Cytomorphological Studies in the Interspecific Hybrids of *Dichanthium* (Willenet)

III. *D. aristatum* (Poir) C. E. Hubb. $\times$ *D. caricosum* (Linn.) A. Camus

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Dichanthium is a genus of the tribe Andropogoneae, family Gramineae. It has been reported to possess 17 valid species, with the chromosome numbers of $2n=20$, 40, 50 and 60 (Celarier, de Wet and Bakshi 1962). A few species are grown for fodder purpose. With a view to study the relationship between different species of *Dichanthium*, crosses were made between *D. annulatum* ($2n=40$), *D. aristatum* ($2n=20$) and *D. caricosum* ($2n=20$). In this paper account is given of the cytomorphological studies and the genome relationship of the hybrids involving the two species given above having $2n=20$ chromosomes.

Material and methods

The species *D. aristatum* and *D. caricosum* used in this study were diploids (probably polyhaploids) with $2n=20$, and are currently maintained under the numbers Marvel-40 and Marvel-7, in the Botany Section, College of Agriculture, Poona-5. Reciprocal crosses were made between these species in December 1963 and January 1964, and the F_1 generation was raised in the subsequent season. For crossing, the technique described by Richardson (1958) was followed. 98 spikelets of *D. aristatum* and 93 of *D. caricosum* were emasculated and pollinated. From the cross *D. aristatum* $\times$ *D. caricosum*, 30 seeds were obtained out of which 17 germinated but only two plants survived and grew to maturity. Out of ten seeds recovered from *D. caricosum* $\times$ *D. aristatum*, nine germinated and three hybrids survived and attained maturity. The two hybrids of *D. aristatum* $\times$ *D. caricosum* were tetraploids and possessed 40 chromosomes, whereas all the three hybrids of the reciprocal cross were diploids ($2n=20$).

Meiosis of the parents and hybrids were studied from the pollen mother cell smears. Flower buds of proper size were fixed in Carnoy's fluid (6 alcohol : 3 chloroform : 1 propionic acid) for one day and then changed to propiono-alcohol (3 alcohol : 1 propionic acid) to which traces of ferric acetate were added.

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Material was stored in the refrigerator for few days to several months and the meiosis was studied as and when it was convenient. The anthers were first smeared in 1% propiono-carmin and then destained with 45% propionic acid after introducing a drop of lacto-propiono-orcin (Dyer 1963).

Observations

A. Morphological characters :

1. *Parents* : *D. aristatum* and *D. caricosum* are closely related species and exhibited morphological similarity in several characters. Both were erect to decumbent in habit, with thick stem, broad and long leaves. Lower nodes were glabrous and a few top nodes possessed short velvety hairs. The mature inflorescence was pinkish on the dorsal side, and green on the ventral side with big ovate spikelets. However, these species had several minor variations. A clear cut difference between these species was that, *D. aristatum* had thick and hairy peduncles and primary axes, while in *D. caricosum* these parts were thin and glabrous. There were several other minor variations in respect to quantitative characters.

2. *Hybrids* : The two tetraploid hybrids derived from the cross in which *D. aristatum* was used as female parent, were shorter in height, with slender stem, and had reduced vigour. One of the two hybrids was found to be very weak during the first year of its growth (1964) and produced only six tillers. In the second year however, it attained vigour and was as vigorous as the other hybrid. These plants had shorter inflorescences, shorter and thinner spikelets and were relatively smaller and more slender habit of growth.

On the other hand, the three diploid hybrids from the cross *D. caricosum* $\times$ *D. aristatum*, were as vigorous as the parents. The hairiness of peduncles of *D. aristatum* parent was dominant and was present in all the hybrids.

B. Cytological studies :

1. *Parents* : Meiosis in both the parents was regular. In most of the cells analysed, ten bivalents were regularly formed at diakinesis and metaphase (Figs. 1 and 2), and ten chromosomes moved to each of the poles at anaphase of the first meiotic division. In some cells however, a single quadrivalent was observed. It is likely that the quadrivalent association was due to the segmental homology between two bivalents. The bivalents possessed one or two chiasmata and accordingly presented ring or rod shaped configurations. In *D. aristatum*, 60% of the chromosomes associated into ring bivalents, whereas in *D. caricosum* 76% bivalents formed ring shaped configurations.

The second division was regular and synchronous. However, at the tetrad stage occasional cells contained chromosomes which remained at the metaphase I stage and did not proceed further with the meiotic division. Chromosomes in such cells were clumped together and appeared to form restitution nuclei (Fig. 3). The study of pollen grains indicated occasional existence of giant

pollen grains (Fig. 4). These enlarged pollen grains might have been derived from the pollen mother cells which failed to complete the meiotic process.

2. *Hybrids*: In the tetraploid hybrids of *D. aristatum* × *D. caricosum*, the bivalents formed were higher as compared to the other associations of

Figs. 1-9. 1, metaphase I in *D. aristatum* showing 10 bivalents. ×950. 2, metaphase I in *D. caricosum* with 10 bivalents. ×950. 3, PMC of *D. aristatum* forming restitution nucleus. ×950. 4, unreduced giant pollen among normal pollens in *D. aristatum*. ×950. 5, diakinesis in the tetraploid hybrid No. 1 showing 18II 1IV. ×950. 6, metaphase I in the tetraploid hybrid No. 1 showing 16II 2IV. ×950. 7, metaphase I in the tetraploid No. 1 with 20II. ×950. 8, metaphase I in the tetraploid No. 1 with 2I 17II 1IV. ×950. 9, metaphase I in the tetraploid hybrid No. 2 showing 18II 1IV. ×950.

higher order. The average bivalent associations per cell were 16.4 in one hybrid and 14.92 in the second. Univalents were found in 50% of the cells but their number was not more than two per cell. Only one cell in each of these hybrids possessed a single pentavalent. Hexavalent associations in the two hybrids were more frequent and were found in five cells of one hybrid and nine in the other, out of 25 cells analysed, but the number of hexavalents was not more than one per cell (Figs. 5 to 10).

Pairing in the diploid hybrids (*D. caricosum* $\times$ *D. aristatum*) was rather regular. Univalents were absent in all the three hybrids. Most of the cells

Figs. 10-15. 10, metaphase I in the tetraploid hybrid No. 2 with 17II 1VI. $\times 950$. 11, metaphase I in the diploid hybrid No. 3 with 10 bivalents. $\times 950$. 12, diakinesis in diploid hybrid No. 2 showing 8II 1IV. $\times 950$. 13, metaphase I in the diploid hybrid No. 3 showing 7II 2III. $\times 950$. 14, anaphase I showing two dividing laggards in the tetraploid hybrid No. 1. $\times 950$. 15, telophase I in the tetraploid hybrid No. 2 with two laggards moving towards the main bodies of nuclei. $\times 950$.

at metaphase I formed ten bivalents. However, one or two loosely paired bivalents were observed and such bivalents were found to separate precociously at metaphase I (Fig. 11). These were both ring and rod bivalents in almost equal frequencies. One or two quadrivalents were observed in some cells, which appeared to have formed due to the segmental homology between the chromosomes (Fig. 12). Trivalents were absent in all the hybrids except for a single observation in one of the three diploid hybrids (Fig. 13). Formation

of occasional trivalents and quadrivalents indicated that the genomes of *D. aristatum* and *D. caricosum* although very closely related, differed at least by a chromosomal exchange. Anaphase I and the second meiotic division were regular in the diploid hybrids. There was regular and equal distribution of ten chromosomes to each pole.

In the tetraploid hybrids, however, some cells showed one to four lagging chromosomes at anaphase I (Figs. 14 and 15). Out of 100 cells that were examined in each tetraploid hybrid, 56 cells in one and 60 cells in the other showed regular separation of chromosomes at anaphase I. Over 80% of the irregular cells possessed only one or two laggards. At the second division also, the two hybrids, indicated one to four lagging chromosomes in 22 and 42 per cent of the cells. In most of the cells these laggards were found to move towards the poles so as to be included in the daughter nuclei. Chromatin bridges (due to non-disjunction of multivalents) were found only in two and four per cent of the cells in the two hybrids at the first anaphase.

The tetrads formed in the diploid hybrids were normal, but in the two tetraploid hybrids abnormalities to an extent of 5.6 and 2.7 per cent of the tetrads were observed. In all the hybrids, the percentage of filled pollen and seed development was high.

Discussion

There were five hybrids in crosses between *D. aristatum* and *D. caricosum*. Two were derived from the cross in which *D. aristatum* was the female parent and three were from the cross where *D. caricosum* was the female parent. The two hybrids of the first cross were tetraploids, while those of the second cross were diploids. Since the progeny in the second cross consisted of only diploid plants as was expected, further discussion may not be necessary. The tetraploids in the cross of *D. aristatum* $\times$ *D. caricosum* might have arisen either due to (i) the fusion between unreduced egg as well as sperm, or (ii) due to the endomitosis in the first cleavage of zygote which lead to the doubling of chromosomes. Since the embryological studies of *D. aristatum* (reported elsewhere) revealed normal reduction and development of 8-nucleate reduced embryo sacs in the ovaries, and as a few unreduced pollens were found in both the species, possibility of the fusion of male and female unreduced gametes seems to be remote, thus leaving the second as the most probable way by which the tetraploid hybrids were produced. Since these tetraploids were obtained only when *D. aristatum* was used as the female parent, it is very likely that the cytoplasm of *D. aristatum* has played an important role in the doubling of chromosomes.

Celazier, de Wet and Bakshi (1962) stated that the species *D. aristatum* and *D. caricosum* are morphologically very close and differ primarily in a single qualitative character, that is, hairiness of peduncle. The closeness of these species was also indicated by the earlier workers like Pandeya (1953)

and Celarier and Harlan (1955).

In the present investigations, chromosome pairing in the tetraploid hybrids indicated that the genomes formed bivalents within themselves and formed a few quadrivalents as the genomes of *Dichanthium* species were capable of significant autosyndetic pairing (de Wet *et al.* 1961). Pairing in the diploid hybrids was very regular and ten bivalents were usually formed at metaphase I. Although pairing in the tetraploid hybrids was complicated by preferential autosyndetic association, from the regular formation of ten bivalents in the diploid hybrids, it is obvious that the genomes of *D. aristatum* and *D. caricosum* possess very close degree of homology. The present investigations therefore, support the views of earlier workers and offer a cytological proof for the closeness of these two species.

de Wet *et al.* (1961) studied the chromosome relationships of *Dichanthium* species and assigned the genome formulae **AAA'A'** for *D. annulatum*, **DDD'D'** for *D. aristatum* and **CCC'C'** for *D. caricosum* respectively. Recently, Singh (1966) reported the genome relationships of *D. annulatum* and *D. panchganiense* and assigned the symbols **DDD'D'** for *D. annulatum* and **D₃D₃** for *D. panchganiense*. The present investigations showed that there was perfect homology between the genomes of *D. aristatum* and *D. caricosum*. The genomes of *D. annulatum* also indicated homologies with those of *D. aristatum* and *D. caricosum* (Reddy and D'Cruz 1967 and 1968). Evidently, the genomes of *D. annulatum*, *D. aristatum* and *D. caricosum* are closely related and therefore, need to be given common symbols with different subscripts indicating minor differences between their genomes. The genome formulae suggested by de Wet *et al.* (1961) will have therefore, to be modified. The genomes of *D. annulatum* may be assigned the symbols **AAA'A'** being a segmental allotetraploid as suggested by de Wet *et al.* Since *D. aristatum* and *D. caricosum* parents used in this study are presumed to be polyhaploids of segmental allotetraploids, their genomes may be designated as **A₃A₃'** and **A₆A₆'** respectively. As the genomes of *D. annulatum* and *D. panchganiense* have been reported to be distantly related, the symbolization suggested by Singh (1966) will have to be modified, and symbols of **PP** may be assigned to the genomes of *D. panchganiense*.

Summary

Five hybrids were obtained from the reciprocal crosses between the diploid biotypes of *D. aristatum* and *D. caricosum*. Two of them were tetraploids and were derived when *D. aristatum* was used as the female parent, and three were diploids where *D. caricosum* was the female parent. The hybrids were intermediate in regard to their morphological characters as compared to their parents. Meiosis was regular in the parents and the diploid hybrids. Tetraploids had slightly irregular meiotic behaviour and formed some univalents and multivalents at metaphase I. Based on the cytological studies,

it has been concluded that the genomes of *D. aristatum* and *D. caricosum* possessed very close degree of homology. Modified genome formulae have been proposed for some of the species of the genus *Dichanthium*.

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