Basic chromosome numbers and polyploid levels in some South African and Australian grasses (Poaceae)

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ABSTRACT

Chromosome numbers of 46 specimens of grasses, involving 24 taxa from South Africa and Australia, have been determined during the present study. For the first time chromosome numbers are given for *Eragrostis sarmentosa* (Thunb.) Trin. (n = 20), *Panicum aequinerve* Nees (n = 18), *Digitaria argyrograpta* (Nees) Stapf (n = 9) and *D. maitlandii* Stapf & C.E. Hubb. (n = 9). Additional polyploid levels are described for *Diplachne fusca* (L.) Beauv. ex Roem. & Schult. (n = 10) and *Digitaria diagonalis* (Nees) Stapf var. *diagonalis* (n = 9).

B-chromosomes were observed in several different specimens. The presence of B-chromosomes often results in abnormal chromosomal behaviour during meiosis.

INTRODUCTION

Raven (1975) regarded cytogenetics as an important element in the evaluation of relationships and in the determination of phylogenetic sequences in the angiosperms. In South Africa this useful taxonomic tool has not been used widely and plant cytogenetics can be considered to be one of the most neglected fields of botany. Thorough cytogenetic studies are restricted to a few economically important species and the most basic cytogenetic data, the chromosome numbers of the taxa, are not available for the majority of our indigenous species.

In an attempt to increase our cytogenetic knowledge of the South African flora, a cytogenetic study of the family Poaceae was initiated by the Botanical Research Institute during 1986 and is now continued at the National Botanical Institute, the Grassland Research Centre and the Department of Botany and Genetics at the University of the Orange Free State. Results were reported in previous publications in this series (Spies & Du Plessis 1986a, b, 1987a, b, 1988; Spies & Jonker 1987; Spies & Voges 1988; Du Plessis & Spies 1988; Spies et al. 1989). The present paper reports on miscellaneous unpublished chromosome numbers and aims to determine whether this information can contribute to our knowledge on the basic chromosome numbers and polyploid levels present in the South African Poaceae.

MATERIALS AND METHODS

Cytogenetic material was collected in two different ways for the purpose of this study. The material was either collected and fixed in the field, or living material was collected in the field and transplanted in the nursery of the National Botanical Institute, Pretoria, where cytogenetic material was later collected and fixed. The material used and localities of origin are listed in Table 1. Voucher specimens are housed in the National Herbarium, Pretoria (PRE).

Young inflorescences were fixed in Carnoy's fixative (Carnoy 1886). The fixative was replaced by 70% ethanol after 24–48 hours of fixation. Anthers were squashed in aceto-carmine (Darlington & La Cour 1976). Contrast between cytoplasm and chromosomes was enhanced by adding a small droplet of 45% acetic acid, saturated with iron acetate, to the stain immediately prior to making the squash (modification of method used by Thomas (1940)). Slides were made permanent by freezing them with liquid CO₂ (Bowen 1956), followed by dehydration in ethanol and mounting in Euparal. An Olympus Vanox-S photomicroscope and Ilford Pan-F film were used for the photomicrographs. At least ten cells per specimen were studied for each meiotic stage, except where otherwise indicated.

RESULTS AND DISCUSSION

The haploid chromosome numbers observed are listed with the voucher specimen numbers and their localities in Table 1. The classification of subfamilies and tribes follows Clayton & Renvoize (1986). Unless otherwise indicated, meiotic chromosome behaviour was normal.
TABLE 1. — List of species studied, haploid chromosome numbers, voucher specimen numbers and localities according to the degree reference system (Edwards & Leistner 1971)

<table>
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<th>Taxon</th>
<th>Haploid chromosome number</th>
<th>Locality and voucher number</th>
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| **Subfamily Arundinoideae**
| Tribe Aristidae
| Aristida congesta subsp. congesta | 11 | NATAL. — 2940 (Pietermaritzburg): 5 km from Muden to Greytown, (−BA), Du Plessis 137. |
| Stipagrostis obtusa | 22 | NAMIBIA — 2616 (Aus): 10 km east of Aus, (−CB), Spies 2905. |

| **Subfamily Chloridoideae**
| Tribe Arundinoideae
| Aristida congesta subsp. congesta | 22 | NAMIBIA — 2617 (Bethan): near bridge over Fish River on road between Seeheim and Luderitz, (−DD), Spies 2898. |
| Subtribe Elesinusinae
| Deplachne fusca | 10 | CAPE. — 3018 (Kamiesberg): 52 km from Springbok to Loeriesfontein, (−AA), Spies 3373. |
| E. cilianensis | 10 | TRANSVAAL. — 2528 (Pretoria): Soutpan Experimental Farm, (−AC), Spies 3287. |
| E. sarmentosa | 20 | CAPE. — 3140 (Vanrhynsdorp): Gilberg Pass, (−DC), Spies 3065. |

| **Subfamily Panicoideae**
| Tribe Panicaceae
| Panicum sphaerocarpum | 18 | SWAZILAND. — 2631 (Mbabane): 18 km north-east of Mbabane, (−AC), Spies 3255. |
| P. maximum | 16 | SWAZILAND. — 2631 (Mbabane): 55 km from Siteki to Manzini, (−AD), Spies 2603. |
| Brachiaria brizantha | 18 | CAPE. — 3028 (Mattedale): Antelope Park, (−DC), Spies 2528. |
| Urochloa mosambicensis | 14 | TRANSVAAL. — 2528 (Pretoria): cultivated varieties collected at Roodeplaat Experimental Farm, (−AC), Spies 3725. |

| **Subfamily Andropogoneae**
| Tribe Andropogoneae
| Sorghum australiense* | 10 | TRANSVAAL. — 2528 (Pretoria): cultivated in the garden of the National Botanical Institute, Pretoria, (−AC), Spies 1711. |
| S. maturakens* | 5 | TRANSVAAL. — 2528 (Pretoria): cultivated in the garden of the National Botanical Institute, Pretoria, (−AC), Spies 1715. |
| S. stipeoides* | 5 | TRANSVAAL. — 2528 (Pretoria): cultivated in the garden of the National Botanical Institute, Pretoria, (−AC), Spies 1738 & 1741. |
| S. aff. stipeoides* | 5 | TRANSVAAL. — 2528 (Pretoria): cultivated in the garden of the National Botanical Institute, Pretoria, (−AC), Spies 1740. |

* Seed originally collected by M. Andrew in Australia.
Subfamily Arundinoideae Tateoka

Tribe Aristideae C.E. Hubb.

The haploid chromosome number of \( n = 11 \) for *Aristida congesta* Roem. & Schult. subsp. *congesta* (Figure 1A, B) corresponds with published cytogenetic information on this taxon (De Winter 1965; Davidse et al. 1986; Spies & Jonker 1987). The occurrence of \( n = 11 \) as the lowest haploid chromosome number in the genus and the presence of multiples of 11 in other species of this genus (De Winter 1965; Spies & Du Plessis 1986a, 1987b; Viano & Bourreil 1987), support 11 as the basic chromosome number for both the genus *Aristida* L. and the tribe.

The basic number of \( x = 11 \) for the tribe Aristideae is further supported by our observation of a *Stipagrostis obtusa* (Del.) Nees specimen with a haploid chromosome number of \( n = 22 + 0 - 3B \) (Figure 1C). Chromosome laggards were frequently observed (Figure 1D) and it is suggested that the laggards represent the B-chromosomes, undergoing chromatid segregation. This is, to the best of our knowledge, the first report on the presence of B-chromosomes in this tribe. Polyploidy seems to be present in this species with our specimen being tetraploid and the one examined by Reese (1957) diploid (2n = 22).

Subfamily Chloridoideae Rouy

Tribe Eragrostideae Stapf
Subtribe Eleusininae Dumort.

The diploid chromosome number of \( n = 10 \) for *Diplachne fusca* (L.) Beauv. ex Roem. & Schult. (Figure 1E) is the lowest chromosome number yet described for this species and it supports a basic chromosome number of \( x = 10 \) for this species, genus, subtribe, tribe and subfamily. Published results indicate the presence of tetraploidy (Bir & Sahni 1986) and aneuploidy (Spies & Voges 1988).

A basic chromosome number of \( x = 10 \) for *Eragrostis* Wolf is substantiated by the diploid *E. cilianensis* (All.) F.T. Hubb. specimen observed during this study. The presence of diploid and tetraploid specimens of this species is well documented (Fedorov 1969; Moore 1973; Goldblatt

![FIGURE 1. —Meiotic chromosomes in some grass specimens. A, B, Aristida congesta subsp. congesta, Du Plessis 137, n=11; metaphase I; C, D, Stipagrostis obtusa, Spies 2905, n=22, anaphase I with laggards (L); E, Diplachne fusca, Spies 3373, n=10, diakinesis; F, Eragrostis capensis, Spies 3975, n=20, diakinesis. Bar = 10 \( \mu \text{m} \).](image-url)
1983, 1988; Goldblatt & Johnson 1990). The tetraploid *E. capensis* (Thunb.) Trin. specimen (Figure 1F) supports observations indicating different ploidy levels for this species, i.e. diploid (De Wet 1958), tetraploid (Avdulov 1931; Pienaar 1955; Spies & Du Plessis 1986a; Davisdie et al. 1986) and hexaploid (Moffett & Hurcombe 1949; Spies & Voges 1988). To the best of our knowledge the tetraploid level observed for *E. sarmentosa* (Thunb.) Trin. is the first report on a chromosome number for this species.

Subtribe Sporobolinae Benth.

The genus *Sporobolus* R. Br. is cytogenetically complex and basic chromosome numbers of \( x = 6, 9 \) and 10 seem to be present (Davidse et al. 1986). The haploid chromosome numbers of \( n = 12, 18 \) and 24 observed during this study in *S. africanus* (Poir.) Robyns & Tournay (Figure 2) support a basic chromosome number of \( x = 6 \) for this species. Polyploid levels vary from tetraploid \( (2n = 4x = 24) \) to decaploid \( (2n = 10x = 60) \) (Fedorov 1969; Spies & Du Plessis 1986b; Spies & Jonker 1987; Spies & Voges 1988). A thorough cytogenetic investigation of this genus is necessary to determine the phylogenetic relationships.

The presence of B-chromosomes in some specimens impedes the interpretation of the results (Figure 2C). The number of B-chromosomes varied from 0 to 6 per meiotic cell in a single specimen, which indicates that they are mitotically unstable. Occasionally a B-chromosome could be distinguished by its position in the cell but the majority of B-chromosomes resembled the euchromosomes. These chromosomes are considered to be B-chromosomes, judging by the variation in their numbers in different cells.

Tribe Cynodonteae Dumort.

Subtribe Chloridinae Presl.

This report on *Harpochloa fals* (L.f.) Kuntze corresponds with published data on this species (Fedorov 1969; Spies & Du Plessis 1986a) which seems to indicate a basic chromosome number of ten, with all the specimens studied being tetraploids.

Subfamily Panicoideae A. Br.

Tribe Panicae R. Br.

Subtribe Setariinae Dumort.

The chromosome number of \( n = 18 \) observed for *Brachiaria brizantha* (A. Rich.) Stapf during this study, equals the lowest chromosome number reported for this species (Darlington & Wylie 1955; Ornduff 1967–1969; Fedorov 1969; Moore 1970–1977; Goldblatt 1981–1988; Goldblatt & Johnson 1990). In addition to this number, a higher ploidy level of \( n = 27 \) has also been described in Fedorov (1969) and by Spies & Du Plessis (1987b), as well as by Basappa et al. (1987). The basic chromosome number, however, is considered to be \( x = 9 \) (Darlington & Wylie 1955; Ornduff 1967–1969; Fedorov 1969; Moore 1970–1977; Goldblatt 1981–1985; Goldblatt & Johnson 1990). These results contradict the meiotic configuration of \( 12_{12}^1 \), observed by Nath et al. (1970), which suggests a basic chromosome number of 12 for the species.

This seems to be the first report on the chromosome number of *Panicum aequinerve* Nees. The haploid chromosome number of 18 indicates a basic chromosome number of \( x = 9 \) for this genus and species. The absence of multivalents suggests an allopolyploid origin for this specimen (Figure 3A). In contrast to this basic number, we confirm numerous reports of a somatic chromosome number of \( 2n = 32 \) for *Panicum maximum* Jacq. (Ornduff 1967–1969; Fedorov 1969; Moore 1970–1977; Goldblatt 1981–1988; Goldblatt & Johnson 1990). Contrary to these reports several other somatic numbers are reported in the same sources \( (2n = 18, 28, 34, 36, 42, 48, 52, 54) \). We found it very difficult to obtain well-spread meiocytes in this species and this may be a reason for the discrepancies in the chromosome numbers reported for this species. A re-investigation of the phylogenetic relationship between *P. maximum* and other *Panicum* species is necessary.

*Urochloa mosambicensis* (Hack.) Dandy with \( 2n = 4x = 28 \), supports the deviated basic chromosome number of \( x = 7 \) for this species reported by Spies & Du Plessis (1987b). The formation of one ring quadrivalent in almost all diakinesis cells studied (Figure 3B), indicates...
a certain degree of autosyndetic chromosome pairing. The low frequency of multivalents in the presence of a high chiasma frequency (Spies 1984) indicates a segmental allopl oid origin for this specimen.

**Subtribe Digitariinae Butzin**

This is the first report on the chromosome numbers of *Digitaria argyrograpta* (Nees) Stapf (n=9) and *D. maitlandii* Stapf & C.E. Hubb. (n=9 & 36). In addition a new level of ploidy is described for *D. diagonalis* (Nees) Stapf var. *diagonalis* [n=9, in contrast to the 2n=36 described by De Wet & Anderson (1956) and Spies & Du Plessis (1987a)]. This is also the first report on the presence of B-chromosomes in *D. eriantha* Steud., where four of the 15 specimens studied had up to five B-chromosomes (Table 1).

This study included several cultivated specimens of *D. eriantha*. These cultivars are currently being evaluated for possible distribution as fodder crops by the Grassland Research Centre. Two of these 'cultivars' had two different ploidy levels. This indicates the variability present in these specimens and the need for a thorough cytogenetic investigation before these cultivars are released.

A basic chromosome number of x=9 for the genus is supported by the presence of diploid specimens in all the species studied (Figure 3C).

**Tribe Andropogoneae Dumort.**

**Subtribe Sorghinae Bluff**

A basic chromosome number of five in the Andropogoneae is evident from both the literature and some of the *Sorghum* Moench specimens used during this study. A basic chromosome number of x=5 was observed in both *S. mattrankense* Garber & Snyder and *S. stipoideum* Gardner & Hubb. These numbers correspond to the published numbers by Garber (1950, 1954), Garber & Snyder (1951) and Celarier (1956a, 1958). Although meiosis was normal in most specimens (Figure 5A–C), some cells formed micronuclei (Figure 5D).

Abnormal meiotic behaviour was observed in one of the *S. stipoideum* specimens, *Spies 1740*. Six bivalents were formed during diakinesis (Figure 4A). One of these bivalents was smaller than the rest and it is concluded that this bivalent is formed by B-chromosomes. The B-chromosomes seem to be outside the spindle. Different behaviour patterns of the B-chromosomes were observed during metaphase I. They form part of the metaphase plate (Figure 4B); one stays on the metaphase plate while the other moves to one of the poles (Figure 4C); both move towards the same pole (Figure 4D) or they move to different poles (Figure 4F). Precocious chromosome segregation during late metaphase I was observed for one bivalent in one cell (Figure 4E). The result of the different movements of the B-chromosomes is that anaphase II laggards are sometimes observed (Figure 4G). The ultimate fate of the B-chromosomes was not determined by this study.

**Subtribe Ischaeminae Presl**

Cytogenetic studies on *Ischaemum afrum* (J.F. Gmel.) Dandy seem to be restricted to our laboratories (Spies & Du Plessis 1987b). The formation of bivalents only during meiosis and the absence of specimens with a somatic chromosome number of ten indicate that this specimen can be considered to be a diploid (2n=2x=20). These results are substantiated by reported chromosome numbers for other *Ischaemum* species (Darlington & Wylie 1955; Ornduff 1967–1969; Fedorov 1969; Moore 1970–1977; Goldblatt 1981–1985; Goldblatt & Johnson 1990). However, the same reports suggest that x=9 and x=19 should be considered secondary and tertiary basic chromosome numbers respectively in the genus.

**Subtribe Andropogoninae Presl**

The chromosome number of n=10 observed for *Andropogon eucosmus* Nees, corresponds with the number published by Moffett & Hurcombe (1949) and Gould (1956). Previous studies by one of our laboratories revealed two different chromosome numbers for this species, i.e. n=10 and n=20 (Spies & Du Plessis 1987a, b). These numbers, in addition to the presence of multiples of ten in other *Andropogon* species, as well as the absence of 2n=2x=10 specimens in the genus (Darlington & Wylie 1955; Ornduff 1967–1969; Fedorov 1969; Moore 1970–1977; Goldblatt 1981–1985), suggest a basic chromosome number of x=10 for this species and genus. A deviation from this basic chromosome number was reported with x=9 for *A. lacunosus* J.G. Anders., *A. tectorum* Schum. & Thonn., *A. lima* (Hack.) Stapf and *A. distachyos* L.
FIGURE 4.—Meiotic chromosomes in a *Sorghum stipoides* specimen with B-chromosomes, Spies 1740. A, diakinesis with five bivalents and a B-chromosome bivalent; B, metaphase I with five bivalents and both B-chromosomes on the equatorial plate; C–E, metaphase I with five bivalents and both B-chromosomes on one side of the equatorial plate; F, metaphase I with five bivalents and the B-chromosomes on different sides of the equatorial plate; G, two telophase I cells with chromatid segregation of the B-chromosomes. Bar = 80 μm.
FIGURE 5.—Meiotic chromosomes in Sorghum stipoideum. A. Spies 1741, n = 5, diakinesis; B. Spies 1741, n = 5, meta-phase I. C. Spies 1738, n = 5, anaphase I; D. Spies 1738, n = 5, telophase I with micronuclei visible. Bar = 10 μm.

(Celarier 1956b; Gould 1956; Hedberg & Hedberg 1977; Okoli 1982; Spies & Du Plessis 1986a), x = 8 for A. abyssinicus Chippind. (Gould 1956) and x = 7 for A. manni Hook. f. (Davidse et al. 1986).

CONCLUSIONS

Clayton & Renvoize (1986) claim that chromosome numbers can contribute little to the taxonomy of the grasses, since the karyotype is relatively constant. Yet the relevance of cytogenetics to the taxonomy of grasses is apparent in the different basic chromosome numbers present in higher taxa, in the meiotic behaviour of chromosomes and in the mode of polyploidy which may help to unravel the phylogeny of a taxon.

A correlation exists between the basic chromosome number and the tribal classification of the majority of grasses. This study confirms that the following basic chromosome numbers are found in the following tribes: Aristideae x = 11, Eragrostideae (with the exception of the genus Eleusine and some Sporobolus representatives) x = 10, Cynodonteae x = 10, Paniceae x = 9 (with some species or genera having x = 7, 8 or 10) and Andropogoneae has a primary basic chromosome number of x = 5 and, more commonly, a secondary basic chromosome number of x = 10. Although deviations from these basic numbers are known, the phylogenetic development of higher taxa is correlated with a specific basic chromosome number and deviations from these numbers may help to solve relationships in some taxa.

Polyploidy is frequently observed in the grasses (Carnahan & Hill 1961; Goldblatt 1980) and 303 of the 388 specimens studied in our laboratories were polyploids (Spies & Du Plessis 1986a, b, 1987a, b, 1988; Spies & Jonker 1987; Spies & Voges 1988; Du Plessis & Spies 1988; Spies et al. 1989). Detailed cytogenetic studies are necessary to determine the mode of polyploidy as well as the extent of polyploidy in the South African grasses.

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