Cyto-genetics of Millets* (1)

R. P. Chandola

BD 1020 Binayamagar, New Delhi, India

Received February 11, 1957

Introduction

The grass family (Gramineae or Poaceae) is one of the largest families of flowering plants and contains the largest number of individual types. Whenever an attempt has been made to classify a group of related variable species in this family by various systematists, they have always been confronted with considerable difficulty leading to differences in the details of their arrangement in any phylogenetic series.

Brown (1810) was the first to recognise two subfamilies of *Gramineae*; the *Pooideae* and the *Panicoideae*. Till recently this system was followed with slight modifications introduced here and there by Bews (1929), Arber (1934), Hutchinson (1934) and Hitchcock (1950). Brown based his classification on external morphological characters alone. Later, however, anatomical characters of leaf and nature of starch grains acquired a greater taxonomic importance.

Avdulov (1928 and 1931) followed by Hunter (1934) advanced a classification of this family based on karyological studies. From these they came to the conclusion that the *Gramineae* have a monophyletic origin.

The two tribes of *Gramineae* included in this study and to which the millets belong are *Chlorideae* and *Paniceae*. These form very important groups in this family having obviously very close connections. *Chlorideae* and *Agrostideae* are morphologically so similar to each other that sometimes an arrangement is preferred in which some of the genera belonging to *Chlorideae* are transferred to that tribe (Hutchinson 1934). Both of these tribes of *Gramineae* show similarities in many respects to *Phalarideae*, another tribe of *Gramineae*. *Paniceae* including the genera *Setaria*, *Panicum* and *Pennisetum* is the only important tribe of *Panicoideae* having genera with a mixture of both advanced and primitive characters.

From a cytological standpoint the millets have not received much attention. Cytological studies were confined to the determination of chromosome numbers only. Such studies were done on various genera by Avdulov (1931), Church (1929), Morinaga (1929), Rau (1929), Nakajima (1930), Hunter (1934), Krishnaswamy and Ayyanger (1935, 1941 and 1949), Kishimoto (1938), Krishnaswamy (1939, 1940 and 1950), Burton (1942) and

* Thesis approved for the degree of Doctor of Philosophy of the University of Allahabad, India (1955).

Janki Amal (Cf. Darlington 1945). Considering the enormous size of these genera covering the larger part of the globe and in the light of their economic importance they need be studied in greater cytological details. The present work was thus taken up to evaluate the compatibility with investigations of previous workers the conclusions of whom with regard to the basic number of genera *Eleusine*, *Dactyloctenium* and *Setaria* are different. Further our knowledge regarding the origin and fertility of the millets is also scanty. An attempt has therefore been made to arrive at definite conclusions regarding the basic number, mode of origin, original home and fertility of millets and their closely allied species.

Materials and methods

Seeds of *Eleusine coracana* were collected at the Agricultural Institute, Allahabad. Six more varieties were also sent by the Farm Superintendent, Millet Breeding Station, Tarikhet, Almora where breeding work is being intensively pursued between different varieties to obtain new and desirable agricultural forms for breeding. Seeds of *Panicum miliaceum* and *P. crus-galli* were collected at the Agricultural Institute Farm, Allahabad. *S. italica* seeds were received from the District Agricultural Officer, Garhwal to whom my thanks are due. The wild species of these genera together with two species of *Pennisetum* viz. *P. typhoideum* and *P. purpureum* and one more of a different genus-*Dactyloctenium aegyptium*, were collected from the local fields.

The following is the full list of the species and varieties studied cytologically:—

I. Tribe Chlorideae

1. Genus *Eleusine*

- (a) *E. coracana*
(Var. T136, T149, T41,
T63, T121 and T313;
Allahabad local)
- (b) *E. indica*
- (c) *E. verticillata*

2. Genus *Dactyloctenium*

- (a) *D. aegyptium*

II. Tribe Paniceae

1. Genus *Panicum*

- (a) *P. miliaceum*
- (b) *P. crus-galli*
- (c) *P. colonum*
- (d) *P. ischane*
- (e) *P. maximum*

2. Genus *Setaria*

- (a) *S. italica*
(Var. T4, T13 and Garhwal
local yellow and red).
- (b) *S. verticillata*
- (c) *S. glauca*

3. Genus *Pennisetum*

- (a) *P. typhoideum*
- (b) *P. purpureum*

These seeds thus obtained were sown in beds in the Botanical Gardens of the University of Allahabad in the month of July 1952. These flowered in the middle of November, 1952. Some of the seeds of the cultivated species were germinated on moist filter papers in petridishes. Root tips when 3/4 inch long were fixed in various fixatives.

Flower buds for meiotic studies were fixed in various fixatives after pretreatment with Carnoy's fluid (1930), La Cour's 2BE (1931), Navashin's fluid (Maeda, 1930) and Karpenchenko's fluid (1938) were tried. Levitsky's fluid (1930) with a pretreatment with chloral hydrate solution (0.4 %) was also used and proved better in *Panicum* material.

Meiosis was also studied from slides prepared by McClintock's smear method (1930). The smear material was used from flower buds fixed in aceto-alcohol for 8 hours, stored in 70 percent alcohol.

All the drawings were, unless otherwise mentioned, made at table level with the aid of Camera Lucida. An achromatic objective N.A. 1.5 was used in conjunction with Zeiss eye-piece K20, giving a magnification of 3750 diameters. These are further reduced to one-third in reproduction here.

Observations

Tribe *Chlorideae*

The genus *Eleusine* (Pl. VIII, 1-3; Figs. 1-48)

In well fixed materials the chromosomes were generally well spaced out and slender so that their size variations and morphology could be worked out fairly accurately. For the study of meiosis both paraffin and smear methods were used. Chromosome counts in all cases were made from root tip cells. Chromosome numbers of *Eleusine* *Spp.* studied are given in Table 1.

Table 1

Name of plant	Somatic number	No. of bivalents at meiosis
<i>E. coracana</i>	36	18
<i>E. indica</i>	18	9
<i>E. verticillata</i>	18	9

All the varieties of *E. coracana* at metaphase showed the somatic number to be $2n=36$. The chromosomes were similar in size and shape in all (Fig. 1). Chromosome number for *E. verticillata* has been determined for the first time being $2n=18$ (Fig. 2). The numbers ascertained for *E. coracana* and *E. india* (Fig. 3), confirm the counts made by Rau (1929), Avdulov (1931), Krishnaswami (1937) and Krishnaswamy and Ayyanger (1942). In the present work the characteristic morphology of the somatic chromosomes and size variations was studied in considerable detail from a large number of cells at metaphase. The results are given in Table 2.

Figs. 1-27. 1, somatic metaphase from root-tip cells of *E. coracana*, $2n=36$. 2, somatic metaphase from root-tip cells of *E. verticillata*, $2n=18$. 3, somatic metaphase from root-tip cells of *E. indica*, $2n=18$. 4, somatic metaphase from root-tip cells of *D. aegyptium*, $2n=36$. 5, one of the anther locules of *E. coracana* showing the division of the tapetal and pollen mother cells simultaneously. 6, leptotene threads in pollen mother cells of *E. coracana*. 7, early zygotene pairing in *E. coracana*. 8, early diakinesis in *E. coracana*. Three pairs have three chiasmata each, rest having either two or one chiasmata. 9, early diakinesis in *E. indica*. Two pairs have 3 chiasmata while others have either two or one only. 10, early diakinesis in *E. verticillata* having 2 chiasmata in six bivalents and rest have only one in each. Some have opened at one end due to complete terminalization. 11, first metaphase in *E. coracana* drawn after analysis of bivalents from different plates. Three bivalents show interstitial chiasmata, four have two chiasmata at the ends thus forming rings and rest have one chiasma thus having rod shapes. 12, first metaphase in *E. indica* showing interstitial chiasmata in two bivalents, two terminal in each of the two ring bivalents and one in each of the rods. 13, first metaphase in *E. verticillata* showing one bivalent having interstitial chiasmata, two with terminal chiasmata forming ring and rest rod shaped. 14, first metaphase in *D. aegyptium* which shows four bivalents with

Table 2

Shape of chromosomes	Name of plant	No. of chromosome	Length of each chromosome in μ
V shaped with median attachment constrictions	<i>E. coracana</i>	2	3.4
		2	3.1
		4	2.6
		2	1.9
	<i>E. indica</i>	4	3.1
		6	2.5
		4	2.8
		10	1.5
L shaped with sub-median attachment constrictions	<i>E. coracana</i>	4	3.1
		8	2.9
		4	2.7
		2	2.3
	<i>E. indica</i>	4	3.1
I shaped with sub-terminal attachment constrictions	<i>E. coracana</i>	2	2.7
		2	2.3
		2	2.1
		2	1.1
	<i>E. indica</i>	2	1.5
		2	1.0
	<i>E. verticillata</i>	4	1.0

The somatic metaphase plates (Figs. 1-3 show that the largest pairs fall in two categories viz. (i) with V. shapes and (ii) with L shapes. The apex of V is always turned towards the centre. The shortest chromosomes' pairs are distinctly shorter than the rest of the chromosomes. Other intermediate comparable pairs with differences of size and shape exist which make it possible to classify the 36, 18 and 18 chromosomes of *E. coracana*, *E. indica* and *E. verticillata* respectively.

The somatic metaphase in many cells showed that chromosomes were sorted out in pairs lying near each other without being in actual contact. The degree of this pairing, somatic pairing as it is called, between homologous chromosomes is variable in different cells, ranging from a few pairs to many (Figs. 1-3). The characteristic arrangement of the chromosomes

interstitial chiasmata, two terminal chiasmata in each of the two rings and rest have one chiasma in each bivalent. 15, prometaphase in *E. coracana*. The bivalents have gathered in the centre. 16, equatorial view of first metaphase in *E. coracana*. 17, first anaphase in *E. coracana*. 18, first telophase in *E. coracana*. 19, second metaphase in *E. coracana*. 20, first metaphase in *E. indica*. 21, first metaphase in *E. verticillata*. 22, early anaphase I in *E. indica*. 23, polar view of first metaphase in *E. verticillata* showing secondary association of 2(2)-5(1) type. 24, polar view of first metaphase in *E. verticillata* showing secondary association of 2(2)-5(1) types. 25, late telophase I in *E. coracana*. 26, late telophase I in *E. indica*. 27, early diakinesis in pollen mother cell of *D. aegypticum* showing 2 bivalents with 3 chiasmata, nine bivalents with 2 chiasmata each and rest with one chiasma each.

in pairs in all these cases cannot be an accidental one but must be on account of some specific attraction due to homology between paired chromosomes which may exist between parental chromosomes throughout the life cycle of the sporophyte. A paired condition of chromosomes was first found in somatic tissue of plants by Strausberger in 1905. This condition has also been reported in several other plants as in *Oenothera* (Gates, 1912), *Galtonia* (Newton, 1924), *Dahlia* (Lawrence, 1931), *Mathiola* (Huskins, 1932) and *Cajanus* (Naithani, 1941). Koller (1934) assumes that this somatic pairing may be conditioned by the genotype controlling the degree of attraction between homologous chromosomes. On the principle that pairs of paired chromatids repel each other, the two homologues which lie side by side ought repel each other because they are double each of them being composed of two chromatids. In the cases where somatic pairing occurs the forces of attraction between the chromosomes are presumed to overcome the repulsion between the pair of paired chromatids and in cases where the somatic pairing is absent the forces of repulsion are supposed to exceed those of attraction.

Early prophase stages were studied from preparations using McClintock's Method (1931). At the time of division the anthers were rather very small. They were taken out of the flowers with the help of dissecting microscope.

Three species of *Eleusine* have been studied but observations on *E. coracana* will be dealt with in detail below and any differences observed in case of other two *Eleusines* will be pointed out.

In *Eleusine coracana*, the tapetal cells were found dividing simultaneously with the pollen mother cells (Fig. 5). In other two species it was not so.

At leptotene chromosomes appear as single fine threads each having an uneven granular structure, presents the appearance of string of unequal beads strung together (Fig. 6). These threads are dispersed without any orientation throughout the nucleus. They can be followed to a considerable length but their relationships to each other cannot be made out clearly. Some of these threads have become aligned along short distances. The nucleolus is deeply stained. Many of the threads appear to be attached to it (Fig. 6). At the point of connection of the chromosomes to the nucleolus no granular structure is ever observed as has been reported by Latter (1926) in *Lathyrus*; McClintock (1934) in *Zea*; Naithani (1937) in *Hyacinthus* and Nandi (1937) in *Oryza*.

At early diplotene bivalents each consisting of two closely associated chromosomes are seen forming loops (Fig. 7). At this stage each chromosome is double in nature and is presumably composed of two chromatids. The chromosomes become condensed due to spiralization. The paired partners stretch out along their lengths except at various chiasma points. Spiralization continues and the bivalents are now clearly differentiated as 18 bodies in

R. P. Chandola: Cyto-genetics of Millets

E. coracana (Fig. 8). In *E. indica* and *E. verticillata* only 9 bodies in each were observed (Figs. 9 and 10). In none of the nuclei these bivalents showed any connection between themselves. They are free bodies spaced well apart in the nucleus (Figs. 8-10). Varying numbers of chiasmata in different pairs are discernible. These never exceed three. In *E. coracana* three bivalents show three chiasmata each (two interstitial and one subterminal, Fig. 8). In *E. indica* only two bivalents show three chiasmata (Fig. 9) in the largest chromosomes. Smaller chromosomes in these species as well as in those of *E. verticillata* (Fig. 10) form two or one chiasmata (interstitial or subterminal) much depending on the size of the chromosomes. Chiasma frequency has been worked out in detail in various stages.

Table 3. Analysis of chiasmata in *E. coracana*, *E. indica* and *E. verticillata*

Name of plant	No. of nuclei	No. of pairs of biv.	Total no. of chi.	Mean no. of chi. each biv.	No. of interstitial chi.	No. of terminal chi.	Terminal chi. per biv.	Terminalization Coefficient	Highest no. of chi. in any bivalent
DIPLOTENE									
<i>E. coracana</i>	10	180	320	1.75	185	140	0.77	0.44	3
<i>E. indica</i>	10	90	110	1.25	85	25	0.27	0.21	3
<i>E. verticillata</i>	10	90	108	1.20	80	28	0.31	0.25	2
DIAKINESIS									
<i>E. coracana</i>	10	180	312	1.73	130	182	1.01	0.58	2
<i>E. indica</i>	10	90	100	1.10	60	40	0.44	0.40	2
<i>E. verticillata</i>	10	90	96	1.05	30	65	0.72	0.68	2
METAPHASE									
<i>E. coracana</i>	10	180	260	1.44	40	220	1.20	0.82	2
<i>E. indica</i>	10	90	95	1.05	5	90	1.00	0.95	2
<i>E. verticillata</i>	10	90	92	1.02	3	89	0.98	0.92	2

Table 3 shows that the further condensation of the chromosomes leads to complete terminalization with the result that the two chromosomes of a bivalent are held together by terminal connections into rod or ring shaped bodies. On analysing the chiasmata it is seen that some of the chiasmata do not terminalize fully the bivalents retaining interstitial chiasmata at diakinesis and metaphase I (Figs. 11-13). As the contraction of bivalents advances the number of terminal chiasmata increases and interstitial chiasmata decrease in number.

At diakinesis the chromosomes appear to be shortened due to double coiling, the minor and the major coils. Eighteen bivalents in *E. coracana* and nine in each *E. indica* and *E. verticillata* are formed regularly and continue to be evenly distributed throughout the nucleus. No multivalents

have been observed in any of these species. At this stage the two chromatids of the homologous chromosomes are so close to each other that their identity is lost. Three important points may be noticed: First, the longer chromosomes have the greater number of chiasmata; secondary, they have in consequence the greater proportion of the interstitial type of chiasma; and thirdly, apparently also in consequence, it is amongst the shorter types that the loosely paired or paired-at-one-end chromosomes are most frequent. The bivalents are of different sizes, the differences being similar to those of somatic chromosomes. One bivalent is always attached to the nucleolus in all the *Eleusines*.

Nuclear membrane disappears at the inception of prometaphase and a spindle begins to be organised (Figs. 15 and 16). At the end of diakinesis the converging movement of bivalents became more intense resulting in crowding of chromosome pairs in the centre of the nucleus. The bivalents are quite free from each other without any physical connection even though they are so near to each other (Fig. 15). These bivalents now have contracted to their maximum. At this stage the bivalents have begun to pair which manifests itself in a prominent manner in the succeeding metaphase and anaphase. Such pairing of the bivalents has been described as secondary association resulting from a generalized attraction between phylogenetically though distantly related bivalents. It is characteristic of secondary polyploids and is a measure of quantitative and structural changes in a given complement.

In polar as well as equatorial views of metaphase I the bivalents are found grouped in twos, threes and fours. This happens due to a mutual attraction among the closely associated bivalents. Thomas and Revell (1946) in *Cicer areitinum* have shown physical connections between the associated bivalents. It has not been possible to see such connections in *Eleusine* materials and also other millets described later. Analysis of complete side views of metaphase I is found difficult in *E. coracana* owing to the secondarily paired chromosomes lying perpendicular to the microscope plane. In other species, however, full views of metaphase I plates were easily analysed (Figs. 20 and 21).

Since the grouping of bivalents at this stage is due to secondary association, caused by the distant relationship between chromosomes, it will be used here for determination of the original basic composition of the chromosomes in *Eleusine*.

The various groupings of secondary association observed in polar views (some of these are also confirmed in equatorial views) of metaphase I in the three species of *Eleusine* are given in table 4 (Figs. 23, 24 and 28-38).

Every *E. coracana* metaphase I plate examined showed secondarily associated bivalents. A considerably large number of cells were examined in this species but nine groups of two bivalents each were never observed as has been reported by Krishanswamy (1937). His studies do not seem to be

critical as he has based his results on the examination of a few cells in each case. During the present studies it has been noticed that in *E. coracana* groups of three bivalents were frequently met with (Table 4). If the formation and the number of groups are considered, the different combinations of bivalents present suggest a lower basic number than nine.

Table 4. Types of secondary associations observed in metaphase I

Name of plant	No. of sec. assoc.	No. of biv. in assoc.				No. of cases observed	Total	Fig. no.
		1	2	3	4			
<i>E. coracana</i>	1	15	—	1	—	5	5	28
	2	14	2	—	—	13	13	29
	3	11	2	1	—	20	30	30
	3	10	1	2	—	10		31
	4	9	3	1	—	20	35	32
	4	8	2	2	—	9		33
	4	7	1	3	—	6		34
	5	6	3	2	—	7	14	35
	6	3	3	3	—	8		36
	6	2	2	4	—	6		37
	7 (Max.)	—	4	2	1	3	9	38
<i>E. indica</i>	No.	9	—	—	—	30	30	—
	1	7	1	—	—	4	4	—
	2 (Max.)	5	2	—	—	4	4	23
<i>E. verticillata</i>	No.	9	—	—	—	10	10	—
	1	7	1	—	—	5	5	—
	2 (Max.)	5	2	—	—	3	3	24

Anaphase I proceeds normally in all the species (Figs. 17 and 22). During the separation in univalents, secondary association persisted. At this stage it is difficult to have any definite idea about the different associations particularly in *E. coracana* where the number of univalents for one pole is eighteen.

Telophase I is quite regular in all these species and two nuclei are always formed at the end of division I (Figs. 18, 25 and 26).

At metaphase II also chromosomes were seen secondarily associated. The following patterns of groupings were generally met with (Fig. 19).

Secondary association of univalents was much pronounced at metaphase II. This has also been observed by Lawrence (1931) in *Dahlia*; Müntzing (1933) and Nandi (1935), both in rice, where a more intensive association is met with in metaphase II.

In none of the metaphase plates observed was more than one group of four chromosomes observed. In both smeared and sectioned preparations it was also noticed that the associated bivalents or univalents involved in any

secondary association group did not strictly show identity in size but varying sized chromosomes were also frequently found associated (Figs. 28-38).

Table 5. Types of secondary associations at metaphase II

Name of plants	No. of secondary association	No. of univ. in assoc.				No. of cases observed
		1	2	3	4	
<i>E. coracana</i>	1	16	1	—	—	10
	2	14	2	—	—	20
	3	11	2	1	—	8
	5	6	3	2	—	8
	6 (Max.)	—	4	2	1	4*
<i>E. indica</i>	2 (Max.)	5	2	—	—	1
<i>E. verticillata</i>	2 (Max.)	5	2	—	—	1

* Fig. 43.

Figs. 28-48. Figs. 28 to 45. Polar views of metaphases I and II showing varying degrees of secondary association of bivalents and univalents. *E. coracana* (Metaphase I). 28, 1(3)-15(1). 29, 2(2)-14(1). 30, 1(3)-2(2)-11(1). 31, 2(3)-3(2)-10(1). 32, 1(3)-3(2)-9(1). 33, 2(3)-2(2)-8(1). 34, 3(3)-1(2)-7(1). 35, 2(3)-3(2)-6(1). 36, 3(3)-3(2)-3(1). 37, 4(3)-2(2)-2(1). 38, 1(4)-2(3)-4(2). *D. aegyptium* (Metaphase I) 39, 2(3)-12(1). 40, 2(3)-1(2)-10(1). 41, 3(3)-1(2)-7(1). 42, 4(3)-3(2). 43, polar view of second metaphase in *E. coracana* showing a maximum association of 1(4)-2(3)-4(2). 44, polar view of second metaphase in *D. aegyptium* showing a maximum association of 4(3)-3(2). 45, second metaphase in *D. aegyptium*, $n=18$. 46, three pollen grains of *E. coracana* (700 $\times$). 47, three pollen grains of *E. indica* (700 $\times$). 48, three pollen grains of *E. verticillata* (700 $\times$).

The maximum associations observed consist of one group of four chromosomes, two groups of three chromosomes and four groups of two chromosomes in *E. coracanna* (Fig. 38). In *E. indica* and *E. verticillata* both a maximum association is brought by two groups of two chromosomes and five chromosomes remaining free (Figs. 23 and 24). Therefore the basic chromosome number in *Eleusines* is seven. The present number nine has been derived from number seven by secondary polyploidy.

The genus *Dactyloctenium*

Genus *Dactyloctenium* bears a close resemblance to *Eleusine* in morphological characters and has the similar geographical distribution in the world. It is a monotypic genus having only one species viz. *D. aegypticum*. Chromosome number of this species was obtained from the somatic mitosis in root tip cells. It has $2n=36$ (Fig. 4). The chromosomes are almost of the sizes found in *E. coracana*. Avdulov (1931) reported somatic number

Table 6. Analysis of chiasmata in *Dactyloctenium aegypticum*

No. of nuclei	No. of pairs of bivalents	Total No. of chi.	Mean no. of chi. each biv.	No. of interstit. chi.	No. of terminal chi.	Terminal chi. per biv.	Terminaliz. coefficient	Highest no. of chi. in any of the biv.
<i>Diplotene</i>								
10	180	328	1.8	185	143	0.79	0.43	3
<i>Diakinesis</i>								
10	180	312	1.73	130	182	1.01	0.58	2
<i>Metaphase</i>								
10	180	265	1.47	45	220	1.20	0.81	2

as 48 while Krishnaswamy (1931) gave a probable number of 34. A careful and critical study of the chromosomes of *D. aegypticum* from a number of metaphase plates in polar view shows that it has 36 chromosomes. A detail study of these revealed that 10 chromosomes have median constrictions. In these four are of 2.8 microns length and six are 2.5 microns long. 12 have submedian constrictions of which four are 2.9 microns and eight are 2.5 microns long. The rest are with subterminal constrictions among which six are 2.5 microns, four are 2.1 microns and four are 1.0 micron long (Fig. 4).

Meiotic studies of *D. aegypticum* reveal that the division of chromosomes is quite regular. At diplotene two bivalents had 3 chiasmata each and the rest had either two or one depending on the size of the chromosomes.

At early diakinesis different sizes of bivalents with different shapes can be seen (Fig. 27). Two bivalents seem to be attached to the nucleolus.

At metaphase I the bivalents, 18 in number assume various arrangements due to secondary association of chromosomes. These results are summarised

in table 6 (Figs. 39-42). Presence of eighteen bivalents confirm that the $2n$ number of this species is 36.

Table 7. Types of secondary association observed at metaphase I and II in *D. aegepticum*

No. of secondary association	No. of bivalents in association				No. of cases observed	Total	Fig. nos.
	1	2	3	4			
At metaphase I							
2	12	—	2	—	3	3	39
3	11	2	1	—	2	7	—
3	10	1	2	—	5		40
4	8	2	2	—	3	6	—
4	7	1	3	—	3		41
5	6	3	2	—	2	2	—
6	3	3	3	—	4	4	—
7	1	4	3	—	7	9	42
7 (Max.)	—	3	4	—	2		
At metaphase II							
3	10	1	2	—	3	3	—
4	7	1	3	—	2	2	—
5	6	3	2	—	3	3	—
7 (Max.)	—	3	4	—	2	2	44

The maximum association in this species consisted of four groups of threes and three groups of twos (Fig. 44). Secondary association studies have been done for the first time in this genus. These results lead to the conclusion that the basic number in this genus too is seven.

Metaphase II is normal in this species (Fig. 45).

Tribe *Panicaceae*

The genus *Panicum* (Pl. VIII, 9; Figs. 55-66)

The chromosomes are too small to permit a critical study of morphology and details of early meiotic prophase in the genus *Panicum*.

Panicum miliaceum

Counting of chromosomes from the root tip cells revealed that this species has $2n$ number as 36 (Fig. 48). This is in confirmation with counts made by Avdulov (1931) and Hunter (1934).

Counts made during diakinesis reveal in some cases much less than eighteen bodies and as much as 7 or 8 have also been counted (Fig. 55). The average number seems to be somewhere between 10 and 12. These bodies markedly differed in size and as diakinesis proceeds the larger ones are seen to be compound, their components giving rise to characteristic multivalent associations at diakinesis (Figs. 55-57). Figure 55 illustrates a cell at diakinesis with $1_{VII}-2_{VI}-3_{IV}-2_{II}$. A different and very rare configuration

or $3_{VI}-3_{II}-3_{IV}$ is also observed (Fig. 56). In some cases only quadrivalents are observed to occur in almost innumerable variations although certain simple forms predominate.

Figs. 49-66. 49, *Panicum miliaceum*. Somatic chromosomes from root tip cells ($2n=36$). 50, *Panicum cruss-galli*. Somatic chromosomes from root-tips ($2n=36$). 51, *Panicum colonum*, Somatic chromosomes from root tips ($2n=36$). 52, *Panicum ischane*. Somatic chromosomes from root tips. ($2n=36$). 53, *Panicum maximum*. Somatic chromosomes from root tips ($2n=32$). Four chromosomes have satellites. 54, polar view of metaphase I in *Panicum miliaceum* showing 18 bivalents. 55, diakinesis in *P. miliaceum* showing an association of $1_{VII}-2_{VI}-3_{IV}-2_{II}$. 56, diakinesis in *P. miliaceum* showing an association $3_{VI}-3_{IV}-3_{II}$. 57, diakinesis in *P. miliaceum* showing six quadrivalents and other chromosomes. 58, side view of metaphase I in *P. cruss-galli*. 59, second metaphase in *P. miliaceum*. 18 bivalents on each side are noticed. 60, diakinesis in *P. cruss-galli*. $1_{VI}-2_{IV}-1_{II}$ are noticed. 61, anaphase I in *P. cruss-galli*. Four bivalents are seen on the equator. 62, diakinesis in *P. colonum* showing $1_{VI}-3_{IV}-II$. 63, anaphase I in *P. colonum* showing regular division. 64, diakinesis in *P. maximum*. 65, polar view of metaphase I in *P. maximum*. 66, second metaphase in *P. maximum*.

In side views it can be seen that the multivalents arrange themselves on the equator in haphazard fashion. Multivalents involving the same number of chromosomes do not always combine in the same manner e.g. in some rings are found while in others chains are present (Figs. 55-57). Bivalents are invariably rod shaped and disjoin most easily at anaphase I. About 60

per cent plates showed regular formation of bivalents at metaphase I (Fig. 58).

At anaphase the multivalent chromosomes disjoin irregularly in all the cases. Probably this is an immediate consequence of the chromosomes failing

Table 8. Types of configurations seen at metaphase I of *P. miliaceum*

VIII	VI	IV	III	II	I	No. of cells examined
1	3	—	—	4	2	2
1	3	—	1	2	3	1
—	1	5	—	4	2	20
—	4	—	—	4	4	11
—	—	9	—	—	—	10
—	—	—	—	18	—	30
						Total 74

to assort themselves regularly on the equatorial plate at metaphase. As a result the chromosomes fail to separate though nothing can be said about their forming restitution nuclei. This irregularity is observed in 30 per cent cases. Division II is much more regular than division I. A normal metaphase II plate is illustrated in Fig. 59.

Pollen sterility in this species is 32 per cent.

Panicum cruss-galli

In somatic metaphase from root tip cells the $2n$ number was found to be 36 (Fig. 50). This number is reported by Krishnaswamy also (1939).

At diakinesis (Fig. 60), multivalent formation of chromosomes is prevalent though to a lesser extent than in *P. miliaceum*. The configurations commonly met with are summarised in the table 9.

At anaphase unpaired chromosomes are sometimes left behind on the equator though they were not seen dividing (Fig. 61). Since some unpaired ones that have

Table 9. Frequency of multivalent formation in *P. cruss-galli* at metaphase I

VI	IV	II	I	No. of cases observed
1	2	11	—	30
—	6	4	4	7
—	8	2	—	5
—	9	—	—	2
				Total 44

been lying on one side of the equator probably always pass to the poles without division, it is possible that the laggards are partly the results of breaking up of the hexavalents and quadrivalents. Thus anaphase I is irregular in this species.

Pollen sterility in this species is 40 per cent.

Panicum colonum

Somatic number in this species is 36 (Fig. 51). This has also been observed by Rau (1929).

Figs. 67-98. 67, somatic metaphase in the root tip cells of *S. italica*, $2n=18$. 68, somatic metaphase in the root tip cells of *S. verticillata*, $2n=36$. 69, somatic metaphase in the root tip cells of *S. glauca*, $2n=36$. 70, diakinesis in pollen mother cells of *S. italica*, $n=9$. 71, diakinesis in pollen mother cells of *S. verticillata*, $n=18$. 72, diakinesis in pollen mother cells of *S. glauca*, $n=18$. 73, prometaphase in *S. verticillata*. 74, prometaphase in *S. glauca*. 75, side view of metaphase I in *S. italica*. 76, polar and equatorial view of metaphase I in *S. verticillata*. Note some of the bivalents show secondary association. 77, side view of metaphase I in *S. glauca*. 78, no secondary association in *S. italica*. 79, secondary association of 1(2)-7(1) in *S. italica*. 80, secondary association of 2(2)-5(1) in *S. italica*. 81, a maximum secondary association of 1(3)-2(2)-2(1) in *S. italica*. 82, secondary association in *S. verticillata*. 83, secondary association in *S. glauca*. 84, telophase I in *S. italica*. 85, telophase I in *S. verticillata*. 86, anaphase I in *S. glauca*. 87, metaphase

This species also shows marked multiple association of chromosomes at the first division (Fig. 62). At metaphase I quadrivalents, hexavalents and bivalents occur in approximately this order of frequency in the plates examined, bivalents being by far the least frequent. Side views of metaphase plates exhibit regularity in the line up for division. At anaphase the movement of the chromosomes to the poles proceeds with much uniformity, laggards have never been seen (Fig. 63). It seems that the irregularity is in the second division as the pollen fertility is very low.

Pollen sterility is 45 per cent.

Panicum ischane

Somatic number in the root tip cells was found to be 36 (Fig. 52). Avdulov (1931) recorded $2n$ as 18 but Krishnaswamy (1940) gives the count as 36.

Metaphase I plates show multivalents formation and the pollen count also confirms that the meiotic irregularity is the main cause of sterility. In many instances four hexavalents, two quadrivalents and two bivalents were observed in the same P.M.C. Various other combinations are also met with (Fig. 64).

Pollen sterility is 25 per cent.

Panicum maximum

In *P. maximum* the somatic number has been observed to be $2n=32$, this being contrary to the reports of Burton (1940) who counted $2n=36$. There are two pairs of satellited chromosomes in the somatic complement of this species (Fig. 53). One pair is large and other consists of two small chromosomes. Probably in this species a reduction of two pairs in the somatic number has taken place. Thus this species has chromosome number four less than the tetraploids. This is almost a case like genus *Dahlia* where addition of four chromosomes gave rise to species $2n=36$ (*D. merkii*; Lawrence, 1929), while other species have chromosomes as $2n=32$ or in other multiples of eight.

At metaphase I polar views, multivalents, quadrivalents and bivalents are observed (Fig. 65). Sixteen bivalents were, however, ascertained in many instances in metaphase II (Fig. 66). The development of the quadrivalent bodies is naturally variable. The pairing between the components each to each varies and probably results from differences of chiasma formation and further development.

II in *S. verticillata*. 88, metaphase II in *S. glauca*. 89, pollen grains of *S. italica*. 90, pollen grains of *S. verticillata*. 91, pollen grains of *S. glauca*. (700 $\times$). 92, somatic metaphase in *Pennisetum typhoideum*. Six chromosomes have median constrictions, four have sub-median and rest have sub-terminal constrictions. 93, diakinesis in *Pennisetum typhoideum*. 94, polar view of metaphase I in *P. typhoideum*. 95, polar view of metaphase II in *P. typhoideum*. 96, somatic metaphase in *Pennisetum purpureum*, $2n=28$. 97, a cut cell showing multivalents in *P. purpureum*. 98, a cut cell showing some more multivalents of *P. purpureum*.

Lagging chromosomes are seen at Anaphase I.

Metaphase II plates showed in a few cases an unequal number in the divided nucleus.

Pollen sterility is 30 per cent.

In addition to the cytological studies in the different species of *Panicum*, the fertility of pollen grains, setting of seeds in flowers and germination percentage of seeds were also observed. These are summarised in table 12.

The pollen grains in all species are of the same size. They varied in number of good pollen, a result to be expected in view of the intensity of irregular meiosis in each species. The normal pollen are uniform in size and structure and take a deep stain. These apparently represent functional ones. Percentage of such pollens is 70 in *P. miliaceum*, 60 in *P. cruss-galli*, 75 in *P. ischane*, 55 in *P. colonum* and 70 in *P. maximum* (Table 12). Non-viable pollen grains are identified with their shrivelled and empty nature and these also do not take good stain. Setting of seed varies significantly in different species being 88 per cent in *P. miliaceum*, 42 per cent in *P. cruss-galli*, 60 per cent in *P. colonum*, 80 per cent *P. ischane* and 30 per cent in *P. maximum*. Among these species a direct correlation between percentage of good pollen and the number of seeds-set is marked in *P. miliaceum*, *P. colonum* and *P. ischane*. No such relation is, however, maintained in case of *P. cruss galli* and *P. maximum* because these species show a poor seed setting ability inspite of higher percentage of pollen fertility. Germination tests show that the species having a high seed setting capacity produce a larger percentage of viable seeds.

The genus *Setaria* (Pl. VIII, 4-7; Figs. 67-91)

Somatic studies were done in three species viz. *S. italica*, *S. verticillata* and *S. glauca*. The chromosomes of all varieties of *S. italica* were similar in all respects and are larger than those of *S. verticillata* and *S. glauca*. Somatic number was ascertained from the metaphase of the root tips as $2n=18$ in *S. italica* (Fig. 67), $2n=36$ in each of *S. verticillata* (Fig. 68) and *S. glauca* (Fig. 69). Krishanswamy and Ayyanger (1935) and Wet (1954) have on the other hand reported the $2n$ number in *S. verticillata* as 18. Avdulov (1928), however, gives the somatic number as verified here.

The size differences and morphology could be fairly worked out in *S. italica*. In, *S. verticillata* and *S. glauca* no such study could be done as in these species constrictions could be discerned in very few chromosomes and these too were not well marked to allow their use for an exact chromosome analysis. Therefore in the latter two species length of individual chromosome was determined and the results are given in Table 10.

Setaria italica chromosomes present a range from 2.2 to 1.1 microns. *S. verticillata* had chromosomes varying from 1.3 to 0.6 microns. In *S. glauca* it was revealed that chromosome lengths varied from 1.0 to 0.5

Table 10

Shape of chromosomes	Length in μ	No. of chromosomes	Total length in μ
<i>S. italica</i>			
V with median attach. constr.	2.2	2	4.4
	1.5	4	6.0
	1.8	2	3.6
L with submedian attach. constr.	1.1	4	4.4
	2.2	2	4.4
	1.8	2	3.6
I with subtermin. attach. constr.	1.1	2	2.2
			Total 28.6 μ
<i>S. verticillata</i>
1.3	8	10.4
	1.0	10	10.0
	0.8	12	9.6
	0.6	6	3.6
			Total 33.6 μ
<i>S. glauca</i>
1.0	6	6
	0.7	8	5.6
	0.5	22	11.0
			Total 22.6 μ

microns. Thus the length of largest chromosome in the diploid *S. italica* is approximately double the length of that longest in either *S. verticillata* or *S. glauca*. The same relation is noticed among the shortest chromosomes' lengths.

No satellites or secondary constrictions have ever been observed.

Pollen mother cells in the genus *Setaria* after the premeiotic division were distinctly round. They were

loosely packed up in the locules. The leptotene threads seemed to have some connection with the nucleolus though no definite points of attachments or any special structure on the chromosomes could be traced out in any of the species studied. No budding or fragmentation of nucleolus was ever observed as has been reported in *Lathyrus odoratus* by Latter (1926), in *Oryza sativa* by Mazmudar and Datta (1935) and Nandi (1936). The early meiotic stages could not be studied due to very delicate nature of the chromosomes.

At diakinesis the chromosomes in all the species of the genus *Setaria* are thick and short (Figs. 70-72). As further shortening proceeds the adjoining loops between chiasmata were pushed towards the ends of the chromosomes. The compact bivalents are seen evenly distributed throughout in the nucleus. The medium sized chromosomes form two chiasmata which terminalize by metaphase I to give ring bivalents with two terminal chiasmata or the chiasma in one side sometime may be incompletely terminalized (Figs. 70-72). Small chromosomes form a chiasma in one side and by metaphase I the chiasma completely terminalizes resulting in a rod bivalent with one terminal connexion (Fig. 75).

In *Setaria italica* only one bivalent is found attached to the nucleolus. In *S. verticillata* and *S. glauca* due to their being tetraploids are with two

bivalents in each of them attached to the nucleolus.

No multivalents or univalents have been observed in any of the species.

The bivalents came close to each other in the centre of the cell at pro-metaphase (Figs. 73-74). Nucleolus completely disappeared and a spindle was formed in the central region at this stage.

In *S. italica* nine bivalents become more conspicuous at metaphase I (Fig. 75). They were in condensed stage and all of them could be seen on one plane placed on the equator of the spindle. In each of the *S. verticillata* and *S. glauca* eighteen bivalents were regularly formed (Figs. 76 and 77).

The bivalents at metaphase I were seen secondarily paired in polar as well as equatorial views in all the three species (Figs. 78-83). A detailed study of secondary association could only be taken in *S. italica* alone. Table 11 shows the various groupings of bivalents at metaphase I.

Table 11. Types of secondary associations in metaphase I of *S. italica*

No. of 2ndry assoc.	No. of bivalents in assoc.			No. of cases	Fig. nos.
	1	2	3		
No	9	—	—	8	78
1	7	1	—	15	79
2	5	2	—	32	80
3	2	2	1	7	81

Some of the metaphase plates do not show secondary association (Fig. 78). The nine bivalents in such cases were more or less evenly dispersed with two bivalents in the centre and seven on the periphery. Nandi (1936) also recorded the same type of ar-

range in *Oryza sativa* and showed that such bodies always arrange themselves on definite patterns governed by the principle of floating magnets. The metaphase plates in other two species also show that secondary association does occur in them (Figs. 82 and 83). A detailed study, however, with a view to show the definite grouping and original basic number could be undertaken in *S. italica* only.

Thus the maximum grouping in *S. italica* consists of groups as $1_3-2_2-2_1$, thus the basic number coming to 5. Therefore the original basic number in this genus is five and not nine as supposed by Avdulov (1928). By assuming that structural changes have taken place in the original complement, the haploid genom of *S. italica* can be shown to have the following chromosomes:—

AA₁A₂, BB₁, CC₁, D, E

Anaphase I and telophase I stages are quite normal. The univalents go to poles in these species in balanced numbers and form two normal daughter nuclei (Figs. 84-86). At metaphase II also the univalents showed secondary association though less intensively (Figs. 87 and 88). In *S. italica* the maximum association of $1_3-2_2-2_1$ is confirmed at this stage also.

Pollen grains in these species were always normal and took a deep stain.

This was because of the fact that their normal contents were included in them during their formation (Figs. 89-91).

The genus *Pennisetum* (Pl. VIII, 8; Figs. 92-98)

P. typhoideum

Uniform distribution of chromosomes on the metaphase plates in the root tip cells revealed fourteen as the $2n$ number (Fig. 92). Rau (1929) and Krishnaswamy (1940) have also reported this number. Among these 7 pairs three V shaped, two L shaped and two rod shaped were observed. This is, however, in difference to Rau's observations who found four rod shaped pairs, two L shaped and one V shaped pairs. Seven bivalents at diakinesis were observed (Fig. 93). Out of these five are ring shaped and two X shaped. Nucleolus is not persistent. At metaphase I seven bivalents are noticed (Fig. 94). These exhibit variations in size.

The anaphase I separation of bivalents into univalents and their tendency to move to poles is observed to be regular. Laggards have been reported by Church (1929 a and b), Asse (1930), Parathasarathy and Ramanujam (1934), Ragavan and Venktasubban (1934) Myers and Hill (1940) and others.

At metaphase II the chromosomes are smaller than chromosomes of metaphase I and are crescent shaped (Fig. 95).

Telophase II is normal and shows regular division.

P. purpureum

Mitosis in root tip cells presented metaphase plates with chromosome number as $2n=28$ (Fig. 96). This is in agreement with the results of Burton (1942) and Krishnaswamy and Ayyanger (1949). Four chromosomes were seen attached to the nucleolus. For meiosis only the metaphase I plates could be made available for study. These showed presence of multivalents and univalents (Figs. 97 and 98). Maximum number of quadrivalents in any of the 10 available plates was three (Fig. 97).

The cytological examination of millets and their allied species has made the problem of relationship and phylogeny easier to solve. It was deemed fit to examine the species in the above mentioned context in order to find out the perceivable effects of chromosome changes in the different species. Such changes certainly were found to influence the various plants in many characters which has been given in Table 12.

From the following table it is evident that *E. coracana*, *D. aegypticum*, *S. verticillata* and *S. glauca* are tetraploid species. In these genera *E. indica*, *E. verticillata* and *S. italica* are the diploid species each having $2n=18$. The tetraploid nuclei are from about $1\frac{1}{2}$ to $2\frac{1}{2}$ times bigger than the diploid nuclei. Same is true when a consideration of many other readings is done. This increase in specific parts of plants primarily happens due to increase in chromosome numbers. Lindstrom and Humphry (1933) and Humphry (1934) observed such a tendency in tetraploid tomatoes. Smith (1943) also came to similar conclusion by studying haploid, diploid, tetraploid and octoploid

Table 12. Linear morphological and morphological studies in the millets

Name of plants	Haploid number	Cell size from mitotic metaphase	Diameter of cell at diakinesis (μ)	Diameter of nucleus at diakinesis (μ)	Total chromosome lengths (μ)	Pollen grain size (μ)	Viable pollen ¹ per-centage	Seed size (mms)	Seed ² set per-centage	Seed ³ germination per-centage	Size of stomata from leaf epidermis (mms)	Height of plants (Ft)
<i>E. coracana</i>	18	13.6	11.0	3.7	96.8	18.0	98	2.00	95	95	3	3.5
<i>E. indica</i>	9	8.4	6.6	2.7	27.4	7.3	98	0.75	95	98	1.6	2.0
<i>E. verticillata</i>	9	8.5	6.3	2.7	30.0	7.5	98	0.85	90	78	1.65	2.0
<i>D. aegyptium</i>	18	12.8	10.0	3.5	85.6	18.0	95	1.25	90	90	2.5	2-3.5
<i>S. verticillata</i>	18	10.4	8.8	4.4	33.6	18.0	95	0.7	90	98	3.15	5.0
<i>S. glauca</i>	18	8.5	7.9	3.2	23.6	16.0	90	1.2	95	90	3.2	1.5
<i>S. italica</i>	9	5.1	4.1	5.1	28.6	8.2	96	2.0	98	98	1.8	5.3
<i>P. miliaceum</i>	18					15-18.2	70	3.5	88	96		
<i>P. crus-galli</i>	18					"	60	3.2	42	48		
<i>P. colonum</i>	18					"	55	3.0	60	86		
<i>P. ischane</i>	18					"	75	2.6	80	85		

1. Pollen grains of these species were examined in a drop of lactophenol containing cotton blue. 1000 pollen grains have been counted in each case.

2. This has been counted out of 1000 flowers in each species.

3. Germination tests were done in each individual species by growing them on moist filter papers in petridishes. For this 100 seeds of each species were taken.

series in *Nicotiana langsdorffii*. Exception to this general rule is found in *S. glauca*. When the plant sizes are taken into account the conclusion differs in this respect. The plants of tetraploid *S. glauca* are much smaller than those of other two *Setarias*. *S. verticillata* another tetraploid has smallest seeds in the genus. This shows that certain structural features undergo modifications proportionate to the degree of chromosome doubling while other factors such as seed size and plant size fail to express the above mentioned consistent correlation with the double number of chromosomes. *S. glauca* and *S. verticillata* though have the same chromosome number but still one grows lavishly and with other, the growth is restricted considerably under natural conditions. Dwarfing of amphidiploid *Nicotiana rustica* $\times$ *N. glauca* was reported by Kostoff (1935a and b) and in *N. cotophora* and *N. tabaccum* populations of Goodspeed and Bradley (1943). Kostoff attributed the lower vitality of his amphidiploids due to too great an increase in chromosome numbers. This, however, does not seem to be true in case of *S. glauca* as the species *S. verticillata* has the same chromosome number and is a vigorously growing plant with bigger size. Probably the quantitative chromosome alterations have accounted for this decrease in plant size in this case. Genetic factors can suppress the robustness which is sometimes associated with chromosome doubling. Blacklee (1939), Straub (1940), Randolph (1941), Pirche (1942) and Larsen (1943) examined the effects of artificially induced polyploid on pure species of various types. A survey of their studies brings home the fact that the only generalization which may be made about the morphological and physiological effects of polyploidy is that these depend greatly on the nature of genotypes. The popular conception that polyploidy usually produces gigas types which are larger than their diploid ancestors is perhaps true in special instances, particularly if the original diploid is heterozygous as is found of the progenitors of the first known gigas tetraploid of *Oenothera lamarckiana* (Gates, 1909). Under these circumstances it can be ascertained that polyploidy can increase cell size in the meristematic tissue, but the eventual size of individual organs and of the plant as a whole depends as much on the amount of cell elongation and on the number of cells; produced during growth as it does on the initial size of the cell. Both of these latter processes can be adversely affected by polyploidy.

S. italica although is a diploid species but has the largest seeds, This is perhaps due to selection by human beings. This being a cultivated species is known to be grown for food since times immemorial. The seed size factor as in other several other cultivated crops seems to have been modified greatly by culture.

Stomatal and pollen grain sizes are valuable criteria for the detection of polyploid plants in genera *Eleusine*, *Setaria* and *Pennisetum*. These sizes are proportional to the degree of polyploidy.