Cytogenetic Studies of Members of the Family Moraceae in Benue State, Nigeria

Akesa, T.M.

Abstract
Cytogenetical studies were carried out on members of the Family Moraceae in Benue State, Nigeria to investigate and establish the cytogenetic characters between June and September,2015. These studies were carried out to determine the number and characterization of chromosome complements that are arrested at mitotic stage through a series of treatments and to reveal the chromosomes of related species of plants that vary in several ways, which are collectively summed as karyotype. Data on chromosomal work showed that both *Ficus thonningii and Artocarpus heterophyllus* had the highest chromosomal number of 2n = 28(i.e.basic number of 14). This was followed by *Ficus exasperata, Ficus ingens, Ficus platyphylla, Ficus polita, Ficus trichopoda, with* chromosomal number of 2n = 26 (i.e.basic number of 13). *Ficus sur* had the lowest chromosomal number of 2n = 18 (i.e.basic number of 9).

Keywords: *Artocarpus* chromosome Cytogenetical, *Ficus* mitotic Moraceae

1. INTRODUCTION

Cytogenetic studies include determination of number and morphological characterization of chromosomal complements that are arrested at mitotic metaphase stage through series of treatments. Such studies have revealed that chromosomes of related species may vary in several ways, which are collectively summed as karyotype. These cytogenetic details have been found extremely useful at all levels of the taxonomic hierarchy as well as resolving controversies on biogeography, taxonomy and evolution of different taxa (Stace, 1980).

The term karyotype has been given to the group of characteristics that identifies a particular set of chromosomes (Singh, 2004). These characteristics include chromosome numbers, relative size, centromeric position, arm length, secondary constrictions and satellites (Verma and Agarwal, 1974). Karyotypic variation is indispensable as genetic variability, as well as species differentiation (Urdampilleta *et al.*, 2007). The chromosome morphology includes the centromere, secondary constriction, satellite and telomeres of a chromosome (Stace,1980). The different aspects of chromosome morphology are usually studied at the metaphase stage of mitosis at which chromosomes have become thickened and shortened to the maximum extent, and when they are most easily stained in which the principal landmarks may be seen (Stebbins,1971).

Centromere is a constricted region of chromosomes to which the spindle fibres are attached and involves in chromosome segregation during cell division (Kidwell, 2005). Chromosomes of most organisms contain only one centromere and they are said to be monocentric chromosomes (Singh,2003b). Unless it is located at chromosome end, the centromere divides a chromosome into two distinct regions, referred to as chromosome arms (Verma and Agarwal, 1974). In a given chromosome, the centromere is localized in one particular region, whereas its relative position varies from chromosome to chromosome (Singh, 2004), and thus the difference in centromeric position among chromosomes is used to classify chromosomes into several categories: Telocentric chromosomes are those which have the centromere at their end and such chromosomes have only a single arm (Stebbins, 1971); Acrocentric chromosomes are those having the centromere nearer to one end and thus possess one very short arm and another one relatively long arm (Singh,2003b); Sub-metacentric chromosomes have a centromere located somewhat away from the middle portion of the chromosome and thus forming two unequal arms, but not as extreme as in the case of acrocentric chromosomes (Verma and Agarwal, 1974); Metacentric chromosomes contain a centromere at or near the region and thus form two equal or nearly equal arms (Singh, 2003b).

Alteration in the position of the centromere which converts metacentric to sub- metacentric or acrocentric chromosomes or vice versa occurs through inversion of chromosomal segments containing the centromere or through unequal translocation between non-homologous chromosomes (Thompson, 2005). Metacentric chromosomes can also arise by the Robertsonian fusion of two telocentrics (Sharma and Sharma, 1999). Conversely, horizontal breakage through the

centromere of a biarmed chromosome can give rise to two telocentric chromosomes. The latter two types of chromosomal rearrangements lead to change in centromeric position, but respectively decrease or increase chromosome number without affecting the total number of arms and total amount of the genetic material.
Another useful aspect of chromosomal gross morphology is the position of a secondary constriction. Secondary constriction is a thin undercondensed region also known as nucleolus organizer region (NOR) (Singh, 2004). The secondary constriction also delimits distally a condensed chromosome segment that is referred to as a satellite. The satellite is connected to the rest part of the chromosome via the secondary constriction (Stace, 1980; Sumner, 2003). Chromosomes possessing the satellites are also designated as sat- chromosomes and only one pair or a few pairs of satellited chromosomes are present in diploid chromosomes (Verma and Agarwal, 1974).
Genome size refers to the total DNA content of the nucleus of a particular of an individual or a species (Stebbins,1971). The DNA amount in the unreplicated haploid nuclear chromosome complement is known as C-value. Since species vary in their C-values, it can serve as a significant biodiversity feature essential in species characterization (Bennett and Leitch, 2005a).

Chromosome size refers to the entire length of individual chromosomal complements, also determined as relative chromosome size (Stebbins,1971). Monocots generally possess larger sized chromosomes than dicots. Plants in general have larger-sized chromosomes in comparison to animals (Verma and Agarwal, 1974). Chromosomes of woody angiosperms are usually small with little size differences between related species and genera, whereas chromosomes of herbaceous angiosperms show great size difference between different genera of the same family, sometimes even between different species of the same genus (Sharma and Sen, 2002). The chromosomal complements or karyotypes of most of the plant species consist of chromosomes which are comparable to each other in size. Difference in relative chromosome size can be brought about by unequal translocation, deletion or duplication of chromosomal segment (Stebbins, 1971). Chromosomes are characterized by their relative sizes as small, medium and large chromosomes (Singh, 2004).

Chromosome number is usually constant for a particular species (Krawiec, 2003), but may vary between different species. Variations in chromosome number are of two major types; aneuploidy and euploidy (Stebbins, 1971). When chromosome numbers vary in multiples of the complete chromosome set basic to a species, it is termed as euploidy. When the number varies in less than a complete set, it is termed aneuploidy (Sleper and Poehlman, 2006). In addition to the normal constant chromosome complements, many plant species contain a variable number of chromosomes known as accessory or supernumerary or B-chromosomes (Camacho, 2005). They are genetically dispensable since they do not have any known significant effect on the morphology or physiology of the host plants (Singh, 2004).

Polyploidy is an aspect of euploidy in which an individual or cell possesses more than two sets of chromosomes. The term "ploidy" or "ploidy level" refers to the number of complete sets of chromosomes, a set being denoted by an "x" (Ranney, 2006). Therefore, polyploidy is defined as the heritable condition of possessing more than two complete sets of chromosomes (Ramsey and Schemske, 1998), and it is a prominent and significant force in plant evolution (Adams and Wendel, 2005). The frequency of polyploidy is high in both angiosperms and pteridophytes but it is more prevalent in pteridophytes than in angiosperms (Otto and Whitton, 2000). In the case of angiosperms, polyploidy is more common in the monocots than in the dicots, and within each of these groups the frequency of polyploids varies significantly among families and genera (De Wet, 1971). It has been established that around 60 percent of all plant species are polyploids (Ennos and Sheffield, 2000).
Classification or grouping of the plants in the past was focused mainly on the morphology of the plants without involving other parameters. For this reason, there is the need to include other means by which plants can be distinguished such as cytological parameters which can help in giving a more clear-cut evolutionary relationship between the species rather than using only the morphological traits. At present, comprehensive information on the cytological parameters of members of the

Family Moraceae in Benue State, Nigeria is lacking.

2 MATERIALS AND METHODS

2.1 Growing of the plants and Harvesting of the Root-tips.

Stem cuttings obtained from the study sites, of plant species belonging to the family Moraceae were used for propagation in polythene bags filled with saw dust. The polythene bags were properly labeled with the names of plant species that they contained for reference purposes. The plants were watered twice a day (morning and evening) for three weeks. Fresh roots appeared within the said period.The larger roots having firm, shiny tips with slight yellowish tinge which would give many cells suitable for study were selected. Roots having dull, brown or flaccid tips were avoided as they do not produce good cells. The root-tips were collected by removing the terminal (5-10mm) of the roots with fine pointed forceps. The root tips were collected between 08.00am and 11.00am, which is the time for highest active cell division according to Haskell (1986).

2.2 Fixing of the Root-tips

The harvested root-tips were transferred with forceps directly into test tubes nearly filled with Carnoy's fixative and stoppered to prevent evaporation or hydration. The test tubes were labelled with the names of the plant species. Root tips were fixed for a minimum of 3 hours, although, they could remain in the fixative up to 48 hours at room temperature. The root tips not used within 48 hours were prevented from deteriorating by preserving them for future use in a solution of 70% alcohol in tightly stoppered tubes and kept in the refrigerator at about 10^{o}C. The fixed root tips immediately were then rinsed a couple of times in the ethyl alcohol to remove the acetic acid from the fixative. Acetic acid, if not removed, could reduce the stainability of the chromosomes. The acetic acid acts as neutralizer of stains.

2.3 Hydrolysis for Softening the Root-tips

Hydrolysis involves the maceration of the tissues in hot hydrochloric acid to soften the tissues or to hydrolyse deoxyribonucleic acid (DNA) of the chromosomes in the root-tips. The links between the cellulose walls of plant cells are broken down by the treatment with hydrochloric acid. This ensures that the stain can penetrate the cells and allows the tissue to be squashed to spread out a layer one-celled thick under the cover slip on a glass slide.

Hydrolysis is often done prior to orcein staining. The fixative was decanted from the test tube containing the fixed root tips and was replaced with 10% HCL. The test tubes were then transferred to an oven preset at 60^{0}C for30 minutes after which hydrolysed root tips were transferred into specimen tubes and washed several times to remove the hydrochloric acid which otherwise would interfer with the staining process.

2.4 Staining of Root-tips

Formo-lacto-propiono-orcein stain is very useful in chromosomal studies as it is partly selective in its action and so enables chromosomes to be stained differentially from the other cell contents. The hydrolysed and washed root-tips were transferred into test tubes containing Formo-lacto-propiono-orcein stain and left for 30minutes for staining. Root-tips left for longer periods in the stain became unusable owing to over-staining of the cytoplasm

2.5 Preparation of Slides for Microscope work

The stained root tip was placed on a slide and viewed under a dissecting microscope. The end of the root- tip behind the root cap which is the region of active mitotic activity was isolated from the rest of the root tip using a sharp dissecting knife. Using mounting needles, the root tip was broken into small bits and flooded with one drop of Formo-lacto-propiono-orcein stain. It was then covered with a cover slip and placed on a paper towel which was then folded over the cover slip and pressed as hard as possible with the thumb. This forced the cells to separate from each other giving a single layer of cells making it possible to observe the cell inclusion together with the chrososomes at different stages of mitotic division.

The number of chromosomes per cell for each species of the Family Moraceae studied was estimated from the mitotic stages studied. Efforts were made to as much as possible cover all the area covered by the root tip so as to observe as

much as possible all the mitotic activity per root tip. A Carl-Zeiss Jena NU microscope equipped with an Olympus Camedia C 2000 Z camera was used for the study. This enabled observations to be made using the 100x oil immersion objectives for chromosomal counts and other chromosome characteristics. Photomicrographs were made of the chromosomes at different mitotic stages.

2. RESULTS AND DISCUSSION

Table 1 illustrates the Chromosomal number of plant species belonging to Moraceae Family in Benue state. Number of chromosomes varied among plant species of the Family Moraceae. Both *Ficus thonningii and Artocarpus heterophyllus* had the highest chromosomal number of 2n = 28(i.e.basic number of 14. This was followed by *Ficus exasperata, Ficus ingens, Ficus platyphylla, Ficus polita, Ficus trichopoda, with* chromosomal number of 2n = 26 (i.e. basic number of 13). *Ficus sur* had the lowest chromosomal number of 2n = 18 (i.e. basic number of 9. Chromosome counting is a relatively easy task which produces reliable and highly reproducible data, unless the chromosome number of the specimen is very high and /or clump together (Guerra,2008). The findings of the present study have shown that the chromosomes of Moraceae plants were clumped together in most of the cells undergoing mitosis, so it was difficult to count them precisely. Even in those very rare cases where chromosomes were well spread and countable, the cell might have lost some of its chromosomes in the process of slide preparation and so it was not always possible to get complete number of chromosomes. It was a challenging and tedious task to obtain the precise chromosome counts for all the species included in the study. Although, in such cases according to Guerra (2008), approximate count would be enough for cytotaxonomic purposes. Therefore, an approximate chromosome count nearest to the possible somatic chromosome number was recorded in the present study. In most cases, more than one intact cell were counted for each sample plant, except for a few of the sample plants for which a count was obtained only from a single cell.

The findings of this study have shown that the basic chromosome number of Moraceae is, x = 9, 13 or 14. Several cytogenetic studies established that the species of family Moraceae have a base number of *x = 13 or 14* (Condit, 1928 & 1933; Bawa, 1973). Compared to the base number, the chromosome counts in the present study indicated that these identified species were not polyploids, they were all diploids. The findings of the present study are also in line with Pimienta (1995), whose review indicated diploidy as a common phenomenon within the family Moraceae. In this study, the basic number of x = 9 was obtained in *Ficus sur.* This finding is contrary to the findings of Condit (1928 & 1933) and Bawa (1973) who found the basic number x of 13 and 14. Several authors have reported variations in chromosomal numbers in plants of the same kind that are vegetatively propagated. They include Avav and Kalu who worked on the Ethnobotanical Identification and Characterization of White Guinea Yam grown in Benue Valley of Nigeria, and Muthamia *et al.*(2014) who worked on Determination of ploidy level among Yam (*Dioscorea* spp.) landraces in Kenya by flow cytometry. Others are Miege (1954), Paghavan (1958) and Martin and Ortiz (1966). Another possible explanation for this low basic chromosome number is that chromosomes were clumped together in most of the cells undergoing mitosis, so it was difficult to count them precisely. Even in those very rare cases where chromosomes were well spread and countable, the cell might have lost some of its chromosomes in the process of slide preparation and thereby making it impossible to get the complete number of chromosomes. This is subject to confirmation

Photomicrographs of the root tip cells of the various species of Moraceae plants in Benue state are shown below including the various stages of mitotic cell division : Plate 1 (*Ficus exasperata* root tip cells), Plate 2 *(Ficus ingens* root tip cells), Plate 3 (*Ficus platyphylla* root tip cells), Plate 4 *(Ficus polita* root tip cells), Plate 5 *(Ficus sur* root tip cells), Plate 6 *(Ficus thonningii* root tip cells), Plate 7 *(Ficus trichopoda* root tip cells) and Plate *8 (Artocarpus heterophyllus* root tip cells)

Table 1: Chromosomal number of plant species belonging to Moraceae Family in Benue state.

S/No	Plant Species	Chromosomal number
1	*Ficus exasperata*	2n =26
2	*Ficus ingens*	2n =26
3	*Ficus platyphylla*	2n =26
4	*Ficus polita*	2n =26
5	*Ficus sur*	2n =18
6	*Ficus thonningii*	2n =28
7	*Ficus trichopoda*	2n =26
8	*Artocarpus heterophyllus*	2n =28

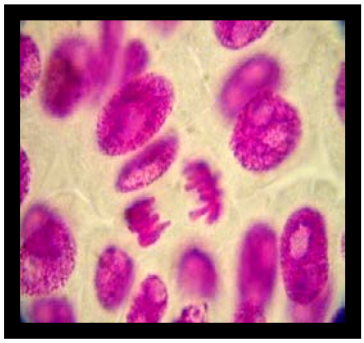

PLATE NO.1 : *Ficus exasperata* root tip cells showing early anaphase prophase

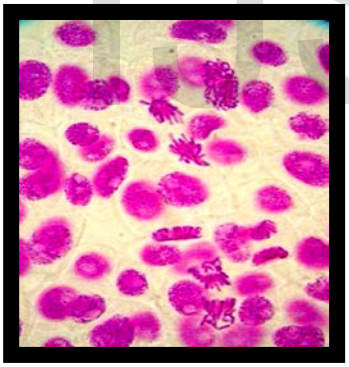

PLATE NO.2 : *Ficus ingens* root tip cells showing early and late prophase, early and late anaphase, and telophase

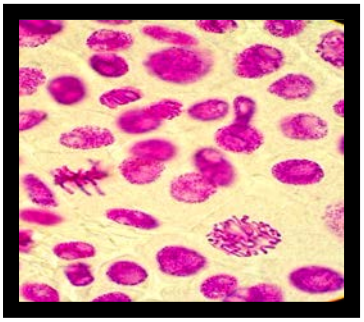

PLATE NO.3 : *Ficus platyphylla* root tip cells showing early and late prophase, metaphase and telophase.

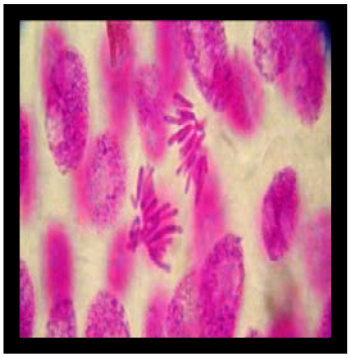

PLATE 4 : *Ficus polita* root tip cells showing early prophase and late anaphase.

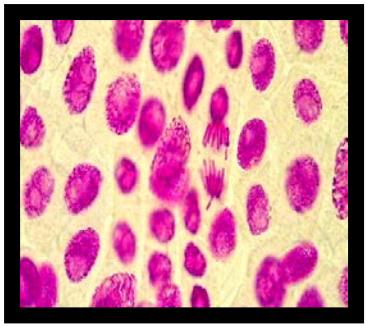

PLATE 5 : *Ficus sur* root tip cells showing prophase and late anaphase

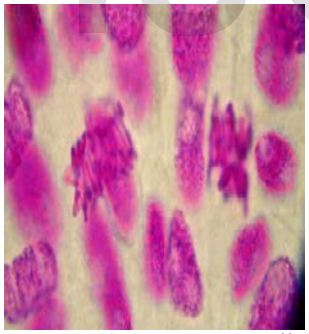

Plate 6: *Ficus thonningii* root cells showing early metaphase

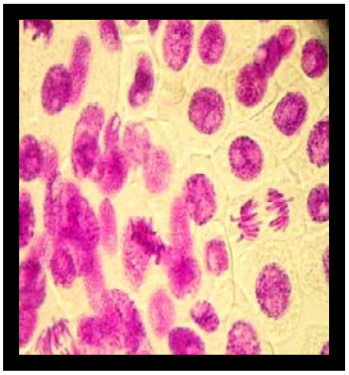

PLATE 7 : *Ficus trichopoda* root tip cells showing early and late anaphase and metaphase.

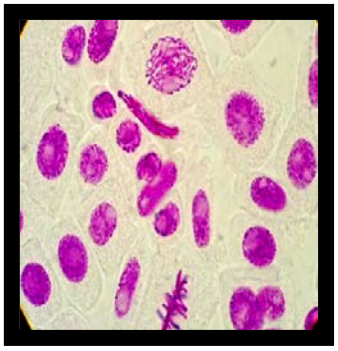

PLATE 8: *Artocarpus heterophyllus* root tip cells showing prophase and metaphase

REFERENCES

Acquaah, G. (2007). *Principles of Plant Genetics and Breeding*. Blackwell Publisher. United States of America pp. 214-230

Adams, K.L. and Wendel, J.F. Polyploidy and genome evolution in. plants. *Curriculum. Opinio*n. *Plan*t. *Biology*. 8 (2005),135–141

Agishi, E. C. Etulo, Idoma, Igede, Tiv and Hausa names of plants. Chammer publishers, Makurdi. (2004),188pp.

Aiyelero, O.M., Ibrahim, Z. G. And Yaro, A.H.(2009).Analgesic and Anti-Inflammatory Properties of the Methanol Leaf Extract of *Ficus ingens* (Moraceae) in Rodents. *Nigerian Journal of Pharmaceutical Sciences 8(2):79-86* .

Akesa, T. M.(2010). Flowering patterns of woody plants at Agan Forest Reserve, Makurdi. Unpublished *M.Sc. Dissertation*, Biological Sciences Department, Benue State University, Makurdi. 97pp.

Alemayehu, M. (2007). Chromosome study on seven endemic *Aloe* species (Aloaceae) of Ethiopia. *M.Sc. Thesis*, Biology Department, Addis Ababa University.

.

Avav, T. and Kalu, B. A. (1989). The Ethnobotanical Identification and Characterization of White Guinea Yam grown in Benue Valley of Nigeria. *Tropical Agriculture*, 41(5): 23-56.

Baranska, M., Schulz, H., Kruger, H., and Quilitzsch, R. (2005). Chemotaxonomy of aromatic plants of genus Origanun via Vibrational Spectroscopy. *Analytical Bioanalytical Chemistry*, 381:1241-124

Bawa, K.S. (1973). Chromosome numbers of tree species of a lowland tropical community. *Journal Arnold Arbor*. 54 : 422-434.

Bennett, M.D. and Leitch, I.J. (2005a). Plant genome size research: A field in focus,Overview. *Ann. Bot.* 95**:** 1–6.

Bradham, P.E. and Doherty, M.J. (1998). Genome size variation in the Aloaceae, an angiosperm family displaying karyotypic orthoselection. *Annals of Botony*. 82a: 67-73.

Camacho, J.P.M. (2005). B-chromosomes. In**:** *The Evolution of the Genome*. (Gregory, T.R. ed.). Elsevier Academic Press. pp. 223-286. .

Condit, I.J. 1928. Cytological and morphological studies in the genus *Ficus.* I. Chromosome number and morphology in seven species. Univ. California *Publ. Bot*. 11 : 233- 244.

Condit, I.J. 1933. Cytological and morphological studies in the genus *Ficus.* I1. Chromosome number and morphology in thirty-one species. Univ. California pp 559-567

Corner, E. J. H.(1962) . The classification of Darlington, C.D. and La Cour, L.F.(1976). The Handling of Chromosomes(Six

edition). George Allen and Unwin Ltd, London. 201pp.

Cramer, C.S. (1999). Laboratory techniques for determining
ploidy in plants.
HortTechnology. 9(44): 594-596..

De Wet, J.M. (1971). Polyploidy and evolution in plants. *Taxon.* 20(1): 29-35.

Elutade, A. O. and Bem, A. A. (2003). Cytogenetics of some male sterile cowpeas, *Vigna unguculata* (L.WALP) Lines. *Benue Valley Journal of Interdisciplinary Studies*. 2: 131 -137).

Erdtman, H.(2006). Some aspects of chemotaxonomy. Institutionen for OrganiskKemi. Pp 679-706.

Fankhausser,D.B.(2011).ChromosomesinRoottip file:/// C:Users/User/Documents/root-tip-chromosomes2.hyml

Fikadu G.(2009). Chromosome study in five endemic *Kniphofia* species(Asphodelaceae) of Ethiopia. *M.Sc. Thesis*, School of Graduate Studies, Biology Department, Addis Ababa University.

Fukui, K., and S. Nakayama., Eds. (1996). Plant Chromosomes: Laboratory Methods. CRC Press.

Gregory, T.R. (2005). Genome size evolution in animals. In**:** *The Evolution of Genome*. pp. 1-71 (Gregory, T.R. ed.). Elsevier Academic Press, United of America.

Guerra, M. (2008). Chromosome numbers in plant cytotaxonomy: concepts and implications. *Cytogenet. Geno. Res*. 120:339-350.

Gupta, P.K. (2008). *Genetics*. Rastogi Publications, New Delhi, India. pp. 46-60.

Haskell, G. and Willis, A. B.(1986). Primer of chromosome practice. Plant and Animal chromosomes under the microscope. Gordon and Breach Science pu blishers, New York

Jones, J.R., Ranney, T.G. and Lynch, N.P. (2007). Ploidy levels and relative genome sizes of diverse species, hybrids, and cultivars of *Rhododendron*. *J. Amer. Rhod. Soci.* pp. 200-221.

Kidwell, M.G. (2005). Transposable elements. In**:** *The Evolution of the Genome*.(Gregory,

Krawiec, S. (2003). Genome. In**:** *Genetics. Vol-2,* pp. 112-117. (Robinson, R. ed.)Macmillan Reference, United States of America

Levin, D.A. (2002). *The Role of Chromosomal Change in Plant Evolution*. Oxford University Press, New York, United States of America. pp. 47- 144.

Mable, B. K. (2004). Why polyploidy is rarer in animals than in plants: Myths and mechanisms. *Biol. J. Linn. Soc.* 82: 453–466.

Martin, F. W. and Ortiz, S.(1966). Origin in anatomy of tubers of *Dioscorea floribunda* and *Dioscorea spiculiflora. Botaical Gazzette. 124: 416 - 421.*

Muthamia, T. A. (2014) Determination of ploidy level among Yam (*Dioscorea* spp.) landraces in Kenya by flow cytometry. Field crop research, 11(7): 22-45.

Negron-Ortiz, V. (2007). Chromosome numbers, nuclear DNA content, and polyploidy in Consolea (Cactaceae), an endemic cactus of the Caribbean Islands. *American Journal of Bot*any 94(8):1360–1370.

Obermayer, R., Leitch, I.J., Hanson, L. and Bennett, M.D. (2002). Nuclear DNA C-values in 30 species double the familial representation in Pteridophytes. *Annals of Botany,* 90: 209-217.

Otto, S.P. and Whitton, J. (2000). Polyploid incidence and evolution. *Annual Revised Genetics* 34:401–37.

Paghavan, R.S. (1958). A chromosome survey of Indian Dioscoreas. *Proc Indian Academic Science Section B*. 48, 59-63.

Pimienta (1995). Determination of ploidy levels within the family Moraceae. *Annu. Rev. Ecol. Syst.* 40: 46–50.

Ramsey, J. and Schemske, D.W. (1998). Pathways, mechanisms, and rates of polyploidy formation in flowering plants. *Annu. Rev. Ecol. Syst.* 29: 467–501.

Ramsey, J. and Schemske, D.W. (2002). Neopolyploidy in flowering plants. *Annu. Rev. Ecol.Syst*. 33:589–639.

Ranney, T.G. (2006). Polyploidy: From evolution to new plant development. *Combined Proceedings International Plant Propagators' Society.* 56: 137-141.

Sharma, A. and Sen, S. (2002). *Chromosome Botany*. Science Publisher, Inc. United States of America.Pp.41-52.

Sharma, A.K., and A. Sharma(1965). Chromosome Techniques, Theory and Practice. Butterworths, London.105pp.

Sharma, A.K. and Sharma, A. (1999). *Plant Chromosomes: Analysis, Manipulation and Engineering*. Overseas Publisher Association. Netherlands, Amsterdam. pp. 4-39.

Stebbins, G.L. (1971). Chromosomal Evolution in Higher Plants. Edward Arnold Publishers Ltd., London

Sumner, A.T.(2003). Chromosomes: Organization and Function. Blackwell publishing company, United Kingdom. pp. 133-142.

Tezera, T. (2008). Chromosome study of eleven endemic *Aloe* species of Ethiopia with emphasis on the satellites and nucleoli. *M.Sc. Thesis*, School of Graduate Students, Department of Biology, Addis Ababa University.

Thompson, J.N., Nuismer, S.L. and Merg, K. (2004). Plant polyploidy and the evolutionary ecology of plant/animal interactions. *Biol. J. Linn. Soc.* 82: 511–519.

Urdampilleta, J.U., Ferruci, M.S. and Forni-Martins, E.R. (2007). Chromosome studies of some *Thinouia* species (Sapindaceae) and the taxonomic implications. *Ann. Bot.Fennici*. 45: 68-73.

Verma, P.S. and Agarwal, V.K. (1974). *Cell Biology, Genetics, Molecular Biology, Evolution and Ecology*. CHAND and Company Ltd., Ram Nagar, New Delhi, pp. 257-279.

Akesa, T.M. is currently a lecturer at Adamawa State University, Mubi – Nigeria