



### Short Communication

## CHROMOSOME COUNTS AND CYTOMORPHOLOGICAL STUDIES OF NINE SPECIES OF *Crotalaria* Linn. FROM NORTHERN NIGERIA

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### ABSTRACT

Chromosome counts and morphological studies of some leguminous taxa of genus *Crotalaria* from wild populations in Northern Nigeria were carried out to determine their chromosome numbers, phenology and pollen fertility. Results from chromosome counts confirm the previous reports of  $n=8$  for *C.mucronata*, *C.retusa*, *C.senegalensis*, *C.naragutensis*, *C.microcarpa*, *C.lachnosema*, *C.falcata*, while the chromosome number of  $n=8$  were recorded for the first time in *C.graminicola* and *C. macrocalyx*, in the area studied. B-Chromosome was also recorded for the first time in *C.microcarpa*. All the species investigated are diploids with high pollen fertility. The phenological data of investigated taxa are presented for the first time.

**Key Words:** Chromosome, *Crotalaria*, Morphological, Phenology

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## INTRODUCTION

The family Fabaceae is more or less equivalent to Gramineae in economic importance due to its known as potential as food plants and a diverse of other uses. With about 700 genera and nearly 20,000 species, they constitute the third largest Family of flowering plants after Compositae and Orchidaceae. 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 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).

*Crotalaria* is a large genus comprising 550 species (Willis and Airy- Shaw, 1973) which are distributed in tropical and sub-tropical parts of the world. In West Africa, it is represented by 51 species and of these about 35 species are found in Nigeria. Members of this genus are herbs or shrubs and are grown as green manure crop, fodder for livestock feed, fertilizers, medicinal, chemicals and horticultural varieties (Lewis *et la.*,2005). They are also used for reducing root knot nematodes (Richard *et al.*, 2001). The base numbers of 7, 8 and 9 have been recorded by Goldblatt (1981). Little cytological information is available on the Northern Nigeria genus of

*Crotalaria*. Nine species were presently investigated.

## MATERIALS AND METHODS

The living materials of all investigated taxa were collected from the fields all over the three Ecoregions of Northern Nigeria. Young flower buds from the wild were collected and fixed in 1:3 glacial acetic and alcohol for 24 hours and then preserved in 70% alcohol at 4°C. Staining of the chromosomes was improved by saturating the acetic acid in the fixative with ferric acetate. The anthers were squashed in 2% aceto-orcein stain and finally chromosome number was confirmed from at least 20 well spread cells. Meristematic growing root-tips were pre-treated with 0.5% solution of colchicine for 1-2 hours at room temperature. They were then 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 bath for about 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-orcein stain and chromosome counts were confirmed from at least 20 well spread cells.

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.

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. 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: *C. falcata*: 010, *C. lachnosema*: 099, *C. microcarpa*: 064, *C. naragutensis*: 045, *C. senegalensis*: 076, *C. retusa*: 023, *C. graminicola*: 095, *C. macrocalyx*: 085, *C. mucronata*: 063.

## RESULTS

*Crotalaria falcata* are shrubby, diffusely branched, about 1m high, often found in coastal sands and flowers from September to November in Northern Nigeria. A haploid chromosome counts of  $n=8$  was determined at Metaphase 1. Meiosis was regular with percentage pollen stainability of 97% and average pollen grain size of 24.5 $\mu$ m.

*C. lachnosema* are woody under-shrubs of 21.5m high, flowers yellow with orange veins. It flowers from June to July around Zaria. It usually occurs in acidic soils among tall grasses in gardens and along road sides in Savannah area. At metaphase 1, eight haploid chromosomes were orientated and counted. Meiosis was regular and normal with 98% filled pollen and average pollen grain size of 25.1 $\mu$ m.

*C. microcarpa* are variable diffusely branched herbs, woody at the base, flowers fused together in a cluster on a slender peduncle with deep yellow to orange colour. It occurs in acidic to alkaline soils in the Savannah. A haploid count of 8+1B was observed at the metaphase 1. This was observed in about 25% of the cells in the two populations studied. Meiosis was normal with 99% filled pollens and average pollen size of 26.0 $\mu$ m.

*C. naragutensis* are erect, branched under-shrubs, up to 1m high, flowers yellow in racemes up to 30cm long. It grows in open Savannah. At early anaphase, eight bivalents were counted. Meiosis was normal with 96% filled pollen grain and the average pollen grain size was 24.6 $\mu$ m.

*C. Senegalensis* are Sub-erect or prostrate, half woody, up to 0.6m high, flower yellow turning orange. It occurs in dry fields and flowers from September to December in Northern Nigeria. At anaphase 1, eight haploid chromosomes were counted. Meiosis was normal with percentage pollen

stainability of 95% and an average grain size of 22.0 $\mu$ m.

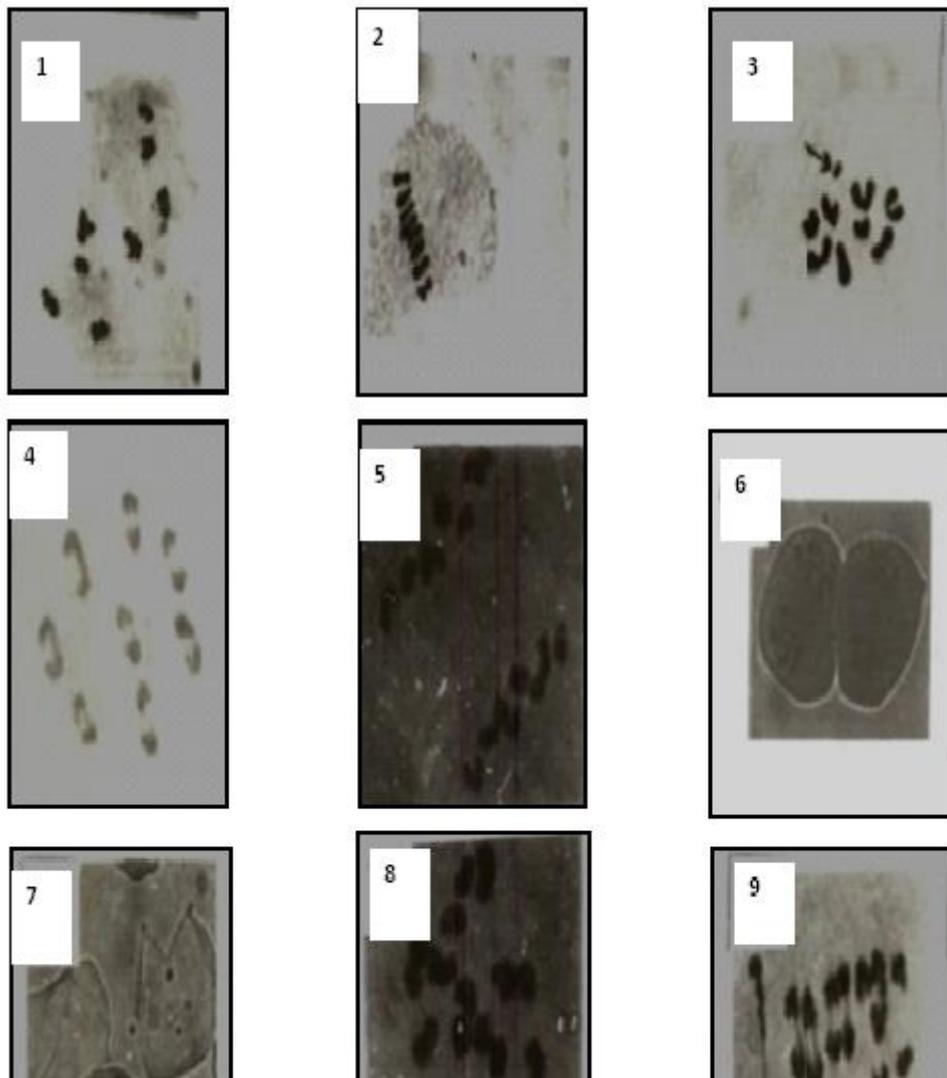
*C. retusa* are half shrubby herbs, 1.5m high with stout striated branches. It occurs in acidic soils around roadside and waste places. Flowering is sporadic throughout the year. The three populations investigated in this work proved to have a haploid count of  $n=8$  at anaphase 1.

*C. graminicola* are perennial herbs, usually found in burnt grassland, with numerous nearly simple stems 6-10m high. It flowers from February to March in Zaria. Three populations were investigated in this work and a haploid chromosome count of  $n=8$  were observed at metaphase 1. This is a new chromosome count reported for this species. Meiosis was normal with percentage filled pollen grain of 98%

while the average grain size was 23.0 $\mu$ m.

*C. macrocalyx* are erect or procumbent herbs, up to 0.5m high, with woody taproot and flowers yellow to orange. It occurs in the Savannah and flowers from September to October in Zaria. A diploid count of  $2n = 16$  chromosomes was observed at metaphase 1. This is a new count for this species. Meiosis was normal with 90% filled pollen and the average pollen size of 22.5 $\mu$ m.

*C. mucronata* are erect under-shrubs, up to 1.5m high with yellow flowers. A haploid count of 8 was observed at early anaphase 1. Meiosis and pollen formations were normal with 89% filled pollen. The average pollen grain size was 24. 0 $\mu$ m.



**Plate I.** Photomicrographs showing chromosomes and their numbers in the Family *Fabaceae* from Northern Nigeria

1. *Crotalaria falcata* (M)(n = 8)      2. *C. lachnosema* (M)(n = 8)      3. *C. microcarpa*  
 (M) (n = 8 + 1B)      4. *C. naragutensis* (Early A)(n=8)      5. *C. senegalensis*  
 (A) (n = 8)  
 6. *C. retusa* (A) (n = 8)      7. *C. graminicola* (M)(n = 8)      8. *C. macrocalyx* (M)(n = 8)  
 9. *C. mucronata* (Early A)(n = 8)

Table 1. Summary of Cytological Data of *Crotalaria* from Northern Nigeria

S/N	SUB-FAMILY: <i>CAESALPINIOIDEAE</i>	ACCES NO.	LOCALITY	CHROM NO	POLL FERT %	POLL SIZE $\mu m$	BASE NO.	SOIL pH	PLOIDY LEVEL.	PREVIOUS REPORTS
Tribe: <i>Crotalariaeae</i> .										
1	<i>Crotalaria falcata</i> Vahl	010	Bauchi rd, Jos	8	97	24.5	8	6.0	Diploid	Verma <i>et al</i> (1984) n=8  Husaini & Iwo (1992) n=8
2	<i>C. lachnosema stapf.</i>	099	Zango, Zaria	8	98	25.1	8	6.2	Diploid	Husaini & Gill (1985) n=8  Husaini & Iwo (1992) n=8
3	<i>C. microcarpa</i> Hutch ex Benth	064	Bauchi Rd, Jos	8+1B	99	26.0	8	5.2	Diploid	New report
4	<i>C. naragutensis</i> Hutch.	045	Naraguta, Jos	8	96	24.6	8	5.9	Diploid	Boulter <i>et al</i> (1976) n=8  Husaini & Iwo (1992) n=8
5	<i>C. senegalensis</i> (Pers) Bacle ex DC.	076	Bauchi rd, Jos	8	95	22.0	8	6.2	Diploid	Frahm-leliveld (1960) 2n=16  Boulter <i>et al</i> (1970) 2n=16
6	<i>C. retusa</i> Linn.	023	Zango, Zaria	8	97	22.0	8	5.9	Diploid	De olivera <i>et al</i> , (1999) 2n=16  Gill & Abubakar (1975) n=7  Gill & Husaini (1984) n=8  Gupta & Gupta (1978) n=8  Sharma, (1970) n=8
7	<i>C. graminicola</i> Taub ex Bak. F.	095	A.B.U. Dam, Zaria	8	98	23.0	8	6.1	Diploid	New Report
8	<i>C. macrocalyx</i> Benth.	085	Toro, Bauchi	8	90	22.5	8	6.2	Diploid	New Report
9	<i>C. mucronata</i> Desv	063	Vom Rd, Jos	8	89	24.0	8	6.1	Diploid	Husaini & Iwo (1992) n=8  Gupta & Gupta (1978) n=8  Verma & Raina (1983) n=8

## DISCUSSION

The Nine species from the genus *Crotalaria* presently investigated from Northern Nigeria are diploids with chromosome number  $n = 8$ . This is in agreement with earlier chromosome numbers report given by authors as indicated in Table 1. The count of  $n=8$  and the presence of B- chromosome in *C. microcarpa* is being reported in the studied area for the first time. B-Chromosomes were reported in *Indigofera hirsuta* and *I. tinctora* 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 key factor in plant evolution and speciation and the origin of novel adaptation (Levin, 2002). The chromosome number of  $n=8$  for *C. graminicola*

and *C. macrocalyx* is also a new report for Northern Nigerian species (Table 1). The base numbers of 7, 8 and 9 suggested earlier by (Goldblatt, 1981) seems to be fundamental in this tribe. The counts of  $n=8$  confirmed earlier report for *C. mucronata*, *C. retusa*, *C. senegalensis*, *C. naragutensis*, *C. lachnosema* and *C. falcata*. The genus *Crotalaria* are widely distributed within the South west and central part of the country with 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 $\mu$ m to 28.5 $\mu$ m. The

high pollen fertility in all investigated taxa shows their high susceptibility of growth and adaptation in various ecological zones of Nigeria. Phenological results indicate that all investigated species flowers and fruit within the month of July and November. This will serve as a pointer to plant collectors as to when these plants can be collected for further studies.

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