Accessory Chromosomes and Their Meiotic Behaviour in Hybrids of Grain Sorghum and Johnson Grass

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The occurrence, cytological behaviour and genetical effects of accessory or B-chromosomes are now well known (Müntzing 1966, 1967). In a majority of plants with B-chromosomes, the number of accessories present at meiosis is the same as that found in the root tips. A few cases are also known where the accessories are eliminated from the somatic tissues and are only retained in the central parts of the plant. The first case of this kind was observed in a *Para-Sorghum*, *S. purpureosericeum* (Janaki Ammal 1940) and subsequently in *Poa alpina* (Müntzing 1946, 1948) and *Panicum coloratum* (Swaminathan and Joginder Nath 1956). Another *Para-Sorghum*, *S. nitidum* and a species of *Eu-sorghum* viz., *S. halepense* (Johnson grass) were also found to belong to this category (Raman and Krishnaswami 1960, Raman *et al.* 1964, 1965 and 1966). Diploid and tetraploid forms of *S. nitidum* ($2n=10$ and 20) and *S. halepense* ($2n=20$ and 40) have been found to be prevalent in natural populations (Garber 1950, Krishnaswamy and Raman 1953, 1953a, Krishnaswamy *et al.* 1956). Plants with acc. chromosomes are found only in diploid populations of the two species. The acc. chromosomes in *S. nitidum* ($2n=10+2\text{ B's}$) paired regularly and behaved normally at meiosis while those of *S. halepense* were inconstant ($2n=20+4-6\text{ B's}$) and aberrant in behaviour. Data presented below provide information on the cytological properties of the extra chromosomes present in the hybrids of *S. subglabrescens* and *S. halepense*.

Material and methods

The race of *S. halepense* possessing 4–6 extra chromosomes besides its normal complement of 20 was originally collected in the Nilgiris and subsequently maintained as a clone. Pollen of this plant was used in cross pollinations with the grain sorghum species, *S. subglabrescens*. Artificial crosses were effected after hand emasculation of the spikelets. For the study of meiosis, young panicles were fixed in Carnoy's fluid (3:4:1) and the smears of pollen mother cells stained in acetocarmine. The somatic chromosomes were also studied by Feulgen-acetocarmine squashes of root tips after pretreatment with 8 oxyquinoline for 3 hours and hydrolysis in NHCl for 25 minutes.

Observations

The availability of diploid hybrids between three different grain sorghums and the normal *S. halepense* facilitated a good comparison of these with the F_1 s pos-

sessing acc. chromosomes. The divergence between the species of grain sorghum and *S. halepense* is significant both in morphology and in the development of isolation barriers. There is a distinct difference in the time of anthesis and natural habitat of the two. The percentage of seed set on spikelets of *S. nervosum* pollinated with pollen of *S. halepense* was 57.7 while in the reciprocal, it was 18.4. In crosses with *S. subglabrescens*, the percentage of seed set was 56.9 and the reciprocal gave a value of 14.3. Sixty four percent of spikelets of *S. Roxburghii* gave viable seeds on cross pollination, while pollen of this species on the emasculated spikelets of *S. halepense* could induce the development of only a few shrivelled seeds. The variation in the percentage of seed setting, and occurrence of inviable seeds on

Figs. 1-3. Parents and hybrid. 1, *S. subglabrescens* ($2n=20$). 2, F_1 with 20 A plus 4-6 B-chromosomes. 3, *S. halepense* ($2n=20+4-6$ acc. chromosomes). Fig. 1. 1/8 'nat' size. Fig. 2. 1/25 'nat' size and Fig. 3. 1/20 'nat' size.

cross pollination are indicative of the operation of incompletely developed cross incompatibility and failure of embryo development as isolation barriers.

The hybrids between the grain sorghums and *S. halepense* referred above have regular chromosome pairing and no evidence of gross structural differences in chromosome complements of the species was obtained from a study of the meiotic behaviour of the hybrids (Fig. 4). However, in spite of this regular meiosis, the F_1 s showed a reduction of 24 to 36 per cent in their pollen fertility compared with the parents. Seed sterility in the hybrids ranged from 42 to 48 per cent. The F_2

progenies showed predominance in the expression of characteristics of the wild species, their meiotic behaviour was similar to the hybrids and were also relatively

Figs. 4-14. Meiosis in hybrids possessing 4-6, 6-8 and 8-10 acc. chromosomes. 4, precocious disjunction of two bivalents at MI in hybrid between *S. subglabrescens* and *S. halepense* devoid of acc. chromosomes. 5, different configurations presented by the B1 and B2 bivalents. 6, eleven bodies at diakinesis comprising 10 A-bivalents plus a unit where the B1 and B2 pairs are held in close approximation. 7, fourteen bodies at diakinesis. One A-bivalent shows precocious disjunction. Of the five B-pairs, one is free and the rest approximate to form two groups. 8, a chain of eight acc. chromosomes and one free B-bivalent. 9, prometaphase. Note the approximation of one B-bivalent with two A-pairs. A second B-pair and a B-quadrivalent are also seen. 10, AI showing the sticky B1 and B2 bivalents away from the spindle and the precocious separation in some of the A-pairs. 11, AI. The B-quadrivalent disjoins as 2/2 and three A-bivalents showing delayed disjunction. 12, AI. The B-quadrivalent migrating *en bloc* to one pole. 13, distribution of 14/16 at TI resulting from the non-disjunction of a B-bivalent in F_1 with 10 acc. chromosomes. 14, non disjunction in one of the three B-bivalents leading to 14/12 at TI in hybrid plant with 6 B-chromosomes. Figs. 4 and 5 $\times 1600$. Figs. 13 and 14 $\times 900$ and the rest $\times 1400$.

fertile. No evidence of hybrid breakdown was apparent. The parental species appear to have genomic differentiation limited to genic and small structural changes (Krishnaswamy *et al.* 1956a, Ananthasayana 1964, Mahudeswaran and Raman 1972, Sethupathi Ramalingam and Raman 1974).

The clone of *S. halepense* with 4-6 acc. chromosomes resembled its normal counterpart in external morphology but differed in being more vigorous and pro-

Table 1. Disjunctional patterns of three B-bivalents at early AI

Pattern number	Manner of disjunction of accessory bivalents			No. of PMCs
	B ₁	B ₂	B ₃	
1	1/1	1/1	1/1	5
2	1/1	1/1	2/0	11
3	1/1	0/2	1/1	7
4	1/1	1/1	Eliminated	6
5	2/0	2/0	1/1	13
6	2/0	2/0	Eliminated	12
7	2/0	1/1	-do-	9
8	2/0	1/1	1/1	11
9	2/0	2/0	2/0	6

Table 2. Distribution of B-chromosomes at T1

Cells without laggards				Cells with lagging chromosomes										No. of PMCs
0/6	1/5	2/4	3/3	0/2	0/3	0/4	0/5	1/1	1/2	1/3	1/4	2/2	2/2	
2	3	3	4	4	2	4	4	4	3	4	3	4	4	48

Table 3. Variation in size of three classes of pollen grains and their frequencies in hybrids with 4-6 acc. chromosomes ($\times 3.1$ for μ).

Category	Diameter of pollen grains (in oc. mic. divisions)				Mean	S.E.	CV (%)
	9-11	12-14	15-17	18-20			
A. Stainable (fertile)	—	116	80	4	14.32	0.095	9.35
B. Non-stainable (sterile)	164	34	2	—	10.57	0.064	8.51
C. Germinable	—	128	65	7	14.19	0.101	10.07

ducing about 60 per cent of ill-filled and deformed pollen grains. Seed set under open pollination was reduced to nearly one third of that realised from panicles of the plant devoid of the acc. chromosomes.

Twelve hybrids were derived from cross pollination of the spikelets of *S. subglarens* with pollen from the B-race of *S. halepense*, the percentage of success being 48 per cent (Figs. 1-3). Six of them showed only 4 accessories, two had 4-6,

in two others the B's ranged from 6–8 and in one from 8–10. In the last F_1 , the PMCs carried 6, 8 and 10 B chromosomes. The number of chromosomes in the root tip cells determined by the rapid squash technique was only 20 in all the hybrids. The F_1 s carrying 8 and more accessories in addition to the normal complement of 20, were found to be less vigorous and exhibited a reduction in vegetative and floral organs. Compared to the male parent, a progressive reduction in fertility accompanied an increase in the number of B-chromosomes in the hybrids.

Meiosis: The behaviour of the B-chromosomes in the hybrid with 4–6 accessories, described below, is similar to that observed in the meiosis of the male parent. In the hybrid, the number of bodies noticed at diakinesis was not constant. Two A-bivalents formed the nucleolar chromosomes. The 6 accessories presented themselves as three individual pairs in less than 10 per cent of PMCs. In a majority of cases, they were seen as two extra bodies, one being constituted of two B-bivalents in close approximation while the other was a free bivalent (Fig. 5). The former is referred in the text as the B_1 and B_2 bivalents and the latter as the B_3 pair. The B_3 bivalent was absent in about 20 per cent of cells exhibiting eleven bodies (Fig. 6) and in such cells the B_1 and B_2 pairs gave distributions of 2/2, 0/2 and 0/4 at AI.

With regard to stainability, the B_3 was less deeply stained than the A-bivalents. The intensity of staining of the A-bivalents and that of the B_1 and B_2 pairs was, however, the same. The differential staining was more apparent, in the A and B-univalents, the stainability of the latter being somewhat less. Compact MI plates are formed in a majority of cases. Secondary association is pronounced and the B-pairs are seen to approximate themselves with the A-bivalents (Fig. 9). The A and B bivalents exhibited precocious and delayed disjunction. The stickiness of the B_1 and B_2 pairs to constitute the multivalent structure was evident. Spindle abnormalities are not much pronounced. The accessories also take up positions away from the spindle and get eliminated or revealed a tendency for non-disjunction to be incorporated in the daughter nuclei (Figs. 10–12 and 13–14).

The disjunctional behaviour of the three B-bivalents at early AI is presented in Table 1. It may be noted that normal disjunction and *en bloc* movement is characteristic of all the pairs while the B_3 gets eliminated in about thirty four per cent of the cells analysed. Most AI figures showed whole and dividing univalents. Seventy five per cent of the cells showed 1–4 laggards at TI (Table 2). In 25 per cent of the cells they get incorporated in the daughter nuclei, the distribution being 0/6, 1/5, 2/4 and 3/3 and these could be matched with the disjunctional behaviour of the bivalents, indicated against pattern numbers 9, 5, 8 and 1 respectively. The frequencies of cells with the four different distributions at TI were considerably reduced compared to their corresponding values at early AI. That the initial disjunctional behaviour of the accessory pairs is no indication of their ultimate distributions is evident from the high frequency of cells with laggards. Lagging of the half B-univalents was noticed at AII and bridges at this stage were exceptional. Where four telophase groups are organised in a regular way, fusion of adjacent nuclei was noticed occasionally. Apparently good pollen was ascertained by counting eight hundred and five grains. Fifty eight percent of well-filled pollen grains

were produced and their size ranged from 37.2 to 58.9 μ . Pollen and seed fertility in hybrids of similar parentage but lacking the acc. chromosomes are 76 and 56 per cent respectively. The variation in size of fertile and sterile pollen and that of the microspores germinated *in vivo* is presented in Table 3. The differences in frequencies under the size classes of the stainable pollen and of microspores germinated on the stigma are not marked. The sterile grains are smaller, eighty two per cent of these conformed to the range in size from 28 to 34 μ which is much lower than the minimum level of expression of the fertile grains. The difference between the mean size of pollen grains of the two categories A and C is not significant as revealed from the 't' test, while the average size of microspores of the category B was found to be significantly different from the corresponding values of A and C at probability 0.01 level.

Hybrids with 4 B's always exhibited two independent bivalents. In F_1 s with 6-8 accessories, the B_1 , B_2 and B_3 pairs were present in addition to either one of the former two. The most frequent configuration was an association of three pairs plus a free bivalent. Occasionally, a quadrivalent and two independent bivalents were also noticed. Either an association of four pairs and a free bivalent or two groups consisting of two pairs each plus a free pair was characteristic of the F_1 with 8-10 B chromosomes, indicating thereby the duplication of the B_1 and B_2 bivalents (Figs. 7 and 8). Pollen mother cells showing 6 and 8 chromosomes in these hybrids probably lacked the B_3 pair as a result of elimination during pre-meiotic mitosis and the frequency of cells with the minimum number of accessories was around 20 per cent. In the last hybrid, 6-10 acc. chromosomes were present in the same anther. Twenty seven per cent of the PMCs revealed 6 acc. chromosomes and twenty per cent carried 10 such chromosomes; eight B-chromosomes were found in 53 per cent of cells. The ten accessories either formed a long chain of eight plus a free pair or presented two configurations each consisting of two bivalents in approximation and leaving an independent pair. Cells with 6 and 8 acc. chromosomes probably do not exhibit the B_3 pair and in the former case, the B_1 and B_2 bivalents occur with an additional unit of either one of them. Degeneration of PMCs was noticed in hybrids with 8 and 10 B-chromosomes. Gametes resulting if at all, from PMCs devoid of the B_3 are probably inviable as evidenced from the non-recovery of hybrids carrying the B_1 and B_2 pairs alone. The B_3 therefore, appears to be a companion pair. The quantity of apparently good pollen in the hybrids with 6-8 and 8-10 accessories was 42 and 28 per cent respectively and in the F_1 with 6-10 chromosomes it was 16 per cent. A higher percentage of seed sterility than that of pollen occurred in these hybrids. The seeds were rather small and shrivelled.

Discussion

Darlington and Thomas (1941) reported three kinds of B-chromosomes in *S. purpureosericeum*, viz., large standard chromosome (M), large iso-chromosome (L) and short chromosome (S). Again, Garber (1950) observed that of these, two (M and S) were present in the sub-species *decanense* and *typicum*. The two accessories in *S. nitidum* paired with each other and because of their equal size, they were

classified under the standard type (Raman and Krishnaswami 1960). The supernumerary chromosomes of *S. halepense* can also be grouped under the standard category since they are seen only as pairs and are not appreciably larger in size than the A-chromosomes. Further, the B's exhibited the property of stickiness and variable in their number in the reproductive cells. Numerical increase is a phenomenon specific for the acc. chromosomes in grasses and in this respect, the B-race of *S. halepense* is no exception. The chimaerical PMCs with regard to the minimum and maximum number of accessories were also observed in only half the number of hybrids tested. In such cells with lower number of B's the B_3 pair seemed to be absent. The three pairs in 'normal' cells exhibited independence in segregation, and their co-existence in gametes appeared to be necessary as evident by their presence in the hybrids. The nature of approximation of the B_1 and B_2 pairs in the 8 and 10 chromosome F_1 s indicated the partial homology of the chromosomes of the two sets and this property, probably facilitated the 'stickiness' exhibited by them. The acc. bivalents of *S. halepense*, therefore, seem to be of three kinds of which two show partial relationship. The sterility appears to result mainly from the behaviour of accessories during meiosis and a linear relationship can be found between the level of sterility and the number of acc. chromosomes present in the hybrids. In view of the limited number of progeny obtained, it is unsafe to draw definite conclusions regarding the relative competitive ability of male gametes of the B parent in fertilisation. An evaluation of the frequency of B-chromosomes in large hybrid populations and selfed progenies of the B-race of *S. halepense* should provide more reliable estimates on the rate of transmission of the accessories from the male and female sides. A survey on the distribution of acc. chromosomes in natural populations of the species is also called for. The resemblances and differences between the B-chromosomes of the *Para* and *Eu-sorghums* both in morphology and behaviour in reproduction are of phylogenetic importance. Infecting the diploid grain and grass sorghums with the acc. chromosomes of *S. halepense* is a field of study, the findings of which will supply interesting and useful information from the fundamental and applied cytogenetic points of view.

Summary

Crosses between the grain sorghum species, *S. subglabrescens* ($2n=20$) and a diploid race of *S. halepense* ($2n=20+4-6$ B's) yielded F_1 s exhibiting constant and oscillating number of accessories only in the PMCs. Six hybrids showed only 4, two F_1 s had 4-6, in two others the accessories ranged from 6-8 and in one from 8-10. In the last hybrid, 6, 8 and 10 B chromosomes could be found in PMCs of an anther.

The size of the accessories was not appreciably large compared to the A-chromosomes and the former occurred only in pairs. They segregated independently of each other and exhibited normal separation, delayed disjunction, elimination and *en-bloc* movement at AI. Lagging of the B-chromosomes also occurred at AI and AII leading to their expulsion. Three B-pairs, B_1 , B_2 and B_3 could be recognised of which the former two presented configurations involving four or more chromosomes depending on their numerical strength. The presence of the B_3 in all hybrids

emphasises the importance of coexistence of accessories in gametes of the male parent for successful cross pollinations.

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