Abnormal Meiosis in the Grain Sorghums

II. The cytogenetics of the progeny of the desynaptic plant—2

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Two types of desynaptic plants were described in grain sorghums (Krishnaswamy and Meenakshi 1957) of which the second type (Dsy-2.) produced more seeds. This plant was peculiar in producing almost no seed when selfed but when dusted with pollen from a tetraploid grain sorghum plant (Krishnaswamy 1957) a large number of seeds was obtained. The meiosis showed 0-3 bivalent formation and a high frequency of 20-0 distribution at the first division. When the panicles were dusted with pollen from a normal (synaptic) diploid plant only a few seeds set. At this time it was suspected that the seeds which set when panicles were enclosed in a selfing paper bag, may be apomictic. The panicles were dusted fully with pollen from *Pennisetum typhoides* and also from a $4n$ -maize plant. In both cases very few seeds set.

The seeds were all sown during 1956 main season and transplanted into the field. Most pollinations with $4n$ -sorghums resulted in hybrids. The others turned out to be either selfed or crossed with other diploid sorghums. The present report deals exclusively with the latter plants.

Materials and methods

All seedlings were raised in seed pans and when about a month old transplanted into the field. Before sowing the well filled and shrivelled seeds were separated. Such seeds which were very poorly developed and small were placed between moist blotting papers and allowed to germinate in petri dishes. To prevent mould development they were treated with ceresan before sowing.

All finer measurements were done with a pair of callipers with a vernier reading to 1/10 mm. Other measurements like height etc., were done with meter rods and scales. The diameter of pollen grains was measured with a Spencer $\times 10$ micrometer ocular. For meiotic studies the buds were fixed in acetic alcohol 1:3 mixture and smeared in acetocarmine or propionocarmine. Mostly temporary preparations were used in the study. Microphoto-

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graphs were taken with a Zeiss Kolibri camera on either Kodak Verichrome 127 or Gevart Gevachrome 127 films.

Observations

The seedlings were found generally slower in growth than those of the control. In some cases they often developed a reddish purple colour in the coleoptile and the first leaf which later on disappeared. This was especially prominent in those seedlings which failed to grow beyond the second leaf stage. Out of 78 seeds sown 18 failed to germinate, 35 survived and grew to maturity, while the rest died at different stages of growth.

Fig. 1. a-f. a, 25-, b, 28-, c, 30-, and d, 40-chromosomed plants. e, desynaptic $2n$. f, normal $2n$ -parent. a, $\times 1/11$. b, $\times 1/24$. c, $\times 1/36$. d, $\times 1/34$. e, $\times 1/36$. f, $\times 1/50$.

The trials of pollinations mentioned above gave some interesting results. Pollen from the normal diploid sorghum did not produce any tubes while that from the $4n$ -plant gave better germination. There was hardly any stimulation to growth in pollen from $4n$ -maize. The pearl millet pollen gave a high percentage of germination and produced long healthy tubes but none penetrated into the papillae of the stigma. All the tubes ramified entirely on the surface of the stigmatic cells.

The thirty five plants progeny surviving, consisted of one each of 25-, 28-, and 30- and the rest of 40-chromosomed plants (fig. 1). All of them were similar in morphological characters and resembled closely the normal parent, A.S. 681 (Fig. 1f). The general description is as follows:

Plants with medium thick smooth stems, with slight bloom at the nodal

¹ The term 'normal' is used in this paper to denote the condition existing in a diploid synaptic fertile plant.

region, nodes glabrous, green, not pigmented stalks sweet, pithy, 1-2 tillered; leaves broad, wavy, midrib white, leaf junction green, leaf sheath reddish purple, axil purple. Panicles medium compact, conical, spikelets reddish purple, wrinkled, awns long, anthers yellow, drying to red, stigma yellow; grains umbonate, exposed, red, not shattering.

In the fertile parental panicles the grain set is complete while in the desynaptic few to nil. The anthers remain undehiscent but fully exerted in the sterile plants and the stigmas are viscid and fully exerted. The time of anthesis is as in the fertile plant but it usually is prolonged.

Data regarding some of the important quantitative characters are given in table 1. In 40-chromosomed plants the values are means of 20 plants, similarly; in the normal-20, of 11 plants.

A comparison of the length of the stomata gave longer ones in the 25-, 28-, 30- and 40-chromosomed plants than in the diploids. Table 2 gives the frequencies of of 50 cells at different lengths.

Fig. 1, g. Showing disparity in growth in seedlings of Dsy-2. $\times$ ca. 1/8.

Table 1. Measurements of some morphological characters

Character	2x chromosome number of plants					
	20 normal	20 Dsy-2	25	28	30	40
Height in cms.	276.8	205.0	143.0	136.0	207.0	212.6
No. of leaves	12	8	11	6	6	10
4th leaf length in cms.	72.8	60.6	52.8	48.5	61.0	69.0
4th leaf breadth in cms.	7.6	6.4	5.2	4.0	4.7	6.8
Peduncle thickness in cms.	0.9	0.88	0.8	0.6	0.6	0.87
Panicle length in cms.	21.8	30.5	12.2	15.0	31.5	21.4
Panicle breadth in cms.	8.4	6.5	2.9	7.0	4.0	4.5
Awn length in mms.	7.9	5.3	3.4	5.09	6.8	6.2
Glume length in mms.	5.2	4.7	4.1	4.89	6.42	6.1
Glume breadth in mms.	2.3	2.2	2.5	3.01	3.38	2.8

Meiosis

1. 40-chromosomed plants: The chromosome counts were all done in the P.M. cells. The anthers at this stage are normal. The mother cells are spherical, turgid with a prominent nucleus as in the normal ones. A prominent nucleolus is also present.

The P.M.Cs., show a stage similar to synapsis of the normal fertile plants but usually the threads are long, single and thin. Staining reaction is normal. At a stage corresponding to early diplotene (Fig. 2) the threads

Table 2. Frequency of stomatal lengths in Oc. micrometer divisions (multiplication factor= $\times 1.6$ for μ)

Lengths of stomata	2x chromosome number of plants					
	20-normal	20-Dsy-2	25	28	30	40
20	3	—	—	—	—	—
21	4	10	—	—	—	—
22	4	9	—	1	—	—
23	3	10	—	—	—	—
24	7	10	4	1	—	—
25	16	11	3	3	1	1
26	4	—	11	4	—	4
27	7	—	5	5	10	3
28	2	—	11	11	5	9
29	—	—	3	7	6	11
30	—	—	7	9	6	11
31	—	—	3	4	11	6
32	—	—	3	5	7	1
33	—	—	—	—	2	4
34	—	—	—	—	1	—
35	—	—	—	—	1	—
Total	50	50	50	50	50	50
Mean	24.36	23.06	27.72	28.5	29.78	29.22

are thicker and shorter but they show semimitotic characters having tapering ends. Frequently the chromosomes also show longitudinal splits. At this stage a bivalent and sometimes a quadrivalent (figs. 2 and 3) are seen in some of the cells. The pairing is loose and the bivalents appear to be joined at one end as though terminalisation has taken place. The other chromosomes lie suspiciously near one another to remind of somatic pairing. The majority of the chromosomes remain unpaired. From this it becomes possible that at $4n$ -level more chromosomes are conjugated at pre-diakinetic stage than at the $2n$ -level.

The contraction of the chromosomes is normal and a stage corresponding to the late diplotene can be recognised. The chromosomes here, are seen only half as long as in early diplotene and are thicker at the middle. The ends are pointed and tapering (fig. 3). The bivalents and quadrivalents, if any, become more rounded and appear thicker than the univalents.

Towards diakinesis all the chromosomes become much contracted and spherical in appearance (figs. 4, 5 and 6). At times it looks possible that there is distance conjugation. No chiasma is found. It is possible that, here, there is a conjugation effect without actual conjugation taking place. At this stage semimitotic appearance is completely lost and the chromosomes become fully spherical as in normal fertile plant. The maximum association found is $2_{IV} + 1_{III} + 1_{II} + 27_I$ (Table 3a).

Metaphase—I: The chromosomes in general congress at the equatorial

Figs. 2-20. Explanation given in text. All figures relate to meiosis. 40-chromosomed plants.
 2-4, 11 d, $\times 480 \rightarrow 2B$ size. 5-11 a, b, c, 12-18, $\times 200 \rightarrow 2B$ size. 19, 20, $\times 125$.

plate. A normal bipolar spindle is formed. The metaphase plate is stretched as in the diploid (desynaptic) parent but less compact and regular (Fig. 9a and 9b). The arrangement of the chromosomes is more haphazard. P.M.Cs. showing several univalents scattered on the spindle without congression are frequent (fig. 9b). In some P.M.Cs. the plate may be very compact and not much stretched resembling more the plate of normal diploid.

A somewhat reduced persistent nucleolus is usually found either in the cytoplasm or at one of the poles and rarely on the spindle.

Table 3. Frequency of chromosome associations (counted at Diakinesis and M I)

a) 40 chromosomed					b) 30 chromosomed					c) 28 chromosomed					d) 25 chromosomed				
IV	III	II	I	No. of cells	III	II	I	No. of cells	III	II	I	No. of cells	III	II	I	No. of cells			
2	1	1	27	1	0	14	2	1	1	9	7	1	1	10	2	1			
1	2	2	26	1	2	10	4	1	3	9	1	1	1	8	6	1			
1	—	2	32	1	3	7	7	1	3	8	3	2	2	8	3	1			
—	3	2	27	1	3	9	3	2	3	7	5	2	2	7	5	1			
—	2	2	30	2	3	8	5	1	3	6	7	2	3	7	2	2			
—	2	1	32	1	4	8	2	1	4	7	2	3	3	6	4	5			
—	1	1	35	1	4	7	4	1	4	6	4	4	3	5	6	1			
—	—	3	34	4	4	6	6	1	4	5	6	3	3	4	8	1			
—	—	2	36	2	5	7	1	2	4	4	8	1	4	6	1	2			
—	—	1	38	2	5	6	3	3	5	6	1	1	4	5	3	1			
—	—	—	40	9	6	5	2	2	6	5	0	1	4	4	5	1			
					6	4	4	1	6	4	2	1	4	3	7	1			
					7	4	1	3	7	3	1	2	5	5	—	2			
					7	3	3	3	8	1	2	1	5	4	2	3			
					8	3	—	1					5	3	4	1			
					8	2	2	1					6	2	3	1			
Total	4	13	31	883	25	127	150	70	25	105	146	93	25	89	137	84	25		
Mean	0.16	0.52	1.24	35.32		5.08	6.0	2.8		4.2	5.82	3.72		3.56	5.48	3.36			
% of chromosomes paired				11.7				90.7				86.4				86.6			

Anaphase—I: The early anaphase is normal and reductional (fig. 10). Equational division does not take place. The unpaired univalents are not passively moved to the poles but appear to show active migration since there is good congression at the plate. However, in cases where the univalents are found distributed on the spindle they fail to come to the equatorial plate but get included in the nearest pole. No laggards or chromatin bridges are found. Normally the bivalents undergo disjunction and the quadrivalents show 2-2 distribution. However, rarely, in some P.M.Cs. the bivalents and quadrivalents fail to disjoin and move to a single pole. Owing to the random movement of the univalents, the number of chromosomes distributed to each pole varies considerably. Table 4 gives the frequency of distribution at late anaphase and early telophase.

Table 4. Frequency of P.M.Cs. showing distribution of chromosomes

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total
$\frac{0}{40}$	$\frac{1}{39}$	$\frac{2}{38}$	$\frac{3}{37}$	$\frac{4}{36}$	$\frac{5}{35}$	$\frac{6}{34}$	$\frac{7}{33}$	$\frac{8}{32}$	$\frac{9}{31}$	$\frac{10}{30}$	$\frac{11}{29}$	$\frac{12}{28}$	$\frac{13}{27}$	$\frac{14}{26}$	$\frac{15}{25}$	$\frac{16}{24}$	$\frac{17}{23}$	$\frac{18}{22}$	$\frac{19}{21}$	$\frac{20}{20}$	
45	7	6	3	—	2	—	1	—	—	3	—	4	1	3	4	1	6	3	2	1	92

Thus in 48.9 % of the P.M.Cs. studied all the chromosomes get included in the same nucleus. The nucleolus is seen to persist in this stage also.

The telophase and interkinesis: Telophase is normal and clean. Generally the univalents show the chromatid split by the time they reach the poles. Very rarely one or two univalents may be found lagging. Persistent nucleolus is found here also. Cytokinesis proceeds normally, often quite independently of the condition of the nucleus. Cases were found in which the nucleus had not yet been released from its prophasic condition yet the cytokinesis had been completed. Sometimes the laggards get cut by the wall so that at Metaphase-II or interkinesis they are found stretched between the two daughter cells.

When 0-40 distribution takes place, it so happens that the chromosomes remain very much in M-I condition and placed below or above the equator of the spindle and cytokinesis takes place just at the equator cutting the cell into two, one without any chromosome at all.

The cytokinesis appears more like cleavage of the cytoplasm rather than actual wall deposition. However, evidences of cell wall deposition sometimes occur. The signs of cytokinesis begin with the appearance of a region thinly stained or whitish (fig. 11a). The cytoplasm appears as if it were shrinking away from this region (Fig. 11b, c and d). This gradually extends along the line and the cytoplasm is divided into two.

The second metaphase is very nearly normal with a bipolar spindle. Fig. 12 shows P.M.C. from division-I with laggards connecting the two cells. Fig. 13 shows M-II in a 0-40 distribution in P.M.C. The spindles in the sister cells are naturally dependent on the number of chromosomes they have inherited. Unequal distributions seem to occur at the second division also. In the case of the 0-40 distribution normal equational division takes place (fig. 14). The sister cells form no spindle but may become irregularly divided even prior to the division of the cell with chromosomes (fig. 15). Fig. 16 shows a telo-II in a 0-40 cell with a few laggards. Telo-II shows abnormal cytokinesis, sometimes, the daughter cells still connected (fig. 17). As a consequence of these cytokineses the cytoplasm sometimes appears to be cut into portions rather small and not including any chromatin at all. This leads to formation of more cells than four. It appears that cytokinesis in a cell with chromosomes dividing induces the sister cell without chromosomes also to divide. Often the first division is skipped over and an equational division with cytokinesis occurs giving 40-40 distribution of chromosomes. In most cells tetrad division is completed. Fig. 18 shows a tetrad with two daughter

cells each having only one chromosome. The persistent nucleolus sometimes gets included in the daughter cells and remains in the cytoplasm.

The pollen is of various sizes and shapes. Owing to incomplete cytokinesis dyads, monads and abnormal shapes arise (fig. 20). Pollen sterility is very high (88 %). Table 5 gives the frequencies of the diameters of the

Figs. 21-35. Explanation given in text. All figures relate meiosis. 30-chromosomed plant. 21-25, 28-33, $\times 480 \rightarrow 2B$. 26, 27, 34, $\times 200 \rightarrow 2B$. 35, $\times 125$.

sterile and fertile pollen grains. While the sterile pollen is completely empty and often having wavy depositions and yellow disorganised nucleus, the fertile pollen shows contents mostly granular and sometimes non-granular (fig. 19). The fertile grains are mostly large sized while the steriles are of mixed sizes.

Table 5. Frequency of pollen grains diameters (multiplication factor=X. 1.66 for μ)

Plant no. and ploidy.		11-13	14-16	17-19	20-22	23-25	26-28	29-31	32-34	35-37	38-40	41-43	44-46	47-49	50-52	53-55	56-58	Total	Mean	S.D.
A.S. 681																				
Fertile ($2n=20$)		—	—	—	—	2	7	49	42	—	—	—	—	—	—	—	—	100	30.9	2.07
Dsy. 2 $2n=20$																				
Fertile		—	—	—	2	14	25	24	14	10	10	—	1	—	—	—	—	100	30.3	4.93
Sterile		1	6	25	27	22	8	9	2	—	—	—	—	—	—	—	—	100	22.0	4.41
Do. progeny $2n=25$																				
Fertile		—	—	—	1	26	33	23	12	5	—	—	—	—	—	—	—	100	27.1	3.50
Sterile		1	1	7	76	15	—	—	—	—	—	—	—	—	—	—	—	100	21.1	1.78
Do. progeny $2n=28$																				
Fertile		—	—	—	1	12	32	24	9	11	6	5	—	—	—	—	—	100	30.3	5.00
Sterile		—	—	—	3	55	35	5	2	—	—	—	—	—	—	—	—	100	25.1	2.2
Do. progeny $2n=30$																				
Fertile		—	—	—	1	10	21	27	13	17	8	3	—	—	—	—	—	100	30.1	4.72
Sterile		—	—	—	16	57	19	5	2	1	—	—	—	—	—	—	—	100	24.7	2.76
Do. progeny $2n=40$																				
Fertile		—	—	—	1	10	17	22	8	13	9	5	7	—	7	—	1	100	33.1	8.01
Sterile		4	5	8	27	28	17	2	—	4	2	2	—	—	—	1	—	100	24.1	6.69

Shape abnormality occurs both in fertile and sterile grains. Formation of sperm cells are usually observed in fertile grains.

The same degree of fertility seems to occur on the pistil side also. Though no embryological examinations were done it is possible to conclude this through pollination trials and the resulting seed set. As already stated before, almost no seeds set on selfing (about 8 seeds set out of 40,000 spikelets from 20 panicles i.e. .02 %). Pollination from normal diploids as also from tetraploids did not improve seed set.

2. 30-chromosomed plant: Synapsis is normal (fig. 21 and 22 in pachytene stage). There appears to be greater tendency for pairing than in the 40-chromosomed plant. The diplotene (fig. 23) shows the chromosomes shortened and with normal repulsion. Trivalents, bivalents and univalents are observed. The chromosomes do not exhibit the semi-mitotic character as in the parental desynaptic plant or in the desynaptic $4n$ -plant described above. They are completely meiotic. Univalents vary from one to six. By diakinesis the chromosomes become much reduced and condensed (fig. 24). Maximum association noted was $8_{III} + 3_{II}$ (table 3b). Desynapsis may occur at this stage especially in the shorter sets. By prometaphase the reduction in length is completed and the chromosomes appear spherical (fig. 25).

Metaphase-I is normal with a bipolar spindle. Congression of the chromosomes at the equatorial plate is normal. Sometimes 2-3 univalents are found on the spindle (Figs. 26-28 spread out M-I showing trivalents).

Anaphase-I shows normal disjunction and movement of the chromosomes. Invariably some univalents are found left at the equator after the disjoined chromosomes have moved away from it (fig. 29; 5_1 seen). The number is variable. Generally the movement of the daughter bivalents is synchronous but rarely P.M.Cs. are found with spread out univalents.

Telophase-I: The daughter bivalents reach the poles and form a normal telophasic nucleus (fig. 30). Laggards are found on the spindle and frequently they fail to get included in the nuclei (fig. 31). No anaphase is free of such laggards. Towards interkinesis these laggards appear to become less chromatic. Thus the univalents become unequally distributed. They do not appear to undergo equational division. Interkinesis shows univalents lagging in the cytoplasm (fig. 32) and they sometimes get cut by the interkinetic wall.

Metaphase-II is generally normal with compact plates and bipolar spindles (fig. 33). Univalents are found to remain wherever they were at the time of the interkinetic wall formation and do not appear to migrate to the second metaphase plate. They also do not undergo equational division along with the rest of the chromosomes. In general they get included into the daughter cells to which they are nearest. Very rarely they are found forming separate small spindles and undergo equational division. Though generally the second anaphase is more regular than the first (fig. 34) and there are fewer laggards, P.M.Cs. are met with which show laggards at Telo-II. Telo-II and tetrad

formation are normal. Since there is high pairing there are no cases of unreduced gamete formation. Unequal distribution and the lagging of chromosomes lead to a high degree of pollen sterility (fig. 35). Micronuclear formation is rare.

The pollen grains are of mixed sizes large, medium and small (fig. 35). Occasionally unincluded chromatin is found in the pollen grains. Pollen sterility is 42 %. The diameters of the sterile and fertile grains are given in table 5. There is not much size difference between the sterile and fertile grains. Only, the sterile grains are completely devoid of contents but yet the shape is spherical, with a pore just as in the fertile ones. Though gametic fertility is about 58 % the number of seeds set is small. The seed is red and similar to the parental A.S. 681 normal.

3. 28 chromosomed plant: Synapsis shows the homologues closely paired (fig. 36 and 37). At diplotene multivalents are seen (fig. 38a-c). By diakinesis the reduction in the length of chromosomes is normal (fig. 39). The common chromosome associations are trivalents, bivalents, and univalents. The trivalent configurations are the usual chains, rings, sauce-pan shape, Y-form etc. The frequency of the associations is given in table 3c. At diakinesis and earlier prophase also, in addition to the single large nucleolus there appeared one to 4 smaller nucleolar-like bodies sometimes attached to the ends of non-nucleolar chromosomes (figs. 37, 38a, b and 39). These bodies stain fainter than the normal ones. The maximum association observed is $8_{III} + 1_{II} + 2_I$.

Metaphase-I forms a normal bipolar spindle with compact plate (fig. 40-pro-metaphase). The univalents are aligned with the bivalents and trivalents (fig. 41). Occasionally P.M.Cs. are seen in which the univalents may be away from the plate but on the spindle. The number of univalents may vary upto a maximum of six.

Anaphase-I is regular. The bivalents and trivalents disjoin first and move to the poles (fig. 42). The univalents remain at the equator even when the daughter bivalents have reached the poles. These then lag without undergoing equational division and exhibit lethargy in their movements. The enumeration of the chromosomes distributed to the poles is rendered difficult owing to these lagging univalents. However, 13-15 and 16-12 distributions were observed.

At telophase-I laggards are common (fig. 43). Clean anaphase-I is rare. Fragments are rare. However, one P.M.C. was found to show a fragment without a bridge. It may be that there are possibilities of inversions of chromosomal segments. Interkinesis shows the lagging univalents (fig. 44). In rare cases restitution nuclei were seen. Persistent nucleoli are found in all the stages of meiosis. (figs. 41 and 46a).

Metaphase-II is normal with bipolar spindle and compact plate (fig. 45a and b). Usually the division in the two cells is synchronous. Exceptions

Figs. 36-49. Explanation given in text. All figures relate to meiosis. 28-chromosomed plant. 36-46b, 48, $\times 480 \rightarrow 2B$. 49a, b, $\times 125$.

however, occur both as to time and plane of division. Univalents unincluded in the interkinesis nuclei may be found sometimes outside the M-II plate,

remaining wherever they happen to be during the end of the first division (figs. 46a and b). At this stage also the univalents do not seem to undergo any division at all. They get included into the nearest pole just as they are. Fig. 47 shows the ana- and telophase-II. Tetrad is formed and invariably an unincorporated chromatin is found in the microsporocytes (fig. 48). This seems to be absorbed before the pollen grains become mature. Micronuclei occur very rarely.

All pollen grains are spherical. Size differences occur but not appreciable (fig. 49a, b). The pollen shows higher fertility than in the 30-chromosomed plant (39 % sterility; 61 % fertility). Usually the fertile grains are larger than the sterile. The frequency of the diameters of the pollen grains is given in table 5. The seed set is as in the 30-chromosome plant. Grains are red, umbonate.

4. 25-chromosome plant: The prophase shows normal synapsis. At diplotene pairing of chromosomes in threes, twos and singles is observed. Usually a single nucleolus is observed. At diakinesis (fig. 50a- $6_{III}+2_{II}+3_I$; 50b- $1_{III}+8_{II}+6_I$) the condensation and shortening of the chromosome is normal. In some P. M.Cs. 10 to 12 rounded small nucleolus-like bodies, staining much fainter than the normal

Figs. 50-55. Explanation given in text. 50, 52-55, $\times 750$. 51, $\times 950$.

one are found (fig. 50b). The frequency of the chromosome associations is give in table 3d. The configurations of the trivalents are the usual ones. In one P.M.C. $5_{III}+5_{II}$ were found showing a high bivalent formation. Extreme terminalisation of chiasma is found at times.

Metaphase-I is normal with a regular bipolar spindle and a compact equatorial plate (fig. 51- $4_{III}+5_{II}+3_I$). Rarely univalents are found off the plate. The nucleolar-like bodies noted at the prophase are not found at this stage. Anaphase-I is regular, the paired chromosomes first disjoin and move

off to the poles. The univalents then become arranged at the equator (fig. 52). The number of univalents varies but 1-5 are common. They do not generally divide equationally but become random distributed to the poles. Frequently they are found to remain at the equator even when the rest have reached the poles. Telophase again shows them remaining still at the equator and sometimes, however, there is complete migration of all chromatin. Distribution of chromosomes to daughter cells thus depends on the haphazard placement or movement of the univalents. In some P.M.Cs. 10-15 and in other 10-5-10 etc., have been noted. The univalents are invariably present. Cytokinesis is regular.

Second division is regular. Compact metaphase plates with bipolar spindles are formed. The laggards and unincluded chromatin from the first division are frequently found distributed on the spindle and cytoplasm remaining more or less wherever they were left at telo-I (fig. 53). These do not congress at the plate. Anaphase and telophase are regular. However, here also a few chromosomes may fail to get included in the daughter nucleus (figs. 54 and 55). Persistent nucleolus is seen in this stage also. Cytokinesis is completed and tetrad formation takes place. Unincluded chromosomes and fragments are very common in the daughter cells. Microcytes also show these chromatin outside the nucleus.

The pollen grains are spherical. Abnormal shapes are rare. Pollen sterility is 36.5 % and fertility 63.5 %. The fertile grains are slightly larger than the sterile. The diameters of the grains are given in table 5. Seed set is better than in the other plants. Grains are red and umbonate.

Discussion

From the data presented above it is seen that the progeny does not vary much in regard to many of the morphological characters. Statistical analysis of the data from all plants could not be done as the 25, 28 and 30-chromosomed groups were represented by single plants. In the case of the $4n$ -group however, the analysis showed that they had significantly longer and broader spikelets than the $2n$, while the latter one was significantly better in height, number of leaves, panicle breadth and awn length and the differences were not significant in the length and breadth of leaves, and length and thickness of peduncle. All the types showed wrinkling in the upper glume and exhibited also the peculiar character of bleaching of the red pigmentation of the glumes towards maturity. These two factors have been dominant even in hybrids in which the $4ns$ (Krishnaswamy l.c.) were utilized as the pollen parent. None of these plants exhibited any strong tendency towards perenniality inspite of their ploidy. In fact the 40-chromosomed and the 25-chromosomed plants showed the least tendency towards ratooning.

Increase in chromosome number generally produces a corresponding effect in at least some of the phenotypic characters compared to the diploid parents.

This of course is not always valid. It depends much on the genic composition of the diploid before duplication. It has also been noted that polysomy at diploid level is more effective than at tetraploid and higher ploidy levels. Genic balance comes to play at these higher levels (Sinnot et al 1950). It is however, to be expected that the duplication or deletion of chromosomes should either exaggerate or appreciably reduce the effects of gene located in such chromosomes of a diploid. Polysomy thus can serve as a means of locating the genes of a chromosome and help in their mapping. This evidently becomes difficult in polyploids because of compensating effect. Of the several characters analysed in the plants reported here it is interesting to note that

Pollen Grain Sizes in Micro. Divns.

Fertile

Normal (A.S. 681)

Dsy-2

Progeny— $2n=25$

$2n=28$ — x — x —

$2n=30$ x

$2n=40$ x

a

Pollen Grain Sizes in Micro. Divns. Sterile

Dsy-2

Progeny—

$2n=25$

$2n=28$ — x — x —

$2n=30$ x

40 x

b

Fig. 56

there was a depressing effect in the 25 and 28-chromosome plants in the case of plant height, length and breadth of leaves, panicle length, breadth and awn length. Though the diploids are normal in behaviour when compared to the other sorghums of the same group, these characters appear to be more influenced by environmental factors since even in the $3n$ and $4n$ plants they do not differ very much from their immediate diploid parents. The characters that seem to reflect more closely any increase in chromosome numbers appear to be the length, and breadth of spikelets and length of stomata. With regard to the pollen grains the normal diploid shows a narrower dispersion around the mean and is more homozygous. In all the other plants of the progeny, owing to the meiotic unbalance and consequent differences in the distribution of the chromosomes much variation is seen in the sizes of the

pollen grains. However, the fertiles show a greater tendency for larger sizes and the steriles to a smaller size. Fig. 56a and b, give the diameters of the pollen grains graphically represented. The 28- and 30-chromosomed plants show a narrower range of variation both in the fertile and sterile grains.

It is interesting to note that the fertility of the gametes as estimated in the pollen grains increases with greater approximation to the diploid level. This probably has a relationship with the pairing of the chromosomes in these different plants. The mean association shows that there is a greater percentage of pairing in $2n=30$, 28 and 25 than in 40 (table 3), and a lower frequency of univalents. This is evidently due to the following causes: the 40-chromosomed plant has arisen through the fertilization of two unreduced gametes of the diploid desynaptic parent. Evidences for such gametes to arise are plenty in the diploid. The other plants are probably hybrids since they were obtained from panicle which were open pollinated. Since there were no tetraploid sorghum progeny near the diploid parent it is possible that the neighbouring diploids (some of the F_4 progeny of the cross A.S. 681 $\times$ A.S. 4003 which were dull midribbed) may have fertilized giving rise to the plants from the gametes of the desynaptic plant with chromosome numbers 20, 18, and 15. If all the progeny had survived it would have afforded a chance for estimating the frequency of such gametes. However, deliberate pollinations done with the diploid gave very few seeds as compared to the dusting with pollen from a $4n$ -sorghum. In one case (Dsy-2 $\times$ Co. 13 diploid) the parents were enclosed in bags after pollination and four seeds obtained, two of which only germinated and turned out to be auto- $4n$ -plants. Here the gametes from the second parent have failed to fertilize where as the unreduced gametes of Dsy-2 have functioned.

That these are hybrids gains further support in the pairing affinity. The frequency of the quadrivalents, trivalents and bivalents in the auto- $4n$ just repeats the behavior of the diploid where the frequency of bivalents was very low. The desynaptic effect is not in any way altered by duplication. On the other hand the introduction of a genome from a normal, synaptic plant has increased the pairing percentage in the other plants of the progeny. A single plant progeny of the 25-chromosomed plant showed dull midrib, and stem leaf and panicle characters resembling in some respects the *S. caudatum*. This plant was a diploid, with normal bivalent formation and high degree of fertility and seed set, thus showing that the 25-chromosomed plant itself was a hybrid. The 30-chromosomed plant inspite of its being a triploid with a fairly high frequency of trivalent formation exhibits much higher fertility than an autotriploid which was noted in *S. roxburghii* by the third author (unpublished). The latter produced no seeds. Thus the triploid under discussion exhibits a meiotic behaviour comparable to an allotriploid.

The 25 and 28-chromosomed plants can also be considered as simple trisomics viz., $2n+5$ and $2n+8$ respectively. Endrizzi and Morgan (1955)

observed in a trisomic derivative of haploid *S. vulgare* plant, chain trivalents and at metaphase I either $10_{II}+1_I$ or $9_{II}+3_I$. Ten to 11 and 10-10 with lagging chromosomes distributions were noted. Brown (1943) in a haploid obtained from a hybrid between black kaffir $\times$ fetterita found in one P.M.C. 3_{II} , in two 2_{II} and in thirteen, 1_{II} (total P.M.Cs. examined 150). Kidd (1952) reports a single trivalent in one P.M.C. and one to two bivalents in others in a haploid sorghum plant. It could be assumed from this, taking the most frequent bivalents formations, that at least one chromosome is duplicated in the haploid set of grain sorghums or three if maximum bivalent formation is taken into consideration.

In the aneuploid plants under discussion the multivalent associations noted are trivalents and where trivalents have failed high percentage of bivalents occurred. Reading this along with observations on the haploids it is of interest to note the absence of quadrivalent formation. The 28-chromosomed plant in its multivalent formations almost approaches the autotriploid and triploid noted above. In the 25-chromosomed plant a maximum of six trivalents are formed thereby giving one trivalent in excess of the expectation in a trisomic of $2n+5$. This extra trivalent may arise either by autosyndesis or because of the duplication of the genome of sorghum itself as indicated by the analysis of the haploids. While reporting on trisomics derived from a triploid $\times$ diploid *S. vulgare*, Price and Ross (1955) stress the value of trisomic especially in this crop in which only four plural linkage groups have been studied. They also state that these trisomics could be propagated vegetatively and immediately used in test crosses to determine the nulliplex, simplex, duplex and triplex nature of single locus and the trisomic stocks can be produced in given lines by a repeated back crossing.

The meiotic behaviour of the desynaptic diploid parent viz. Dsy-2 and its autotetraploid, the 40-chromosomed plant under discussion is peculiar. In spite of the failure of pairing and the high frequency of univalent formation the spindle gains complete control over them so that, normal congression of chromosomes, regular bipolar spindle and anaphasic movements are observed. This differs markedly from the desynaptic plants reported in Maize, *Crepis*, *Pisum*, *Oryza* etc. Only case which to a certain extent resembles the present one is that reported by Levan (1940) in *Allium amplexans*.

The pollination of the desynaptic sorghum with pollen from unrelated species had various effects. This experiment was intended to verify if there was any tendency for apomixis in this strain. Where panicles were protected the progeny has been exclusively auto-4ns. A diploid desynaptic resembling the mother was not recovered thus ruling out any possibility of apomixis in this plant.

In this desynaptic plant it appears as though there has been some physiological change in the stigmas as judged from the response of n and $2n$ -pollen, the latter more successful. The high tendency for the production of

the unreduced gametes i.e. a $2n$ -gamete in the ovary appears to have a controlling effect or selective influence in the stigma. It is interesting to note that when the panicles of the $4n$ -desynaptic plants were dusted with pollen from a $4n$ -normal plant there was very little pollen germination and very few seeds set. Here also there is tendency for unreduced gamete formation. The female gamete would be $4n$ and this probably requires another $4n$ male gamete to give the same effect as that which was obtained by the $2n$ female gamete of desynaptic $2n$ -plant which was pollinated with the $2n$ -male gamete from the $4n$ hybrid plants. Dusting of the stigma of these $4n$ desynaptic plants with pollen from a normal diploid resulted in no seeds. Plants from the few seeds that were obtained in open pollinated panicles from these desynaptic $4n$ -plants have been raised. In this progeny apart from a few obvious hybrids the others appear to be from selfed seeds. It is possible that these plants may yield octoploids, hexaploids and aneuploids. The progeny is very vigorous in growth. The plants closely resemble the normal diploid parent. Seeds have been gathered from the 30- and 28-chromosomed plants and await sowing.

Summary and conclusions

1. The progeny of the desynaptic plant-2 ($2n=20$) consisted of one each of 25, 28 and 30 chromosomed plants and a larger number of 40-chromosomed ones. Their morphological descriptions have been given. They bear a close resemblance to the parent plant and also the normal diploid which gave rise to the desynaptic plant.

2. The meiosis has been described in detail. From the behaviour it is concluded that while the $4n$ is a result of self-fertilization the others are evidently hybrids, a reduced gamete of a normal diploid having fertilized the 15, 18 and 20 chromosomed gametes of the desynaptic plant.

3. The meiotic behaviour of the 25 and 28 chromosomed plants resemble the trisomics $2n+5$ and $2n+8$, while the 30-chromosomed one is more like the allotriploid.

4. The $4n$ exhibits lower pairing percentage than the others. This is attributed to the synaptic gene introduced into the latter while the $4n$ is an auto- $4n$ and desynaptic gene is continued.

5. In spite of the trisomic nature and polyploidy of the progeny there has been no marked effect in the phenotype of these plants as compared to the diploid except for a few characters such as the size of the spikelets, stomata and pollen grains.

6. These plants are considered to be of value in recovering a number of other trisomic and polysomic plants and study their genetic effects.

These plants are all growing at the Millets and Pulses Breeding Station, Coimbatore.

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