

A CYTOGENETICAL SURVEY OF THE GENUS
CYNODON RICH.

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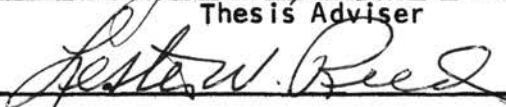
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INTRODUCTION

The genus Cynodon Rich is relatively small, containing some 23 species and about 60 synonyms, but is very widely distributed around the world. One of the species, Cynodon dactylon, is highly cosmopolitan, found in each of the six major continents and ranges from above 40° latitude north to below 40° latitude south. The immense power of acclimatization, adaptability, and vigor of this species have become matters of considerable interest to the taxonomists and plant breeders. A cytological investigation on the genus from the available material collected locally and from different parts of the world has been undertaken to help understand the nature of the germ plasm involved.

There are two schools of thought concerning the basic chromosome number of the genus, namely 9 and 10. Cytological and genetical evidence is as yet not sufficiently critical to permit a confident decision between the two interpretations on the basic number of chromosomes of the Cynodon genus. A basic knowledge of the chromosome numbers of the different species of Cynodon will be of great use in the development of plant breeding methods to utilize selected members of this grass for production of intraspecific and interspecific hybrids for new combinations with desirable agronomic qualities.

Most of the types used for lawns in Oklahoma are rather sterile or are at best poor seed producers. Consequently, seeds are imported from some of the neighboring states of this country. Cytological studies have become essential to investigate whether the sterility of the locally

cultivated varieties is due to any genetic causes or due to environment only.

REVIEW OF LITERATURE

History and Distribution of the Genus Cynodon Rich.

The genus Cynodon Rich. belongs to subtribe Chloridineae Pilger, tribe Chlorideae Kunth and subfamily Eragrostoideae Pilger of the family Gramineae.

Hartley and Slater (1960) opine that the very wide distribution of the subfamily and of its constituent taxa, as well as the close relationship between the distribution pattern and climate, suggests that the subfamily is a very old one. Geographical and taxonomical evidences indicate that it may have originated in tropical or subtropical Africa at least as early as the Oligocene.

In ancient time Cynodon was celebrated in the sacred Vedas as the shield of India, (Moore 1960), around which a flourishing civilization, religion and culture developed and it is still considered the sacred Durva grass of the Hindus. Bor (1960) reports that Cynodon is esteemed as a lawn grass in India. Cynodon dactylon, the most widely distributed species is known as Bermudagrass in America, Kweek grass in S. Africa and Couch grass in Australia. According to Wilson (1961), "records of the United States Department of Agriculture indicate that in terms of official records Bermudagrass seeds were perhaps first brought to the United States from India during 1888."

Burton (1962) suggests that although most early writers expressed the opinion that it probably originated in India, much greater diversity in African types indicate that Africa, rather than India may be regarded

as the primary center of origin of the grass.

Bogdan (1958) lists two species from Kenya, namely, C. dactylon and C. plectostachyus. Chippindall (1955) adds C. transvaalensis, C. polevansii, C. magennesii, C. bradleyi, C. hirstus and C. incompletus and C. notatus from South Africa. Camus (1960) describes C. coursii as an endemic to Madagascar and C. leptochloides as an endemic to Nossibé island. Sultan Ahmed (1959) finds C. dactylon in West Pakistan. C. aracuatus is described by Bor (1960) to have its distribution in India, Burma and East Pakistan although originally described from Luzon. Abrams (1940) shows the distribution of C. dactylon in the New World from Virginia to Argentina and also in California, and it overwinters as far north as Michigan. Love and Löve (1961) describe the distribution of C. dactylon in the cool-temperate areas of Germany, Holland, Belgium, Great Britain, Norway, Sweden. Spillman (1905) describes the distribution of Bermudagrass in the U.S.A. and esteems it to be the best pasture grass in the Southern States. Jisaburo (1941) mentions C. dactylon var. Niponica in Japan. Ran and Chandrasedaran (1947) describe C. plectostachyus, an East African species tested extensively in Coimbatore since 1940. C. barberi is endemic to Madras in India. A list of other apparently valid species of the genus Cynodon together with their distribution is given in Table I.

Cytology

The first important work of Avdulov (1931) on the cytology of grasses reveals the transcendent role of cytology in grass taxonomy. In this study, he classifies grasses on the basis of the number and size of their chromosomes and formed a system strikingly similar to that based on

anatomy and histology. He states "the numerical transformation of the karyotype in the gramineae has proved to be of two types, the usual "polyploid" chromosome multiplication and that of the "basic numbers" themselves. The higher basic number (12-10) and small chromosomes are characteristic of the more primitive tribes, while low basic numbers (7-5) with large chromosomes are characteristic of the more developed tribes."

Stebbins (1958) is of opinion that the relationship between chromosome size, cellular metabolism and growth is a subject which certainly deserves attention and about which new discoveries of considerable evolutionary significance might be made. He, however, does not agree with Avdulov's suggestion and opines that large chromosome size evolved as an adaptation to winter cold is, however, hard to justify on the basis of any known facts of chromosome chemistry or cellular physiology. The intriguing speculation has some parallel when it is observed that in the family Commelinaceae, Liliaceae, Leguminosae, the largest chromosomes are found in species inhabiting temperate climates. But it has been also observed that species with consistently small chromosomes in many families namely Cyperaceae, and Juncaceae, are dominant in the same temperate regions. Noggle (1946) in studying the physiology of polyploidy in plants shows a correlation between stomatal length and chromosome number which has also been shown by Muntzing and Akdik (1948) in Secale. DeWet (1954) finds that stomatal size in Danthonia is dependent on chromosome number as well as geographical distribution and morphological type.

Hurcombe (1946) studies one diploid species Cynodon bradleyi endemic to South Africa, where he observes the chromosomes in the pollen mother cells are all oblong or pyriform in shape and differ only slightly in size. In the somatic chromosome study she observes that in a single

root cell the chromosomes vary in length from one to two microns, and are rod shaped, v-shaped, kidney shaped or club shaped structures.

The classical studies of Navashin (1912), Newton (1921), Taylor (1924), Hunter (1934) and Krishnaswamy (1940) demonstrate that a more or less definite constancy of chromosome morphology and number exists in each genus. The relative size and number of the chromosomes are important characteristics.

Lewitsky (1931) and Lewitsky and Araratian (1931) find that different species of a genus are characterized by different and constant karyotypes and a general karyotype is maintained throughout a genus. The karyotype often undergoes a complete transformation when passing from one genus to the other. In Crepis, Babcock (1947) shows a continuous range of gross morphological types from primitive to advanced ones.

In Chlorideae there is some evidence by Hurcombe (1947) and Snyder and Harlan (1953) suggesting that both Bouteloua and Cynodon show intra-generic variations in basic number of chromosomes.

Tateoka (1953 and 1954) presents important data on chromosome morphology in Gramineae and subdivides the family into three major groups. The festucoid group has large chromosomes mostly multiples of $n = 7$; the panicoid-chloridoid group is characterized mostly by small chromosomes in multiples of $n = 9$ or 10 and the arundinoid-danthonioid group has medium small chromosomes, mostly in multiples of $n = 12$.

Several investigators have reported the occurrence of chromosome fragments, accessory, supernumerary or B-chromosomes in grass species. These extra chromosomes, though differing in some respects, have certain characteristics in common, such as shorter length and a greater proportion of heterochromatin than is characteristic of the normal or "A" chromosome

complement. In Aegilops speltoides, the satellite chromosomes are similar to those of emmer as suggested by Pathak (1940).

Such extra chromosomes sometimes vary in number in different parts of the plant and sometimes undergo non-disjunction in pollen mitosis. At present, the origin and role of these extra chromosomes are not understood. Extra chromosomes of this type have been reported in an increasing number of the Gramineae by Darlington and Wylie (1955), including 30 species in 18 genera. Other authors like Burton (1947), Bosemark (1957) and Hurcombe (1948) have found 16 species including C. dactylon.

Cytological studies of 8 Cynodon species found in two or more countries in a continent have been confined to the determination of chromosome numbers only, while no cytology is known on the rest of the endemic species. Table I shows the chromosome numbers of some of the species studied and the name of the authority, and their geographic distribution.

In the cosmopolitan species C. dactylon, which is tetraploid, Avdulov (1931) finds the somatic chromosome numbers $2n = 36$, which is supported by Brown (1950), Burton (1951) and Forbes in Carnahan and Hill (1961). Forbes and Burton (1963) also study the meiosis of 'Costal' Bermudagrass C. dactylon and show the chromosome numbers to be $2n = 36$. Darlington and Janaki Ammal (1945) also find the chromosome numbers in C. diploideum to be $2n = 18$. Delay in 1950 finds the same number. Hurcombe (1946) and Forbes and Burton (1963) report diploid meiotic behavior of C. bradleyi Stent and the chromosome number is found to be $2n = 18$. Regular diploid meiosis is also observed by Forbes and Burton (1963) in one or more accessions of C. incompletus, C. transvaalensis, C. plectostachyus. Moffett and Hurcombe (1949) report the chromosome

number in C. plectostachyus to be $2n = 54$. All these studies indicate the basic chromosome number of the genus to be 9.

Hurcombe (1947 and 1948) and Moffett and Hurcombe (1949), from a study of root tip smears, show the somatic chromosome number in C. hirsutus, C. plectostachyus and C. transvaalensis to be $2n = 20$, and in C. dactylon to be $2n = 40$.

Tateoka (1953) also finds the chromosome numbers in C. dactylon to be $2n = 40$.

Hunter's (1934) finding of chromosome number $2n = 30$ for C. dactylon supports Hurcombe's assumption that 10 should be the basic number of the Cynodon genus. But Hurcombe finds C. dactylon to be tetraploid whereas Hunter finds it to be triploid.

In C. x magennisii which is a triploid and supposed to be a natural hybrid between C. dactylon and C. transvaalensis, Hurcombe (1949) finds the somatic chromosome number to be $2n = 30$, while Burton (1936) finds the somatic chromosome number to be $2n = 27$, indicating the basic number may be 10 or 9. In this species, until recently, no meiosis had been studied. Forbes and Burton (1963) observe in C. plectostachyus, C. incompletus, and C. x magennisii that large satellites frequently resemble whole chromosomes and give the appearance of extra chromosomes. In one of the C. transvaalensis x C. dactylon, chance hybrids, the maximum possible number of 9 trivalents is observed at diakinesis, indicating that the genomes in the two parents are homologous. They also observe that during meiosis, chromosome stickiness is a fairly common irregularity in Cynodon.

TABLE I
DISTRIBUTION OF THE VALID SP. OF CYNODON AND
PREVIOUS WORK DONE ON CYTOLOGY

Species	Chromosome No. $2n$	Authority	Distribution
<u>C. aracuatus</u>			India, Burma, S.E.
Presl.			Asia
<u>C. barberi</u> Rang. et Tad.			India
<u>C. bradleyi</u> Stent.	18	Hurcombe (1947)	S. Africa
	18	Forbes & Burton (1963)	
<u>C. ciliaris</u> Benth.			Australia
<u>C. coursii</u> A. Camus.			Madagascar
<u>C. dactylon</u> (L.) Pers.	36	Avdulov (1931)	In almost all countries of the world
	30	Hunter (1934)	
	40	Hurcombe (1947)	
	36 \neq f's	Burton (1947)	
	40, 37 \neq 4 f	Hurcombe (1948)	
	40	Moffett & Hurcombe (1949)	
	36	Brown (1950)	
	36	Burton (1951)	
	40	Tateoka (1953)	
	36	Forbes, in Carnahan and Hill (1961)	
	18, 36	Forbes & Burton (1963)	
<u>C. diploideum</u>	18	Darlington & Janaki	Tropical Africa
Janaki Ammal		Ammal (1945)	
	18	Delay (1950)	

TABLE I (Continued)

Species	Chromosome No.	Authority	Distribution
	2n		
<u>C. glabratus</u> Steud.			North Africa
<u>C. hirsutus</u> Stent.	18	Covas (1949)	South Africa
	20	Hurcombe (1948)	
<u>C. incompletus</u> Nees.			Australia
<u>C. leptochloides</u> Steud.			Nossibé
<u>C. notatus</u> Nees.			Australia
<u>C. magennisii</u> Hurcombe	30	Hurcombe (1947)	S. Africa
<u>C. parviglumis</u> Ohwi			Malayasia
<u>C. pascuum</u> Nees.			Brazil
<u>C. plectostachyus</u>	20	Hurcombe (1948)	S. Africa
(Schum.) Pilger.	18	Sampath & Ramanathan (1949)	
	18,54	Moffett & Hurcombe (1949)	
	18	Burton (1951)	
	18	Forbes & Burton (1963)	
<u>C. polevansii</u> Stent.			S. Africa
<u>C. radiatus</u> Roth ex Roem. et Schult.			India
<u>C. tenellus</u> R. Br.			Australia
<u>C. tener</u> Presl.			Luzon
<u>C. ternatus</u> A. Rich.			Ethiopia
<u>C. transvaalensis</u> Burtt-Davy	20	Hurcombe (1947)	S. Africa
	18	Forbes, in Carnahan & Hill (1961)	
	18	Forbes & Burton (1963)	
<u>C. virgatus</u> Nees.			India

MATERIALS AND METHODS

The plant materials under investigation were collected as seed and plant accessions by Dr. Jack R. Harlan¹ and Dr. Wayne W. Huffine¹ from different parts of the Old World and were grown in the field of Oklahoma Agricultural Experiment Station, as outlined by Celarier and Harlan (1958).

Locally cultivated strains in Stillwater were selected at 8 different locations and plant materials were collected for study.

Herbarium specimens have been preserved for all the accessions and the local collections, for morphological studies.

For making cytological studies, Cynodon spikes at the proper stage of development, were fixed in freshly mixed Carony's fluid B i.e. 95% Ethyl alcohol, glacial acetic acid and chloroform in 6:3:1 proportion (Smith, 1947). Fixations were done for the most part between 10 a.m. and 12:30 p.m. The fixed material was stored in a refrigerator until smear preparations could be made. The smear preparations of the microsporocyte cells in acetocarmine were made according to the standard method. The spike under study was removed from the fixative fluid and was placed in a petri dish with a small quantity of the fixative. One of the anthers in a spikelet was dissected out with a fine pointed glazed iron needle, placed it on a clean glass slide of size 25 x 75 mm and a drop of acetocarmine added to it. The anther was squashed on the slide with the curved point of a needle and the pollen mother cells were forced out. First, the microsporocytes were briefly examined under low power of the

¹Professor of Agronomy, Oklahoma State University.

microscope and when the desired stages of cell division were observed, a clean cover slip of size 18 sq. mm. was quickly put on. The other two anthers of the spikelet having the proper stages were squashed similarly and mounted. The cover slip was tapped with a pencil to get good spreading of the cells, and finally pressure was applied with the thumb to remove any excess of the stain and to flatten the cells. Sometime before the pressure was applied, the slide was slightly warmed on a spirit lamp to get better staining of the cells. After cooling, the cover slip was carefully sealed with a mixture of equal parts of paraffin and gum mastic tears. The prepared slides were stored for a day or two in a refrigerator in order to obtain a better contrast between the chromosomes and the cytoplasm and then they were analysed.

Detailed analysis were always made using an oil immersion objective at a magnification of 12.5 x 97. For photography, high contrast Kodalith Ortho Type 3 film was used.

Some of the accessions did not flower and meiosis could not be studied.

EXPERIMENTAL RESULTS

A summary of observations on the meiotic chromosome behavior and morphological characters of Cynodon species with the chromosome numbers is presented in Tables II and III. Photographs of the inflorescences of some Cynodon species are presented in Plates I and II. Photomicrographs of the meiotic chromosomes of several Cynodon species are presented in Plates III and IV. A more detailed morphological description of the plant materials together with additional cytological observations are given below.

Cynodon dactylon accessions found to be tetraploid with chromosome numbers $2n = 36$:

A. 8801 - From Lashkar Gah, Afganistan, very fine dark green leaves, blade quite narrow. Plants growing by stolon only. Ligule is hairy. Upper glume is equal to spikelet length. In 8801 the frequency of chromosome fragments was greater and a satellite chromosome was observed in one microsporocyte cell. Average number of univalents and bivalents were 1.42 and 17.29 respectively. Chromosome stickiness was observed. Metaphase showing 18 bivalents is shown in Plate III, Fig. 9.

A. 8802 - From south of Kandahar, Afganistan. Plants growing by stolon and rhizome. Glumes comparatively shorter than spikelets. The nucleolar chromosome was readily recognizable, it being always darkly stained and occasionally with satellite attached or broken off. (Plate III Fig. 5). The average association of univalents was 0.90 and bivalents 17.55.

LEGEND TO PLATE I

Inflorescences of Cynodon dactylon showing variation in spike number and length, spikelets, glumes and distance of flag leaf from the whorl point.

Figure 1. P.I.267985 - A tetraploid from Pakistan showing one spike below the main whorl point.

Figure 2. A 9230 - A diploid from Ethiopia showing long spike .

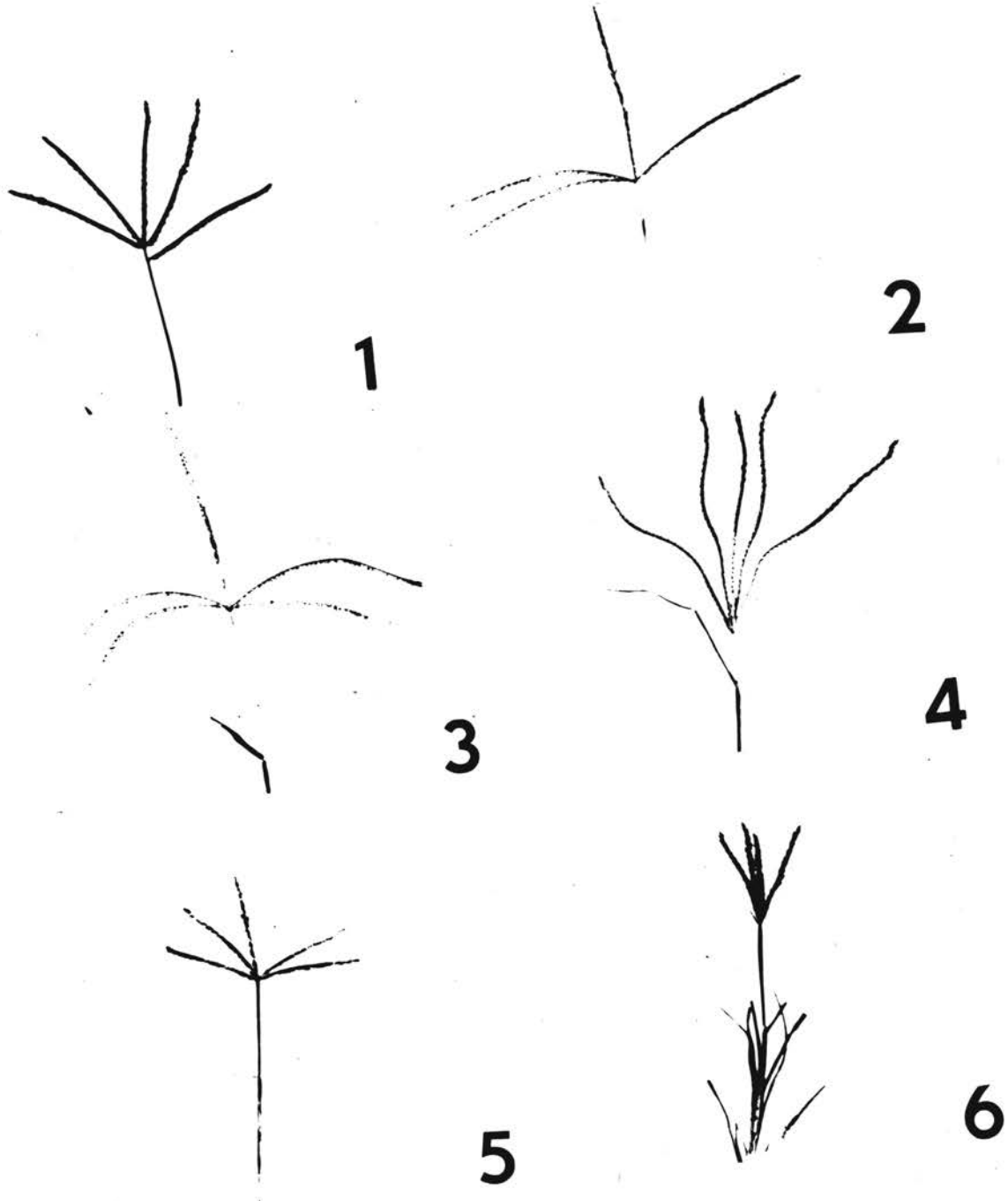
Figure 3. A 9226 - A tetraploid from Ethiopia showing long spike.

Figure 4. Collection No. 5 - A tetraploid from Stillwater showing large inflorescence

Figure 5. P.I.268324 - A tetraploid from India showing short inflorescence.

Figure 6. Collection No. 6 - A tetraploid from Stillwater, showing short inflorescence with prominent glumes.

PLATE I

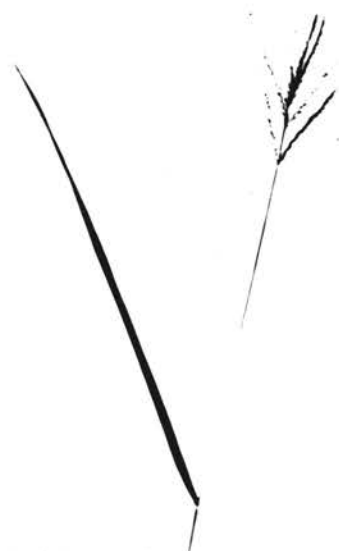


LEGEND TO PLATE II

Inflorescences of Cynodon dactylon, Cynodon plectostachyus and C. x magennisii showing variation in spikes, whorl numbers, glumes, spikelets, and flagleaf distance from the whorl point.

- 1 A 9233 Cynodon dactylon - A diploid from Ethiopia showing large inflorescence, one spike below the main whorl and prominent glumes.
- 2 A 9235 Cynodon plectostachyus - A diploid from Ethiopia showing spikes in three different whorls.
- 3 C. x magennisii - A triploid from South Africa showing very short inflorescence.
- 4 A 9234 C. dactylon - A diploid from Ethiopia showing large inflorescence, one spike below the main whorl, and flagleaf very near to whorl point.

PLATE II

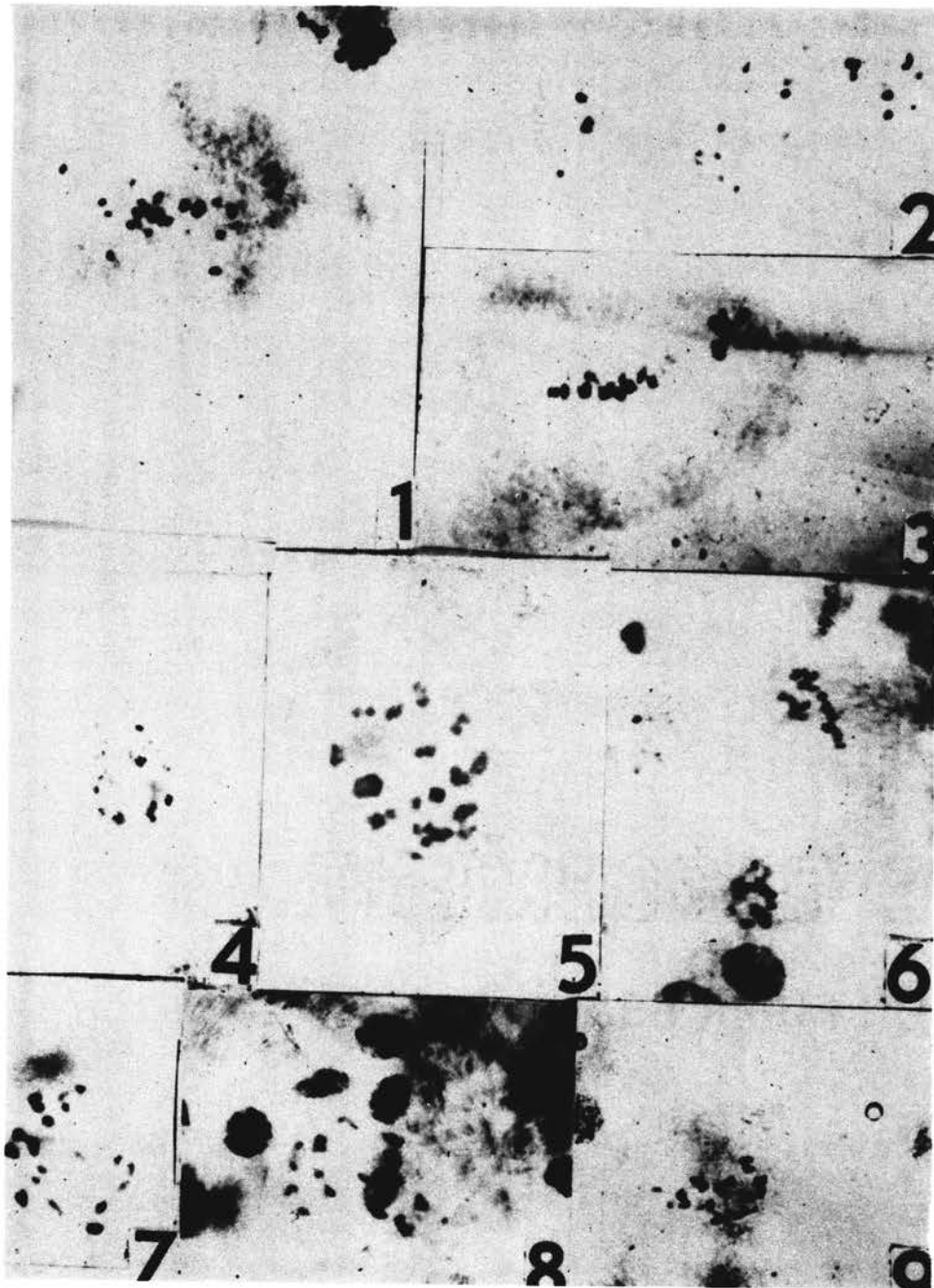
**1****2****3****4**

LEGEND TO PLATE III

Chromosomes of Cynodon dactylon showing variation in size, shape and numbers of chromosomes amongst the tetraploids collected locally and abroad.

- Fig. 1 Local Collection No. 3 - showing metaphase I with a chromosome number $2n = 40$ and fragments.
- Fig. 2 Local Collection No. 6 - showing metaphase I with a chromosome number $2n = 40$.
- Fig. 3 Local Collection No. 6 - showing metaphase I with 20 bivalents.
- Fig. 4 Local Collection No. 1 - showing late diakinesis stage with a chromosome number $2n = 36$.
- Fig. 5 A 8802 - from Afganistan - showing diakinesis stage with a chromosome number $2n = 36$. Big satellite of the nucleolar chromosome is observed.
- Fig. 6 Local Collection No. 6 - showing late Anaphase with 20 chromosomes at each of the two poles.
- Fig. 7 Local Collection No. 7 - showing diakinesis stage with 18 bivalents and a satellite of the nucleolar chromosome.
- Fig. 8 P.I.266697 - From Iran - showing diakinesis stage with 18 bivalents.
- Fig. 9 A 8801 - From Afganistan - showing diakinesis stage with 18 bivalents.

PLATE III

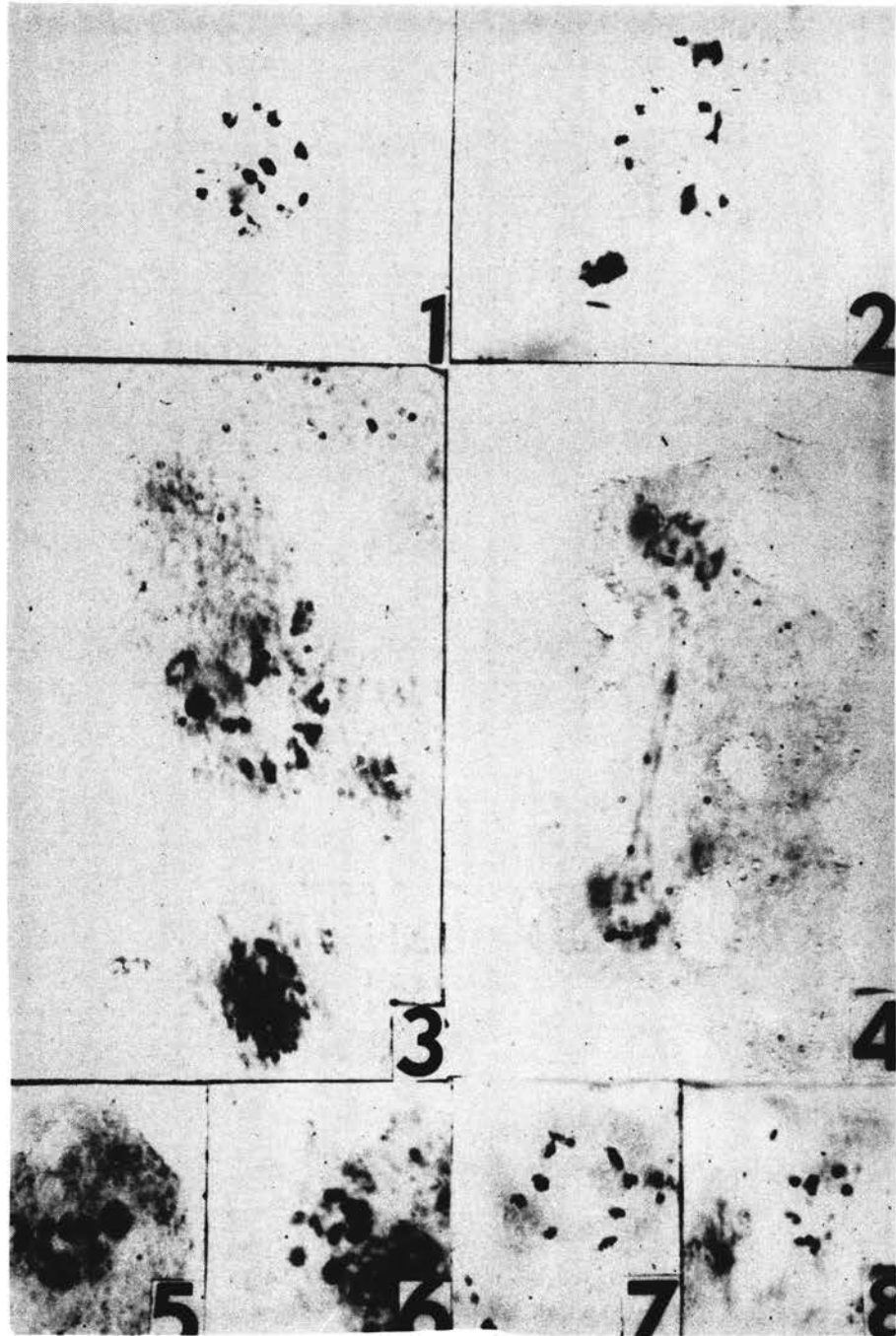


LEGEND TO PLATE IV

Chromosomes of Cynodon species showing variation in chromosome size, shape and number amongst the diploids and triploid collected abroad.

- Fig. 1 C. x magennisii from South Africa, showing a chromosome number $2n = 27$. A diakinesis stage with 13 bivalents and 1 univalent is shown.
- Fig. 2 C. x magennisii, showing a late diakinesis stage with two nucleoli and 13 bivalents and 1 univalent association.
- Fig. 3 A 9235 Cynodon plectostachyus - from Ethiopia, showing a diakinesis stage with 7 bivalents and 4 univalents.
- Fig. 4 C. x magennisii, showing extreme stickiness of chromosomes in Anaphase stage.
- Fig. 5 & 6 A 9233 Cynodon dactylon from Ethiopia showing 9 bivalents. Chromosomes are quite large.
- Fig. 7 - A 9230 Cynodon dactylon from Ethiopia showing a diakinesis stage with 9 bivalents chromosomes more or less elongated in shape.
- Fig. 8 A 9234 Cynodon dactylon from Ethiopia, showing a late diakinesis stage with 2 univalents and 8 bivalents. Chromosomes are quite small as compared to A 9233 under the same magnification.

PLATE IV



A. 9226 - From S. E. Debrazeit, Ethiopia. Robust, stoloniferous plants. Glabrous leaf with expanded leaf blade. Ligule with long hairs. Rachilla when elongated bears reduced floret. Plate I, Fig. 3 shows the inflorescence with five spikes of 6-8 cm. length.

Meiosis was almost regular except occasional occurrence of multivalents, Anaphase I was regular except in one microsporocyte cell, one undividing bivalent was observed.

P. I. 266697 - From N. Kermanshah, Iran, tall growing, stoloniferous rhizomatous. Leaf and ligule both with hairs. Upper glume length is equal to spikelet. Meiosis was quite regular with 18 bivalents. A late diakinesis is shown in Plate III, Fig. 8.

A. 9217 - From Alemaya, Ethiopia, tall growing with spreading habit, stoloniferous, no rhizome present. Leaf dark green, stiff and silicated with rough surface. Ligule with few long hairs. Fragment and satellite were observed. Average univalent numbers were 1.52 and bivalents 17.24. Anaphase I was regular with 36 chromosomes.

P. I. 267985 - From Mastuj Pakistan, (elevation - 8,000 ft), short growing. Stoloniferous and rhizomatous. Hairy leaf and hairy ligule. Leaf light green with medium expansion of blade. Leaf sheath also hairy. Inflorescence in two whorls, one spike below the main whorl point (Plate I, Fig. 1). Meiosis is almost regular except the occurrence of 0.53 univalents. Chromosome No. $2n = 36$.

P.I. 268324 - From 9 miles above Pahalgam, elevation - 9,500 ft., India. Very fine narrow leaf, soft and glabrous. Plants growing both by rhizomes and stolons. Ligule is hairy. Produced rachilla sometimes bearing reduced floret. Plate I, Fig. 5 shows the short inflorescence. Meiosis is quite regular with 18 bivalents.

Cynodon dactylon accessions found to be diploid with chromosome numbers $2n = 18$:

A. 9230 - From Erea Valley, E. of Alemaya, Ethiopia. Tall, robust and erect plant growing by rhizome and stolons. Dark green leaf, quite rough and rigid blade much expanded, leaf sheath hairy, ligule with prominent long hairs, nodes green, inflorescence - green and yellow. Upper glume as long as spikelets, sometimes a little longer. Plate I, Fig. 2 shows the inflorescence.

Meiosis was almost regular with occurrence of occasional univalents. In 22 microsporocyte cells 9 bivalents were observed. Anaphase I was quite regular with 18 chromosomes. Plate IV, Fig. 7 shows diakinesis with 9 bivalent chromosomes, more or less elongated in shape.

A. 9233 - From Erea Valley, 27 miles E. of Alemaya, Ethiopia. Tall, spreading type of plants growing by stolons and rhizomes. Leaf rough, medium green and glabrous, leaf blade - with medium expansion. Nodes and inflorescence pink. Very inconspicuous ligule with practically no hair. Glumes are approximately of the same length of the spikelet. Plate II, Fig. 1 shows one spike below the main whorl. Meiosis is quite regular with 9 bivalents. Anaphase I is also regular with 18 chromosomes. Chromosomes are quite large having round and oblong shape (Plate IV, Fig. 5 and 6).

A. 9234 - From Erea Valley, Alemaya, Ethiopia, tall erect, growing by stolons. Long leaf with rough surface, light green in color. Nodes - some are pink and some green. Leaf sheath hairy, leaf with hairs and ligule with prominent long hairs. Big inflorescence - some are pink and some green. Large number of spikes in the inflorescence, generally 5-8, sometime one spike below the main whorl (Plate II, Fig 4).

Meiosis is almost regular except the very rare occurrence of tetra-valents and univalents. Plate IV, Fig. 8 shows a late diakinesis stage with two univalents and 8 bivalents. Chromosomes are quite small as compared to that of 9233 under the same magnification.

Cynodon plectostachyus accession found to be diploid with chromosome numbers $2n = 18$:

A. 9235 - From Fanfan valley, 62 miles E. of Alemaya, Ethiopia. The plants are tall erect growing by dense net work of stolons. Leaf is very soft of medium length and expanded blade. Leaf and leaf sheath have prominent long erect hairs. Ligule is hairy and conspicuous. Inflorescence - green and yellow. Spikes - 3 to 6 in number and all in different whorls. Glumes are quite small as compared to the spikelets, sometimes one third its length. (Plate II, Fig. 2).

Meiotic study shows 0.64 average univalents and 8.68 bivalents in association. Plate IV, Fig 3 shows a diakinesis stage with four univalents and seven bivalents. The nucleolar chromosome is dark stained and has a big satellite. Anaphase I is found with 18 chromosomes, one bivalent undergoing division. Satellite of the nucleolar chromosomes are observed in four microsporocyte cells and fragments are found in two cells.

Cynodon x magennisii from S. Africa was found to be triploid with chromosome numbers $2n = 27$.

It is supposed to be a sterile hybrid between C. dactylon and C. transvaalensis and does not set seed. The plants are stoloniferous, very short growing with fine, short and glabrous leaves and possess excellent lawn qualities. Ligule is hairy and very prominent. Spikes are 3 to 4 in number with short length up to 2.5 cm. Spikelets - 2 mm

long. Glumes are half the length of the spikelets. Plate II, Fig 3 shows the short inflorescence.

Meiosis is quite irregular. A maximum of 13 bivalents and 1 univalent was observed in diakinesis and sometimes two nucleoli (Plate IV, Figs. 1 and 2). In Anaphase I, one laggard univalent was occasionally observed. Stickiness is fairly common. Extreme form of stickiness of chromosome is shown in Plate IV, Fig. 4.

Cynodon dactylon from local collections found to be tetraploid with chromosome numbers $2n = 36$ and $2n = 40$.

Collection No. 1 - Short growing, stoloniferous and rhizomatous. Fine narrow leaf - glabrous; prominent hairy ligule, inflorescence pink. Spikes 3 to 4 in number and 2.5 to 4 cm. long. Glumes are half the length of spikelet; rachilla bearing reduced floret.

Some univalents were found at meiosis. Plate III, Fig. 4 shows 4 univalents and 16 bivalents.

Collection No. 2 - Short growing, stoloniferous and rhizomatous. Leaf fine, glabrous with hairy ligule. Inflorescence green.

Meiosis shows frequent univalents and satellites of the nucleolar chromosomes. Chromosome numbers found $2n = 36$.

Collection No. 3 - Short growing plant with fine leaf and hairy ligule. Both stolon and rhizome present. Inflorescence green and pink.

Meiosis is found with chromosome number $2n = 40$. Plate III, Fig 1 shows metaphase with 20 bivalents and 3 fragments.

Collection No. 4 - Tall and medium plants growing by rhizome and stolon. Leaf fine - glabrous with hairy ligule. Inflorescence green and pink. Spike little longer than that of Collection 2.

Meiosis is quite irregular with frequent univalents and chromosome fragments and in one case with the occurrence of a tetravalent.

Chromosome number found to be $2n = 26$.

Collection No. 5 - Tall growing plant rhizomatous and stoloniferous, leaf glabrous, ligule hairy, inflorescence - pink and green. Spikelet - 2 mm. Upper glume is of same length as spikelet. Plate I, Fig. 4 shows the long inflorescence with spikes of 4-6.5 cm. length.

Meiosis is almost regular with 18 bivalents except in one microsporocyte cell, 2 univalents were observed. Anaphase I is quite regular with 36 chromosomes.

Collection No. 6 - Short growing plant with rhizome and stolon, leaf-fine, glabrous, ligule-hairy. Inflorescence - all pink. Glumes are showy and of the same length as the spikelet (Plate I, Fig. 6).

Meiotic study of some inflorescences (Table II 6a) shows chromosome number $2n = 36$ with average association of 1.0 univalent 16.50 bivalents and 0.5 tetravalents.

Meiotic study of some other inflorescence (Table II 6b) shows chromosome numbers $2n = 40$ with average association of 1.42 univalents, 19.15 bivalents and .07 tetravalents. Plate III, Fig. 2 and 3 shows metaphase stage with 20 bivalents. Fig. 6 shows late Anaphase.

Collection No. 7 - Short growing plant with rhizome and stolon. Fine, narrow leaf, glabrous, ligule-hairy. Inflorescence - green and light pink. Spikelets - 2 mm. Glumes are half the length of spikelet.

Meiosis is slightly irregular with average association of 0.74 univalents and 17.63 bivalents. Anaphase I is found with one undivided bivalent and 17 chromosomes at each of the two poles. Plate III, Fig. 7 shows a diakinesis stage with 18 bivalents and satellite of the nucleolar

chromosome.

Collection No. 8 - Medium growing plant with spreading habit. Stoloni-ferous and rhizomatous. Leaf-fine, narrow, glabrous. Ligule with long hairs. Inflorescence - green and light pink. Produced rachilla some-time bearing reduced floret.

Melosis is found with an average association of 0.62 univalents and 17.69 bivalents.

TABLE II
Cytological Data of Cynodon

Accession No.	Place	Species	Chro.No. 2n	Chromosome Association*			
				I	II	III	IV
8801	Laskar Gah, Afganistan	<u>C. dactylon</u>	36	1.42 0-4	17.29 16-18	0	0
8802	South of Kandahar Afganistan	do	36	0.90 0-6	17.55 15-18	0	0
9217	Alemaya, Ethiopia	do	36	1.52 0-4	17.24 16-18	0	0
9226	S.E. Debrazeit Ethiopia	do	36	0	17.38 16-18	0	.31 1-3
9230	Erea Valley, E. Alemaya, Ethiopia	do	18	.40 0-4	8.80 7-9	0	0
9233	27 miles E. Alemaya, Ethiopia	do	18	0	9	0	0
9234	do	do	18	.18 0-2	8.73 7-9	0	0.09 0-1
9235	Fan Fan Valley E. Alemaya, Ethiopia	<u>C. plecto- stachyus</u>	18	.64 0-6	8.68 6-9	0	0
PI.266697	N. Kermansah, Iran	<u>C. dactylon</u>	36	0	18	0	0
PI.267985	Mastuj elev. 8000 ft Pakistan	do	36	0.54 0-4	17.73 16-18	0	0
PI.268324	Pahalgam, elev. 9,500 ft. India	do	36	0	18	0	0
-	S. Africa	<u>C. magennisii</u>	27	2.77 0-15	12.07 6-13	.03 0-1	0

TABLE II (continued)

Collection No.	Place	Species	Chro.No. 2n	Chromosome Association*			
				I	II	III	IV
1	2 miles E. of Univ. campus Stillwater	<u>C. dactylon</u>	36	0.88 0-6	17.55 15-18	0	0
2	1 mile S. of Univ. campus	do	36	2.80 0-6	16.60 15-18	0	0
3	2½ miles S.E. Univ. campus	do	40	0	20	0	0
4	W. Univ. campus	do	36	0.48 0-4	17.66 14-18	0	0.05 0-1
5	2 miles S. Univ. campus	do	36	0.40 0-2	17.80 17-18	0	0
6a	1 mile E. campus	do	36	1.0 0-2	16.50 16-18	0	0.5 0-1
b	do	do	40	1.42 0-10	19.15 15-20	0	0.07 0-1
7	1½ miles N.W. campus	do	36	0.74 0-8	17.63 14-18	0	0
8	½ mile E. campus	do	36	0.62 0-8	17.69 14-18	0	0

*Average chromosome association as well as range of chromosome configuration are listed.

I - univalent, II - bivalent, III - trivalent and IV - tetravalent

TABLE III
Morphological Characters of Cynodon

Accession	Species	Growth habit	Leaf Character	Ligule	Flag leaf distance from whorl point
A 8801	<u>Cynodon dactylon</u> $2n = 36$	Tall, stoloniferous	Glabrous	Prominent long hairs	2-4.5 cm.
A 8802	do	Tall, stoloniferous & rhizomatous	do	do	1-2 cm
A 9217	do	Tall, stoloniferous No rhizome	do	Few long hairs	4-7 cm.
A 9226	do	Robust, stoloniferous No rhizome	do	Long hairs	4-6 cm.
PI 266697	do	Tall, stoloniferous & rhizomatous	Hairy	Hairy	3.5-6.5 cm.
PI 267985	do	Short, stoloniferous & rhizomatous	Hairy	Hairy	3.5-6.3 cm.
PI 268324	do	Short, rhizomatous & stoloniferous	Glabrous	Hairy	1.5-2.5 cm.
A 9230	<u>C. dactylon</u> $2n = 18$	Tall, erect rhizomatous & stoloniferous	do	Prominent hairs	1.5-8 cm.
A 9233	do	Tall, stoloniferous and rhizomatous	do	Inconspicuous ligule with practically no hair	2.5-5.5 cm.
A 9234	<u>C. dactylon</u> $2n = 18$	Tall, stoloniferous No rhizome	With little hairs, leaf sheath hairy	Prominent long hairs	.5 cm.

TABLE III (continued)

Accession	Species	Growth habit	Leaf Character	Ligule	Flag leaf distance from whorl point
A 9235	<u>C. plectostachyus</u> $2n = 18$	Tall, erect dense stolons	Long erect hair on leaf & leaf sheath	Conspicuous hairy ligule	3-7 cm.
Collection from S. Africa	<u>C. magennissii</u> $2n = 27$	Short, stoloniferous	Glabrous	Prominent hairs	.7-1.5 cm.
1 Local Collection	<u>Cynodon dactylon</u> $2n = 36$	Short, stoloniferous & rhizomatous	Glabrous	Prominent hairs	1.5-3.5 cm.
2 do	do	Short, stoloniferous & rhizomatous	Glabrous	Hairy	1-2 cm.
3 do	$2n = 40$	do	Glabrous	Hairy	2 cm.
4 do	$2n = 36$	Medium, stoloniferous & rhizomatous	do	do	3-7 cm.
5 do	$2n = 36$	Tall, stoloniferous & rhizomatous	do	do	6 cm.
6 do and	$2n = 36$ $2n = 40$	Short, stoloniferous & rhizomatous	do	do	.5 cm.
7 do	<u>Cynodon dactylon</u> $2n = 36$	Short, stoloniferous & rhizomatous	Glabrous	Hairy	1.5-4 cm.
8 do	do	Medium, stoloniferous & rhizomatous	do	Long hairs	2 cm.

TABLE III (continued)

Accession	Species	Color of Inflorescence	Whorl No.	Spike Nos.	Spike Length	Spikelet	Glumes	Reduced floret on rachilla
8801	<u>Cynodon dactylon</u> <u>2n = 36</u>	Green & yellow	1	4-5	3-6 cm.	2-2.5 mm.	2-2.5 mm.	-
8802	do	do	1	4-5	3.5 cm.	3 mm.	2 mm.	-
9217	do	do	1	4	3-6 cm.	2 mm.	2 mm.	-
9226	do	do	1	4-5	6-8 cm.	2 mm.	2 mm.	✓
PI 266697	do	Pink & green	1	4-5	4-8 cm.	2-3 mm.	2-3 mm.	-
PI 267985	do	Pink	2	4-5	3-4 cm.	1-2 mm.	1.5-2 mm	-
PI 268324	do	Pink & green	1	4-5	1-3 cm.	1-2 mm.	1-1.5 mm	✓
9230	<u>2n = 18</u>	Green & yellow	1	4-7	4.5-6 cm.	2-2.5 mm.	2-2.5 mm.	-
9233	do	Deep Pink	1	4-8	3-6 cm.	2 mm.	2 mm.	-
9234	<u>2n = 18</u>	Pink & green	2	5-8	3-5 cm.	2 mm.	1.5 mm.	-
9235	<u>C. plectostachyus</u> <u>2n = 18</u>	Green & yellow	3-6	3-6	2-3 cm.	3 mm.	1 mm.	-
Collection from S. Africa	<u>C. magennisii</u> <u>2n = 27</u>	Green & pink	1	3-4	1.5-2.5 cm.	2 mm.	1 mm.	-
1 Local Collection	<u>Cynodon dactylon</u> <u>2n = 36</u>	Pink	1	3-4	2.5-4 cm.	2 mm.	1.5 mm.	✓
2 do	do	Green	1	4	2-4 cm.	2 mm.	1.5 mm.	-
3 do	do <u>2n = 40</u>	Green & Pink	1	3-5	2-3.5 cm.	2 mm.	1-1.5 mm.	-
4 do	do	Green & pink	1	4	3-5.5 cm	2 mm.	2-1.5 mm.	-

TABLE III (continued)

Accession	Species	Color of Inflorescence	Whorl No.	Spike Nos.	Spike Length	Spikelet	Glumes	Reduced floret on rachilla
5 Local Collection	<u>Cynodon dactylon</u> <u>2n = 36</u>	Green & pink	1	5-6	4-6.5 cm.	2 mm.	2-1.5 mm.	-
6 do and	do <u>2n = 36</u> <u>2n = 40</u>	Deep pink	1	3-5	1.5-3.5 cm.	2 mm.	2 mm.	-
7	<u>2n = 36</u>	Green & pink	1	3-5	1.5-3.5 cm.	2 mm.	1.5 mm.	-
8	do	Green & light pink	1	4-6	3-4 cm.	2 mm.	1.5 mm.	✓

DISCUSSION

In this study the taxonomic treatments of Cynodon used by Chippindall (1955) and Bogdan (1949) were followed in distinguishing the species C. plectostachyus from C. dactylon.

According to Bogdan (1949) the most conspicuous difference between the two species lies in their flowering heads and spikelets. But while Bogdan finds the numerous spikes in C. plectostachyus are in two whorls on a distinct common axis, Chippindall (1955) shows them in more than two whorls. In this study the spikes are seen in more than two whorls (Plate II, Fig. 2).

Metcalf (1960) notes that leaf hairs (macro hairs) are generally of specific diagnostic value in taxonomy. He, examining three herbarium specimens of C. dactylon, finds variation in the quality and distribution of leaf hairs on the abaxial surface of the leaves. In this study, C. plectostachyus is readily differentiated from C. dactylon by having very prominent long erect hairs on the leaf, leaf sheath and ligule. Within C. dactylon, the tetraploids from Afganistan, Iran and Pakistan have hairy leaves, while the very fine, short growing tetraploid species from India has glabrous leaves. The tetraploid strains collected locally also have glabrous leaves. In the diploid races of C. dactylon from Ethiopia, the accessions 9230 and 9233 have glabrous leaves, while 9234 has short hairs on the leaf and the leaf sheath is hairy. Hairs in the ligule are very prominent in the accessions 9230 and 9234 while in 9233 practically no hair is noticed in the inconspicuous ligule. In the

triploid C. x magennisii, the fine narrow leaves are glabrous.

Cross (1939) claims that the length and structure of rachilla prolongation are diagnostic characters in differentiating various forms of C. dactylon. In this study some of the plants of C. dactylon show a prolonged rachilla bearing reduced floret. However, on the basis of rachis length, the different accessions and local collections of C. dactylon fall broadly under two categories namely, length varying from 1-3 cm. which are short growing plants with narrow leaves representing one morphological type. The other type has spike length from 3 to 6 cm. representing the tall type.

Hurcombe (1947) is of the opinion that the only reliable and constant characters are the presence or absence of rhizomes, the nature of rachilla, and the length of the glumes in relation to that of the spikelet. In the present context, all the plants of C. dactylon are stoloniferous, some are rhizomatous, and C. plectostachyus grows only by dense net work of stolons. Glume characters are found to be of considerable importance diagnostically. Some locally collected tetraploid C. dactylon show prominent glumes as long as the spikelets (Plate I, Figs. 4 and 6). In some accessions also, glumes have been found to be of the same length as the spikelet. In C. x magennisii glumes are found to be 1/2 the length of spikelet. According to Hurcombe (1947) the glume lengths in relation to spikelet length in C. dactylon, C. x magennisii and C. transvaalensis are 1, 1/2 and 1/4 respectively, indicating the intermediate position of the hybrid C. x magennisii. In C. plectostachyus the glume length is found to be 1/3 the length of spikelet.

Bogdan (1949) studied the morphology of C. dactylon species and observed two ecotypes - one is riparian type of low growth, usually six

to twelve inches high with fine leaves and stems, bright green and somewhat yellowish in color. The other is dry ground type, widely distributed, taller, comparatively robust. In the present study two morphological types have been observed in the tetraploid species of C. dactylon. The accessions from Ethiopia, and Afganistan are the robust, erect type growing by stolons whereas two accessions from India and Pakistan are short type with fine narrow leaves and growing both by stolons and rhizomes.

The tall type has been observed in the diploid species of C. dactylon from Ethiopia. In the local collections of Cynodon dactylon, which are tetraploid, both types namely short and tall have been observed.

The present cytological study revealed that Cynodon has diploid species with the chromosome number $2n = 18$, triploid species with $2n = 27$ and tetraploid species with chromosome number $2n = 36$ and 40 . A previous study (Table I) indicated that Cynodon has one hexaploid race with chromosome number $2n = 54$.

In the present study it was observed that the C. dactylon tetraploids, with two distinct morphological types namely short and robust growing, have varied distribution in India, Pakistan, Iran, Afganistan, Ethiopia and in the local place Stillwater. The tall growing diploids of C. dactylon and C. plectostachyus have their distribution in Ethiopia.

Various sizes and shapes of meiotic chromosomes had been observed in the present context. In accession 9233 a diploid C. dactylon from Ethiopia, a subhumid region, very large size of chromosomes (Plate IV, Fig. 5 and 6) were noticed, whereas in accession 9234, which is also a diploid C. dactylon from the same place, chromosomes observed were comparatively smaller under the same magnification (Plate IV, Fig 8). In

another diploid accession 9230 from the same area medium size chromosome, more or less oblong shaped were observed (Plate IV, Fig 7). In the Stillwater collection the tetraploid C. dactylon shows medium and small chromosome size having round, oval and oblong shape (Plate III, Figs. 1, 2, 3, 4 and 7).

The origin and significance of the chromosome fragments are not completely understood. The fragments may originate from reciprocal translocation breakage as proposed by Swanson (1958) or they may be actually univalent chromosome themselves and not fragments. In Cynodon Forbes and Burton (1963) pointed out another possibility that these fragment chromosomes could have been the satellites of the nucleolar chromosomes. In the present study the tetraploid C. dactylon from Afganistan, Ethiopia and Stillwater collections have large satellites of the nucleolar chromosomes. There is also a greater frequency of fragment chromosomes which may be the satellites broken off the nucleolar chromosome during the cytological preparations. In the diploid C. dactylon from Ethiopia, namely, accessions 9230, 9233 and 9234 satellites of the nucleolar chromosomes were not observed and fragments were never present. This study is strikingly similar to that of Forbes and Burton (1963) in which one can find from the table of meiotic chromosome behavior in some diploid Cynodon species (p. 76) that the accessions of C. dactylon from Afganistan, S. Africa, Kenya and Rhodesia have no satellite chromosomes. But in one case, namely NK-37, a highly fertile rhizomatous form which is naturalized in the Southwest United States, there were satellites of the nucleolar chromosomes giving the appearance of being extra whole chromosomes.

Further studies of satellite chromosomes reveal some interesting facts. Forbes and Burton (1963) studied four different diploid Cynodon sp. namely C. bradleyi, C. incompletus, C. transvaalensis and C. plectostachyus in which satellites of nucleolar chromosomes were present. In the present study of the C. plectostachyus from Ethiopia, the nucleolar organising chromosome is readily recognizable, it being darkly stained and very often with large satellite chromosomes (Plate IV, Fig 3).

The diploid and tetraploid species of Cynodon have considerable affinity for each other and hybrids are obtained by crossing them as well as some natural hybrids are formed. These hybrids are triploid and completely sterile. C. x magennisii, a completely sterile natural hybrid, has been studied in the present context and the cytological observations may reveal some facts of phylogenetic interest. Plate IV, Fig 1 shows diakinesis in C. x magennisii with 13 bivalents and 1 univalent. Two pairs of nucleolar chromosomes with big satellites can be easily recognized. In Fig. 2 of the same plate two different nucleoli each having one pair of nucleolar chromosomes can be seen. These suggest that considerable homology exists between the chromosomes of the parental species which are supposed to be C. dactylon with chromosome number $2n = 36$ and C. transvaalensis with chromosome numbers $2n = 18$.

In the present study the tetraploid C. dactylon from two local gardens in Stillwater showed chromosome number $2n = 40$. In the accessions and other local collections tetraploid C. dactylon had a chromosome number of $2n = 36$. In the accessions the diploid C. dactylon and C. plectostachyus the chromosome number was found to be $2n = 18$, and a triploid C. x magennisii, which originated in S. Africa had a chromosome number of $2n = 27$. Thus, either Cynodon has two basic chromosome numbers,

9 and 10, or there are aneuploid races present.

According to Forbes and Burton (1963), all of the chromosome numbers in multiples of 10, as reported by other authors, were based on plants with $2n = 18, 27$ or 36 , in which satellites of the nucleolar chromosomes were counted as whole chromosomes and so the existence of a second basic chromosome number $x = 10$ is questionable.

Hurcombe and other authors in C. dactylon, C. hirsutus, C. x magennisii, and C. plectostachyus found the chromosome numbers to $2n = 40, 20, 30$ and 20 respectively suggesting a basic number $x = 10$. In C. bradleyi a chromosome number of $2n = 18$ was recorded, which Hurcombe explained on the assumption that C. bradleyi is an aneuploid resulting from nondisjunction or unequal distribution of the chromosomes, and gametes with one chromosome less than the usual number may arise, and the union of two such $n-1$ gametes would result in a form with one pair less than the usual somatic complement. According to Sharp (1926) such aneuploid forms differ more from the normal diploid than do polyploid forms. Church (1929) coined the term "disploids" for these "irregular deviations from the fundamental haploid base in a polyploid series" and discovered that these "disploides" had an obvious hybrid origin. He concluded that hybridity and disploidy were frequently correlated. The fact that C. bradleyi is completely sterile suggests that this grass may also be of hybrid origin. Such an origin might certainly account for its unusual chromosome number. So Hurcombe considered C. bradleyi to be an aneuploid species derived from an ancestor with a basic number of $x = 10$ rather than a diploid species with a basic number of $x = 9$. Again, Moffett and Hurcombe (1949) found a somatic chromosome number of $2n = 18$ in diploid C. plectostachyus and also a somatic chromosome number of $2n = 54$ in hexaploid C.

plectostachyus from South Africa, suggesting the basic chromosome number to be $x = 9$. So it is quite probable that Cynodon has two basic chromosome numbers $x = 9$ and 10 which have given the genus more variability and adaptability. There is every likelihood, as observed in the present study of local collections, that Cynodon might have added germ plasm to it from other species as it has invaded more cooler temperate regions in the Americas. A study of Bothriochloinae by Harlan et al. (1961) revealed the same fact that Bothriochloa intermedia also picks up germ plasm from other species like B. ischaemum as it increases its range. Bowden's (1960) study also revealed that some genera of Canadian grasses such as Agrostis, and Calamagrostis have more than one basic chromosome number.

The cytological study in the present context indicates that irregular meiosis leading to genetic dysfunctioning of cells may be a cause of sterility in the local population of Cynodon dactylon.

SUMMARY

Cytological and morphological studies were made on twelve accessions including one natural hybrid and eight local collections of Cynodon species.

Three accessions from Ethiopia were found to be diploid Cynodon dactylon with chromosome numbers $2n = 18$. One accession from Ethiopia was found to be diploid C. plectostachyus with a chromosome number $2n = 18$. Other accessions from Afganistan, Ethiopia, Iran, Pakistan and India were found to be tetraploid C. dactylon with chromosome numbers $2n = 36$. Two local collections from Stillwater were found to be tetraploid C. dactylon with chromosome numbers $2n = 40$ and six other local collections were found to be tetraploid C. dactylon with chromosome numbers $2n = 36$. C. x magennisii, a natural hybrid which originated in S. Africa, was found to be triploid with chromosome number $2n = 27$. This study indicates that Cynodon has two basic chromosome numbers namely, $x = 9$ and 10 or has some aneuploid races.

Meiotic study reveals that the tetraploid C. dactylon collected locally and abroad and also the diploid C. plectostachyus have distinct satellites of the nucleolar chromosome, and chromosome fragments appear in greater or lesser frequency. The diploid C. dactylon accessions show no such satellite of the nucleolar chromosome or chromosome fragments. Meiotic irregularity observed more or less in the natural population from Stillwater may be one of the causes of genetic malfunctioning of the cells leading to incapability of the plants for producing viable seeds.

The diploid Cynodon accessions have been found to be quite regular in their meiotic behavior and the tetraploid Cynodon accessions also show more or less regular meiosis.

Meiotic studies of the natural hybrid C. x magennisii indicated that there is considerable homeology of the chromosomes in the parental species. This may suggest that in the remote past, the first polyploid series of Cynodon started from a lesser basic chromosome number and after years of reversion and natural hybridization the present segmental allopolyploid series of Cynodon has evolved.

On the basis of morphological characters the different accessions and local collections of Cynodon dactylon fall broadly under two categories namely the short growing type and the tall type.

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