



Karyotype analysis in *Machaerium lunatum* (Linn. f.) Ducke

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ABSTRACT: The paper gives an account of the results of karyological investigation on *Machaerium lunatum* (Linn. f.) Ducke using root squash technique. Root tips (approximately 1 cm) were excised and pretreated in 0.002M 8-hydroxyquinoline for 3–3.5 hrs, fixed in 3:1 ethanol-acetic acid for 24 hrs, hydrolysed with 5% HCl and squashed in drops of FLP orcein on clean glass slides. Mitotic chromosome slides were observed under research microscope and photomicrographs of 10 good quality metaphase plates were taken and recorded. A chromosome number of $2n = 30$ was recorded for the taxon and the chromosomes varied from 0.83 – 2.71 μm in length with karyotype of $7m+4sm+4st$. The length of the long and short arms ranged from 0.64 – 1.66 μm and 0.19 – 1.05 μm respectively. The chromosomes varied from metacentric to submetacentric. This is the first report of the karyotype and chromosome of this species from Nigeria.

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Machaerium lunatum (Linn.f.) Ducke (syn. *Drepanocarpus lunatus* (Linn. f.) G.F.W. Mey) belongs to the family Fabaceae-Papilionaceae (Hutchinson and Dalziel 1954). It has about 130 species distributed from Mexico to Argentina which are difficult to delimit taxonomically (Rudd 1987) and 150 species distributed from Mexico to South America, and the West Indies (Keay 1985; Airy-Shaw 1985) and coastal West Africa (Hutchinson and Dalziel 1954). In West Africa, it has monotypic species *M. lunatum* (Hutchinson and Dalziel 1954) which grows along the tropical belt from the Democratic Republic of Congo through Angola, Cameroun, and Nigeria to the Gambia all along the West African coast (Nyananyo 2006). The plant is perennial and is found along fresh and brackish river banks especially salt marshes and tidal portions. The leaves are compound 5-7 foliate. Leaflets are oblong, elliptic and about 5cm long, 2cm wide and rounded at each end. It is well endowed with recurved stipular thorns (Nyananyo 2006). The inflorescence of *M. lunatum* is a panicle consisting of more than one flower, and usually comprises distinct individual flowers. The flowers are purple and are found at the axial or terminal portions of the stem. The fruit is in a pod and is flat and sickle shaped green when unripe and brown when ripe and about 3cm in diameter. It is said to flower between the months of December and March (Husaini and Gill 1986). Traditionally, Ugiomoh and Anyanwu (2016) reported that the root of the plant is used in the treatment of diarrhea, dysentery, edema, gout and stomach ulcers.

Existing literature on the chromosome number and cytological studies on the members of this genus is

scanty (Carlos et al. 2002), however a basic chromosome number (n) equals 10 and 9 has been reported among the genus (Husaini and Gill 1986; Carlos et al. 2002). Coleman and De Menezes (1980) reported $n = 10$ in *M. aculeatum* Raddi; Husaini and Gill (1986) reported $n = 9$ in *M. lunatum* (L.f.) Ducke; Bandel (1974) $2n = 20$ in *M. acutifolium* Vogel and *M. paraguayense* and Carlos et al. (2002) reported $n = 10$ in *M. opacum*, $2n = 20$ in *M. brasiliense* Vogel, *M. lanceolatum* (Vogel) J. F. Macbr., *M. mucronulatum* Mart. ex Benth., *M. oblongifolium* Vogel, *M. opacum* Vogel, *M. triste* Vogel, *M. acutifolium* Vogel, *M. fulvovenosum* H.C. Lima, *M. pedicelatum* Vogel, *M. punctatum* Pers. *M. stipitatum* Vogel, *M. villosum* Vogel, *M. scleroxylon* Tul., *M. uncinatum* Benth. and *M. aculeatum* Raddi., $2n = 40$ in *M. nycitans*, *M. hirtum* and *M. sericiflorum* and basic chromosome number $x = 10$ in the genus.

In some other members of Fabaceae family the chromosome number, length and size including karyotypes have been described (Tabur et al. 2009; Adesoye and Nnadi 2011) but this information is lacking in the genus *Machaerium*. Also, the chromosome number of the West African species of *M. lunatum* is not yet determined. Therefore this work presents the first report on the chromosome number and karyotype of *M. lunatum* from Nigeria, West Africa.

MATERIALS AND METHODS

The materials used for this work were sourced from brackish and fresh river bodies south-south Nigeria where *M. lunatum* grow namely Ogoni and

Rumuchem in Rivers State and Ogbia in Bayelsa State. The seeds were first sterilized for 10 min in 10% ethanol solution, rinsed properly in deionised water, plated under sterile conditions in Petri dishes and incubated till germination occurred. Young primary roots (about 1cm long) were excised and pretreated in 0.002 M 8-hydroxyquinoline for 3–3.5 hr, Fixed in 3:1 ethanol-acetic acid for 24 h and the roots were stored in 70% ethanol solution before squashing. When required the roots were removed from the 70% ethanol, hydrolyzed in 5% HCl and squashed in a drop of FLP orcein (Osuji 2003). Mitotic chromosomes were observed under research microscope and photomicrographs of 10 good quality metaphase plates were taken and recorded with Leica photomicroscope equipment fitted with a digital camera. The long arm (l), short arm (s) and the total chromosome length (c) of each chromosome were measured on enlarged microphotographs. The relative lengths, arm ratios ($r = l/s$) and centromeric index ($I = s/c \times 100$) were calculated and used to classify and determine homologous chromosomes (Gomurgen et al. 2010). For karyotype description the chromosomes were arranged in groups according to the position of the centromere (median, m; submedian, sm; subtelocentric, st) and in order of decreasing size in each class. Chromosome nomenclature followed Levan *et al.* (1964). The variation in chromosome length and chromosome arm ratio within the karyotype was estimated by calculating mean and standard deviation (SD) of these parameters using Microsoft Excel 2010.

RESULTS AND DISCUSSION

A mitotic chromosome count of $2n = 30$ was recorded in *M. lunatum* (Figure 1). This suggests that this species is triploid with basic chromosome number $x = 10$. The basic chromosome number observed in this

species supports existing information of $n = 10$ reported in the genus (Carlos et al. 2002) but in contrast with Husaini and Gill (1986) who reported $n = 9$ in *M. lunatum*.



Fig 1: Somatic metaphase chromosomes of *M. lunatum* ($2n = 30$)

The average sizes of the chromosomes in this species varied from 0.83 μm to 2.71 μm (Table 1). This is within the values previously reported among some members of Fabaceae family. Adesoya and Nnadi (2011) reported chromosome size of 0.54 – 1.84 μm in *Sphenostylis stenocarpa* (Hochst. ex A. Rich), Mercado-Ruaro and Delgado-Salinas (2009) reported 0.70 – 1.60 μm in *Phaseolus* L., Mercado-Ruaro and Delgado-Salinas (1998) reported maximum length of 2.36 μm in *Phaseolus* L. while Osuji and Edeoga (2005) reported 2.50 – 5.0 μm in *Vigna subterranean* (L.) Verdc. all in Fabaceae family. The ranges of chromosome size observed in this study are small according to Osuji and Edeoga (2005) suggesting that *M. lunatum* is advanced phylogenically (Stebbins, 1971). The length of the short arms ranged from 0.19 – 1.05 μm while the long arms varied from 0.64 – 1.66 μm . These arm lengths are relatively smaller than 1.04 – 1.96 μm for short arm and 1.84 – 3.84 μm for long arm recorded in *Phaseolus vulgaris* L. cultivars (Mirela *et al.*, 2005).

Table 1: Morphometric data of chromosomes of *M. lunatum*

Chromosome pair number	Chromosome length (μm)	Short arm (μm)	Long arm (μm)	Arm ratio	I	CM
1	2.71 \pm 0.08	1.05 \pm 0.01	1.66 \pm 0.10	1.59	38.75	m
2	2.35 \pm 0.07	0.98 \pm 0.08	1.38 \pm 0.01	1.42	41.43	m
3	2.24 \pm 0.06	0.90 \pm 0.02	1.34 \pm 0.08	1.50	40.07	m
4	2.12 \pm 0.06	0.57 \pm 0.04	1.52 \pm 0.08	2.69	26.60	sm
5	2.00 \pm 0.06	0.85 \pm 0.10	1.15 \pm 0.16	1.37	42.76	m
6	1.88 \pm 0.06	0.83 \pm 0.08	1.06 \pm 0.02	1.29	43.84	m
7	1.77 \pm 0.05	0.65 \pm 0.10	1.12 \pm 0.05	1.75	36.67	m
8	1.65 \pm 0.05	0.70 \pm 0.13	0.95 \pm 0.18	1.42	42.39	m
9	1.53 \pm 0.04	0.38 \pm 0.02	1.16 \pm 0.02	3.10	24.41	sm
10	1.41 \pm 0.04	0.38 \pm 0.04	1.04 \pm 0.01	2.82	26.25	sm
11	1.30 \pm 0.04	0.36 \pm 0.04	0.94 \pm 0.07	2.67	27.50	sm
12	1.18 \pm 0.04	0.26 \pm 0.02	0.92 \pm 0.06	3.65	21.67	st
13	1.06 \pm 0.03	0.21 \pm 0.06	0.74 \pm 0.07	3.59	20.00	st
14	0.94 \pm 0.03	0.21 \pm 0.05	0.86 \pm 0.15	4.25	21.59	st
15	0.83 \pm 0.02	0.19 \pm 0.04	0.64 \pm 0.01	3.50	22.50	st

Note: Mean \pm SD, I = Centromeric index (Ratio of short arm to the total chromosome length \times 100), CM = Centromeric position: m = Metacentric, sm = Submetacentric, st = Subtelocentric.

From our study, the karyotype formula of *M. lunatum* is $7m+4sm+4st$ (seven of the chromosomes were metacentric, four submetacentric and four subtelocentric) as shown in Table 1 and Figure

2. There is slight variation in the chromosome morphology however the chromosomes are predominantly metacentric types. Osuji and Edeoga (2005), Sinha and Sinha (1980) have inferred that this

is an indication of advanced phylogeny. Also the presence of metacentric and submetacentric chromosomes in the karyotype of *M. lunatum* is a common feature of Fabaceae family (Osuji and Edeoga 2005; Mirela *et al.* 2005; Adesoya and Nnadi 2011). Decrease in total chromatin length can be used to infer the evolutionary trend of plant species (Stebbins 1950). In *M. lunatum* the total chromatin length is 49.94 μ m and the metacentric frequency is 46.67%. The high percentage of metacentric chromosomes in plant is an indication of symmetric karyotype which is primitive in nature (Stebbins 1950). Furthermore, we observed subtelometacentric chromosomes in *M. lunatum* karyotype. This corresponds with the works of Mercado-Ruaro and Delgado-Salinas (1998) who reported subtelometacentric chromosomes in *Phaseolus chiapasanus*, Zheng *et al.* (1991) in *Phaseolus vulgaris* and Tabur *et al.* (2009) in *Vicia*, *Coronilla*, *Trifolium* and *Spatium*. The presence of subtelometacentric chromosomes suggests that *M. lunatum* is highly advanced (Oziegbe and Eludini 2013). Finally, it is also important to note that a symmetric karyotype does not necessarily implies primitivity as assumed by earlier students (Lorenzo and Halil 2013) and is evident in *M. lunatum* due to the presence of metacentric and submetacentric chromosomes.



Fig 2: Karyotype of *M. lanatus* ($2n = 30$)

The findings of this work represent the first report on the chromosome number of $2n = 30$, karyotype description and formula of in $7m+4sm+4st$ (seven of the chromosomes were metacentric, four submetacentric and four subtelometacentric) *M. lunatum* from Nigeria.

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