

A POLYHAPLOID OBTAINED FROM A HYBRID DERIVATIVE
OF *SORGHUM HALEPENSE* × *S. VULGARE*
VAR. *SUDANENSE*

B. N. DUARA AND G. L. STEBBINS, JR.

*Department of Agriculture, Jorhat, Assam, India and University
of California, Davis, California*

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ALTHOUGH the origin of new and stable forms of plant species by means of chromosome doubling is well known to cytogeneticists, the reverse process of reduction in chromosome number in polyploids, such as the occurrence of haploids from diploids by parthenogenesis, is a rare phenomenon. This paper reports the occurrence of a diploid or polyhaploid plant in a population of tetraploid *Sorghum* of hybrid origin. This plant was a chance discovery; its sister plants were all tetraploids, and no other polyhaploids have yet been detected in the numerous cultures of tetraploid *Sorghum* grown at the University of California, Berkeley, where the present study was made. For these reasons, the discovery was of sufficient interest to warrant the cytological and morphological study of which the results are reported in this paper.

ORIGIN AND HISTORY OF THE POLYHAPLOID CULTURE

As has been reported by many authors (MYERS 1947), all forms of *Sorghum vulgare*, sens. lat., including var. *sudanense*, or Sudan Grass, are diploid, with $2n = 20$ chromosomes; while *S. halepense*, or Johnson grass, is tetraploid with $2n = 40$. This difference in chromosome number makes hybridization between the two species very difficult, and the triploid F_1 hybrids are sterile. On the other hand, hybrids between *S. halepense* and autotetraploid *S. vulgare* are easily made, and are fertile. The present material is descended from a tetraploid hybrid made by DR. L. F. RANDOLPH of Cornell University between artificially produced autotetraploid plants of Sudan Grass (*S. vulgare*) and the natural tetraploid species *S. halepense*. According to RANDOLPH (in litt. to G. L. STEBBINS, JR.), the female parent was a male sterile strain of *S. halepense* which was exposed to the pollen of tetraploid Sudan grass. The cultures which were studied for the present report were obtained from two different plants of the F_5 generation, grown in the garden of the Genetics Division, University of California, and descended from seeds of F_2 plants obtained from DR. RANDOLPH. One of these two F_5 cultures (No. 802) contained only tetraploid plants, while the other, no. 801, consisted entirely of diploids. The parent of culture no. 801 was a single F_5 plant, which probably arose from its tetraploid F_4 ancestor by means of parthenogenesis. Unfortunately, this F_5 plant had already been destroyed when the diploid nature of culture no. 801 was discovered. After this discovery, 30 plants each of the F_3 , F_4 , F_5 , and F_6 generations were grown, but no diploids were found among

them. Evidently parthenogenesis is a rare occurrence in this material, and its detection was a lucky chance. Since culture no. 801 differed from all other diploids grown in our garden, and possessed certain morphological characteristics typical of its tetraploid relatives, it could not have resulted from accidental contamination of the parental culture.

EXPERIMENTAL PROCEDURE

The two strains, diploid and tetraploid, were compared under uniform conditions in two different experiments. The first was in the greenhouse, in a large wooden soil bin, which had a depth of about 50 cm. The seeds were planted in October, 1947, and the plants reached maturity in January and February, 1948. For the second, seedlings were started in the greenhouse in April, were planted in the field in May, and matured between August and September. Under greenhouse conditions, the seeds were planted in adjacent rows 1 foot apart, and the young seedlings thinned so that the plants which matured were spaced about 1 foot apart in the rows. For the field experiment, the seedlings were transplanted into rows 3 feet apart, and spaced the same distance apart in the rows. In this instance, there were planted in addition to the two F_6 cultures, tetraploid cultures of the F_3 , F_4 , and F_5 generations, obtained from seed harvested in previous years.

Somatic chromosomes were studied from root tips fixed in RANDOLPH'S "Craf" solution, sectioned and stained with iodine-gentian violet in the usual manner for this laboratory. Meiotic chromosomes were studied from acetocarmine squash preparations of material previously fixed in a 3:1 absolute alcohol acetic acid solution. The percentage of good pollen was determined from mature anthers squashed and mounted in a solution of lactic phenol stained with cotton blue. At least 400 grains from 2 different flowers, were counted for each plant.

GENERAL MORPHOLOGY

During early stages of growth, both in the greenhouse and in the field, the polyhaploid and the natural diploid, Sudan 23, grew more slowly than the tetraploid hybrid derivatives of the F_5 and F_6 generations. At maturity, however, the tetraploid was not larger or more vigorous than the diploids. Table 1 shows the differences between the three strains in respect to the four most conspicuous morphological characteristics, and in pollen fertility. The polyhaploid is seen to be the tallest, the tetraploid intermediate, and Sudan 23 the shortest. On the other hand, the polyhaploid strain has significantly fewer tillers and broader leaves than the other two which do not differ significantly in these respects. The only reproductive character included was the nature of branching of the panicle. The length and number of nodes of the panicle was about the same in all three strains, but the polyhaploid bore a larger number of branches at the lower nodes than the other two. This difference was best estimated by counting the total number of branches per panicle.

One character not recorded in the table is the shape and color of the spikelets at maturity. In the tetraploid, they resemble those of *S. halepense* in being

relatively narrowly ovate, black, and shining. Sudan 23, however, has broader, yellowish spikelets which are somewhat duller. In this respect, the polyhaploid was indistinguishable from its tetraploid progenitors and differed from all other diploid strains of Sorghum known to us.

In perenniality, the polyhaploid strain was intermediate. Under conditions at Berkeley, Sudan 23 dies after maturing its seed, and never regenerates. The tetraploid hybrid derivatives, on the other hand, produce new shoots freely, and survive for several seasons. Most of the polyhaploid plants produced new shoots during the early winter after their first flowering season, but these were killed by an unusually heavy January frost, which also destroyed many of the tetraploids. After this time, no new regeneration took place.

TABLE 1

Comparison of certain morphological characters and pollen fertility in the polyhaploid, the tetraploid F₂, and diploid Sudan 23 under field conditions.

	Polyhaploid (822)	Tetraploid F ₂ (821)	Diploid Sudan 23 (823)
Number of plants	28	30	30
Height of plant (cm)			
Range	152-254	86-218	116-187
Mean	207 ± 5.3	173 ± 5.6	149 ± 2.9
Number of tillers			
Range	22-119	43-196	39-134
Mean	65.0 ± 5.4	93.0 ± 11.0	83.3 ± 3.9
Width of leaves (mm)			
Range	17-40	13-48	14-27
Mean	27.2 ± 1.2	22.6 ± 1.4	21.0 ± 0.6
Number of panicle branches			
Range	21-56	17-54	14-34
Mean	34.8 ± 1.5	26.5 ± 1.2	24.4 ± 0.9
Pollen fertility (%)			
Range	71-88	7-70	70-96
Mean	75.6	41.4	84.0

The tetraploid hybrid derivatives were more variable in all characteristics than either of the diploid strains. This is shown in table 1 by the greater range of variation recorded for this type, and the larger size of the standard errors. In this respect, the polyhaploid strain was intermediate, though somewhat nearer to the tetraploid than to Sudan 23.

CYTOLOGY AND FERTILITY

Somatic divisions were normal in all three strains, under both greenhouse and field conditions. In meiosis, the diploid Sudan 23 was also normal in every respect, and as shown in table 1, its fertility was high and rather constant from plant to plant.

In the tetraploid hybrid derivatives, meiosis was essentially similar to that reported by HUSKINS and SMITH (1934) and GARBER (1944) for *S. halepense*. Bivalents and quadrivalents occurred in every sporocyte, while uni-

valents and trivalents were not infrequent. No association higher than quadrivalent was observed. The maximum number of quadrivalents per sporocyte was five, as found by GARBER in *S. halepense*. Later stages of meiosis were frequently irregular, and individual chromosomes were often eliminated from the microspores. This probably accounts for the high percentage of pollen abortion in many of the tetraploid plants.

The 20 chromosomes of the polyhaploid strain were usually paired normally as 10 bivalents, but occasional sporocytes contained 9 bivalents and 2 univalents (Figures 1, 2). Because of these univalents, a small percentage of irregularity was found in later stages of meiosis. This was enough to account for the slight reduction in pollen fertility of this strain, as compared to Sudan 23.

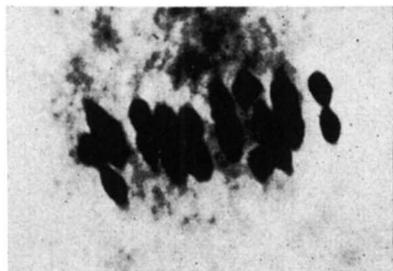


FIGURE 1.

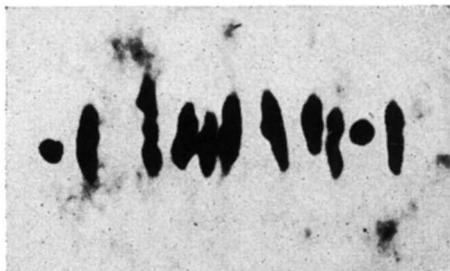


FIGURE 2.

FIGURE 1.—A cell of the parthenogenetic diploid strain showing the 20 chromosomes paired normally into 10 bivalents. ($\times 2500$.)

FIGURE 2.—Another cell of the parthenogenetic diploid strain showing 9 bivalent and 2 univalent chromosomes. ($\times 2500$.)

DISCUSSION

The production of a fertile diploid from a tetraploid was first reported by HÅKANSSON (1924), who found a 14-chromosome plant in the progeny of the 28-chromosome autotetraploid *Oenothera gigantea*, a derivative of *O. Lamarckiana*. RANDOLPH and FISCHER (1939) found 23 parthenogenetic maternal diploids in a population of 17,165 individuals of autotetraploid maize. KATAYAMA (1935), who found haploid plants in progeny of the allopolyploid *Aegilotriticum*, observed that they were sterile. He concluded that the character of parthenogenetic derivatives with the reduced chromosome number could provide information as to whether the parental form was auto- or allopolyploid.

STEBBINS and KODANI (1944) found that a parthenogenetic haploid, or polyhaploid, produced from 72-chromosome guayule was weak, sterile, and had slightly irregular meiosis, and from this concluded that the parental type was at least partly allopolyploid. GERSTEL and MISHANEC (1950) reported the occurrence in this species of several such polyhaploids, some of which were relatively vigorous and fertile, indicating that 72-chromosome guayule is partly autopolyploid. ELLIOTT and WILSIE (1948) found a fertile polyhaploid in *Bromus inermis*, and assumed from this that the parental species is largely autopolyploid, but with some differences of homology among the parental genomes.

The meiotic behavior and fertility of the present polyhaploid strain are similar to that found in ELLIOTT and WILSIE's polyhaploid of *Bromus inermis*. In the present instance, the progenitor of the polyhaploid was a descendant of an artificial hybrid between a synthetic autotetraploid and a second natural tetraploid which possesses many autotetraploid characteristics. Although both HUSKINS and SMITH (1934) and GARBER (1944) have suggested that the 20-chromosome types of the *Sorghum vulgare* complex might be ancient tetraploids they now behave essentially like diploids, and can be considered such for purposes of the present argument. On this assumption, the hybrid which gave rise to the present polyhaploid could be considered an autotetraploid only on the assumption that *S. halepense* is also an autotetraploid, and that its two genomes are completely homologous with the genome of *S. vulgare* var. *sudanense*. The considerable differences in external morphology between *S. vulgare* and *S. halepense* suggest that at least one of the two genomes of the latter species was derived from a species different from *S. vulgare*, a conclusion also reached by HUSKINS and SMITH. Furthermore, the slight meiotic irregularities found in the polyhaploid suggest that its two genomes are not completely homologous with each other, although they are very nearly so. That this strain contains some genes derived from *S. halepense* is evident from the appearance of its mature spikelets and its tendency toward the perennial habit. This whole situation could be explained on the basis of the following hypothesis. Assume that the genomic constitution of *S. vulgare* var. *sudanense* is V_1V_1 , and that *S. halepense* originated as a segmental allopolyploid (STEBBINS 1947), containing the V_1 genome plus one designated as V_2 , which was largely homologous with V_1 , but differed from it with respect to a number of chromosome segments. The evolution of *S. halepense* after its origin would have involved heterogenetic association between V_1 and V_2 chromosomes, with natural selection for fertility tending to establish a balance which approached autopolyploidy in respect to factors affecting fertility, but which was still allopolyploid to the extent that morphological characters of the V_2 species were retained. The genomic constitution of modern *S. halepense* might therefore be designated as $V_{12}V_{12}V_{12}V_{12}$. This would agree with the external morphology, fertility, and nearly autopolyploid cytological behavior characteristic of *S. halepense*. The original hybrid between autotetraploid *S. vulgare* var. *sudanense* and *S. halepense* would have received two V_1 genomes from the former, and $V_{12}V_{12}$ from the latter, and so would have the constitution $V_1V_1V_{12}V_{12}$. This constitution would give it a slight degree of genetic unbalance, and so would explain the high degree of variability of its offspring in both external morphology and fertility. Most of its gametes with the reduced chromosome number would be non-functional, and polyhaploids would be expected only rarely, as was found to be the case. Furthermore, the V_1 and V_{12} chromosomes would have exchanged genes through crossing over during the four generations between the original F_1 hybrid and the progenitor of the present polyhaploid, so that one would expect the latter to have received mostly chromosomes of the V_1 type, but a few of them which contained blocks of genes derived from V_{12} . The polyhaploid, therefore, would be expected to appear and behave like a

strain of Sudan grass which had obtained genes by introgression from *S. halepense*. This is exactly the case, and therefore supports the assumptions which have been made concerning the genetic nature of *S. halepense*, and the events which took place prior to the formation of the polyhaploid.

SUMMARY

1. One culture of the F_6 generation from a hybrid between autotetraploid *Sorghum vulgare* var. *sudanense* ($2n = 40$) and *S. halepense* ($2n = 40$) consisted entirely of plants with $2n = 20$ chromosomes, and presumably arose from a tetraploid F_5 plant through the parthenogenetic development of an egg with the reduced chromosome number.

2. In general morphology, the polyhaploid plants resembled tetraploid plants of the same generation, but were taller, had fewer tillers, broader leaves, more numerous panicle branches, and were more weakly perennial.

3. The chromosomes usually paired normally into 10 bivalents at meiosis, but occasional univalents were observed, leading to irregularity at later stages of meiosis, and somewhat reduced pollen fertility.

4. The morphology and chromosome behavior of the polyhaploids suggests that their F_5 parent was nearly autotetraploid, but contained some differences between its four genomes with respect to chromosome segments. This indicates that *Sorghum halepense* originated as a segmental allopolyploid containing two genomes derived from *S. vulgare* and two genomes derived from some related species with chromosomes partly homologous to those of *S. vulgare*.

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