

Cytogenetic relationships between some species of *Setaria L.*(Poaceae)

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Mitotic karyotype structure, meiotic behavior, pollen characteristics and seed setting of six species of genus *Setaria* were investigated in six species of *Setaria L.* *Setaria verticillata*, *S. italica*, *S. viridis* and *S. glauca* were found diploid ($2n = 18$) and *S. sphacelata* and *S. plaicatilis* are tetraploids ($2n = 36$). Karyotype measurements of the diploid species, to some extent, reflect their genetic stability except *S. italica*. This species has the longest chromosomes among the investigated species with degree of variation between chromosomes. A degree of variation was also observed in the karyotype of tetraploids. Irregular meiosis, chain trivalents and univalents were observed in *S. italica* and *S. sphacelata*. Other diploid species show regular meiosis. Tetraploids show various types of meiotic chromosome associations. Tetraploids also showed large pollen compared with diploids. Pollen fertility and seed setting of diploid species are higher compared to that recorded for tetraploids.

Key words: Karyotype structure, Meiotic behaviour, *Setaria*.

Introduction

The genus *Setaria L.* belongs to Poaceae with more than 100 species distributed throughout tropical, subtropical and warm regions (Clayton & Renvoize 1986). Many species of this genus are of economic importance (Wang *et al* 1995).

South West part of Saudi Arabia is rich with many species and genera of the grass family (Poaceae). *Setaria* is represented by six species mostly common in that region (Chaudahry 1989). Compared to other grasses, *Setaria* has been subjected to few recent studies (Haroun 1997, Chennaveeraiah *et al* 1991 Devos *et al* 2000; Le Thierry *et al* 1998, Wang *et al* 1995, Khan 1997), with no record of any studies on the area of South West of Saudi Arabia. However, this genus is still badly in need of modern revision for its cytogenetics. This investigation has been undertaken in order to gain insight into its cytogenetics and species relationships of six species in this part of the world.

Parameters investigated in the present study are mitotic karyotype structure, meiotic chromosome behavior, pollen characters and seed setting. This work is also part of a program aimed to study the cytogenetics of grass family.

Materials and methods

Plant material for morphological description were collected from natural habitats at Abha, South South West Saudi Arabia, and kept as herbarium specimens at the Department of Biological Sciences, College of Science, King Khalid University, Abha, Saudi Arabia. Check for names was based on Chaudahry (1989) and Migahid (1978).

For karyotype studies, excised root-tips of germinated seeds were pretreated in % 0.25 colchicine for 3h, fixed in 1:3 v/v acetic alcohol for 24h, hydrolyzed in I N HCl at 60° C for 10 min., washed in distilled water and stained in Feulgen reagent for 3h. Three to five

well-spread, fully contracted metaphase plates were used to construct karyograms. Cells were photographed at a magnification of $x = 1200$ using X30 Olympus photomicroscope. Karyotypes were compared using a karyotype similarity index (KSI) (Richards 1972, Booth & Richards 1976). This is a numerical technique designed to estimate the degree of resemblance between two karyotypes of any chromosome numbers.

The score calculated as follow:

Chromosome type	2x	4x	Score
A	2	2	1
B	2	4	2
C	4	8	4

The maximum score of identical karyotypes always equal the dipliod chromosome count. KSI of the studied species were compared and the comparisons are plotted on a kulcinski square, grouping similar ploidy levels together.

For meiosis and pollen characteristics, young buds were fixed in fresh fixative solution at early morning. Anthers were macerated and squashed in 2% acetocarmine using iron mordant needle for good preparation. Dividing cells were examined at diakinesis and metaphase I stages for chromosome association and chiasma frequency.

Pollen stainability was assessed using 2% acetocarmine. Unstained pollens were considered sterile. Diameters of about 50 pollen were measured using calibrated eye piece and calculated as mean value for each species.

Seed setting was estimated at natural open pollination condition by examining five panicle for each species. About 100 florets were examined for each panicle and seed setting was calculated as percentage.

Results and discussion

A. Morphology

1. *Setaria verticillata*

This species is tufted annual grass, about 40 cm tall. Leaf blade up to 20 cm long and 4-10 wide. Panicle more or less up to 10 cm long with spikelets of 1-2.5mm long. Lower glume shorter than upper glume. This species is widely distributed in all regions especially in cultivated areas. The present collections were from Najran and Abha, South West Saudi Arabia. No morphological differences were recorded in samples from the two places. Compared to the same species investigated from Egypt (Haroun 1997), no significance differences are evident except for plant height and leaf blade length.

2. *Setaria italica*

Plants of this species are tall up to 1.5 meter, rhizomatous and perennial. Leaf blade up to 50 cm long and about 15mm wide. Panicle more dense and up to 30 cm long. Spikelets about 2.5 mm long. Upper and lower glumes shorter than spikelet. The upper is longer than the lower. Lower lemma thin and sterile. The species is widely distributed in cultivated areas.

3. *Setaria viridis*.

Annual grass, up to 30 cm tall. Leaf blade about 15 cm long and about 12 mm wide. Panicle about 8 cm long with spikelets of 1.5 mm. Lower glume about one third of the spikelet, the upper one as long as the spikelet. Lower glumes sterile and palea is as tall as the length of lower lemma. The species is common and widely distributed.

4-*Setaria glauca* (pumila)

Tufted annual grass scattered in agricultural areas. Plants are 50-100 cm tall. Leaf blade of about 20 cm long and 7mm wide. Panicle dense and cylindrical. Spikelets ovate, about 2-5 mm long, Glumes shorter than spikelet. Lower and upper lemma are staminate with palea almost the same length as the lemma.

5. *Setaria sphacelata*

Perennial grass with rhizomes. Culms about 1.5 m tall and glabrous. Leaf blade flat up to 40 cm long and 5-15 mm wide. Panicle dense and cylindrical, up to 50 cm long. Spikelets purple 2.5 mm long . Lower glume shorter than the upper one. Both are shorter than spikelet.

6. *Setaria plaicatilis*

Tufted perennial grass, up to 1m tall. Leaf blade of about 30 cm long and 20 mm wide. Panicle 25 cm long. Spikelets about 2.5 mm long, lower glume up to the length of spikelet, upper glume is slightly taller than lower. Lower lemma as long as the spikelet. Upper lemma smooth and shiny

B - Karyology

The karyotype of the investigated species are presented in Fig. 1. Four are diploids with $2n = 18$. This number is common to many genera and species of *Poaceae* (Haroun 1991, 1997, 2000, Devos *et al* 2000, Le Thierry 1998, Bala Sachdeva 1990). Except for *Setaria italica*, diploid species do not differ markedly in the mean chromosome length. This species has significantly longer chromosomes (1.47 μm) compared to other species (Fig.1-b). This result agrees with that previously recorded by Chandola (1959). The mean value of chromosome length recorded for diploids being 1.18 μm against 1.29 μm for tetraploids (Table 4). This finding is in contrast to that previously recorded by Haroun *et al* (1992) for the same ploidy levels for species of the genus *Panicum* (*Poaceae*).

The karyotype measurements recorded for *S. verticillata* are significantly high compared with that previously recorded for the same species from Egypt (Haroun, 1997). No satellited chromosomes or secondary constrictions are observed in the chromosome set (Fig. 1.a). Karyotype morphology and measurements of this species also differ significantly from that recorded for *S. italica*. This result indicates that the two species are cytogenetically different as previously stated by Poirier and Pernes (1986). Based on life form, perennial species of *Setaria* tend to have slightly longer chromosomes than annuals, in the present study three of the four diploids are annuals and the two tetraploids are perennials.

With regard to centromere position as assessed by arm ratio, all species have metacentric, submetacentric and telocentric types of chromosomes (see Table 1; Fig. 1). Variation in chromosome length within the karyotype set of *S. italica* and the high values of standard errors recorded for this parameter, and also for arm ratio reflect, to great extent, the heterogeneity of karyotype structure of the species. This is in agreement with Devos *et al* (1998), who observed some sort of rearrangement in the genome structure of this species. This rearrangements is probably due to wide distribution of the species in cultivated areas where possibilities of partial crosses with unknown compatible grasses is available and even highly expected.

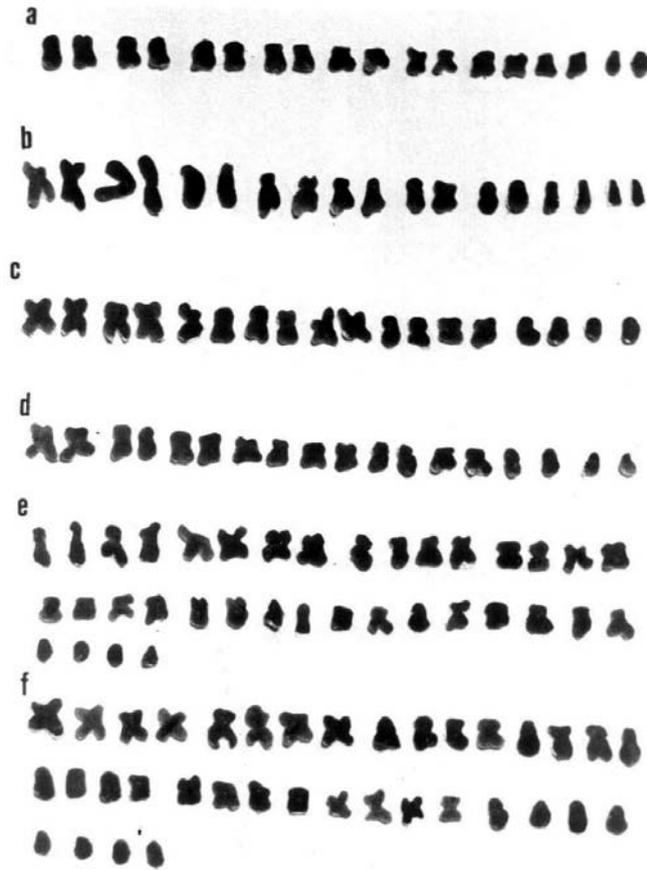


Fig. 1(a-f): Karyotypes of six species of *Setaria*. a, *S. verticillata* ; b, *S.italica*; c, *S. viridis* ; d, *S. glauca*; e, *S. sphacelata*; f, *S. plaicatilis*

Table 1: Chromosome number, mean chromosome length, mean arm ratio and karyotype formula of the *Setaria* species examined.

Species	Life form	2n	Chromosome Length \pm S.E	Arm Ratio \pm S.E.	Karyotype Formula
<i>Setaria verticillata</i>	Annual	18	0.99 \pm 0.07	1.27 \pm 0.07	12m + 4sm + 2t
<i>Setaria italica</i>	Perennial	18	1.47 \pm 0.11	1.34 \pm 0.18	10m + 6sm + 2t
<i>Setaria viridis</i>	Annual	18	1.31 \pm 0.08	1.30 \pm 0.80	14m + 2sm + 2t
<i>Setaria glauca</i>	Annual	18	1.15 \pm 0.09	1.24 \pm 0.09	12m + 4sm + 2t
<i>Setraia sphacelata</i>	Perennial	36	1.21 \pm 0.13	1.11 \pm 0.12	22m+ 10sm + 4t
<i>Setria plaicatilis</i>	Perennial	36	1.37 \pm 0.09	1.19 \pm 0.15	28m + 4sm + 4t

Table 2: Karyotype similarity matrix coefficient for the six studied species of *Setaria*.

<i>S. verticillata</i>	100					
<i>S. italica</i>	23	100				
<i>S. viridis</i>	56	67	100			
<i>S. glauca</i>	67	39	67	100		
<i>S. sphacelata</i>	34	39	50	39	100	
<i>S. plaicatilis</i>	39	45	39	45	39	100
Species	<i>S. vert.</i>	<i>S. ital.</i>	<i>S. viri.</i>	<i>S. gla.</i>	<i>S. sph.</i>	<i>S. plaic.</i>

Based on karyotype structure and measurements it is of interest to note that the two species *S. italica* and *S. viridis* are highly similar (Fig.1- b & c respectively). This points to a close genetic relationship between the two species as previously recorded by Wang *et al* (1995) who found no isozymatic differences between them. Li *et al* (1948) in fact had earlier concluded that *S. viridis* is the probable mediate progenitor of the cultivated *S. italica*. The karyotype morphology (Fig.1-d) and measurements of *S. glauca* reflect its genetic stability as suggested for most diploids.

Regarding tetraploids, karyotype morphology (Fig. 1- e & f), measurements and standard error values (Table 1) indicate high degree of variation between chromosomes in the set of *S. sphacelata* but not in that of *S. plaicatilis*. This, to great extent, reflects the heterogeneity of its genome. In this species, a degree of reorganization between chromosomes in the complement, accompanied with asymmetrical arm ratios has probably occurred. Based on these findings, it is predicted that this species is allotetraploid and a possibility of incorporation of two different diploid genomes in its structure is highly expected.

Different types of chromosomes were identified in the species investigated. The length ranged from 0.8 to 2.4 μ m and the centromere positions varied from median to telocentric. Satellites or accessory (B) chromosomes were not identified (Fig.1).

Comparison of the karyotype of the species species by means of karyotype similarity index (KSI) and karyological relationships are displayed in table 2. The lowest degree of similarity (23%) is recorded between *S. italica* and *S. verticillata*. The former also shows low similarity with all species except *S. viridis* (67%), which support the close genetic relationship between the two species (Wang *et al* 1995). The same value of similarity was also recorded between the two annual species *S. verticillata* and *S. glauca*. The two tetraploids recorded a similarity of 39%, which reflect their different origin and distinct karyotype structure.

Based on life form and habit the lowest degree of similarity recorded (23%) was between an annual and a perennial species. This to some extent suggests that this character is cytogenetically controlled. The highest value is recorded between diploids and tetraploids was 50% and is recorded for *S. viridis* and *S. sphacelata*. As the latter shows karyologically derived origin, it could be said that one of diploids (probably *S. viridis*) participated in the parental genome of this species.

C. Male meiosis and pollen characteristics.

Among diploids, *S. italica* recorded the highest value of univalents. Chain bivalents and multivalents were also observed (Fig. 2-a). As a consequence of this behavior low chiasma frequency is expected. This finding supports the suggestion of Devos *et al* (1998) that rearrangements in genome structure of this species has occurred. Meiosis of other diploids is almost regular with a low frequency of univalents(Fig. 2-b, c, d). Bivalents ratio varied from

species to another (Table 3) and seems likely to affect chiasma frequency. Diploid species recorded a mean ratio of 1.05 against 1.21 for tetraploids. This result is in contrast to that previously recorded by Haroun *et al* (1992) for some species of *Panicum* (*Poaceae*).

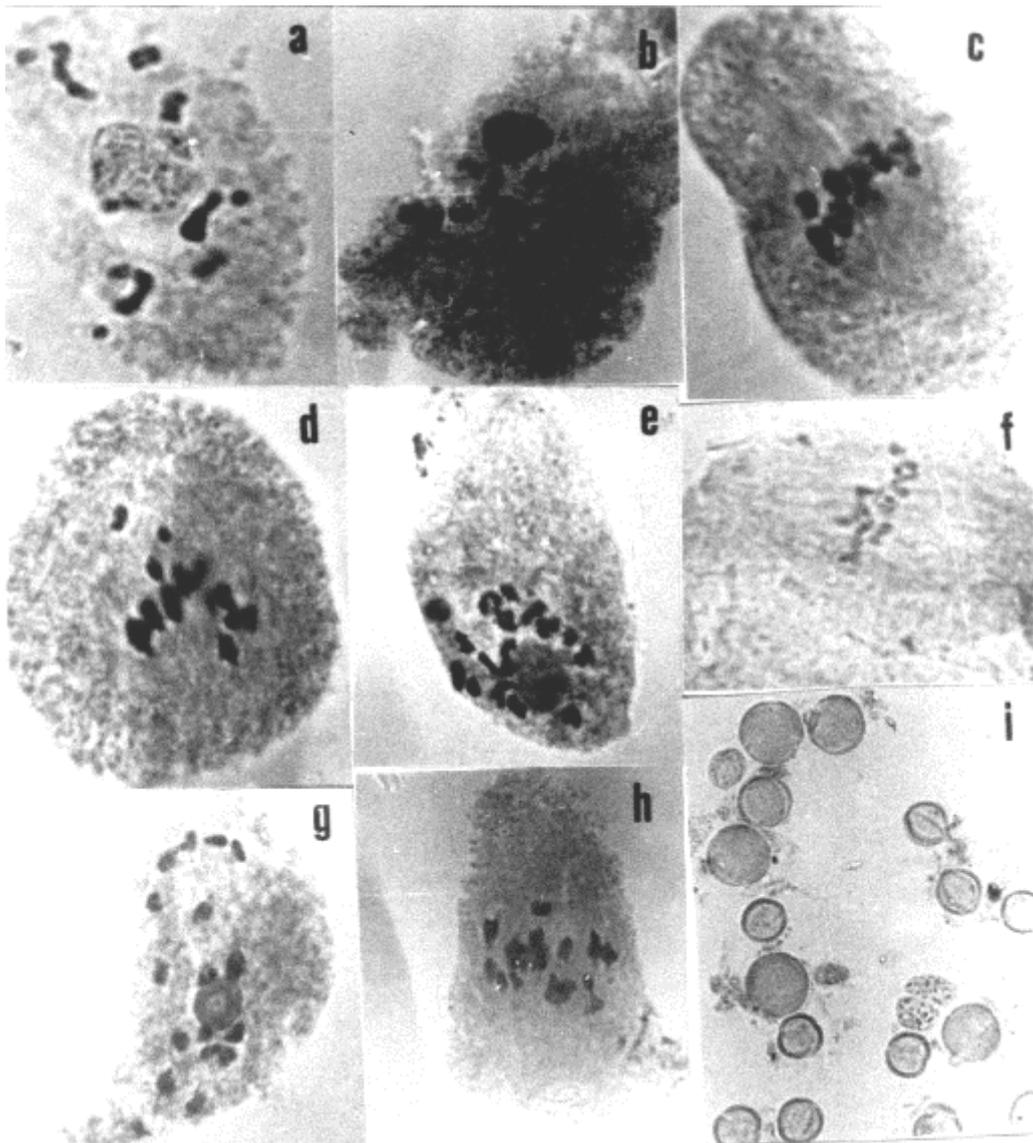


Figure 2(a-i): a, Diakinesis of *S. itlica* showing univalents and rod multivalents, b, diakinesis of *S. verticillata*; c, metaphase I of *S. viridis*; d, diakinesis of *S. glauca*; e, diakinesis of *S. sphacelata* showing trivalents and rod bivalents; f, metaphase I of *S. sphacelata* with lagging chromosomes ; g,h, diakinesis and metaphase I of *S. placiatis*; I, pollen grains of *S. sphacelata* with different size and sterile pollen.

Table 3. Number of pollen mother cells (PMC,s) observed, chromosome association and chiasma frequency per bivalent for six species of *Setaria*.

Species	No PMC's	Chrom. Associatio ⁿ				Chias . freq . ± S.E
		I	II	III	IV	
<i>S. verticillata</i>	33	1.4	8.30	0.0	0.0	1.07 ± 0.03
<i>S. italica</i>	41	1.9	6.80	0.8	0.0	1.04 ± 0.05
<i>S. viridis</i>	25	1.7	8.15	0.0	0.0	1.71 ± 0.03
<i>S. glauca</i>	16	1.2	8.40	0.0	0.0	1.01 ± 0.07
<i>S. sphacelata</i>	27	1.9	8.20	3.5	1.8	1.16 ± 0.09
<i>S. plaicatilis</i>	40	1.7	8.95	1.6	2.9	1.26 ± 0.09

Table 4. Percentage of abnormal PMC's, pollen fertility, seed set and pollen diameter of six species of *Setaria*.

Species	%Abn. PMC'S	% Pollen fertility ± S.E	Pollen diameter		% Seed set ±S.E.
			Range	mean ±S.E.	
<i>S. verticillata</i>	5.1	91.6±1.34	25 – 29	25.7±1.21	89.7±1.87
<i>S. italica</i>	7.6	89.9±1.42	22 – 35	31.1±2.11	92.4±2.12
<i>S. viridis</i>	4.5	96.2±1.93	20 – 27	25.3±1.76	75.2±2.34
<i>S. glauca</i>	6.5	77.8±1.82	22 – 28	27.2±2.01	83.6±1.44
<i>S. spacelata</i>	7.1	71.8±2.10	29 – 41	35.6±1.65	70.1±1.72
<i>S. plaicatilis</i>	5.2	87.3±2.34	33 – 42	37.2±1.34	79.2±1.89

Not surprisingly, *S. italica* recorded low chiasma frequency inspite of its relatively longer chromosomes because abnormal meiosis was observed. On the other hand *S. viridis* recorded the highest value of chiasma among diploids as it has normal meiosis and long chromosomes compared to other two diploids (*S. verticillata* and *S. glauca*). Except *S.italica* it could be said that meiosis of diploids is regular. The value of chiasma frequency recorded for *S. verticillata* is significantly high compared to that previously recorded for the same species from Egypt (Haroun, 1997). However, this indicates the genetic stability of the species and could be regarded as evolutionary response to the challenge of normal pairing as previously suggested by Chandola (1959).

For the two tetraploids observation of multivalents is expected and associations in the form of trivalents and tetravalents were observed. To some extent *S. plaicatilis* shows higher ratio of bivalents and quadrivalents (Fig. 2-g & h) compared to *S. sphacelata*. On the other hand, high level of trivalents and rod bivalents (Fig.2- e) recorded for *S. sphacelata* reflect its genetic instability and allopolyploid nature as previously stated by Poirier and Pernes (1986).

Based on meiotic configurations the treatment of the two tetraploid species as autotetraploid or allotetraploid is not justified. The two species represent an intermediate situation for the two types with high frequency of bivalents and some univalents, multivalents and quadrivalents. Such intermediates suggest that the two tetraploids are segmental allopolyploid as previously stated by Stebbins (1950), where the two genomes are rather alike or unlike but rather in between. The assumption that the two species are segmental allopolyploids is further supported by pollen fertility and seed set.

Some irregularities were observed including lagging and unoriented univalents (Fig.2-f). Diploids recorded 5.92 mean value of these forms against 6.15 for tetraploids (Table 5). It

seems likely that such irregularities affect normal pairing and cause a decrease in the ratio of chiasma frequency.

A positive relationship is clear between the percentage of seed setting and pollen fertility (Table 4). Diploid species, to some extent, show high pollen fertility compared to tetraploids (88.9% vs 79.6%). The lowest proportion of pollen fertility (71.8%) is recorded in *S. spaelata*. The sterile pollen were always seen as small and unstained (Fig. 2-i). The mean values for meiotic parameters recorded for diploids and tetraploids show that the former have higher pollen fertility and seed set than the latter (Table 5).

Table 5. Mean values for chromosome length, arm ratio, chiasma frequency, abnormal PMC's, pollen fertility, pollen diameter and percentage of seed setting in diploids and tetraploids species of *Setaria*.

Ploidy level	Chr.L.	A.R.	Chias. Freq.	% Ab. PMC's	%Fert.	Poll. Diam.	% seed set
2n	1.17	1.28	1.13	5.92	88.9	27.3	85.2
4n	1.29	1.15	1.21	6.15	79.6	36.4	74.7

Not surprisingly tetraploids have larger pollen than diploids (36.4 vs 27.3 μm). Pollen diameters of diploids ranged between 25.3-31.1 μm against 35.6-37.2 μm for tetraploids. Based on pollen measurements it seems likely that the ratio between diploids and tetraploids (1: 1.3) does not follow the ploidy level. Although there is no overlap in pollen diameter between diploids and tetraploids, this parameter could not accurately predict the ploidy level.

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