

Cytological and Morphological Evaluation of Some Species of the Tribe Mimoseae and Mimozygantheae from Northern Nigeria

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ABSTRACT-- Cytological and morphological evaluations were carried out on some species of the tribe mimoseae and mimozygantheae from northern Nigeria so as to determine their chromosome numbers, phenology and their relationships with the soils on which they were found growing. Results from chromosome counts has confirmed the previous reports of $n=14$ for *Entada africana* and *E. abyssinica*, $n =52$ for *Leuceana leucocephala*, $n=13$ for *Mimosa pigra* and $n=22$ for *Mimosa pudica*. The counts of $n=52$ and $n=12$ have also confirmed previous report for *Neptunia oleraceae* and *Schrankia leptocarpa* respectively while the count of $n=14$ is a new report for *Prosopsis africana*. While *E.africana*, *E.abysinica* and *Leuceana leucocephala* are tetraploids, *Mimosa pigra* and *M.pudica* are diploids. Chromosome number in this tribe has remained stable for long period of time. The distribution maps of the investigated taxa and their phenological data are presented for the first time.

Keywords-- Chromosome, Morphological, Mimoseae, Mimozygantheae, Phenology

1. INTRODUCTION

Legumes are economically and culturally important plants. They are well recognized for their extra ordinary diversity and abundance, the wide variety of edible vegetables they represent and the variety of uses they can be put to: in horticulture and agriculture, as a food, for the compounds they contain that have medicinal uses and for the oils and fats they contain that have variety of uses Graham and Vance, (2003).

The tribe Mimoseae comprises 37 genera distributed in tropical and sub- tropical and most abundantly in tropical America and Africa. The members are trees, shrubs, climbers, rarely herbs and occasionally aquatic; armed or unarmed, leaflets bipinnate, pinnate, few to many, alternate or opposite; petiolar glands sometimes present, flowers numerous in heads, spikes or racemes, actinomorphic, fruits 2 valves usually winged, curved, membranous to woody, indehiscent or dehiscent along one or both margins, occasionally with the valves separating with persistent margin and breaking into seeded segment. There are little information available on the cytology of this tribe. The only notable contributions are by Mangenot and Mangenot (1962), Freitas *et al.*, 1991, Sorenson *et al.*, 1994, Gill and Husaini (1986) and Bir and Kumari (1975). The base numbers of 12, 13, and 14 have been reported for the tribe by Goldblatt (1981). It was based on these facts that the cytological evaluation of some Northern Nigerian species were carried out.

2. MATERIALS AND METHODS

The living materials of all investigated taxa were collected from the fields all over the three Eco-regions of Northern Nigeria which include the Northern Guinea Savanna, Sudan Savanna and Southern Sahel Savanna. The taxa studied along with their locations, accession numbers, chromosome numbers, base numbers and ploidy level are summarized in Table 1.

3. CYTOLOGICAL TECHNIQUES

Chromosome counts were made from young flower buds and meristematic region of the root (root tip). The following methods were employed:

Schedule for Meiotic Study

Young flower buds from the wild as well as cultivated populations were collected and fixed in Carnoy's fluid (Acetic acid, chloroform and absolute alcohol, in ratio 1:3:6) for 24 hours and then transferred to 70% alcohol. Staining of the chromosomes was improved by saturating the acetic acid in the fixative with ferric acetate. The anthers were then squashed in 2% aceto- carmine stain and finally chromosome number was confirmed from at least 20 well spread cells. Permanent slides were made by passing the prepared slides through 70% alcohol for three minutes and absolute alcohol for two minutes and finally mounted in "euparal".

Schedule for Mitotic Study

Meristematic growing root-tips were pre-treated with 0.5% solution of colchicine for 1-2 hours at room temperature. They were then washed in tap water and fixed directly in 1:3 glacial acetic acid and alcohol for 24 hours Darlington and La-Cour, (1962). The materials were then hydrolyzed in 1N hydrochloric acid in water for 10 minutes at 60°C to soften the tissue. After rinsing with quick changes of tap water, the root tips were squashed in 2% aceto carmine stain and chromosome counts were confirmed from at least 20 well spread cells. Permanent slides were made using the methods already described under [a]

Schedule for Pollen Fertility Determination

Pollen fertility was determined from the ability of pollen to stain with 50% glycerol-aceto carmine. The anthers from matured flower buds were squashed in a drop of glycerol-aceto carmine. Pollen grains which were filled with stained protoplasm were considered to be fertile, while small, shrunk and unstained ones were counted as sterile. The grains were observed under 50 different microscopic fields, counted, measured using graduated eye piece graticle and finally percentage pollen fertility was calculated by dividing the number of well filled stained pollen by the total number of cells observed multiplied by hundred.

Schedule for Soil pH Determination

For pH determination, the soil samples were taken from the exact site of the plant collection a little bit far from the plant stand where soil is available and the soil pH was determined by testing the dissolved soil in test tubes using Philips Pw 9418pH meter. Soils at point of plant sample collection were tested to see if it has any effect on plant morphology.

Preparation of Distribution Maps

For the plotting of the distribution maps, a base map designed by Agboola (1979) was used. The distribution and abundance of all studied plant specimens and those available at Forest Research Institute, Ibadan were inserted in the base map. This will aid plant collectors in knowing where the species of plants are available and the quantity.

Photography

The photomicrographs were taken under a NIKON L-50 orthomat microscope with an automatic Ricoh XR – X 3000 camera attachment and oil-immersion contrast objective of X100 and compensating ocular x 10. Panchromatic high contrast copy films with a speed of 120 to 135 millimeters were used.

Voucher Specimen

Voucher specimens from which chromosome counts have been made were deposited in the herbarium of the Biological Sciences, Department, Ahmadu Bello University, Zaria with the following references/ accession numbers: MAA 019: *Entada africana*, MAA 081: *E. abyssinica*, MAA003 : *Leuceana leucocephala*, MAA 042: *Mimosa pigra*, MAA 028 : *M. pudica*

4. RESULTS AND DISCUSSION

Entada. africana is a Savannah tree which grow up to 7m high; nervation of leaflets distinct on both surfaces; leaflets 8 – 24 pairs, linear or oblong linear, 9-45mm long, 3-15mm broad, glabrous or sometimes puberulous; spikes axillary

among the leaves; flowers creamy white; fruits up to 38cm long and 5-7.3cm broad. It flowers from May to July in Northern Nigeria.

Fourteen haploid chromosomes were counted at metaphase 1. Miege (1962) reported $n = 16$ while Mangenot and Mangenot (1962) recorded $2n = 28$. Miege's report may be as a result of increased aneuploidy. Meiosis was normal with an average pollen grain size of $30.5\mu\text{m}$.

E. abyssinica is a. Savannah tree which grow up to 14m high; flowers cream; fruit straight, 15 – 39cm long, leaflets numerous in pairs, linear, mucronate. it grows well in moist areas especially in eastern and central parts of tropical Africa.

A haploid chromosome count of $n = 14$ was observed at metaphase 1. Mangenot and Mangenot (1962) also recorded a count of $2n = 28$. Meiosis was regular with percentage pollen stainability of 90% and on soils with pH 6.3.

Leuceana leucocephala. is a little slender shrub or trees which grow up to 15m high. It has feathery leaves and conspicuous white flowers in globose heads. It is Widespread in warm countries throughout the world. It has only one introduced species represented in Nigeria.

A haploid chromosome count of $n = 52$ was observed at metaphase 1 which is in line with $n = 52$ earlier reported by Freitas *et al.*, (1991) and Sorenson *et al.*, (1994). Goldbaltt (1981) also suggested a base number of $x = 13, 14$ which indicates that *Leuceana* is a tetraploid. Meiosis was normal with percentage pollen stainability of 90% and an average pollen grain size of $22.5\mu\text{m}$.

Mimosa pigra is a prickly shrub with sensitive leaves and pale mauve flower-balls, forming dense thickets on river banks; pinnate 6 – 16 pairs petiole shorter than leaf rachis; branchlets and leaf rachis prickly and roughly hirsute; fruits densely bristly all over, 4-6cm long, segments narrow and numerous. It grows on alkaline soils and flowers from June to September.

A haploid number of $n = 13$ was observed at metaphase 1 from the two populations investigated. This is in agreement with earlier reports of $n = 13$ by Husaini and Gill (1985). Meiosis was normal with percentage pollen stainability of 92% and an average pollen grain size of $23.8\mu\text{m}$.

Mimosa pudica is a straggling prickly sensitive plant introduced and rather local leaves are pinnate 1-2 pairs, petiole much longer than leaf-rachis; leaflets sensitive, linear-oblong, about 1.5 cm long, more or less bristly-hairy; branchlets and petioles bristly pilose or almost glabrous; stamens 4; fruits densely clustered. It grows along roadside and flowers from May to August in Northern Nigeria.

Three populations were studied and all proved to have a haploid count of $n = 22$. The present report is in agreement with earlier reports of $2n = 22$ by Husaini and Gill (1985) but, differs from the previous recorded number of $2n = 32$ by Bandel (1974), $2n = 26$ by Bir and Kumari (1975) and $2n = 26$ by Yeh *et al.*, (1986). The genus shows a decreasing aneuploidy i.e from 14, 13, 12, and 11. Meiosis was normal with percentage pollen stainability of 90% and an average pollen grain size of $24.5\mu\text{m}$. It grows well in acidic to alkaline soils with pH range of 5.5 - 6.5.

Neptunia oleraceae is an aquatic herb with thicken stems rooting at the nodes; leaves sensitive, bipinnate; pinnate 2-3 pairs, opposite; leaflets in 8 or more pairs, linear-oblong, rounded at apex; flowers capitate on elongated stout peduncles up to 20cm long, sepals and petals glabrous stamens 10; anthers with a deciduous apical gland. It is widespread in tropical Africa and flowers in August.

A haploid count of $n = 14$ was determined at metaphase 1. Meige (1960) reported a count of $2n = 54$. Turner and Fearing (1960a) have shown quite conclusively that $x = 14$ is the basic number for *Neptunia* and that the counts of $n = 52, 54,$ and 78 are incorrect. Meiosis was normal with the percentage pollen stainability of 85% and the average pollen grain size was $22.0\mu\text{m}$.

Prosopis africana is a Savanna tree which grows up to 12 – 18m high and up to 1m in girth, often branching very low down and up to crooked limbs forming an irregular open crown, but with clean bole in forest condition. Bark blackish, strongly fissured, flaking off in ragged patches leaving light brown scars; slash reddish darkening to red brown.

A haploid count of $n = 14$ was observed at metaphase 1. from the two populations investigated. This is a new chromosome report for this species which agrees with earlier base number of $x = 14$ suggested for the genus by Goldblatt (1981). Meiosis was normal and regular with a pollen grain size of $26.5\mu\text{m}$. It grows well on slightly acidic soils with pH of 6.1

Schrankia leptocarpa is a straggling perennial herb resembling the “sensitive plant” but with continuous not jointed pods. It was introduced into Nigeria from tropical America; leaves alternate, bipinnate; pinnate 2-3 pairs and stem quadrangular. It flowers from April to June. Two populations were investigated and both proved to have a haploid number of $n = 12$.

This corresponds with the earlier report of $n = 12$ by Gill and Husaini, (1986). Meiosis was regular and normal with percentage pollen stainability of 80% and an average pollen grain size of $25.0\mu\text{m}$. It grows best on soils with pH of 6.0.

From the five species of the tribe Mimoseae presently investigated from Northern Nigeria Four species namely *Entada africana* $n=14$, *E. abyssinica* $n=14$, *Leuceana leucocephala* $n=52$ and *Mimosa pudica* $2n=22$ are tetraploids, while only *Mimosa pigra* with $n=13$ is diploid. *Neptunia oleraceae* $n=14$, *Schrankia leptocarpa* $n=12$ and *Prosopis africana* $n=14$ are all diploids. The count of $n=14$ for *Prosopis africana* is a new record for Northern Nigerian species (Table 1). The lack of B- chromosome in the two tribes investigated indicates the genetic stability of the tribe. B- Chromosomes were reported earlier in some species of the genus *Indigofera* from Northern Nigeria Adelanwa *et al.*, (2004). They were considered to be genetically inert but now, they are known to increase variability and have been a pivotal factor in plant evolution and speciation and origin of novel adaptation (Levin, 2002). From the distribution map it is quite evident that the Tribe Mimoseae are widely distributed within the South west and Central part of the country with only few populations found around the far North and South eastern part of Nigeria. Meiosis was normal in all species studied with filled pollen grains ranging from 75% to 88% and average pollen grain size ranging from $20.8\mu\text{m}$ to $28.5\mu\text{m}$. Phenological results indicate that all investigated species flowers and fruit within the month of July and November. This will adequately serve as a lead to plant collectors as to when these plants can be collected for further investigations.



FIG. 19: Map showing the distribution of *Entada africana* and *E. abyssinica* in Nigeria (Agboola, 1979)

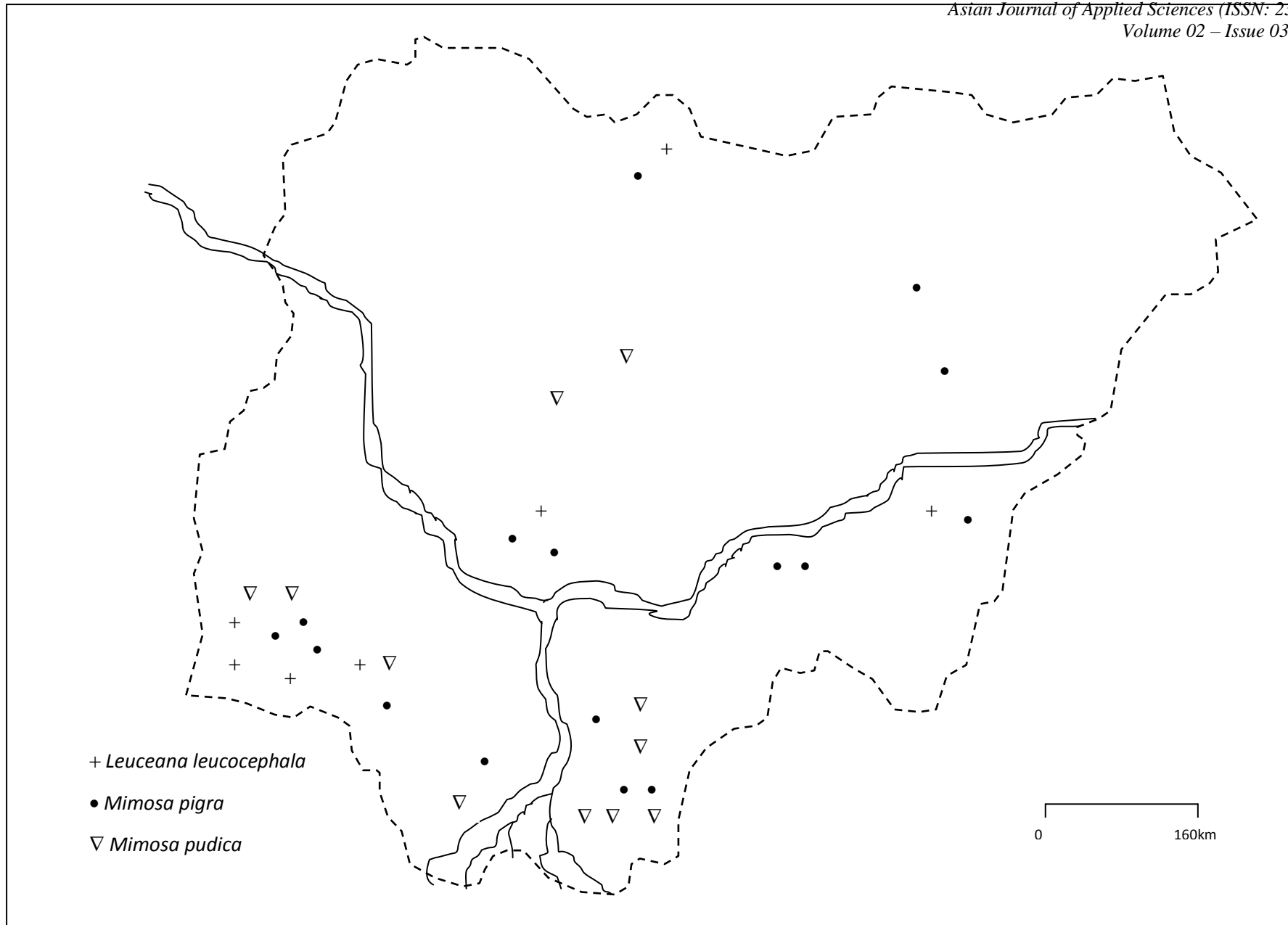


FIG. 20: Map showing the distribution of *Leuceana leucocephala*, *Mimosa pigra* and *M. pudica* in Nigeria (Agboola, 1979)

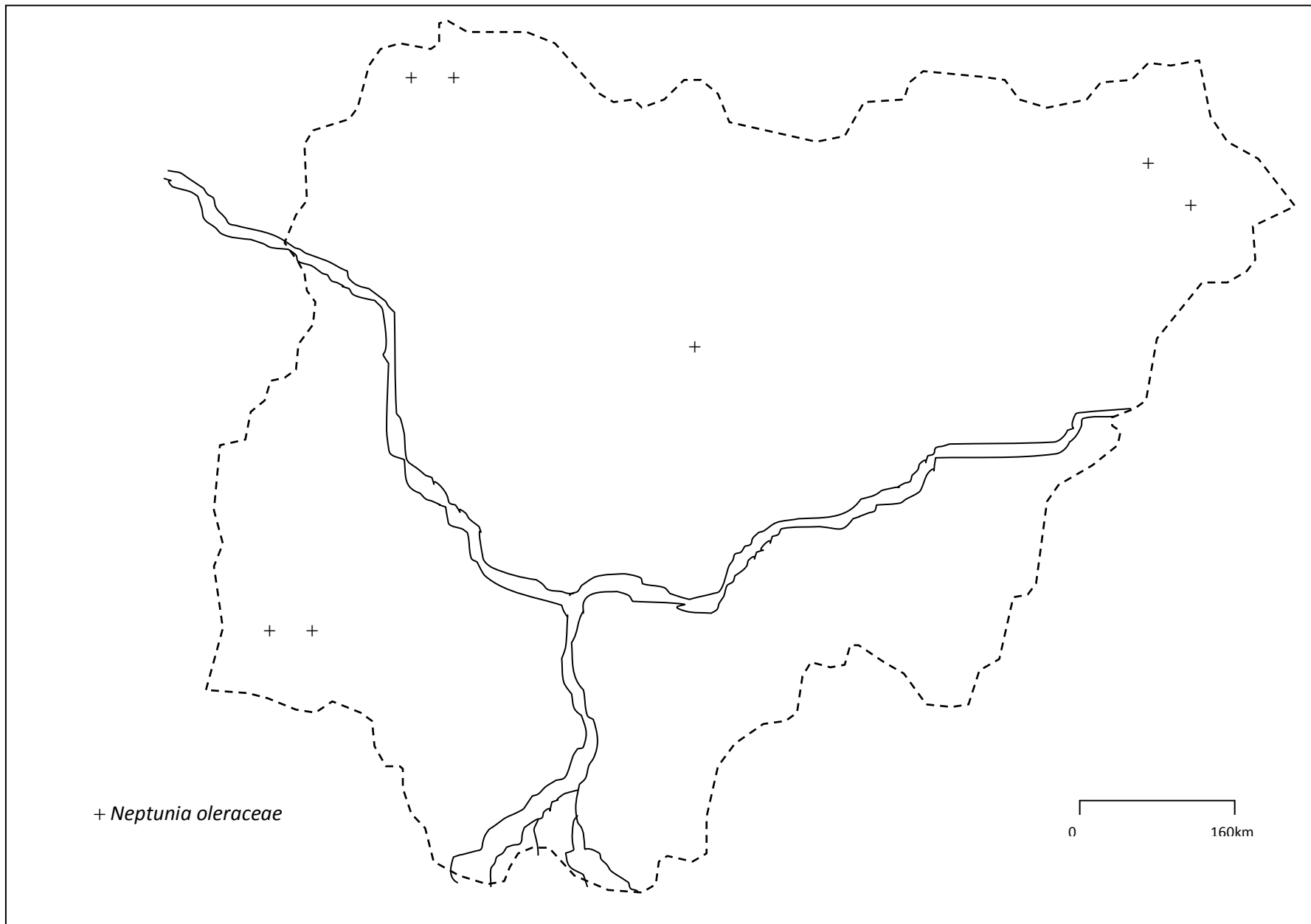


FIG. 21: Map showing the distribution of *Neptunia oleraceae* in Nigeria

(Agboola, 1979)

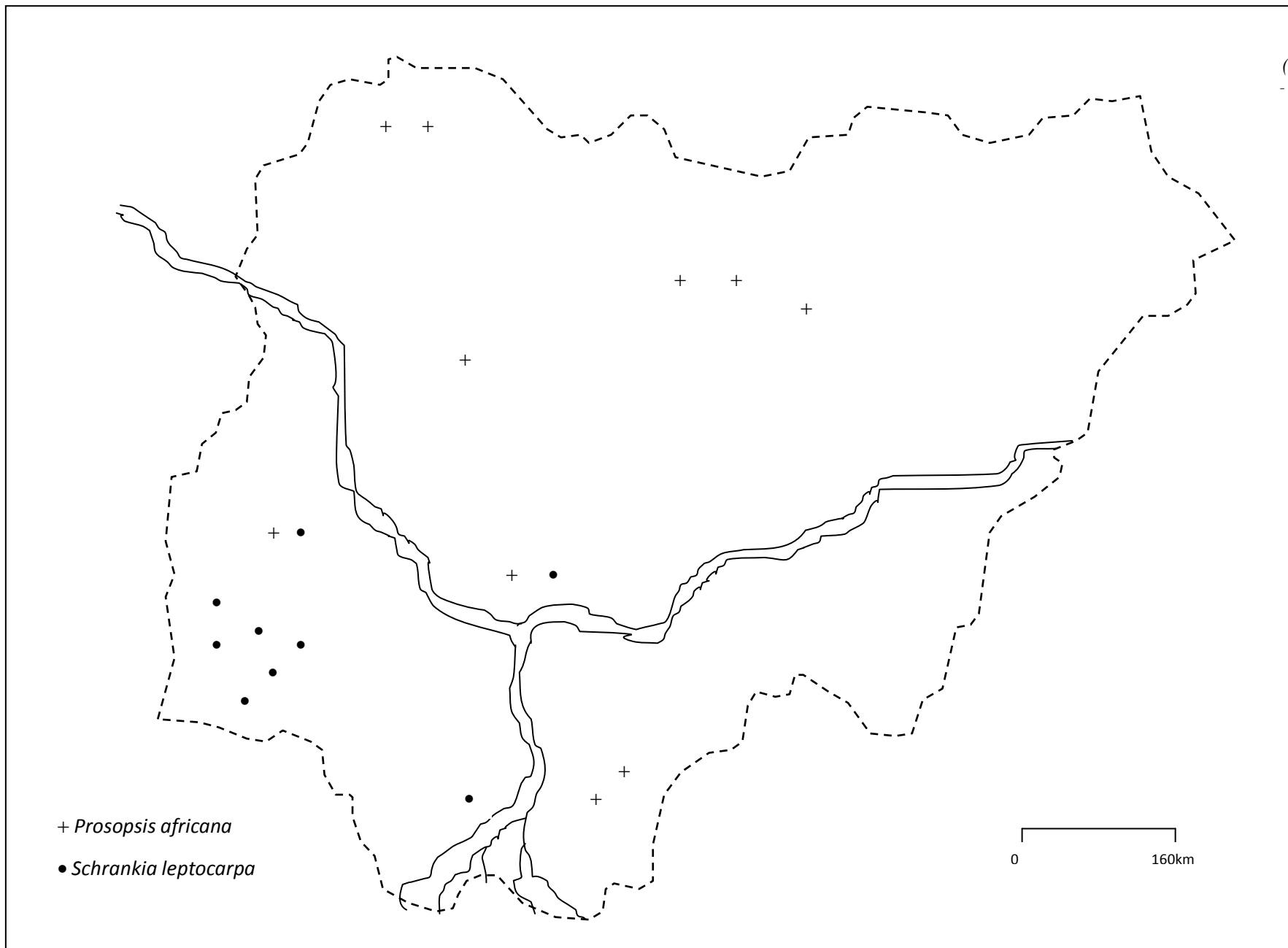


FIG. 22: Map showing the distribution of *Prosopis africana* and *Schrankia leptocarpa* in Nigeria

(Agboola, 1979)

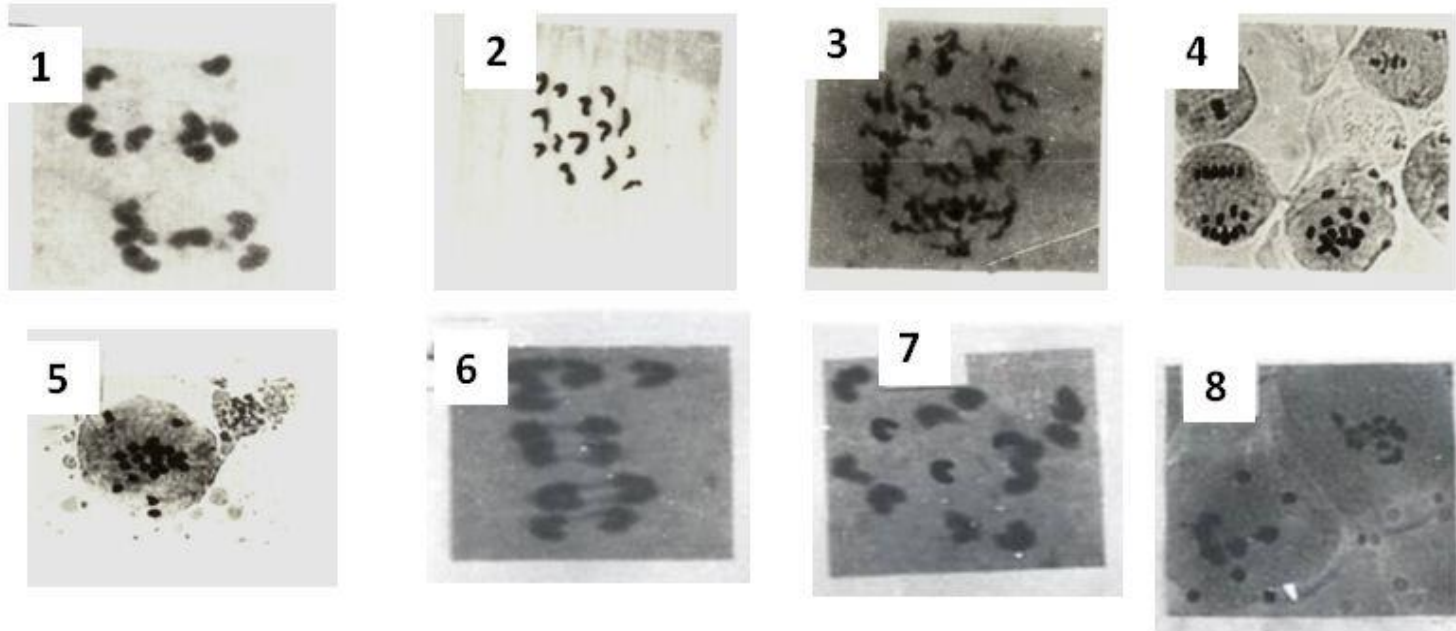


Plate 1. Photomicrographs showing chromosomes and their numbers in the tribe *Mimosaceae* from Northern Nigeria

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|--|---|--|
| 1. <i>Entada africana</i> (M) (n = 14) | 2. <i>E. abyssinica</i> (M) (n = 14) | 3. <i>Leuceana leucocephala</i> (D) (n = 52) |
| 4. <i>Mimosa pigra</i> (M) (n = 13) | 5. <i>M. pudica</i> (M) (n = 22) | 6. <i>Neptunia oleraceae</i> (M) (n = 121) |
| 7. <i>Prosopis africana</i> (M) (n = 14) | 8. <i>Schrankia leptocarpa</i> (M) (n = 12) | |

Table 1. Summary of the Cytological Data of the Northern Nigeria Mimoseae and Mimozygantheae

S/N	SUB-FAMILY: <i>CAESALPINIOIDEAE</i>	ACCES NO.	LOCALIT Y	CHROM NO	POLL FERT %	POLL SIZE μm	BASE NO.	SOIL pH	PLOIDY LEVEL.	PREVIOUS REPORTS
Tribe: <i>Mimoseae</i>.										
1.	<i>Entada africana</i> Guill & Perr.	019	Dumbi, Zaria	14	91	30.5	7	6.5	Tetraploid	Miege (1962) n = 16 Mangenot & Mangenot (1962) 2n=28
2.	<i>E. abyssinica</i> steud ex Rich.	081	Jaji, Kaduna State	14	90	29.5	7	6.3	Tetraploid	Mangenot & Mangenot (1962) 2n=28
3.	<i>Leuceana leucocephala</i> L. Benth.	003	Tatabu, Niger State	52	90	22.5	7	6.1	Tetraploid	Freitas. <i>et al.</i> , (1991) n=52 Sorenson <i>et al.</i> , (1994) n=52.
4.	<i>Mimosa pigra</i> Linn	042	Funtua, Katsina State	13	92	23.8	13	5.9	Diploid	Husaini & Gill (1986) n = 13 Gill & Husaini (1986) n=13
5.	<i>M. pudica</i> Linn.	028	Dutse-wai, Kaduna State	22	90	24.5	11	5.8	Tetraploid	Bir & Kumari (1975) 2n = 52 Husaini & Gill (1986) n = 22 Yeh <i>et al.</i> , (1986) 2n = 52
Tribe: <i>Mimozygantheae</i>.										

6.	<i>Neptunia oleraceae</i> Lour	088	Rayfield, Jos	14	85	22	7	6.2	Diploid	Miege (1960) 2n = 52
7.	<i>Prosopsis africana</i> (Guill ex Perr) Taub.	043	Zaria road, Kano State	14	80	26.5	7	6.1	Diploid	New Report
8.	<i>Schrankia leptocarpa</i> DC.	022	Tatabu, Niger State.	12	80	25.0	12	6.0	Diploid	Gill & Husaini (1986) n=12

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