ABSTRACT: The paper gives an account of the results of karyological investigation on *Machaerium lunatum* (Linn. f.) Duck 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.

**Keywords:** Chromosome number, Fabaceae, metacentric, submetacentric, *Machaerium*, karyotype.
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 1 cm 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 the 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 \( M. lunatum \).

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).

| Chromosome pair number | Chromosome length (µm) | Short arm (µm) | Long arm (µm) | Arm ratio | I (Centromeric index) | CM |
|------------------------|------------------------|---------------|---------------|-----------|-----------------------|-----|
| 1                      | 2.71 ± 0.08            | 1.05 ± 0.01   | 1.66 ± 0.10   | 1.59      | 38.75 (m)             |     |
| 2                      | 2.35 ± 0.07            | 0.90 ± 0.08   | 1.38 ± 0.01   | 1.42      | 41.43 (m)             |     |
| 3                      | 2.24 ± 0.06            | 0.89 ± 0.02   | 1.34 ± 0.08   | 1.50      | 40.07 (m)             |     |
| 4                      | 2.12 ± 0.06            | 0.57 ± 0.04   | 1.52 ± 0.08   | 2.69      | 26.60 (sm)            |     |
| 5                      | 2.00 ± 0.06            | 0.85 ± 0.10   | 1.15 ± 0.16   | 1.37      | 42.76 (m)             |     |
| 6                      | 1.88 ± 0.06            | 0.83 ± 0.08   | 1.06 ± 0.02   | 1.29      | 43.84 (m)             |     |
| 7                      | 1.77 ± 0.05            | 0.65 ± 0.10   | 1.12 ± 0.05   | 1.75      | 36.67 (m)             |     |
| 8                      | 1.65 ± 0.05            | 0.70 ± 0.13   | 0.95 ± 0.18   | 1.42      | 42.39 (m)             |     |
| 9                      | 1.53 ± 0.04            | 0.38 ± 0.02   | 1.16 ± 0.02   | 3.10      | 24.41 (sm)            |     |
| 10                     | 1.41 ± 0.04            | 0.38 ± 0.04   | 1.04 ± 0.01   | 2.82      | 26.25 (sm)            |     |
| 11                     | 1.30 ± 0.04            | 0.36 ± 0.04   | 0.94 ± 0.07   | 2.67      | 27.50 (sm)            |     |
| 12                     | 1.18 ± 0.04            | 0.26 ± 0.02   | 0.92 ± 0.06   | 3.65      | 21.67 (st)            |     |
| 13                     | 1.06 ± 0.03            | 0.21 ± 0.06   | 0.74 ± 0.07   | 3.59      | 20.00 (st)            |     |
| 14                     | 0.94 ± 0.03            | 0.21 ± 0.05   | 0.86 ± 0.15   | 4.25      | 21.59 (st)            |     |
| 15                     | 0.83 ± 0.02            | 0.19 ± 0.04   | 0.64 ± 0.01   | 3.50      | 22.50 (st)            |     |

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

From our study, the karyotype formula of \( M. lunatum \) is \( 7m+4sm+4st \) (seven of the chromosomes were metacentric, four submetacentric and four subtelometacentric) 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
Karyotype analysis in *Machaerium lunatum* (Linn. f.) Ducke 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 49.94µm 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.

![Karyotype of M. lanatus (2n = 30)](image)

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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