploidy status of six yam genotypes using chromosome counting technique was carried-out at the botany department, University of KwaZulu-Natal, South Africa during early summer 2010. The six genotypes were selected from 52 accessions, which had been characterized based on morphological and ploidy level using flow cytometry. A total of ten minisetts of each genotype, including two genotypes, ER 07/030 and ER 07/036, from *D. alata* and four genotypes, NR 07/060, NR 07/071, SR 07/072 and TDr 95/18544 from *D. rotundata*. Each minisett weighing 20 g was grown in quarter liter pots containing vermiculite medium. The materials were grown for about four weeks at 25°C day/night and 70% relative humidity. The vermiculite moisture level was well managed to prevent limited oxygen availability and reduced root growth by over wetting and over drying medium respectively.

Sample preparation for chromosome and raphide analyses

A protocol slightly modified from Fukui and Nakayama (1996) was used for sample preparation and analyis. Root tips each 6 mm long were collected using forceps at 06h00 and treated in small vial of carnoy's (farmers) fixative (3 parts 95% alcohol: 1 part glacial acetic acid) for 48 h at room temperature. Samples were rinsed thoroughly in 70% alcohol to remove acetic acid, which presence could hinder the staining of the chromosomes by acetocarmine. Samples were

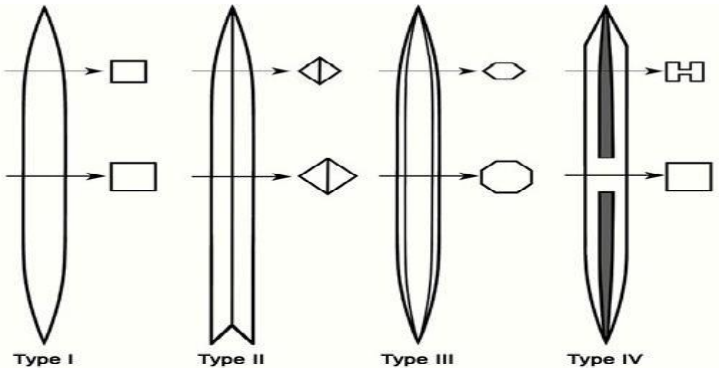

Figure 1. Diagram showing the four basic types of raphides cross-section. (Redrawn from Horner and Wagner, 1995 by Cowther, 2005).

then stored in 70% ethanol at 4°C in a refrigerator untill the day of examination. For mitotic analysis, root tips were hydrolysed in 1N HCl (1 normal Hydrochloric acid) for 90 min at room temperature and then wash in distilled water before staining. Root tips (1 to 2 mm) were excised using a razor blade and stained in a drop of acetocarmine for 15 min on microscope (glass) slide. The cells were covered with a cover slip, carefully mopping excess stain using paper towel. The slide was slighly heated over a spirit burner flame without boiling to prevent cells damage.

The chromosomes were counted in 3 to 5 cells per slide in 5 to 10 root tips per genotype using light microscope (AX70; Olympus Optical Company Limited, Japan) at the 100x objective (1000x total magnification). The well spread chromosomes and raphides at the metaphase stage were photographed and stored using a camera (CC12; soft imaging system, Olympus Optical Company Limited, Japan) connected to a personal computer equipped with image filing software (soft imaging system (SIS) analysis® 3.0 Co. Ltd., Japan).

Identification and analysis of raphides were based on classification protocol by (Horner and Wagner, 1995) (Figure 1). Raphide analysis from a number of higher plants showed four basic types: Type I is the most common consisting of four-sided single crystals that have two symmetrical pointed ends; Type II raphides are four-sided with one pointed and one bidentate or forked end; Type III raphides consist of six to eight sides with symmetrical pointed ends; Type IV raphides comprise twined crystals with H-shaped cross-sections and symmetrical ends (one wedge-shaped and the sharply pointed).

RESULTS AND DISCUSSION

Chromosome counts

The six genotypes evaluated had chromosome numbers ranging from 20 to 60. One genotype, ER 07/030, had 20 chromosomes; four, ER 07/036, NR 07/060, NR 07/071 and SR 07/072, had 40 chromosomes; and TDr 95/18544 had 60 chromosomes. The genomic number of chromosomes of the six genotypes are a multiple of the basic chromosome number, 10 (Figures 2 and 3).

Based on conventional chromosome the chromosome was determined as x = 10 for the various genotypes studied (Figure 2a). A basic chromosome number, x = 10 was also reported by Dansi et al. (2000a). Generally, the dot-like and clumping nature of the chromosomes made counting difficult. In yams, the occurrence of one or two extra chromosomes in cells of individual genotypes is not rare (Gamiette et al., 1999; Dansi et al., 2000b). However, the presence of the extra chromosomes is often attributed to the B-chromosomes or satellites which are sometimes as large as the chromosomes themselves as opposed to aneuploidy (Dansi et al., 2000a).

The B-chromosomes, which may be involved in directing non-disjunction of chromatids during cell division are dispensable and extra to the basic A-chromosome set (Hasterock et al., 2002). Langdon et al. (2000) also noted that B-chromosome-specific region is possibly occupied by a block of hetero-chromatin at the distal end of the long arm in *Secale cereale* (rye). The results were also in agreement with the ploidy results obtained from FCM, which indicated that FCM was a reliable technique for rapid determination of ploidy level in yams (Dansi et al., 2000a; Norman, 2010).

Both flower buds and root tips are vital materials in karyology for the determination of chromosome numbers by the conventional counting method. The use of flower buds is advantageous in that chromosomes are counted in meiosis, and pairing is visible. It however involves much trial and error, since meiosis occurs very early in flower formation. It also requires more time and aptness at dissection of flower bud to locate cells in meiosis, since the buds will be very small. Root tips method is a quicker means of locating chromosomes in cells in mitosis. Chromosomes are not pairing providing the opportunity to count each one individually. Wang et al. (2010) adduced that variability in chromosome size is possibly influenced by meiosis consequently leading to non-independent assortment of chromosomes. Their findings suggested that insertions and deletions may influence chromosome segregation patterns. The flower bud method was not used in this experiment due to the lack of flower set among genotypes studied. The technique also make counting more difficult due to the pairing of the dot-like and the clumping of yam chromosomes compared to other plant species.

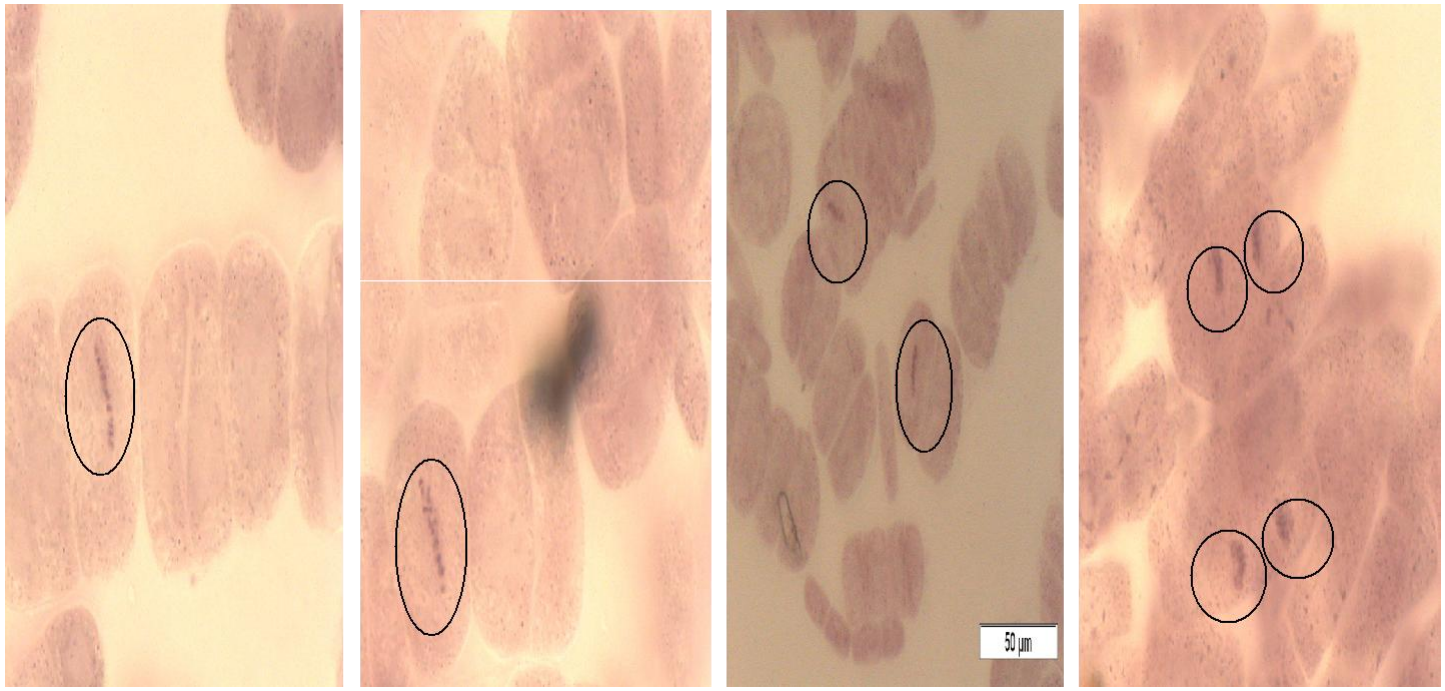

Figure 2. Mitotic chromosomes in root tip cells of the basic number, diploid and tetraploid yams stained with acetocarmine. Metaphase chromosomes in root tip cells of *D. alata*: (a_1 and a_2) examples of countable resolution with the chromosome number x = 10, obtained from ER 07/36, (b) ER 07/030 ($2n = 2x = 20$), (c) ER 07/036 ($2n = 4x = 40$). Circled regions indicate metaphase chromosome.

Raphides

Of the six genotypes studied, only one genotype ER 07/036 exhibited need-shaped raphides, while the remaining had no raphides present (Figure 4). The raphide morphology observed in the root tips of ER 07/036 was of the type III with six to eight sided crystals and symetrical pointed ends. The identification and classification were based on raphide diagrams redrawn from Horner and Wagner (1995) (Figure 1). This raphide type has also been reported in other families of plants such as Agavaceae (Wattendorf, 1996) and Typhaceae (Horner et al., 1981). Raphides mostly occur in bundles of tens to hundreds of crystals in specialised cells known as idioblasts (Barabóe et al., 2004). These cells are formed in extra cellular bundles and within starch granules. Idioblast cells differ in form, structure and content from other cells in the same tissue, and are broadly grouped into defensive and non defensive functions. The defensive cells are smaller in size and suspended in intercellular airspaces compared to the loose arrangement of crystals in non-defensive cells (Barabóe et al., 2004).

The defensive cells are possibly developed in some genotypes of yams as a defense mechanism against herbivores. Horner and Wagner (1995) noted from their anatomical and tissue culture experiments that raphide morphology and formation in specific regions within a plant are geneti-cally controlled. Among key traits used to distinguish raphides included size, cross-section and termination morphology depending on the origin of taxa. However, Franceshi and Nakata (2005) argued that morphological characterization

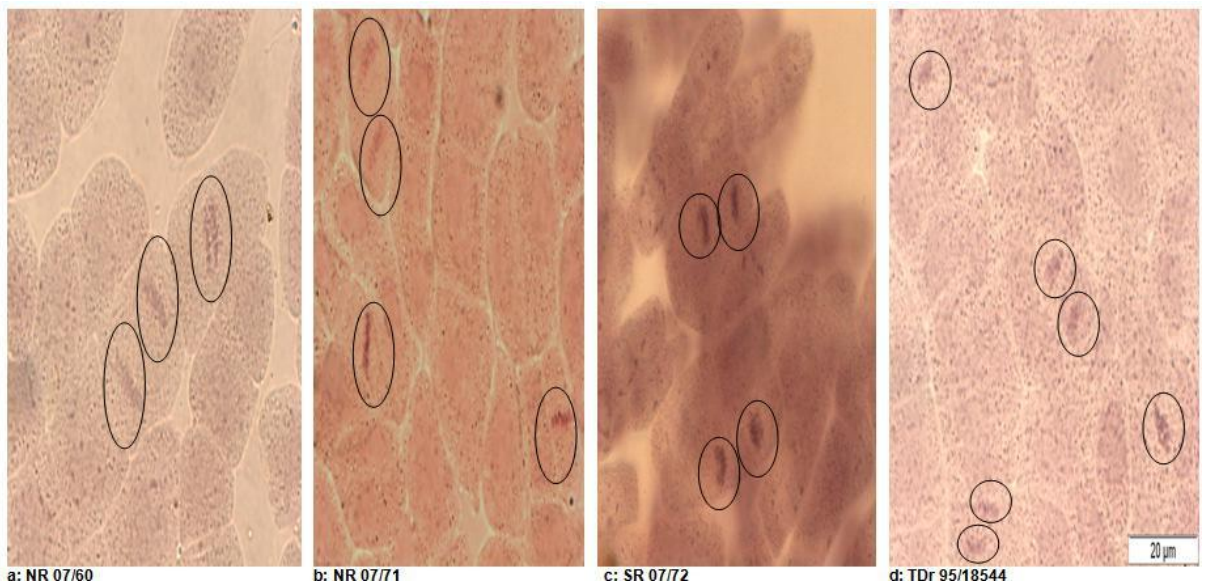

Figure 3. Mitotic chromosomes in root tip cells of tetraploid and hexaploid yam genotypes stained with acetocarmine. Metaphase chromosomes in root tip cells of *D. rotundata*: (a) NR 07/060 (2*n* = 4*x* = 40), (b) NR 07/071 (2*n* = 4*x* = 40), (c) SR 07/072 (2*n* = 4*x* = 40), (d) TDr 95/18544 (2*n*=6*x*=60). Circled regions indicate metaphase chromosomes.

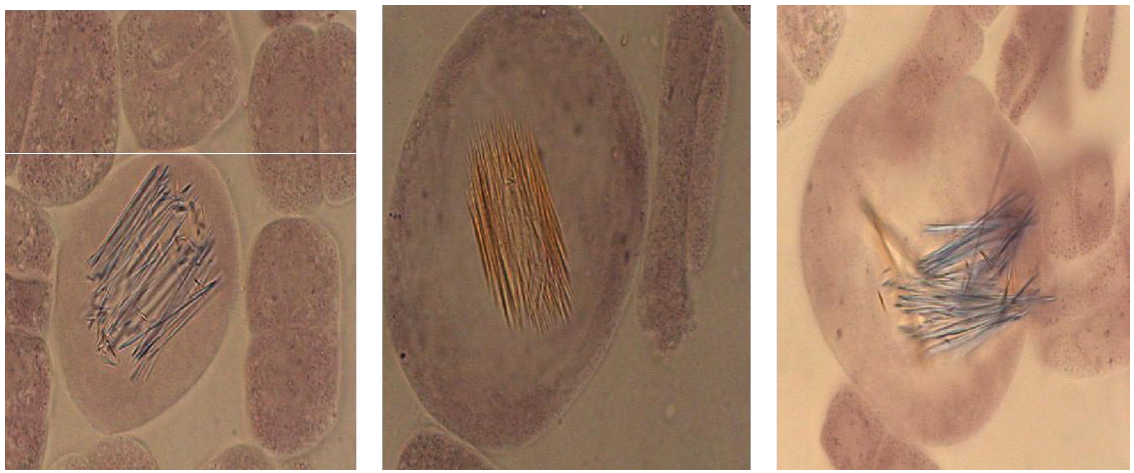

Figure 4. Different spatial arrangements of raphides obsereved in yam root tips. (These are known as type III raphides with six to eight sided crystals and symetrical pointed ends).

of raphides based on size may vary within a species depending partly on genetic variables such as the function of the cell in which the crystal is formed and partly on environmental factors such as the amount of calcium available during calcium formation. The morphological characterization of raphides based on size is yet to be fully captured in yams and could form part of future research.

Conclusions

An adequate knowledge of the chromosome/ ploidy nature of yam is needed for its effective utilization in yam breeding programme. Since yam chromosome counting technique appears to be difficult in accessing the ploidy level of the crop, flow cytometry offers a relatively easy and adequate means of such evaluation. The raphide morphology of *D. alata* constituted the type III with six to eight sided crystals and symetrical pointed ends.

This is the first report of cytogenetic work of yam genotypes from Sierra Leone. It is believed that the information generated from this study would provide guidance in a yam improvement programme both in terms of selection of initial breeding material and choice of breeding methods. The development of flowering genotypes and the exploration of chromosome size and content form part of future research.

REFERENCES

Baack EJ (2004). Cytotype segregation on regional and microgeographic scales in snow buttercups (*Ranunculus adoneus*: Ranunculaceae). Amer. J. Bot., 91: 1783-1788.

Barabóe D, Lacroix C, Chouteau M, Gibernau M (2004). On the presence of extracellular calcium oxalate crystals on the inflorescences of Araceace. Bot. J. Lin. Soc., 146: 181-190.

Cowther A (2005). Starch residues on undecorated Lapita pottery from Anir, New Ireland. Archaeol. in Oceania, 40: 62-66.

Dansi A, Pillay M, Mignouna HD, Dainou O, Mondeil F, Moutairou K (2000a). Ploidy level of the cultivated yams (*Dioscorea cayenensis/ D. rotundata* complex) from Benin Republic as determined by chromosome counting and flow cytometry. J. Afr. Crop Sci., 8: 355-364.

Dansi A, Mignouna HD, Zoundjihekpon J, Sangare A, Ahoussou N, Asiedu R (2000b). Identification of some Benin Republic's Guinea yam (*Dioscorea cayenensis-Dioscorea rotundata* complex) using Random Amplified Polymorphic DNA. Gen. Res. Crop Evol., 47: 619-625.

Dansi A, Mignouna HD, Pillay M, Zok S (2001). Ploidy variation in the cultivated yams (*Dioscorea cayenensis-Dioscorea rotundata* complex) from Cameroon as determined by Flow cytometry. Euphyt., 119: 301-307.

Dolezel J, Bartos J (2005). Plant DNA flow cytometry and estimation of nuclear genome size. Ann. Bot., 95: 99-110.

Franceschi VR, Nakata PA (2005). Calcium oxalates in plants: Formation and Function. Ann. Rev. Plant Biol., 56: 41-71.

Fukui K, Nakayama S (eds.) (1996). Plant Chromosomes: Laboratory Methods. 3rd Edition, CRC Press, Boka Raton, Florida, USA, P. 274.

Gamiette F, Bakry F, Ano G (1999). Ploidy determination of some yam species (*Dioscorea* spp.) by flow cytometry and conventional chromosomes counting. Gen. Res. Crop Evol., 46: 19-27.

Greilhuber J, Doleel J, Lysak M, Bennett MD (2005). The origin, evolution and proposed stabilization of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. Ann. Bot., 95: 255-260.

Hasterock R, Jenkins G, Langdon T, Jones RN (2002). The nature and density of translocated B-chromosome-specific satellite DNA of rye. Chrom. Res., 10: 83-86.

Horner HT Jr., Kausch AA, Wagner BL (1981). Growth and change in shape of raphide and druce calcium oxalate crystals as a function of intracellular development in *Typha angustifolia* L. (Typhaceae) and *Capsicum annuum* L. (Solanaceae). Scan. Elect. Micros., 1981: 251-262.

Horner HT Jr., Wagner BL (1995). Calcium oxalate formation in higher plants. In: SR Khan (ed.), *Calcium Oxalate in Biological Systems*. Boka Raton, CRC Press, Florida, USA, pp. 53-72.

Husband BC, Sabara HA (2004). Reproductive isolation between autotetraploids and their diploid progenitors in fireweed. *Chamerion angustifolium* (Onagraceae). New Phytol., 161: 701-711.

Langdon T, Seago C, Jones RN, Ougham H, Thomas H, Forster JW, Jenkins G (2000). De novo evolution of satellite DNA on rye B-chromosome. Gen., 154: 869-884.

Mwenye OJ, Gondwe WT, Ambali AJT, Msowoya W, Luhanga R (2010). Evaluation of local yam (*Dioscorea* spp.) genotypes for yield and quality in Malawi. In: Mahungu NM (Ed.) *Root and Tuber Crops for poverty alleviation through science and technology for sustainable development*. Proc. of the 10th Symp. of ISTRC-AB, Maputo, Mozambique, 8-12 October, 2007, pp. 268-276.

Nakata PA (2003). Advances in our understanding of calcium oxalate crystal formation and function in plants. Plant Sci., 164: 901-909.

Nashrivah M, Nornasuha Y, Salmah T, Norhayati N, Mohd R (2010). "*Dioscorea hispida* Dennst. (Dioscoreaceae): An Overview", Buletin UniSZA, No. 4, ISSN 2180-0235.

Norman PE (2010). Morphological and cytological diversity of some yams (*Dioscorea* spp.) in Sierra Leone. MSc Thesis, University of KwaZulu-Natal, Peitermaritzburg, South Africa, p. 144.

Obidiegwu JE, Rodriguez E, Ene-Obong EE, Loureiro J, Muoneke CO, Santos C, Kolesnikova-Allen M, Asiedu R (2009). Estimation of the nuclear DNA content in some representative of genus *Dioscorea*. Sci. Res. Essay, 4: 448-452.

Orkwor GC, Asiedu R, Ekanayake IJ (eds.) (1998). *Food Yams. Advances in Research*, IITA and NRCRI, Nigeria, p. 249.

Rosenbaumova R, Plackova I, Suda J (2004). Variation in *Lamium* subg *Galeobdolon* (Lamiaceae) – insights from ploidy levels, morphology and isozymes. Plant System. Evol., 244: 219-244.

Sgorbati S, Labra M, Grugni E, Barcaccia G, Galasso G, Boni L, Mucciarelli M, Citterio S, Iramategui AB, Gonzales LY, Scannerini S (2004). A survey of genetic diversity and reproductive biology of *Puya raimondii* (Bromeliaceae), the endangered queen of Aades. Plant Biol., 6: 222-230.

Staudt G (1989). The species of Frageria, their taxonomy and geographical distribution. *Acta Hort.* 256: 23-33.

Suda J, Krahuleova A, Travnieek P, Krahulee F (2006). Ploidy level versus DNA ploidy level: an appeal for consistent terminology. Taxonomy, 55: 447-450.

Wang J, Chen, PJ, Wang GJ, Keller L (2010). Chromosome size differences may affect meiosis and genome size. Sci. 16 July 2010, 329(5989): 293

Wattendorf J (1996). A third type of raphide crystal in the plant kingdom: six sided raphides with laminated sheaths in *Agave americana* L. Planta, 130: 303-311.