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Botanical and Cytological Studies of *Monodora tenuifolia* Benth.

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Abstract

This study investigated branching pattern, ecology of occurrence, palynology, cytology and phenology of *Monodora tenuifolia* using standard techniques. The branching pattern determines the shape of the canopy, which may be irregular when growing in the shade, or round when growing in the open. The pollens are tetrads, 10.15 µm in diameter, with pollen fertility of 92.24% (determined by using Cotton Blue in Lactophenol) and pollen tube germinability of 65.83%. Two simultaneous cytokineses were studied and the events of meiotic cell division were observed to synchronize. The chromosomes were well paired at pachynema as associations of regular bivalents, paired of ring 11 or rod 11, though univalents were occasionally observed. *Monodora tenuifolia* has the chromosome number equal to 2n = 16, showing a karyotypic formular of 1 acro (large) + 2 submet (medium) + 2 met (medium) + 2 acro (medium) + 1 met (small) chromosome in the 2B Stebbins category. The results indicate that the tetrad pollen grain of *Monodora tenuifolia* is an advantage, since up to four pollen tubes could be seen developing from the some pollen grains, while the karyotype is asymmetrical.

Keywords: chromosome associations, karyotypic formula, pollen grain

Introduction

The genus *Monodora* belongs to the Annonaceae family, which is also called custard-apple family (Kral, 1960). It consists of trees, shrubs, rarely woody vines, deciduous or evergreen, with aromatic bark, leaves and flower (Watson and Dallwitz, 1992). The family consists of 2500 species (Chatrou, 2005). Out of all the reported 130 genera, only 22 genera are represented in Nigeria, out of 24 genera found in West Africa (Hutchinson and Dalziel, 1958). The genus *Monodora* contains approximately 35 species distributed throughout tropical Africa. In Annonaceae, the leaves are simple, petiolate without stipules and are alternately arranged. The leaf blade is pinnately veined, unlobed and the margin is entire. Inflorescences are axillary to leaf scars on old wood, or to leaves on new shoots (Kral, 1960). The primitive angiosperm family, Annonaceae, possesses a remarkable type of pollen that appears unique in its size, and further distinguishes by being polyads, which are compartmentalized individually within septate stamens (Walker, 1971).

Different basic chromosome numbers of x=7, x=8 and x =9 have been reported for the members of the family (Folorunso and Olorode, 2007; Okada and Ueda, 1983; Morawertz and Le Thomas, 1988; Watson and Dallwitz, 1992). Some species of Annonaceae, such as the *Annonas,* are generally consumed as fresh fruits, but are also widely used in semi-processed and processed products (ICUC, 2002). Antibacterial and antifungal metabolites have been identified in *M. tenuifolia* (Oguntimein and Hufford *et al*., 1986), while *M. Myristica,* also called African nutmeg, is used in land conservation and agro-horticulture (Burkill, 1985).

There is no information on botanical and cytological studies of *M. tenuifolia* in Nigeria. This study investigated some botanical characteristics, pollen fertility and morphology, mitotic and meiotic chromosomes of *M. tenuifolia,* in Ile-Ife, Nigeria.

Materials and methods

The flower buds and seeds of *M. tenuifolia* were collected from the wild and seed gardens at Obafemi Awolowo University (N 070 50.689" E 0040 58.439"). The branching pattern, branching index, shape and diameter of the canopy, plant height and ecology of occurrence, pollen fertility, pollen morphology and phenology were studied.

The branching index was calculated as total number of branches / height of the plant. Percentage pollen fertility and germinability were determined; percentage pollen fertility was determined according to Olorode and Baquar (1976). The pollen grains from fresh matured anther were harvested and dusted onto microscope slide, stained with Cotton Blue in lactophenol in order to determin pollen stainability. Pollen tube germination test was determined using the method of Nurhan (2003). Fresh matured pollen grains were dusted on a sterilized culture medium of 25 ml distilled water, 0.5 g agar and 6.25 g saccharose. The culture medium was left for 12 hours at 18 °C, after which the pollen tubes were germinated in vitro. The pollen tubes were then fixed in 1:3 acetic alcohol. One drop of this suspension was set on slide and stained with cotton Blue in Lactophenol. Pollen grains were acetolyzed according to Erdtman (1960). Photomicrographs of the pollen grains

were taken at x400 phase, and oil immersion illumination under Lietz Daialux Research Microscope was done. The phenology, fruiting and foliage of M. tenuifolia were observed once a week over a period of 3 years, from 2008 to 2010.

Flower buds of M. tenuifolia were collected between 9 am-12 pm and fixed in 1:3 acetic-alcohol for meiotic chromosome study. The flower buds of appropriate size were selected through a series of experiments. The slides were prepared by squash techniques and stained in FLP orcein (2 g of orcein in 100 cm3 of solution containing equal parts of formic acid, lactic acid, propionic acid and H2O (Lasebikan and Olorode, 1972). Twenty pollen mother cells were examined and scored for chromosome associations. Mitotic chromosomes were studied from the root tips. The root tips were harvested between 9 am - 12 pm. The root tips were pre-treated in 8-hydroxyquinoline for 3 hours and fixed in 1:3 acetic alcohol. The fixed root tips were hydrolysed in 18% HCl for 25 minutes. The slides were prepared by squash technique and stained in FLP orcein. Good cells were photographed under oil and phase contrast illumination using Lietz Dialux Research Microscope. The mitotic chromosome was karyotyped based on physical analysis of chromosome as described by Torres and Liogier (1970). The short arm length, long arm length, arm ratio (long arm length/short arm), centrometric index, longest/smallest ratio and proportion of chromosome pairs with arm ratio >2 were determined after specific measurements. The chromosome karyotype was further classified into Stebbins category (Stebbins, 1971).

Results and discussion

Monodora tenuifolia is a medium-size tree. The branching pattern determines the shape of the canopy, which could be round when growing in the open or irregular when growing in the shade (Fig. 1A and 1B). The mean branching index is 0.25 cm and 0.1 cm in the open and under the shade respectively. It was observed that the location where the plant grows not only affects the canopy, but also the branching index, as well as leaf size. The canopy and branch angle of those that are growing in the shade was wider than those that are growing in the open.

Monodora tenuifolia showed a pollen fertility of 92.24% and pollen tube germination of 65.83%. There was a positive correlation between pollen fertility and pollen tube germination. The pollen grains of M. tenuifolia are tetragonal tetrad, inaperture, or unipolar tetrad, in which case all four members are in contact at the centre of the tetrad, which has a diameter of 10.15 μm and exine thickness is 0.71 μm (Fig. 2A). *Monodora tenuifolia* flowered from February to August in 2008, from January to September in 2009 and from March to August in 2010. However, fruiting was consistent as interval over the three years of observation, which was between March and July. Defoliation occurred between November and February each year, followed by production of flowers and later the flushing of leaves.

Fig. 1. A) *Monodora tenuifolia* tree grown in the open; B) *M. tenuifolia* tree grown in the shade

During the meiotic chromosome study, two simultaneous cytokineses were observed. The chromosomes were well paired at pachynema and no chromosome aberration was observed. Two nucleoli were sometimes noted. Chromosome association is 2 ring II + 3 rod II + 6I (Fig. 3). The events of cell division were observed to be synchronized.

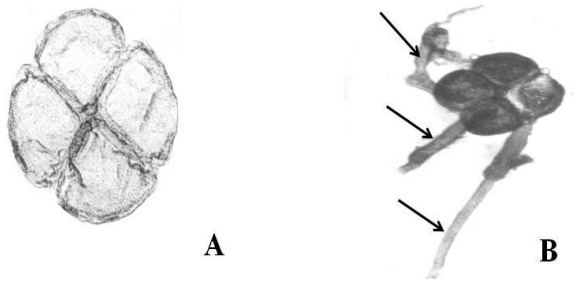

Fig. 2. A) *Monodora tenuifolia* pollen grain; B) Pollen tube germination in *M. tenuifolia* (tubes arrowed)

Mitotic cells of M. tenuifolia showed a chromosome number of 2n = 16. Monodora tenuifolia has a karyotype formula of 1 acrocentric (large)+2 submetacentric (medium)+2 metacentric (medium)+2 acrocentric (medium)+1 metacentric (small) chromosome (Fig. 4A and 4B). The longest / smallest chromosome ratio is 3.82 and the proportion of arm ratio > 2 is 0.2 (Tab. 1). This places the karyotype in 2B Stebbins category.

The difference observed in the branching pattern and canopy of *M. tenuifolia* was supported by the study of

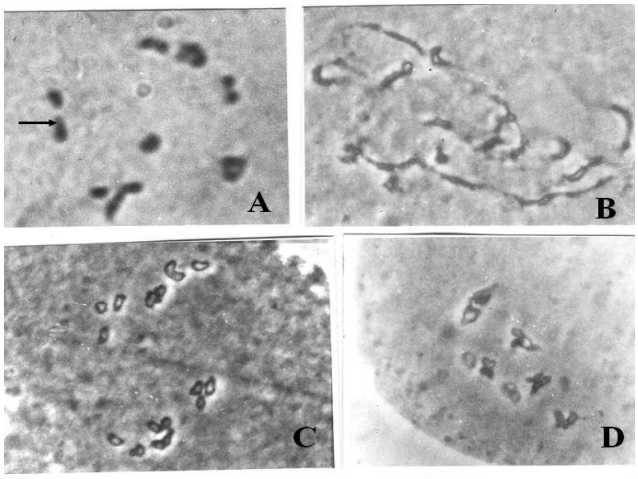

Fig. 2. A) *Monodora tenuifolia* pollen grain; B) Pollen tube germination in *M. tenuifolia* (tubes arrowed)

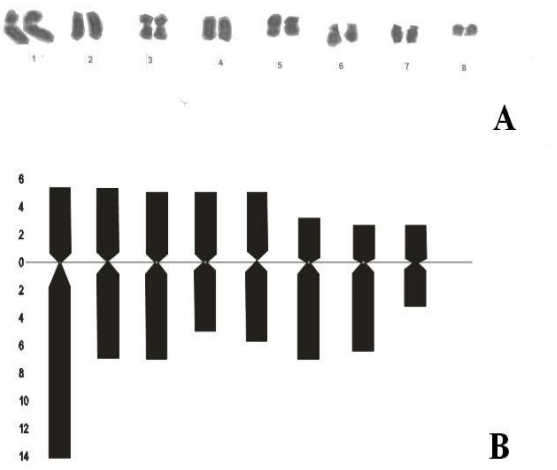

Fig. 4. A) Karyotype of *M. tenuifolia*; B) Idiogram of chromosome complement of *M. tenuifolia*

Tab. 1. Karyotypic data on *Monodora tenuifolia*

Chromosome pair number	Short arm (μm)	Long arm (μm)	Arm ratio	Centrometric index %
1	0.55	1.40	2.55	28.21
2	0.55	0.70	1.27	44.00
3	0.50	0.70	1.4	41.67
4	0.50	0.50	1.00	50.00
5	0.50	0.55	1.10	47.62
6	0.30	0.70	2.33	30.00
7	0.25	0.65	1.30	27.78
8	0.25	0.26	1.04	5.00

Note: Centrometric index is calculated as Small arm/(Small arm + long arm)x100

Heidrum and Josef (1997). They reported that shade-induced changes in the branching pattern of clonal plants could lead to conspicuous modifications of their growth and architecture. Shade significantly slowed down the ontogenetic development of plants and thus resulted in noticeable differences in branching patterns between sun and shade growing, which can be due to purely allometric effect (Heidium and Josef, 1997). Reduction in irradiance reduced the optimal density of leaves per unit area of ground occupied by the canopy, tending to reduce the mass of leaves and branches associated with a given canopy. Thus, given that canopy mass should increase monotonically with the canopys' diameter, shady conditions favour broader canopies (Thomas, 1988). Pickett and Kempt (1980) observed that the branch angle is wider in a closed canopy and this supported the result of wider branch angle observed in *M. tenuifolia* growing in the shade. They also observed that leaves in the field were significantly smaller than those in the forest. Hence, phenotypic and development plasticity were suggested by Pickett and Kempt (1980), as one of the causes of the differences in branching patterns between habitats in the same species.

When percentage pollen fertility and percentage pollen tube germination were compared, it was noted that the former was higher than the latter. This proves that the fact that a pollen grain is well formed and well staine, does not mean it is also viable. There might have been a developmental sterility that is to prevent the pollen grain from being germinated. Hence, the best way to estimate pollen fertility and its viability seems to be through pollen tube germination. This observation conforms to the report of Bhowmik and Datta (2012), which says that pollen grain viability assessment through staining method seem to express the pollen potential, but not its occurrence. It may be explained by the fact that this technique overestimates the percentage of pollen tube formed. In this regards, pollen viability should be used carefully, and rather replaced by the more limited term pollen fertility, as it depends on the staining assay. Stainable pollen grain may vary in size, and thus it can be cytologically unbalanced and not viable. Therefore, pollen fertility rarely corresponds to pollen germination, which is the best index of pollen viability (Bhowmik and Datta, 2012). Well formed, intact, uniformed stained pollen grains were considered fertile and viable, while those that were partially stained or not stained at all, with collapsed outlined, and were scored infertile and non-viable. Hence, there is correlation between pollen fertility and pollen viability; therefore, the higher the pollen fertility, the higher the pollen viability. In this study, there was a considerable production of pollen and pollen stainability, which showed that pollen fertility, as well as pollen viability, was high. Also the pollen germination was generous, which resulted in large fruit production and high seed set.

The tetrad pollen grain structure observed in *M. tenuifolia* was explained by Lora *et al*. (2009) to be a result of the delay in the dissolution of pollen mother cell wall and tapetal chamber, which are the key events that hold the four microspores together in a confined tapetal chamber. These allow them to rotate, and then bind them through the aperture sides by small pectin bridges, followed by joint sporopollein position. Another reason proposed for this permanent binding of pollen in groups of 4 could be a failure in the synthesis of callose layer during microspore separation in the tetrad (Blackmore and Crane, 1988). The tetrad pollens were also reported in *Xylopia aethiopica* and *Annonas*, while some genera, such as *Cleistopholis* in the family Annonceae, have monad pollen grains (Chih-Hua and Yu-Lan, 2002; Folorunso and Olorode, 2007; Le Thomas, 1981). It was noted that up to four pollen tubes could be seen developing from some of the tetrads. This could confine an advantage on tetrad over monad, and is because a monad will produce just a pollen tube, while tetrad can produce up to 4 pollen tubes. Therefore, it has a higher probability of fertilizing the ovule than a monad pollen grain. This view is supported by Harder and Johnson (2008) who stated that the release of aggregated pollen is more advantageous in situation where pollinators are infrequent and in situation of short pollen viability and pollen transport periods, which were reported in *Annona cherimola* (Herrero *et al.,* 2006).

In *M. tenuifolia*, there was resource allocation to flower production before the flushing of leaves. This is supported by the report of Kamaljit *et al*. (2003), who stated that there was temporal separation between flowering and vegetative growth in some rainforest trees. This might be due to the fact that the resources required for flower production are very high compared to what is available, hence allowing flower production before flushing of the leaves. Moreover, Daniel (2006) reported that the pattern of allocation in a species is genetically determined, but not completely fixed.

Mar Arthur and Wilson (1967) and Planka (1970) reported that resources allocation in plants followed k-selection model. Jonasson (1998) explained that defoliation compensated for the losses of nutrient solely by increasing nutrient intake. Flowering branches do not produce leaves, resulting in a decreasing photosynthetic capacity and a reduction of resources for storage and allocation, where there was heavy flowering, as in the case of *M. tenuifolia*. Saulnier and Reekie (1995) also supported the view of resource allocation to flower production at the expense of new leaf production. The peak flowering period in *M. tenuifolia* was between March and April, which was in line with the work of Wright and Calderon (1995), that reported the mean flowering dates concentrated in February and March, in Barro Colorado Island, Panama, which are the driest months of the year, and then in April and May, when the wet season begins.

A chromosome number of 2n=16 was observed in *M. tenuifolia* with basic chromosome number of x=8. This is in line with the previous report of Thomas (2011) that specifies that genus *Monodora* Benth. has a base chromosome number x=8. From the cytological study, no chromosome aberration was observed. The chromosomes were well paired at pachynema and the 8 bivalent pairing of chromosomes at diakinesis confirmed that the chromosome complement of *M. tenuifolia* is a diploid. Folorunso and Olorode (2007) reported chromosome number of 2n=14 for *Anonna muricata, Cleistopholis patens* and *Greenwayodendron suaveolens,* compared to 2n=16 reported for *M. tenuifolia* in this study. The chromosomes of *A. muricata, C. patens* and *G. suaveolens* were grouped into two size classes; each of them were submetacentric, while that of *M. tenuifolia* were grouped into three size classes of acrocentric, submetacentric and metacentric.

Conclusions

In conclusion, the location (in open or shade) where *M. tenuifolia* grows, determines its branching pattern and canopy. The pollen grains of *M. tenuifolia* are tetrads, which have specific advantages over monad pollen grains. The meiosis is regular, the chromosomes paired well at pachynema and the karyotype of *M. tenuifolia* is asymmetrical.

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