Cytological and Morphological Studies in the Genus *Sorghum*

M. L. Magoon¹, P. L. Manchanda and M. S. Ramanna

Division of Botany
Indian Agricultural Research Institute, New Delhi-12, India

Received March 8, 1963

Introduction

The ancestral relationship between different taxa is a fascinating problem in any group of plants; this is particularly so in a complex group like the *Sorghum*, though unravelling process has been one of considerable difficulty. That the conventional approach of studying chromosome pairing at metaphase I and of analyzing the mitotic chromosome morphology has several limitations, has already been amply demonstrated (Magoon and Shambulingappa 1960, Magoon *et al.* 1961, Magoon and Ramanna 1961 a and b). Information of far greater precision can be obtained by the study of pachytene stage in the meiosis of taxa within this complex and in hybrids between them. This is apparent when we consider that in the mitotic metaphase one is dealing with chromosome lengths varying only in a few micra (e. g., about 3μ in *S. ankolib*, Magoon and Shambulingappa 1960) while in the pachytene morphology the magnitude of variation is very high (about 50μ in *S. ankolib*, Magoon and Shambulingappa, 1960) resulting in much less error in classifying the chromosomes on the basis of their size. Several karyomorphological features not observable in the somatic chromosomes such as the chromomeric pattern, extent of eu- and hetero-chromatic segments, nucleolus organizing regions of the chromosomes and in some cases special karyotypic landmarks like the knobs etc. are also available for detailed study.

Further, structural differences that are not normally manifest in the study of metaphase pairing, become very striking at the pachytene stage. Consequently information that would otherwise be not available can be obtained in a study of the early stages of meiosis and if considered critically can be of immense value in deriving homologies and tracing ancestral relationships. While not all plant material are amenable to this approach, valuable information with regard to several *Sorghum* species has already been obtained (Magoon 1961). In order to get a complete picture of the interrelationship amongst the different species in this highly variable group, it is necessary to extend this analysis to as many species as are available and in the present

¹ Present address: Director, Main Tuber Crops Research Station, Sreekariyam, Trivandrum-II, India.

paper some hitherto unanalyzed species and some of their hybrid combination are considered.

Materials and methods

The following species and species hybrids were used in the present study.

1. *S. technicum*, 2. *S. verticilliflorum*, 3. *S. nitens*, 4. *S. virgatum*,
P. I. 209792 I. S. 3036 I. S. 8208 I. S. 106
5. *S. caudatum*, 6. *S. vulgare*, 7. *S. melaleucum*, 8. *S. caffrorum*,
I. S. 3144 I. S. 1517 I. S. 3104 I. S. 3137
9. *S. subglabrescens*, 10. *S. mellitum*, 11. *S. bicolor*, 12. *S. coriaceum*,
P. I. 155143 I. S. 2865 I. S. 3136 MNO 1581
13. *S. halepense*, 14. *S. technicum* × *S. virgatum*, 15. *S. verticilliflorum*
I. S. 3304
- × *S. subglabrescens* 16. *S. nitens* × *S. caffrorum*, 17. *S. vulgare*
× *S. verticilliflorum*, 18. *S. melaleucum* × *S. vulgare*, 19. *S. caudatum*
× *S. nitens*.

All the crosses were effected under controlled conditions. Parents and their hybrids grown in the field as well as those grown in the greenhouse were used for the study of morphology and microsporogenesis. For meiotic studies the propiono-carmin smears (Swaminathan *et al.* 1954 and Magoon *et al.* 1958) were used. Since the pachytene chromosomes in the material under study were found to be somewhat similar to those of tomato, straight line drawings were used for measuring length etc. as suggested by Barton (1950), Gottschalk (1954) and Magoon *et al.* (1961). For the sake of convenience, in comparing the chromosome sizes "Relative length" was used which represents the ratio in percentage of the length of the individual chromosome to that of the longest in the complement (Huziwara 1956).

Results

Chromosome morphology and pairing behaviour

a) *Species*

Based on average values obtained by critical measurements in several nuclei at mid pachytene where all chromosomes could be traced from one to other, the ten pachytene bivalents of each species have been identified (Figs. 1 to 3) and arranged in the decreasing order of their length. Hence chromosome I was the longest and chromosome X was the shortest (Figs. 16a to g). The total length, relative length, the centromeric position, the extent of Eu- and heterochromatin and number and position of chromomeres have been taken into account in depicting the pachytene chromosomes of the species under study. The absolute values concerning average length, relative length and arm-ratio of the chromosomes are presented in Table 1 species-wise. The chief diagnostic morphological characteristics of each of the ten pachytene chromosomes in the haploid complement are given below in ten

different classes pointing out the similarities and differences amongst them. It may be noted, however, that for any given species the comparison is inter-class and for any given chromosome intra-class.

Table 2. Showing classification of chromosome I to X of seven species based on presence of chromomeres

Species	Chromomeres on both arms	No. of chromomeres on long arm		No. of chromomeres on the short arm		No chromomeres
		1	2	1	2	
a) <i>S. halepense</i>	VIII	II, IV, VIII	V, VII	VIII, IX	I, III, VI	X
b) <i>S. melaleucum</i>	III, VIII	II, III, VII, VIII, IX	—	III, VIII	IV, VI	I, V, X
c) <i>S. technicum</i>	II	I, II, IV, IX	—	II, III, V, VIII	VI	VII, X
d) <i>S. mellitum</i>	II, IV, VII, IX	II, III, IV, VII, IX	—	I, II, IV, VII, IX	VI	V, VIII, X
e) <i>S. bicolor</i>	VIII	VIII, IX	III	IV, VIII	II, VI	I, V
f) <i>S. coriaceum</i>	II	II, V, VIII	—	II, IX	VI	I, III, IV, VII & X
g) <i>S. virgatum</i>	II, IV, VIII	I, II, III, IV, VII, VIII, IX	—	II, IV, VIII	VI	V, X

Chromosome I (mean length for different species range from 73.7 μ to 81.1 μ). This longest chromosome of the complement is associated with the nucleolus—the nucleolus organizing region being situated on the short arm. Pycnotic regions are almost equally distributed on both the arms. However, a slight variation is noted with regard to chromomeric pattern in the different species (see Table 2, Figs. 16a to g).

Chromosome II (61.4 μ to 67.5 μ). The second longest of the complement, is asymmetrical in all the species except in *S. bicolor* in which it is symmetrical. Pycnotic regions are distributed more or less equally on both the arms in all the species except in *S. mellitum* where the short arm has a large pycnotic region than that on the long arm and in *S. bicolor* which has a shorter pycnotic region on the short arm as compared to that on the long arm. Variation is noted in the number of chromomeres on the two arms in the different species (Table 2 Figs. 16a to g).

Figs. 1-13. 1-3, pachytene chromosomes ($n=10$) of *S. halepense*, *S. melaleucum*, and *S. technicum* respectively. $\times 1600$. 4-7, ears of parents and hybrids. Left-Female, Middle-Hybrids, Right-Male. 4, *S. nitens* $\times$ *S. caudatum*. 5, *S. nitens* $\times$ *S. caffrorum*. 6, *S. vulgare* $\times$ *S. verticilliflorum*. 7, *S. melaleucum* $\times$ *S. vulgare*. 8-9, pachytene stages in the F_1 of *S. verticilliflorum* $\times$ *S. subglabrescens* showing terminal as well interstitial nonpaired region. ($\uparrow$) $\times 1800$. 10, pachytene stage in the F_1 of *S. nitens* $\times$ *S. caudatum* exhibiting terminal nonpairing regions ($\uparrow$) $\times 1800$. 11, pachytene chromosomes in F_1 of *S. nitens* $\times$ *S. caffrorum* showing small terminal non-paired region and small duplication loop ($\uparrow$). $\times 1600$. 12, pachytene stage in F_1 of *S. vulgare* $\times$ *S. verticilliflorum* showing terminal deletions and unpaired regions ($\uparrow$). $\times 1800$. 13, pachytene chromosomes in the F_1 of *S. melaleucum* $\times$ *S. vulgare* showing normal pairing. $\times 1600$.

Table 1. Showing total length, relative length and arm ratio in the seven "*Eusorghum*" species

Chromosome type	<i>S. halepense</i>			<i>S. melaleucum</i>			<i>S. technicum</i>		
	Total length in μ	Relative length	Arm-ratio	Total length in μ	Relative length	Arm-ratio	Total length in μ	Relative length	Arm-ratio
	a			b			c		
I	79.8 $\pm$ 1.47	100	1:1.2	78.4 $\pm$ 0.86	100	1:1.2	81.1 $\pm$ 1.27	100	1:1.2
II	63.4 $\pm$ 1.13	79	1:1.4	61.4 $\pm$ 0.82	78	1:1.8	67.5 $\pm$ 0.80	83	1:1.4
III	57.9 $\pm$ 1.20	72	1:1.8	58.3 $\pm$ 0.87	74	1:1.3	59.9 $\pm$ 0.85	74	1:1.8
IV	45.2 $\pm$ 1.74	57	1:1.1	49.3 $\pm$ 1.39	63	1:1.2	49.9 $\pm$ 0.97	61	1:1.3
V	38.3 $\pm$ 0.93	48	1:1.3	40.6 $\pm$ 1.20	52	1:1.4	42.1 $\pm$ 0.78	52	1:1.3
VI	35.1 $\pm$ 1.14	44	1:4.3	36.5 $\pm$ 0.79	46	1:4.3	38.4 $\pm$ 1.13	47	1:4.6
VII	32.3 $\pm$ 0.73	40	1:1.2	30.2 $\pm$ 0.86	38	1:1.1	32.7 $\pm$ 0.91	40	1:1.3
VIII	28.3 $\pm$ 0.97	35	1:1.1	27.3 $\pm$ 0.87	35	1:1.3	30.2 $\pm$ 0.63	37	1:1.4
IX	25.3 $\pm$ 1.08	32	1:1.2	23.3 $\pm$ 0.98	30	1:1.3	26.8 $\pm$ 0.61	33	1:1.3
X	22.2 $\pm$ 0.86	28	1:1.1	20.2 $\pm$ 0.82	26	1:1.1	23.2 $\pm$ 0.61	29	1:1.1

(continued)

Chromosome type	<i>S. melliticum</i>			<i>S. bicolor</i>			<i>S. coriaceum</i>			<i>S. virgatum</i>		
	Total length in μ	Relative length	Arm-ratio	Total length in μ	Relative length	Arm-ratio	Total length in μ	Relative length	Arm-ratio	Total length in μ	Relative length	Arm-ratio
	d			e			f			g		
I	76.5 $\pm$ 0.46	100	1:1.2	73.7 $\pm$ 0.99	100	1:1.2	74.4 $\pm$ 0.97	100	1:1.2	78.3 $\pm$ 0.78	100	1:1.2
II	65.8 $\pm$ 0.76	86	1:1.6	62.4 $\pm$ 1.02	85	1:1.3	65.1 $\pm$ 0.78	87	1:1.6	67.2 $\pm$ 0.68	86	1:1.5
III	54.9 $\pm$ 0.74	72	1:1.8	56.1 $\pm$ 0.80	76	1:1.7	59.1 $\pm$ 0.73	79	1:1.8	56.8 $\pm$ 0.68	72	1:1.8
IV	48.9 $\pm$ 0.50	64	1:1.2	47.1 $\pm$ 0.54	64	1:1.5	46.6 $\pm$ 0.96	63	1:1.2	48.5 $\pm$ 0.91	62	1:1.1
V	43.7 $\pm$ 0.85	57	1:1.5	40.2 $\pm$ 0.76	54	1:1.5	39.9 $\pm$ 0.86	54	1:1.3	39.1 $\pm$ 0.60	50	1:1.4
VI	36.5 $\pm$ 0.64	48	1:4.2	35.8 $\pm$ 0.87	48	1:4.6	35.5 $\pm$ 0.36	48	1:4.2	33.4 $\pm$ 0.53	43	1:4.5
VII	30.4 $\pm$ 0.53	40	1:1.1	30.9 $\pm$ 0.83	42	1:1.2	32.2 $\pm$ 0.87	43	1:1.3	31.2 $\pm$ 0.87	40	1:1.4
VIII	26.5 $\pm$ 0.80	35	1:1.2	25.5 $\pm$ 0.60	34	1:1.4	29.1 $\pm$ 1.04	39	1:1.3	28.3 $\pm$ 0.76	36	1:1.2
IX	24.1 $\pm$ 0.86	31	1:1.4	23.1 $\pm$ 1.02	31	1:1.3	26.2 $\pm$ 0.71	35	1:1.2	25.4 $\pm$ 0.56	32	1:1.2
X	20.4 $\pm$ 0.84	27	1:1.1	20.0 $\pm$ 0.96	28	1:1.1	21.6 $\pm$ 0.41	29	1:1.1	20.8 $\pm$ 0.72	26	1:1.1

Chromosome III (54.9μ to 59.9μ). Asymmetrical in all the species except in the case of *S. melaleucum* where it is nearly symmetrical. Pycnotic regions are distributed almost equally on both the arms except in the case of *S. halepense* and *S. technicum* where such regions are found to be larger on the short arm. The pattern of chromomeric variation on the two arms in these species is given in Table 2 (Figs. 16 a to g).

Chromosome IV (45.2μ to 49.9μ). More or less symmetrical in all these species. Pycnotic regions are nearly equally distributed on both the

Figs. 14-15. 14, pachytene chromosomes in the F_1 of *S. technicum* $\times$ *S. virgatum* showing normal pairing. $\times 1600$. 15, metaphase I in the F_1 of *S. vulgare* $\times$ *S. verticilliflorum* showing 10 II ($\times 1600$).

arms except in the case of *S. virgatum* where the short arm has a larger pycnotic region as compared to that on the long arm. The variation noted as regards distribution of chromomeres on both the arms in these species may be seen in Table 2 (Figs. 16 a to g).

Chromosome V (38.3μ to 43.7μ). This is more or less symmetrical in all these species. Pycnotic regions are distributed almost equally on both the arms except in the case of *S. melaleucum* where it is larger on the short arm than that on the long arm. The differences of the chromomeric pattern is given in Table 2 (Figs. 16 a to g).

Chromosome VI (33.4μ to 38.4μ). This is asymmetrical chromosome in the complement in each of the species. Except in *S. technicum* where the pycnotic region on the long arm is larger than that on the short arm, an almost equal distribution of pycnotic regions on both the arms is a characteristic feature of the remaining six species. The pycnotic region occupies about $\frac{3}{4}$ th of the entire length on the short arm. As regards chromomeric pattern, the details are given in Table 2 (Figs. 16 a to g).

Chromosome VII (30.2μ to 32.7μ). This chromosome does not exhibit much variation in respect of relative length, arm-ratio etc. Pycnotic regions are distributed almost equally on both the arms except in *S. technicum* and *S. bicolor* where the pycnotic region is larger on the long arm as compared to that on the short arm. Whereas in *S. virgatum* it is larger on the short arm than that on the long arm. For variation in the chromomeric pattern refer Table 2 (Figs. 16 a to g).

Chromosome VIII (25.5μ to 30.2μ). This chromosome is more or less symmetrical. Except in *S. melaleucum* where the pycnotic region is larger

on the long arm than that on the short arm, the pycnotic region is distributed almost equally on both the arms. The differences amongst the species on the basis of chromomeric pattern in this chromosome are given in Table 2 (Figs. 16 a to g).

Chromosome IX ($23.1\ \mu$ to $26.8\ \mu$). This is a more or less symmetrical chromosome in the species under study. Except in *S. halepense* where two deeply staining regions are present on the short arm, proximal one being larger than the terminal, in all other species the deeply staining region is distributed almost equally on both arms. The differences between species in their chromomeric pattern are given in Table 2 (Figs. 16 a to g).

Chromosome X ($20.4\ \mu$ to $23.2\ \mu$). This symmetrical chromosome is strikingly uniform in all the seven species, being shortest in the complement.

Pycnotic region occupies comparatively larger part of this chromosome on both the arms than in all the other nine chromosomes in the complement. This chromosome is generally devoid of any chromomere.

Fig. 16. a-g, showing idiograms. a, *S. halepense*. b, *S. melaleucum*. c, *S. technicum*, d, *S. mellitum*, e, *S. bicolor*, f, *S. coriaceum* and g, *S. virgatum*

All species under study show more or less similar staining behaviour of pachytene chromosomes. In each pachytene chromosome it was possible to observe a distinctly translucent centromere followed by deeply stained chromatic regions adjacent to it on either side. Pachytene pairing in all the species was found to be complete along the entire length and is apparently normal. Analysis of the pairing properties of the differentially stained regions indicated that synapsis starts from the proximal to the distal end. The separation of the split chromosomes starts from the distal to the proximal.

Ten bivalents were usually present both at diakinesis and M I. Chiasma frequency determined at these stages is presented in Table 3. The later stages of meiosis were also normal accompanied by high percentage of stainable pollen and good seed set.

b) *Species hybrids*

The comparative morphological characteristics of the parents and the hybrids are given in Table 4 (Figs. 4 to 7). For characters such as tillering,

Table 3. Showing chiasma frequency in the species hybrids

Species and stage	No. of cells studied	Bivalents with			Total no. of chiasmata	Average no. of chiasmata per cell
		2 xta	1 xma	0 xma		
<i>S. halepense</i>						
Diakinesis	20	172	28	0	372	18.60
Metaphase I	20	153	47	0	353	17.65
<i>S. melaleucum</i>						
Diakinesis	20	166	34	0	366	18.30
Metaphase I	20	148	52	0	348	17.40
<i>S. technicum</i>						
Diakinesis	20	177	23	0	377	18.80
Metaphase I	20	163	37	0	363	18.15
<i>S. mellitum</i>						
Diakinesis	20	174	26	0	374	18.75
Metaphase I	20	161	39	0	361	18.05
<i>S. bicolor</i>						
Diakinesis	20	175	25	0	375	18.75
Metaphase I	20	158	42	0	358	17.90
<i>S. coriaceum</i>						
Diakinesis	20	161	39	0	361	18.05
Metaphase I	20	152	48	0	352	17.60
<i>S. virgatum</i>						
Diakinesis	20	168	32	0	368	18.40
Metaphase I	20	142	58	0	342	17.10
<i>Species hybrids</i>						
<i>S. technicum</i> ×						
<i>S. virgatum</i>	25	213	37	0	463	18.52
Diakinesis	25	196	54	0	446	17.84
Metaphase I						
<i>S. verticilliflorum</i>						
× <i>S. subglabrascens</i>	25	198	52	0	448	17.92
Diakinesis	25	172	78	0	422	16.88
Metaphase I						
<i>S. nitens</i> ×						
<i>S. cafferorum</i>						
Diakinesis	25	201	49	0	451	18.04
Metaphase I	25	186	64	0	436	17.44
<i>S. vulgare</i> ×						
<i>S. verticilliflorum</i>						
Diakinesis	25	211	39	0	461	18.44
Metaphase I	25	192	58	0	442	17.68
<i>S. melaleucum</i> ×						
<i>S. vulgare</i>						
Diakinesis	25	215	35	0	465	18.60
Metaphase I	25	193	37	0	443	17.72

Table 4. Showing the morphological characteristics of *Sorghum* species and hybrids between them

Characters	<i>S. melaleucum</i>	<i>S. melaleucum</i> × <i>S. vulgare</i>	<i>S. vulgare</i>	<i>S. vulgare</i> × <i>S. verticilliflorum</i>	<i>S. verticilliflorum</i>	<i>S. verticilliflorum</i> × <i>S. subglabrescens</i>	<i>S. subglabrescens</i>
Height of the plant. (tip to earhead). (In inches)	85.2"	106.0"	100.5"	101.5"	90.0"	105.3"	93.5"
No. of leaves/Plant	8-9	8-9	9-10	8-9	8-9	9-10	8-9
Length of leaf (V leaf)	24.2"	25.3"	26.1"	25.3"	26.5"	26.4"	24.3"
Breadth of the leaf (In inches)	2.0"	2.1"	2.3"	2.0"	1.6"	2.6"	2.5"
Inflorescence	Semcompact	Semcompact	Semcompact	Semcompact	Loose	Semcompact	Compact
Pedicellate florets	Non staminate	Non staminate	Infertile	Staminate	Staminate	Staminate	Non staminate
Shape of the spikelet	Obovate	Obovate	Obovate	Oblong	Elliptic	Oblong	Obovate
No. of nerves on the outer glume.	12-13	11-13	11-12	11-12	10-12	10-12	10-12
No. of nerves on the inner glume.	8-10	8-10	8-9	7-9	7-9	8-10	7-9
Lemma.	Awed	Awless	Awless	Awless	Awed	Awless	Awless
Stigmatic surface shape.	Switch type	Compact feathery	Compact feathery	Switch type	Long and uniform	Switch type	Compact feathery
Colour of stigma.	White	Yellow	Yellowish white	Yellow	Yellow	Yellow	Yellowish white
Grain colour	White	White	Light brown	White	Brown	Brown	Brown

(continued)

Characters	<i>S. caudatum</i>	<i>S. caudatum</i> × <i>S. nitens</i>	<i>S. nitens</i>	<i>S. nitens</i> × <i>S. cafrorum</i>	<i>S. cafrorum</i>	<i>S. technicum</i>	<i>S. technicum</i> × <i>S. virgatum</i>	<i>S. virgatum</i>
Height of the plant. (tip to earhead). (In inches)	85.0#	91.3#	90.3#	93.2#	88.0#	91.2#	99.2#	82.0#
No. of leaves/Plant	8-9	9-10	8-10	9-10	8-9	9-10	9-10	8-9
Length of leaf (V leaf)	22.4#	22.1#	23.2#	23.1#	23.3#	23.6#	22.4#	21.2#
Breadth of the leaf (In inches)	2.2#	2.3#	1.9#	2.2#	2.1#	1.8#	2.0#	1.7#
Inflorescence	Compact	Semicompact	Loose	Semicompact	Compact	Semicompact	Semicompact	Semicompact
Pedicellate florets	Non staminate	Non staminate	Staminate	Non staminate	Non staminate	Staminate	Staminate	Non staminate
Shape of the spikelet	Obovate	Obovate	Oblong	Obovate	Obovate	Obovate	Obovate	Oblong
No. of nerves on the outer glume.	10-12	10-11	10-12	10-11	10-12	11-13	11-12	11-12
No. of nerves on the inner glume.	7-8	7-9	7-9	7-8	7-8	9-10	9-10	9-10
Lemma.	Awnless	Awnless	Awnless	Awnless	Awnless	Awnless	Semi-awned	Awned
Stigmatic surface shape.	Switch type	Switch type	Switch type	Switch type	Switch type	Switch type	Switch type	Switch type
Colour of stigma.	White	Yellow	Yellow	Yellow	White	White	Yellow	Yellow
Grain colour	Light brown	Brown	Dark brown	Brown	Light brown	Light brown	Brown	Brown

panicle branching, floret shape, colour of the midrib, stigma and grains, the hybrids were intermediate. But in respect of characters such as plant height, leaf length and number of nodes the hybrids were usually heterotic. The awned condition was found to be recessive to awnlessness and the non-staminate condition of pedicellate florets to staminate condition (see Table 4 for details).

Observations on the synopsis of the chromosomes at pachytene showed deviation from the normal meiosis noted in the parental species in some of the hybrids under study. In the hybrids, *S. verticilliflorum* × *S. subglab-rascens*, two bivalents showed non-paired regions, the unpaired regions being terminal in one and both terminal as well as interstitial in the other (Figs. 8 and 9). Again, in the case of two hybrids, *S. nitens* × *S. caudatum* and *S. nitens* × *S. caffrorum*, one to two bivalents respectively exhibited small terminal non-pairing regions (Figs. 10, 11). In the later hybrid, one bivalent also exhibited a small loop like structure indicating the presence of a small duplication (Fig. 11). In the hybrids, *S. vulgare* × *S. verticilliflorum*, four bivalents showed large terminal non-paired regions as well as small terminal deletions (Fig. 12). In the same hybrid, one bivalent also exhibited interstitial unpaired region (Fig. 12). The pachytene pairing in the remaining two hybrids was complete for the entire length of the chromosomes and apparently normal (Figs. 13 to 14). It is interesting to note that inspite of the existence of such cytologically detectable though minute structural differences in some of the hybrids, the later stages of meiosis were normal in all the hybrids. 10 II were observed both at diakinesis and MI (Fig. 15). The chiasma frequency determined at these stages is presented in Table 5. All the hybrids showed high percentage of stainable pollen and good seed set.

Discussion

It will be obvious from the results obtained in the present investigation and those already reported by Magoon *et al.* (1961) that the *Sorghum* material appears to be exceedingly suitable for pachytene analysis. In none of the species non-homologous pairing or stickiness was observed thereby removing this possible source of error. Further, centromeres are sharply definable in each of the pachytene chromosomes in these species. However, conspicuous morphological land marks such as the presence of deeply staining knobs, were unfortunately not met with in the 20 chromosomes material studied and hence the identification of the individual chromosome was based on total length, relative length, centromeric position, amount and distribution of heterochromatin and number and position of chromomeres.

Considering all the available observations made so far on the morphology of the pachytene chromosomes in many crop plants, pachytene chromosomes may be broadly classified as differentiated and undifferentiated types (see review by Venkateswarlu 1962). The former category is characterized by

proximal darkly staining region and distantly placed lightly staining region. The second type consists of chromosomes which take uniform stain or show chromomere size gradient. In this aspect the species studied in the present investigation and other *Eu-sorghum* species investigated by earlier workers (Venkateswaralu and Reddi 1956, Magoon and Shambulingappa 1960 and 1962 a and b, Magoon *et al.* 1961 a and b, Magoon and Ramanna 1961 a and b) may be classified under the differentiated type. It should be noted, however, that this type is not a characteristic of the entire genus since undifferentiated types of chromosomes have also been reported by some workers in certain species belonging to different sections. Nevertheless, the species of the section *Eu-sorghum* reported here and also studied previously appear to belong to the differentiated group.

The comparative study of pachytene chromosomes in the species under investigation brings out a good similarity in their general morphology. However, some differences are also recorded between them. It would be evident from the data (Tables 1 and 2, Fig. 16 a to g) that in general certain chromosomes do not exhibit much variation except for chromomeric pattern and minor variations in arm-ratios in a few cases. On the other hand, some other chromosomes show comparatively greater variations in their arm-ratio, pattern of pycnotic regions and distribution of chromomeres and thus might have undergone more structural alterations during the course of differentiation of these species. The length measurement between the longest and the shortest chromosome in the complement of these seven species show significant differences; the shortest chromosome (X chromosome) range from 20μ to 23.2μ and the longest chromosome (I chromosome) being 73.7μ to 81.1μ (Table 1). The proportion of the longest chromosome to the shortest in each of the species is little more than 1:3. The karyotype of each species also possesses at least one chromosome, usually VI, whose arm-ratio is more than 1:4. Following the classification proposed by Stebbins (1958), all the seven karyotypes studied here fall under '2 b' type of asymmetry class. Several other *Eu-sorghum* species studied earlier (see Magoon and Shambulingappa 1960, Magoon *et al.* 1961 a and b, Magoon and Ramanna 1961 a and b) could be classed under '2 b' type of asymmetry class.

The species under consideration also show interesting chromomeric pattern (Table 2, Fig. 16 a to g). Four distinct types can be recognized such as a) chromomeres on both the arms, b) chromomeres only on the long arm, c) chromomeres on the short arm, and d) no chromomeres at all on both the arms. It may be interesting to note that each species differ from the other with regard to chromomeric pattern in one or more chromomeres and thus this characteristic, in combination with relative length, arm-ratio etc. has proved to be of great help in characterizing the species. A comparison of the species based on the amount and distribution of heterochromatin is not attempted here since the measurement of relative lengths of the pycnotic

areas could not be made accurately, as their demarcations were not very sharp. However, unequal distribution of pycnotic regions could be clearly observed in some instance, for example, II chromosome in *S. mellitum*, *S. bicolor* and *S. virgatum* and IV chromosome in *S. virgatum*. The data on the characteristics of the pachytene chromosomes being reported in the present study, though very informative is most rewarding when considered along with taxonomic criteria.

The cytological analysis of hybrids in recent years have been of great value in determining the homologies and consequently the probable relationships and origin of many species. A knowledge of cytogenetic affinities between cultivated species and their wild relatives has proved to be useful in utilizing the desirable germ plasm in the most effective manner for the improvement of the cultivated species through hybridization. The species of *Sorghum* belonging to the subsection Arundinacea show a striking similarity in both number and morphology of chromosomes (Magoon *et al.* 1961 a, Magoon and Ramanna 1961 a and b, Magoon 1961). Such studies have revealed the fact that majority of the Arundinacea *Sorghum* appear to be closely related with one another. It must be emphasized, however, that structural differences (minute as well as patent), if any, may not be detectable by simply comparing the karyotypes of different species. Hence analysis of chromosome pairing especially at pachytene in the hybrids is very essential not only to detect such structural changes but will also be helpful in understanding the cytogenetical mechanisms underlying species differentiation among the morphologically identified species in the genus *Sorghum*. This may be of immense value in planning hybridization programme.

It has been emphasized Stebbins (1947) and Magoon *et al.* (1962) that structural differences between the chromosomes could conceivably co-exist with a high degree of regular meiotic pairing if it is assumed that the chromosomes sets, concerned differ by a large number of small structural differences. Such 'cryptic' structural differences may not be able to offer much hindrance to pairing between chromosomes concerned and meiosis would appear to be apparently normal. However, they may be able to effectively prevent the free exchange of genes located within or very close to such regions and thus lay the foundation for the differentiation of the taxa concerned into different species. Consequently the breeder may not be able to recover the recombinant types for which he plans the crosses.

Sharma and Bhattacharjee (1957) reported marked differences in the somatic idiograms of several *Eu-sorghum* species studied by them and attribute species differences as arising out of gross structural changes. On the other hand, Magoon and Shambulingappa (1960), Magoon and Ramanna (1960 a and b) and Magoon *et al.* (1961 b) have demonstrated that the karyotypes of different species do not show much variation and therefore suggested that the species differentiation in the subsection Arundinacea of *Eu-sorghums*

may be largely due to genic and cryptic or small structural changes. The study of species and their hybrids reported here confirm this view. Further, Sharma and Bhattacharjee (1957) have predicted highly irregular meiosis in the species hybrids of the subsection Arundinacea of *Eu-sorghum* on the basis of the marked karyotypic differences noted by them. On the contrary, remarkable regular meiosis, followed by normal pollen and seed fertility appears to characterize several species hybrids studied so far (Magoon and Shambulingappa 1962 a and b). This has also been confirmed by other workers (see Kid 1956, Laubscher 1945, and Endrizzi 1957). These observations would lead one to suspect that species differentiation may be perhaps attributed largely to modifier complexes by which species differ. However, consistent reduction in the mean chiasma frequency at diakinesis and M I in several of the species hybrids reported earlier may suggest a significant role of small structural differences detectable only at pachytene stage. The presence of small structural differences have been recorded in some hybrids reported by Magoon and Shambulingappa (1962 a and b), and the presence of such small structural differences in a few hybrids in the present study provide again direct evidence for the latter view. It, therefore, becomes necessary to look for a sensitive tool to detect minor structural differences, if any, between the chromosomes in the species. The critical analysis of pachytene chromosomes for the pairing behaviour in the hybrids nucleus would appear to be the appropriate tool and is of immense value for such analysis.

Nature of chromosome pairing at pachytene has been studied in six species hybrids in the present study and out of them the following four hybrids (*S. verticilliflorum* $\times$ *S. glabrescens*, *S. nitens* $\times$ *S. caudatum*, *S. nitens* $\times$ *S. caffrorum* and *S. vulgare* $\times$ *S. verticilliflorum*) showed at pachytene stage cytologically detectable though minute structural differences such as terminal and interstitial non-pairing regions, differential segments, terminal deletions and small duplication loop (Fig. 11). In spite of the existence of such structural differences, the later stages of meiosis were generally normal accompanied by high pollen stainability and good seed setting. The chiasma frequency also did not show any significant deviation from those in the parental species thereby indicating a marked extent of homology between chromosomes than would be expected to explain bivalents noted at M I for which even a single chiasma between two chromosomes would suffice. All these factors again point out that the existing chromosomal structural differences are quite small and do not lead to abnormal behaviour of the hybrids either in respect of morphological characters or in the meiotic process. The remaining two hybrids showed complete pairing at pachytene and normal meiosis at later stages, followed by high percentage of stainable pollen and good seed setting. The chiasma frequency also did not show any significant deviation from the parental species. Apparently the differences between the

species are to be sought at the genic level. They might be of the nature of modifier complexes.

Stebbins (1938) found no correlation between the extent of structural differences present in the chromosomes of two *Paeonia* species and hybrid sterility. He observed, for example, that heterozygosity for many small inversions of *Paeonia delavayi*, *P. anomala* and *P. tritermata*, does not at all reduce chiasma frequency at M I in these species. In the hybrid, *P. albiflora* $\times$ *P. tenuifolia*, where there was considerable reduction in chiasma frequency, a significant reduction in pollen fertility and particularly seed setting, showed the presence of only one inversion. Müntzing (1938) reported that interspecific sterility in *Galeopsis tetrahit* $\times$ *G. speciosa* is, mainly, caused by structural differences in the chromosomes. A direct correlation between pollen sterility and the occurrence of univalents at meiosis in *Galeopsis* hybrids was also observed. From this it would appear as if the two phenomena, viz; appearance of univalents at M I and pollen sterility, must be regarded as different manifestation of the same cause, namely structural differences between the forms crossed. However, even in such an instance a certain parallelism between the extent of pollen sterility and degree of meiotic irregularities might be expected, if these phenomena are both proportional to the size and number of structural differences as was recorded by Garber and Gorsic (1956) in *Collinsia*, where the correlation is very weak or absent it must then be attributed to qualitative differences between the different chromosome segments. When certain hybrids exhibit normal meiosis and high sterility then there is reason to assume that the structural differences are small in quantity but important qualitatively. At the lower most level this would be termed as genic differences. In other F_1 combinations, on the other hand, meiosis may be upset by large segmental differences, comprising parts of the chromosomes which, however, are relatively harmless to the viability of the gametes.

In the genus *Sorghum*, while there appears to be a good correlation in some interspecific hybrids between the extent of structural differences present in the chromosomes and the percentage of pollen fertility found in them, no such correlation was observed in some other hybrids (Shambulingappa 1962 and Magoon and Shambulingappa (unpublished)). This apparently indicates qualitative differences in structural hybridity and hence it may be likely that the structural differences present in different species may differ both qualitatively as well as quantitatively. Since in most of the hybrids under consideration, no such correlation between the extent of structural difference present in the chromosomes and pollen fertility is noted, it probably indicates that the structural differences observed in these hybrids are probably not as important qualitatively to affect the viability of gametes.

Interspecific hybrids have been successfully produced and investigated to provide information of cytogenetic and phylogenetic value of possible use in

the improvement of *Sorghum* by hybridization. The results obtained in the present investigation and also reported earlier (Magoon and Shambulingappa 1962 a, b and c) and some unpublished work (Shambulingappa 1962) do suggest the possibility of transferring valuable germ plasm from both wild as well as cultivated species into the commercial variety since a majority of the present species of *Eu-sorghum* do not appear to be separated by any serious barrier to gene-exchange.

Considering the internal mechanisms responsible for speciation in this group of plants, it appears likely that gene mutations or gene substitution, 'cryptic' structural differences, small, though cytologically detectable, polyploidy and probably cytoplasmic differentiation seem to have played a significant role. The part played by the gross structural changes, if any, cannot be very significant. The striking fact, that emerges from a consideration of the crossability relationship of *Eu-sorghum* is that while most of the internal isolating mechanisms mentioned above are present, barriers to crossability between the species appear to be rather feebly developed. This becomes more apparent when we compare the situation prevailing in the section *Para-sorghum* of this genus, where they appear to be very strongly developed. From the point of view of the systematic taxonomist whether species distinctions are attributed to multiple gene substitution or to 'cryptic' or small structural differences is only of academic significance but for a geneticist it is an issue of prime importance. For, upon it depends the degree to which a breeder can hope to transfer a character of potential economic value from one species to another. If species differences are mainly due to multiple gene substitution, the problem is chiefly a matter of growing progenies large enough to secure the required combination. Whereas the success with which a breeder can expect to transfer valuable characters from one species to another will be inversely proportional to the frequency of 'cryptic' or small structural differences in his breeding material since the genes within these regions would tend to be passed on to the progeny enbloc (Magoon *et al.* 1962).

Summary

The morphological characters of nine species and six F_1 hybrids were compared. It was found that with respect to characters such as tillering, panicle branching, floret shape, colour of the midrib, stigma and grains, the hybrids were intermediate but for characters such as plant height, leaf length and number of nodes, the hybrids were usually heterotic. The awned condition was found to be recessive to awnlessness and the non-staminate condition of pedicellate florets to staminate condition.

Critical analysis of chromosomes at pachytene and later stages of meiosis in the seven *Eu-sorghum* species and six F_1 hybrids were made. Pachytene chromosomes are depicted on the basis of total length,

relative length, arm-ratio, amount and distribution of heterochromatin and number and position of chromomeres. The characteristics of each of the ten chromosomes in the haploid complement are described in ten different classes bringing out the similarities and differences among these seven species. Studies on the pairing properties of the differentially stained regions show that synapsis starts from the proximal to the distal and separation of the split chromosomes starts from the distal to the proximal.

Pachytene analysis in the four F_1 hybrids revealed the presence of some minute, though cytologically detectable, structural differences such as terminal as well as interstitial non-paired regions, small duplication and small terminal deletions and differential segments between the parental species. Pachytene pairing was found to be complete and apparently normal in the remaining two hybrids. In spite of the existence of these meiotic irregularities in some hybrids, high percentage of stainable pollen and good seed setting were recorded in all the hybrids under consideration.

The distribution of chiasmata at various stages was studied in both species and species hybrids and the average chiasma frequency did not show any significant deviation from those in the parental species. It is suggested that the existing chromosomal differences are quite small and do not lead to abnormal behaviour of the hybrids either in respect of morphological characters or in the meiotic process in the F_1 generation.

Cytogenetical mechanisms underlying species differentiation in the genus are discussed.

Acknowledgements

The authors are grateful to Dr. B. P. Pal and Dr. M. S. Swaminathan for their keen interest and constant encouragement. Co-operation from the "Rockefeller Foundation", I.A.R.I., New Delhi, is also hereby acknowledged.

References

- Barton, D. W. 1950. Pachytene morphology of the tomato chromosome complement. *Amer. J. Bot.* 37: 639-43.
- Endrizzi, J. E. 1957. Cytological studies of some species and hybrids in *Eu-sorghum*. *Bot. Gaz.* 119: 1-10.
- Garbar, E. D. and Gorsic, J. 1956. The genus *Collinsia* II. Interspecific hybrids involving *C. heterophylla*, *C. concolor* and *C. sparsiflora*. *Bot. Gaz.* 118: 73-77.
- Gottschalk, W. 1954. Die Chromosomen Struktur der Solanaceen unter Berücksichtigung phylogenetischer Fragestellungen. *Chromosoma* 6: 539-626.
- Huziwaru, Y. 1956. Karyotype analysis in some genera of *Compositae*. I. Karyotype of Japanese *Eupatorium*. *Cytologia* 21: 114-123.
- Kid, H. J. 1956. The morphology of the panicle in the cultivated *Sorghums*. Ph. D. dissertation. Washington Univ. St. Louis. (original not seen)
- Laubscher, F. X. 1945. A genetic study of *Sorghum* relationship. Dept. Agric. (Union of South Africa). *Sci. Bull.* 242.
- Magoon, M. L. 1961. Genetical and cytogenetical studies in the genus *Sorghum*. *Proc.*

- 2nd. All. Ind. Millet. Conf., Kanpur.
- , Cooper, D. C. and Hougas, R. W. 1958. Cytogenetic studies of some diploid *Solanums*, section *Tuberarium*. Amer. J. Bot. 45: 207-221.
- , Ramanujam, S. and Cooper, D. C. 1962. Cytogenetical studies in relation to the origin and differentiation of species in the genus *Solanum* L. Caryologia 15: 151-252.
- and Ramanna, M. S. 1961-a. Comparative karyomorphology of *Eu-sorghums*. Caryologia 14 (3): 391-407.
- and — 1961-b. Cytology of some "*Eu-sorghums*". Genet. Iber. XIII 129-144.
- and Shambulingappa, K. G. 1960. Karyomorphological studies in *Sorghum ankolib* var. ankolib red, a *Eu-sorghum*. Indian J. Genet. 20: 166-177.
- and — 1961. Karyamorphology in *Sorghum propinquum* and its bearing on the origin of 40-chromosome *Sorghums*. Chromosoma 12: 460-465.
- , — and Ramanna, M. S. 1961-a. A polyhaploid plant in *Sorghum halepense*. Pers. Current Sci. 30: 347-48.
- , — and —, 1961-b. Chromosomes morphology and meiosis in some *Eu-sorghums*. Cytologia 26: 236-52.
- and — 1962-a. Cytological and morphological studies on some interspecific hybrids in *Eu-sorghum*. Zeitschrift für Vererbungslehre 93: 14-24.
- and — 1962-b. Cytomorphological studies of some interspecific hybrids in the genus *Sorghum*. Der Züchter. 32: 317-324.
- and — 1962-c. A polyhaploid plant of *S. alnum*. Genetica Polonica. 3: 301-306.
- Müntzing, A. 1938. Sterility and chromosome pairing in intra-specific *Galeopsis* hybrids. Hereditas 24: 117-188.
- Shambulingappa, K. G. 1962. Cytogenetical studies and nature of speciation in the genus *Sorghum*. Ph. D. dissertation I.A.R.I., New Delhi.
- Sharma, A. K. and (Miss) Dipiti Bhattacharjee 1957. Chromosome studies in *Sorghum* I. Cytologia 22: 287-311.
- Stebbins, G. L. 1938. Cytogenetic studies in *Paeonia* II The cytology of diploid species and hybrids. Bot. Rev. 11: 463-86.
- 1947. Types of polyploids: their classification and significance. Adv. Rev. 11: 463-29.
- 1958. Longivity, habitat and release of variability in the higher plants. C.S.H.S. Quant. Biol. 23: 365-78.
- Swaminathan, M. S., Magoon, M. L. and Mehra, K. L. 1954. A simple propiono-carminic PMC smear method for plants with small chromosomes. Indian J. Genet. 14: 87-88.
- Venkateswaralu, J. 1962. Some aspects of the study of pachytene chromosomes. 49th Indian Sci. Congr. 1-20.
- and Reddi, R. 1956. Morphology of pachytene chromosomes and meiosis in *Sorghum subglabrescens*, a *Eu-sorghum*. J. Indian Bot. Soc. 35: 344-56.
-