ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2019.891.897

Research Article
Evaluation of Cytological and Morphological Traits of *Morinda lucida* Benth. - An Under-exploited Tropical Species

[1]C.V. Ilodibia, [2]B.E. Okoli and [1]C.U. Okeke

[1]Department of Botany, Nnamdi Azikiwe University, P. M. B 5025, Awka, Anambra State, Nigeria
[2]Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria

Abstract

Background and Objective: *Morinda lucida* Benth. is a multipurpose plant traditionally used in ethnomedicine for treatment of various ailments and for socio-economic purposes that include aesthetics. Information is available on the vegetative and floral characteristics but basic mitotic and meiotic chromosomal investigations are lacking. This study therefore was carried out to evaluate the taxonomic and fertility potentials of *Morinda lucida* with regards to morphological and cytological characteristics. **Materials and Methods:** Mitotic and meiotic cytological investigations were evaluated using root-tip and flower buds squash techniques, respectively. Vegetative and floral characters were studied by visual observations and use of hand lens. **Results:** Cytological result revealed the plant to be 'hexaploid' with mitotic chromosome counts of 2n = 6x = 66 and meiotic chromosome counts to be n = 33. Pollen fertility result showed that the plant is highly fertile. Morphological studies revealed the habit of the plant to be shrub to medium-sized tree, leaf-simple, entire, opposite with interpetiolar stipules. Inflorescence-cymose, flower-stalked, complete, regular, bisexual, epigynous, perianth-5 fused tepals, stamen-pentamerous, carpel-syncarpous, fruit- drupe, seed-endospermic. **Conclusion:** The cytological studies showed that the plant has high fertility and viability level and so could be easily perpetuated and amenable to genetic manipulation. The overall data are also valuable for taxonomic characterization and identification of species in the genus.

Key words: *Morinda lucida*, morphology, cytology, palynology, taxonomy, Nigeria

Citation: C.V. Ilodibia, B.E. Okoli and C.U. Okeke, 2019. Evaluation of cytological and morphological traits of *Morinda lucida* benth. - An under-exploited tropical species. Asian J. Biol. Sci., 12: 891-897.

Corresponding Author: C.V. Ilodibia, Department of Botany, Nnamdi Azikiwe University, P. M. B 5025, Awka, Anambra State, Nigeria Tel: +2348037728771

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Morinda is a genus of flowering plants in the family, Rubiaceae. It comprises about 80 species and occurs throughout the tropics. These species may be trees, shrubs or vines. All *Morinda* species bear aggregate or multiple fruits[1]. In Africa 5 species are found including *Morinda lucida* Benth. *Morinda lucida* is a typical west African rainforest tree commonly known as Brimstone tree[2]. *Morinda lucida* is a medium- sized tree about 15-25 m tall with smooth or rough scaly grey or brown bark, crooked or gnarled branches and shining foliage. *Morinda lucida* is a multipurpose species yielding dyes, timber, fuel and one of the four most used traditional medicines against different ailments in Nigeria[3].

According to Radford[4], the morphological data of plants are easily observable and obtainable and are "thus used most frequently in taxonomic studies." The evidence from external morphology provides the "basic language for plant characterization, identification, classification and relationships"[4]. Pandey[5] reported that it has now been generally accepted by taxonomists that morphological characters alone should not be regarded in systematic classification of plants.

Cytology has been found to be especially useful in solving some of the taxonomical problems by providing additional characters[5,6]. Chromosomal number and homology largely determine pairing behaviour at meiosis, which in part governs the level of fertility of hybrids and hence the breeding behaviour and pattern of variation of populations[7]. Chromosome number is an important and much used taxonomic character and it is, in fact, just about the only biosystematics evidence which is consistently recorded in standard floras and the like[7].

Morinda lucida is a species with immense economic potentials that range from aesthetics to medicinal. However, no comprehensive studies have been done on *Morinda lucida* especially in the area of cytology, hence the need for the present study. Accordingly, the problem and focus of this study is to provide detailed morphological description of the plant organs and to determine the mitotic and meiotic chromosome numbers and pollen characteristics of the plant.

MATERIALS AND METHODS

Experimental site: The experiments were carried out between the years 2014-2017 in the Botany Laboratory Nnamdi Azikiwe University Awka. Field survey was carried out in Awka and Onitsha within these periods.

Procurement and identification of plant materials: Plant materials were procured from Onitsha and Awka, Anambra state.

The identification of plant was done by Prof. B.E. Okoli, a Plant Scientist of Department of the Plant Science and Biotechnology, University of Port Harcourt and Mr. Paulinus Ugwuozor, a Herbarium curator of Department of Botany, Nnamdi Azikiwe University, Awka.

The Voucher specimens were deposited at the Department of Botany Herbarium, Nnamdi Azikiwe University, Awka, Nigeria.

Morphological studies: Observations on vegetative and floral characteristics were studied using samples collected from mature tree. For the leaves, the 3rd and 4th fully opened leaves from the stem tip were used. A meter rule was used to measure the length, breadth dimension of leaves, seeds, fruits, flower and other parts.

Cytological studies: Materials for cytological studies (mitotic and meiotic events) were *Morinda lucida* root tips, immature flower buds, photomicroscope, reagents and stains used were Carnoy's fluid, 1:3 (v/v) glacial acetic acid and 95% ethanol, 70% ethanol, 18% hydrochloric acid, F.L.P. Orcein, distilled water and 0.002 M 8-hydroxy-quinoline.

Procedure for mitotic studies: The seeds were germinated and when several roots have grown, about 1-2 cm of the root tips were cut off with the use of fine forceps. The soil particles were washed off and the root tips separated. The roots were transferred to a corked bottle containing Carnoy's fluid and left overnight at room temperature to fix the material. This was done to coagulate the components of the cell without solution and disintegration of their internal or external spacing. It also reduces the staining of the cytoplasm while allowing the chromosomes to take up stain readily thus improving the optical contrast of the cell's components. The root tips were removed with forceps by grasping the cut end of the root, transferred to a Petri dish containing distilled water and washed for a few minutes to remove the fixative. The root tips were then transferred to a test-tube containing 18% hydrochloric acid for 3 min. This process loosens the cementing substance between cells and allows the cells to spread out during squashing. The root tips were removed from the acid and transferred into a dish containing 70% alcohol and washed to remove the acid.

Using a mounted needle, 1 mm portion of the root tip was cut off onto a clean slide. One drop of F.L.P orcein

stain was dropped on the specimen. A thin cover slip was laid on top of the specimen and the material squashed by gently and briskly tapping the cover slip with the blunt end of a biro. The tapping continued until the material was spread out properly and was hardly visible. The slide was placed between a large filter paper on a hard smooth table surface and thumb pressure applied cautiously on top of the cover slip. Lateral movement of the cover slip was avoided during the process. This technique is called the "squash" technique.

Excess stain was drained off with filter paper and the slides were examined to see the chromosomes under the high powers of the microscope and photomicrographs were taken. This procedure is as outlined by Okoli[8].

Procedure for meiotic studies: Immature flower buds of M*orinda lucida* harvested from 10-12 am were fixed in Carnoy's fluid for 24 h. The fixed materials were removed from Carnoy's fluid and the pollen mother cell teased out from the anther unto a clean slides. Some fixed materials were transferred to 70% ethanol and stored in a refrigerator for use later. One drop of F.L.P orcein stain was dropped on the specimen. A thin cover slip was laid on top of the specimen and the material squashed by gently and briskly tapping the cover slip with the blunt end of a biro. The tapping continued until the material was spread out properly and was hardly visible. The slide was placed between a large filter paper on a hard smooth table surface and thumb pressure applied cautiously on top of the cover slip. Lateral movement of the cover slip was avoided during the process. This technique is called the "squash" technique.

Excess stain was drained off with filter paper and the slides were examined to see the chromosome under the high powers of the microscope and photomicrographs were taken. This procedure is as outlined by Okoli[8].

Pollen fertility: Pollen fertility was determined by teasing out anthers onto a clean slide. The slide was stained with cotton blue and small thumb pressure applied. The deeply stained blue ones were regarded as viable while lightly stained ones were regarded as non-viable. Percentage fertility was calculated as follows:

$$\text{Fertility (\%)} = \frac{\text{No. of viable pollen grains}}{\text{Total number of pollen grains}} \times 100$$

RESULTS

Morphological studies: Observations on the morphology of *Morinda lucida* tree (Fig. 1 and 2) showed the habit to be an ever-green shrub to medium-sized erect tree, perennial, with hard and woody stem with bole and branches and bark roughly scaly and grey to brown in colour.

Observations on the morphology of *Morinda lucida* leaf (Fig. 1 and 2) revealed the following features: Phyllotaxy-decussate, margin-entire, venation-reticulate, apex-acute, base-cuneate, colour-green, lamina-auriculate, form-simple, shape-ovate to elliptical, surface-glabrous, stipule-interpetiolar, width 3.4-7.2 cm and length 7.3-18.2 cm.

Observations on the morphology of *Morinda lucida* flower (Fig. 3-6) revealed the following features: Type- regular,

Fig. 1: *Morinda lucida* tree in its natural habitat

Fig. 2: Twig of *Morinda lucida*

Fig. 3: *Morinda lucida* flower buds

Fig. 6: *Morinda lucida* flower

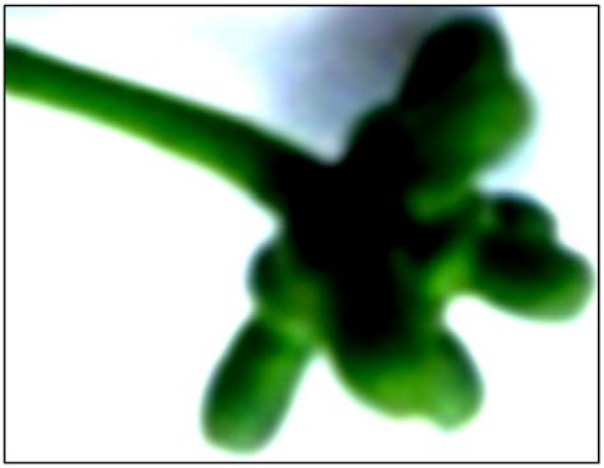

Fig. 4: *Morinda lucida* inflorescence

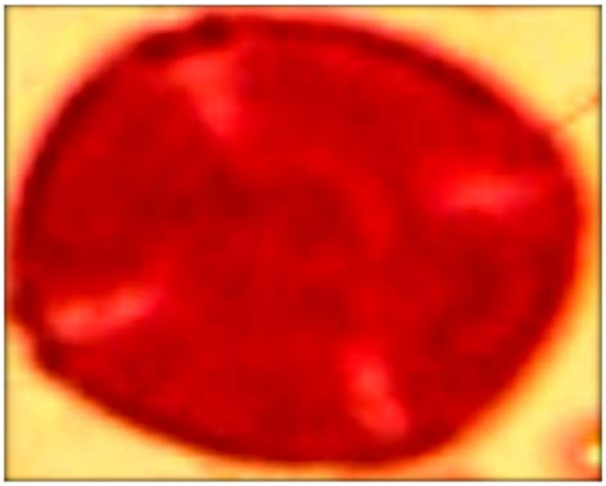

Fig. 7: *Morinda lucida* tetracolporate pollen

Fig. 5: *Morinda lucida* stigma lobes and style

bisexual, ovary-epigynous or inferior, perianth-5-fused tepals, filament-singly, stamen-pentamerous, carpel-syncarpous, stigma-2 bilobate, length 0.4-0.8 cm, Inflorescence-cymose, placentation-axile, aestivation-valvate.

Observations on the morphology of *Morinda lucida* pollen, fruit and seed (Fig. 7-9) revealed the following features: Pollen (Fig. 7) is tetracolporate, the fruit (Fig. 8) is a drupe, fleshy, indehiscent, green in colour and hard when unripe but turns black and soft when ripe. The seeds (Fig. 9) are 0.5-2.0 mm in size, endospermic and triangular in shape.

Cytological studies: The cytological results are as presented in Fig. 10 and 11. The mitotic chromosome number of *M. lucida* is 2n = 6x = 66 and the meiotic chromosome number, n = 33. Hence *M. lucida* is a hexaploid. The

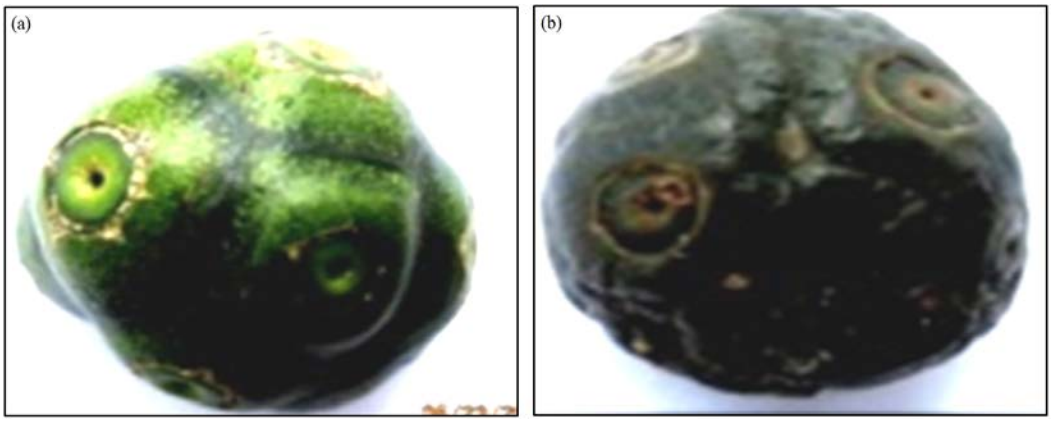

Fig. 8(a-b): (a) Unripe and (b) Ripe fruits of *Morinda lucida*, respectively

Fig. 9: *Morinda lucida* ripe seeds

Fig.10: Mitotic chromosomes (2n = 66)

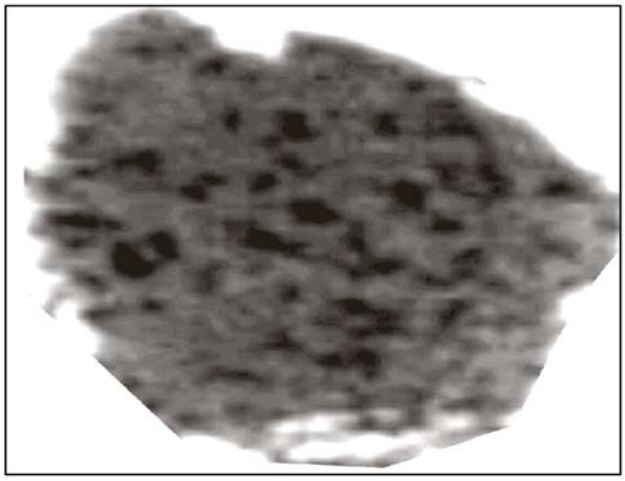

Fig. 11: Meiotic chromosomes (n = 33)

chromosomes exhibited various kinds of chromosomal configurations ranging from univalent to multivalent associations (Fig. 11).

DISCUSSION

The research carried out on external morphology revealed *Morinda lucida* as an ever green shrub to medium-sized tree, perennial, hard and woody, erect or strong with bole and branches bark roughly scaly with grey to brown colour. Leaf morphology showed phyllotaxy-decussate, margin-entire, venation-reticulate, apex-acute, base-cuneate, colour-green, lamina-auriculate, form-simple, shape-ovate to elliptical, surface-glabrous, stipule-interpetiolar, width 3.4-7.2 cm and length 7.3-18.2 cm. Flower morphology showed type-regular, bisexual, ovary-epigynous or inferior, perianth-5-fused tepals,

filament-singly, stamen-pentamerous, carpel-syncarpous, stigma-2 bilobate, length 0.4-0.8 cm. Inflorescence-cymose, placentation-axile, aestivation-valvate, pollen-tetracolpate. Fruit-drupe, fleshy, indehiscent. Seed-endospermic, shape-triangular, size-0.5-2.0 mm (Fig. 1-9). These morphological characters observed strengthen the intra and interspecific relationship and can be used to enhance the plant species identification and characterization. Morphological features according to Pandey[5] provided a sound base in characterizing taxonomic groups. The results tally with an earlier study by Adesida and Adesogan[3], except in those features they did not study. High variability in crop genotypes based on morphological characters has been reported by some authors Aremu[9], Adewale *et al.*[10], Aziagba *et al.*[11] and Ilodibia *et al.*[12].

Cytological studies carried out on *M. lucida* showed that the mitotic chromosome counts was 2n = 6x = 66 this shows that *M. lucida* is a hexaploid (Fig. 10). Consequently, a basic chromosome number of x = 11 is depicted. This basic number is confirmed for this genus so that 2n = 22 for diploids, 2n = 44 for tetraploids etc. This suggested that *M. lucida* is a polyploid and was derived from the diploid ancestors through hybridization or through chromosomal changes. This conforms with the reports of Okoli and Olorode[13] that inequality in sizes of supposedly homologous chromosomes in diploid *Andropogon tectorum* Schum. and Thonn. collection is probably due to a translocation or deletion or addition. Polyploidy induction has been successfully applied to crop, ornamental and medicinal plants in order to obtain lines exhibiting new agronomical characteristics[14]. The meiotic study showed that the haploid chromosome number was n= 33 (Fig. 11). The chromosomes exhibited various kinds of chromosomal configurations ranging from univalent to multivalent associations (Fig. 10 and 11). This conforms with the report of Irimagha[15] that *Gomphrena celosioides* showed various kinds of chromosomal configuration in its meiotic behaviour which ranges from univalent to pentavalent associations. No work has been done on the cytology of *M. lucida* but the chromosome numbers both mitotic and meiotic count agree with those reported in the literature and generally with the basic number (x = 11) for the family (Rubiaceae) and even the genus *Morinda*[16]. The polyploidy species *Amaioua intermedia* has 2n = 66 which had a multiple of 11. With regards to pollen stainability, *M. lucida* pollen showed 75% viability which indicates that it has a high fertility level. This is in line with the report of Sambamurty[1] who reported that the pollen viability of *Digitalis* species was high indicating a stable breeding system. Generally, Adesida and Adesogan[3] reported that morphological characters alone should not be regarded in systematic classification of plants, cytology and others are found to be especially useful by providing additional characters.

CONCLUSION

The data obtained from chromosomal and pollen studies showed that the species has high fertility and viability levels and thus could be easily perpetuated and open to genetic manipulation and variations. The overall data can be used to enhance proper taxonomic characterization and identification of these plant species. The author recommends that anatomy, phytochemistry and palynology should be carried out in order to provide additional characters on the species characterization and identification from other species in the genus.

SIGNIFICANCE STATEMENT

This study discovered a new species polyploid derived from the diploid ancestors through hybridization or chromosomal changes. The study is beneficial to breeders and taxonomist by providing cytological information that could be used as an initial step towards manipulation of the species for ornamentals, medicinal and other agronomic purposes in crop improvement. This study will help the researchers to uncover the critical areas of taxonomic problems that many researchers were not able to explore. Thus a new theory on taxonomic delimitation of the species may be arrived at.

REFERENCES

1. Sambamurty, A.V.S.S., 2005. Taxonomy of Angiosperms. I.K. International Pvt. Ltd., New Delhi, India, ISBN-13: 9788188237166, Pages: 908.
2. Adeneye, A.A. and E.O. Agbaje, 2008. Pharmacological evaluation of oral hypoglycemic and antidiabetic effects of fresh leaves ethanol extract of *Morinda lucida* benth. in normal and alloxan-induced diabetic rats. Afr. J. Biomed. Res., 11: 65-71.
3. Adesida, G.A. and E.K. Adesogan, 1972. Oruwal, a novel dihydroanthraquinone pigment from *Morinda lucida* Benth. J. Chem. Soc.: Chem. Commun., 7: 405-406.
4. Radford, A.E., 1986. Fundamentals of Plant Systematics. Harper and Row, New York.
5. Pandey, B.P., 2007. A Textbook of Botany: Angiosperms. S. Chand and Company Limited, New Delhi, India.
6. Dutta, A.C., 2004. Botany for Degree Student. 5th Edn., Oxford University Press, London, Pages: 708.

7. Stace, C.A., 1980. Plant Taxonomy and Biosystematics. University Park Press, London, ISBN: 9780839141464, Pages: 279.
8. Okoli, B.E., 1992. Field, Herbarium and Laboratory Techniques. Mbeyi and Associates (Nig.) Ltd., Port Harcourt, Pages: 239.
9. Aremu, C.O., O.J. Ariyo and B.D. Adewale, 2007. Assessment of selection techniques in genotype X environment interaction in cowpea *Vigna unguiculata* (L.) walp. Afr. J. Agric. Res., 2: 352-355.
10. Adewale, B.D., C. Okonji, A.A. Oyekanmi, D.A.C. Akintobi and C.O. Aremu, 2010. Genotypic variability and stability of some grain yield components of cowpea. Afr. J. Agric. Res., 5: 874-880.
11. Aziagba, B.O., C.U. Okeke, C.A. Ezeabara, C.V. Ilodibia, T.P. Egboka and I. Obika, 2016. Taxonomic implication of morphology of seven varieties of *Vigna unguiculata* (L.) Walp. cultivated in Awka Anambra State, South Eastern Nigeria. Am. J. Life Sci. Res., 4: 132-135.
12. Ilodibia, C.V., C.U. Arubalueze, S.N. Udearoh, B.I. Okafor and C. Agbanusi, 2018. Assessment of morphological and nutritional attributes of two varieties of *Ipomoea batatas* (L.) utilized in Nigeria. Arch. Agric. Environ. Sci., 3: 394-398.
13. Okoli, B.E. and O. Olorode, 1983. Cytogenetic studies in the *Andropogon gayanus-A. tectorum* complex (Graminae). Bot. J. Linnean Soc., 87: 263-271.
14. Tamayo-Ordonez, M.C., L.A. Espinosa-Barrera, Y.J. Tamayo-Ordonez, B. Ayil-Gutierrez and L.F. Sanchez-Teyer, 2016. Advances and perspectives in the generation of polyploid plant species. Euphytica, 209: 1-22.
15. Irimagha, T.B., 2013. Morphological, anatomy and cytological studies on Khaki weed (*Gomphrena celosioides* Mart). Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria.
16. Morakinyo, J.A. and A.O. Awojobi, 1991. Cytogenetics and morphology of *Digitaria exilis* (kipp). Stapf., *Digitaria horizontalis* wild and *Digitaria leptorhachis* Stapf. Niger. J. Bot., 4: 199-212.