CYTOLOGICAL AND INHERITANCE STUDIES OF A SORGHUM CROSS - (JOHNSONGRASS x 4n SUDANGRASS) x AUTOTETRAPLOID SUDANGRASS

by

ALFRED JACKSON CASADY

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INTRODUCTION

The studies to be presented in this paper grew out of a practical breeding problem concerned with the development of a forage sorghum suitable for pasturage. A type of Sorghum, originating from a cross between johnsongrass (Sorghum halepense (L.), Pers.) and autotetraploid common sudangrass (S. vulgare var. sudanense (Piper), Hitchc.) made by Dr. L. F. Randolph at Cornell University, Ithaca, New York, has been grown in the sudangrass breeding nursery at Kansas State College, Manhattan, Kansas since 1942. This material was subjected to considerable selection, and some very promising lines have been isolated in respect to certain agronomic characteristics such as seedling vigor, plant vigor, leafiness, high degree of tillering, leaf disease resistance, and chinch bug tolerance. This sorghum possesses one character, however, which is considered to be undesirable in a forage sorghum. It has a dry, pithy stalk which lowers its palatability to livestock. The primary purpose of the breeding problem has been to cross the promising selections with juicy-stalked autotetraploid sudangrass in an attempt to combine the juicy-stalked character with the desirable characters of the johnsongrass x 4n sudangrass selections.

In order to carry on an intelligent breeding program with the proposed cross, it was deemed necessary first of all to study the resulting progeny in order to learn the mode of inheritance of specific characters. It also seemed advisable to observe both the parental material and the progeny cytologically. The main purpose of these cytological studies was to observe the behavior of the chromosomes so that the success of the cross could be evaluated. While the cytological studies were being made, the possibility of learning something of the relationship of Sorghum halepense to S. vulgare

Pers. was kept in mind.[1]

Two other studies have been included. Certain characteristics of the autotetraploid sudangrass were observed and compared with similar characteristics of their diploid counterparts. Also the frequency of hybridization between the johnsongrass x 4n sudangrass selections and both the diploid and autotetraploid sudangrass was observed. Although these two studies have no immediate bearing on the problem at hand, they would seem to be of enough general interest to warrant their inclusion. Furthermore, the results obtained would no doubt be of value if crosses were desired between Sorghum halepense and the varieties of S. vulgare other than sudanense.

A REVIEW OF THE LITERATURE

Relationship of the Sorghums

Classified as to chromosome number, the genus Sorghum falls into three groups (15, 22, 48). One group with a haploid number of five chromosomes is represented by the species Sorghum versicolor Anderss. A second group with a haploid number of 10 is represented by the species S. vulgare. A third group represented by S. halepense has a haploid number of 20 chromosomes. Aside from the numerical relationship of the chromosome complements of the three groups, the available evidence indicates that these three chromosome groups represent the diploid, tetraploid, and octoploid forms of the genus. Huskins and Smith (20) and Chin (12) found quadrivalent associations among the meiotic chromosomes of S. vulgare. Huskins and Smith (20) also pointed

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[1] Based on the conclusions of Vinall, Stephens, and Martin (48), Sorghum vulgare as used in this paper will include all Sorghum with a haploid number of 10 chromosomes.

out that the occurrence of duplicate or polymeric factors may, with reservation, be taken as an indication of polyploidy. A number of duplicate factors are known to occur in S. vulgare (16, 21). Further evidence that S. vulgare is the tetraploid form is furnished by Brown (10) as the result of cytological studies of haploid S. vulgare plants. Her studies of 150 meiotic metaphase nuclei revealed one case of three bivalents and four univalents, two cases of two bivalents and six univalents, and 13 cases of one bivalent and eight univalents. In respect to S. halepense, Huskins and Smith (20) observed chromosome associations higher than quadrivalents, which indicates that it is a higher polyploid than S. vulgare. More commonly, however, all the chromosomes of S. vulgare associate as bivalents at meiosis, and most of the chromosomes of S. halepense associate as bivalents with a few quadrivalents (15, 20, 26).[1]

The evolutionary relationship between the three chromosome groups of Sorghum apparently has not been definitely established. Longley (26) considered that S. halepense was derived from Sorghum ancestors, having 10 chromosomes as the haploid number. Huskins and Smith (19), in their studies of the somatic chromosomes of S. vulgare and S. halepense, concluded that it was possible that one of the parental species of S. halepense was a member of some genus other than Sorghum. They based this conclusion on the morphology of one chromosome which was present in duplicate in all varieties of S. vulgare studied, and which was present also only in duplicate in S. halepense instead of quadruplicate as would be expected, if both parents were of the genus Sorghum. In later studies, Huskins and Smith (20) found quadrivalents and higher

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[1] For this reason S. vulgare is usually referred to as the diploid form and S. halepense as the tetraploid form. Unless otherwise indicated these two species will be considered in this manner for the remainder of this paper.

associations of the meiotic chromosomes of S. halepense which gave evidence that both parents were at least of the same genus; however, they suggested the possibility of seven rather than five being the basic chromosome number of the genus. Karper and Chisholm (22) found that there was a decrease in chromosome size as the number increased in respect to S. versicolor, S. vulgare, and S. halepense. The average length for the three species was 4.86, 2.24, and 1.98 microns, respectively. They concluded from these studies, based on the size relation of the chromosomes of the three groups, that, if S. versicolor is the diploid, S. vulgare the tetraploid, and S. halepense the octoploid, the evolution of these species involved processes other than the mere doubling of the chromosomes. Snowden (38) divided the genus Sorghum into two subsections, namely, Para-sorghums with a haploid number of five and Eu-sorghums with a haploid number of 10 with the exception of S. halepense (n = 20). Although he gave no reason, he did not consider the Para-sorghums to have played any part in the evolution of the Eu-sorghums. Garber (15) from his studies of the genus Sorghum concluded that the basic chromosome number is five. He found the chromosome complements in the Eu-sorghums (S. vulgare and S. halepense) to be morphologically similar in all phases of meiosis, whereas he found a striking difference at meiosis between the chromosome complements of the Para-sorghums and the Eu-sorghums in respect to staining with acetocarmine stain, morphology, and size of the chromosomes. He considered these striking differences to indicate that Para-sorghums played no part in the evolution of the Eu-sorghums of higher chromosome numbers. He considered S. halepense to be a polyploid, but he was not certain whether it was an autopolyploid or an allopolyploid. He stated, however, that there was no reason to assume that one of the parents belonged to a genus other than Sorghum.

Some evidence of the evolutionary relationship between Sorghum halepense and S. vulgare, that is, whether or not S. halepense is an autopolyploid or an allopolyploid, has been furnished by the observations made of the meiotic chromosomes in S. halepense, a colchicine-induced autotetraploid S. vulgare, and the F_1 progeny of a S. halepense and autotetraploid S. vulgare var. sudanense mating. Huskins and Smith (20) found that 14 bivalents with the remainder of the chromosomes in quadrivalent or higher associations occurred most commonly in S. halepense. Garber (15) found the maximum number of quadrivalents in S. halepense to be five with a range of 0 - 5 in 500 microsporocytes studied. Chin (12) in a cytological study of a colchicine-induced autotetraploid of S. vulgare var. hegari found three quadrivalents to be the average number per microsporocyte with six being the maximum. Randolph (32) observed the chromosome behavior to be regular in the F_1 progeny of a S. halepense and autotetraploid S. vulgare var. sudanense mating. However, in considering the possibility that S. halepense is the result of chromosome doubling in S. vulgare, it should be pointed out that both Randolph (32) and Karper and Chisholm (22) have stated that the segregation observed in the hybrids (n = 20) of S. halepense and S. vulgare matings for the rhizomatous character of S. halepense indicates that something other than the mere doubling of chromosomes was involved in the evolution of S. halepense.[1]

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[1] Although the hybrids used by Karper and Chisholm had a haploid number of 20, they originated from a natural cross between S. halepense and diploid S. vulgare. Since it was assumed that the vulgare chromosomes had doubled, the hybrids would be essentially the same as if they had come from a S. halepense and autotetraploid S. vulgare mating.

Hybridization of the Sorghums

Much of the speculation as to the relationship of the three chromosome groups of Sorghum has been based on their ability to hybridize with one another. Apparently all the 10-chromosome varieties of Sorghum cross freely with one another (15, 19, 22, 48). Attempts have been made to cross S. versicolor with the two higher chromosome groups, but such attempts have been unsuccessful (15, 22). S. vulgare and S. halepense are known to hybridize occasionally under natural conditions, but such crosses are rare (22, 23, 27, 45). Vinall (46) made numerous experimental attempts to cross S. halepense with several of the more important commercial sorghums in 1912, 1913, and 1914. The 1912 work was conducted in the field, and the 1913 and 1914 work was in the greenhouse. Only one seed was obtained each year, and in all three instances S. vulgare served as the pistillate parent. Harper and Chisholm (22) reported that they obtained only one seed from 217 emasculated S. sudanensis (Piper) Staph. florets pollinated by S. halepense plus 53 reciprocal crosses; they also reported that no successful crosses were obtained in 116 trials to cross S. virgatum (Hack.) Staph. and S. halepense. Randolph (32) reported no difficulty in obtaining cross-fertilized seed in attempts to hybridize S. halepense and colchicine-induced autotetraploid S. vulgare var. sudanense.

Vinall and Getty (45) postulated that the failure of hybridization between Sorghum halepense and S. vulgare was due to "an antagonism or unfavorable reaction between the reproductive organs of the two plants." Huskins and Smith (19) attributed the difficulty encountered in crossing 10 and 20 chromosome Sorghum to the difference in chromosome number. The results of Randolph (32) furnished rather substantial evidence that at least a part of the difficulty encountered in previous attempts to cross 10 and 20 chromosome Sorghum was accountable to the difference in chromosome number.

Thompson (44) stated that, although the incompatability (failure of hybrids to be formed) encountered between species of a polyploid series may be due to the failure of fertilization, a large proportion of the incompatability is due to post-fertilization breakdown of the endosperm with the resulting death of the embryo. Brink and Cooper (8) reported that the cause of incompatability between diploids and their autotetraploid derivatives, in some cases, is due to abnormal pollen tube development which prevents fertilization. They stated, however, that seed abortion is probably the most effective barrier to hybridization between diploids and their autotetraploid derivatives. Brink and Cooper (9) discussed the following facts concerning the role of the embryo and endosperm in the life history of the Angiosperms: (1) The embryo embodies the line of decent and is, therefore, the principle component of the seed, but the conditions essential for growth and differentiation of the zygote are not present in the Angiosperms at the time of fertilization. (2) The significance of the endosperm lies mainly in the fact that it plays a major role in the development and maintenance of a medium suitable for the growth of the young embryo; if the endosperm does not succeed in its function, the embryo which is dependent on it fails also. (3) In normal 2n x 2n matings the chromosome ratio between the embryo, endosperm, and maternal tissue is 2:3:2, and any change in this ratio may result in the breakdown of the endosperm with the ensuing death of the embryo. In their studies of the endosperm development in 2n x 2n, 2n x 4n, 4n x 4n, and 4n x 2n matings of Lycopersicon pimpinellifolium Brink and Cooper (8) found that, although fertilization of the egg and the polar nuclei were parallel events, by the time the zygote divides the endosperm is a rapidly growing tissue in the 2n x 2n matings. It was also found that 4n x 4n matings gave endosperm development similar to 2n x 2n matings, but that 2n x 4n and 4n x 2n matings gave slow endosperm growth from the beginning with

eventual collapse. The mature seed obtained from the various matings in these studies gave supporting evidence to the developing endosperm studies. Almost all the seed from the 2n x 2n matings were plump, and approximately two-thirds of the seeds in the 4n x 4n matings were plump, with the remainder shriveled. In the 4n x 2n matings all the seeds were shriveled and incapable of germination, while in the case of the 2n x 4n matings all the fruits dropped by the end of the eighth day after fertilization. These investigators concluded from these studies that the continued development of the young seed after fertilization occurs was dependent to a considerable degree upon the maintenance of the rapid growth of the endosperm, which required a delicate physiologic balance between the endosperm and the adjacent maternal tissue, and further, that incompatability arose when the chromosome ratio between maternal tissue and endosperm was varied in either direction. In respect to seed development and the chromosome ratio between embryo, endosperm, and maternal tissue, Muntzing (30) observed that shriveled seed from autotetraploid rye gave more aneuploid plants than did plump seed. He concluded from this that even the slight deviation from the 2:3:2 ratio, resulting from aneuploid gametes, can result in endosperm breakdown.

Some Characteristics of Sorghum halepense and S. vulgare var. sudanense, and the Inheritance of the Specific Characters Studied

Morphologically Sorghum vulgare var. sudanense and S. halepense are quite similar with the exception that the latter produces rhizomes and is considered a perennial, while the former has no rhizomes and is ordinarily considered an annual (23, 26, 27, 47), although it lives through the winter readily when brought into the greenhouse. S. halepense is usually more slender and somewhat shorter than S. vulgare var. sudanense (23, 45). S. halepense can also

be distinguished from S. vulgare var. sudanense by the manner in which the sessile spiklet is shed (2). Ayyangar and Ponniya (4) observed that the two species also could be distinguished by their time of anthesis; they found that S. vulgare var. sudanense flowered between 5:30 - 6:00 a.m. while S. halepense did not flower until 8:30 - 9:00 a.m.

Sorghum vulgare var. sudanense has a dry pithy stalk indicated by the white, chalky midrib of the leaves (6, 23). It is also characterized by a blackish purple pigment in the stems, leaves, and mature glumes (6). Tift sudangrass, a variety selected from a cross between S. vulgare var. sudanense and S. vulgare var. leoti, has a distinctive chocolate colored glume which is sometimes obscured by an inhibitor, causing it to fade to a straw color; its general appearance has been modified by the leoti parent (11). S. halepense also has white midribs in the leaves, denoting a dry, pithy stalk (44, 46). It also has the blackish purple pigment in the leaves, stem, and mature glumes (5).

Stephens and Quinby (41) pointed out that the varieities of Sorghum with white, chalky midribs in the leaves have dry stalks, whereas those varieties with a dull or opaque midrib have a juicy stalk. These authors also stated that seedlings and new leaves of all members of the genus Sorghum have dull midribs, but in the dry-stalked varieties the white midrib starts as a narrow streak and spreads toward the margin until all the midrib is white. Even in the juicy varieties there usually occurs a white streak in the midrib before maturity, but it begins much later in the plant's development and the margin of the midrib remains dull. This pair of characters in diploid Sorghum is inherited as a single factor pair with the dry-stalk (D) being dominant to juicy (d) (18, 42).

There are two broad types of plant color expressed in the sorghums,

purple and brown. The former group is divided into two sub-groups, reddish purple and blackish purple (1, 40, 41). Ayyangar, Vijiaraghavan, Pillai, and Ayyar (1) and Stephens and Quinby (40, 41) have studied the inheritance of the plant color and have shown that it is expressed and inherited in the following manner. Plant color can be distinguished in injured or decaying seminal or coronary roots, tissue attacked by insects, tissue surrounding areas attacked by disease, and mature glumes. The purple plant color (P) is a simple dominant over brown plant color (p). Another pair of factors control the two sub-groups of purple. Reddish purple (Q) is a simple dominant over blackish purple (q). Therefore, in the purple group a genotype of P- Q- gives reddish plant color and red glumes, and P- qq gives blackish purple plant color and blackish purple glumes. The Q factor has no effect on the color of the pigment in the leaves, stalks, and roots of the brown group of sorghums. Its effects are manifest, however, in the color of the mature glumes. Plants of the genotype pp Q- have brown plant color with sienna glumes, whereas a genotype of pp qq gives brown plant color and mahogany glumes. According to Stephens and Quinby (41), S. vulgare var. shallu and leoti were originally the only commercial varieties grown in the United States with the brown plant color. Regardless of the genotype for P and Q, glume color is sometimes inhibited and the glumes appear neutral or straw in color; this character is controlled by a single factor pair (Gs, gs), and the inhibitor is recessive to color (27).

Ayyangar, Ayyar, and Rao (3) demonstrated that the P factor for plant color and the gene for the dry-juicy stalk character were linked with a 30 percent crossover value. Stephens and Quinby (41) observed a similar crossover value for these two linked genes, but they found the Q factor to be inherited independently of D and P.

Randolph (32) reported that segregations of the F_2 progeny of a S. halepense and S. vulgare var. sudanense mating indicated that at least several factors are involved in the expression of the rhizomatus character.

The Character, Cytology, and Inheritance of Autotetraploids

Autotetraploids usually differ from their diploid counterparts in a number of morphological characters. The actual chromosome count is the only way to determine an autotetraploid with certainity, but there are certain criteria that may be useful in the preliminary determination of autotetraploids. The morphological differences commonly observed between diploids and their autotetraploid derivatives are as follows: the autotetraploids are characterized by (1) a more stocky habit of growth, (2) later maturity, (3) deeper green color, (4) wider and thicker leaves, (5) larger pollen, (6) larger seeds, (7) larger stomata, and (8) larger somatic and reproductive cells (7, 35). Another characteristic that is almost always present in an autotetraploid is a reduction in seed set (24, 29, 31, 35, 39).

Autotetraploids usually are distinguishable by the behavior of their chromosomes at meiosis. Since there are four homologous genomes present in autotetraploids instead of two as in diploids, univalents, bivalents, trivalents, and quadrivalents may be formed at prophase I, and unequal distributions of the chromosomes may result from the univalents, trivalents, and quadrivalents at metaphase I (13, 35). Also autotetraploids can usually be distinguished from allotetraploids by the behavior of the chromosomes at meiosis. In the typical autotetraploid all or part of the homologues form multivalent groups, whereas in the typical allotetraploid (amphidiploid type) only bivalents are formed (14, 35). However, Sharp (34, p. 350) has pointed out that the chromosome associations in an autotetraploid may vary from all quadrivalents

on the one extreme to all bivalents on the other, depending on the genus, species, variety, or individual concerned. Obviously the occurrence of univalents and trivalents is likely to result in an unequal distribution of chromosomes, but quadrivalent associations do not necessarily give an unequal distribution of chromosomes. It depends to a large extent into what phase of meiosis the quadrivalent condition prevails. If the chromosomes in the quadrivalent association have disjoined into two bivalents by metaphase, equal distributions may be expected (24). There seems to be full agreement among the investigators in this field that the production of aneuploid gametes causes sterility in autotetraploids (24, 28, 31, 35, 39), but the results of several investigators indicate that the degree of homozygosity is also a factor (24, 31, 39). Lindstrom and Humphrey (24) studied the fertility of tomato tetraploids from four different sources: one from a haploid (through a diploid), one from Lycopersicum esculentum, one from L. pimpinellifolium, and one from an F_1 hybrid of L. esculentum and L. pimpinellifolium. They found the meiotic behavior of all these tetraploids to be strikingly similar, but the variation in fertility was 10 percent normal seed set for the tetraploid from the haploid, 40 percent normal seed set for the tetraploids from L. esculentum and L. pimpinellifolium, and 50 percent normal seed set for the tetraploid from the F_1 hybrid. Randolph (31) reported that maize autotetraploids from open pollinated varieties and hybrid stocks were more fertile than those from inbred lines, although the meiotic chromosome irregularities appeared to be no more prevalent in the sterile lines than in the fertile lines. Sparrow, Ruttle, and Nebel (39), working with snapdragons, found intravarietal tetraploids to be relatively sterile and intervarietal tetraploids to be relatively fertile, although meiotic irregularities occurred only slightly more frequently in the intravarietal tetraploids. In respect

to fertility in autotetraploids, both Randolph (31) and Muntzing (29) have demonstrated that selection can increase the fertility to a certain degree.

Muntzing (28) and Dobzhansky (14, p. 226) have reviewed the evolutionary significance of autotetraploids. Sturtevant (42) discussed in some detail one important role autotetraploids may play in evolution. He stated,

> Within one species there may be a series of different but similar genes at any one locus in different members of a population. If one studies the characters conditioned by the various genes at any one locus, they turn out to be related. The impression is that the genes at any one locus are developmentally alike; apparently they are carrying on the same function, but with different degrees of efficiency. . . If genes do not change their functions, but only change the relative efficiency with which they carry out their predestined ones, it follows that organisms also cannot develop new functions, which is obviously contrary to fact, for there can be no doubt that new functions do develop in the course of time. It may be taken as probable that most of the genes present in an organism are performing functions that are advantageous to the organism, otherwise they will not long persist. Most of the genes, then, are needed by the organism, and cannot well be spared for the production of new functions. It seems likely that the most favorable condition for the production of such new functions is one in which some of the usual genes are present in duplicate. Cases of doubled chromosome numbers furnish such an opportunity, for in these cases there is a whole extra set of genes, whereas a single set is all that is needed to carry on the functions normal to such an organism.

Since each chromosome with its genes is represented four times in an autotetraploid, instead of twice as in a diploid, the inheritance of a given character in an autotetraploid is different than the inheritance of the same character in a diploid. Lindstrom (25) has reviewed the methods of inheritance that may be exhibited by tetraploids: Method 1. Preferential pairing of similar chromosomes (autosyndesis). When two chromosomes of a tetrasome are very different genetically from the other two, or when a true allopolyploid is involved, and pairing is conditioned by gene-by-gene attraction, it is apparent that two similar chromosomes should synapse. If then, disjunction is from a bivalent condition, all the diploid gametes should be alike, and the hybrid should breed true. Such is rarely the case, but it has been reported

in tetraploids from very wide species or genus crosses (allopolyploids). Method 2. Preferential pairing of dissimilar chromosomes (allosyndesis). There seems to be no apparent reason for such a condition, if pairing is instigated by a gene-by-gene attraction. Such a condition would give a 15:1 ratio in the F_2 generation. Some early data on the inheritance of tetraploids seemed to fit a 15:1 ratio, but it was better explained later. Method 3. Random assortment of four chromosomes. If the four homologues synapse during prophase in a quadrivalent condition and later emerge as bivalents, a 1AA + 4Aa + 1aa assortment of diploid gametes would result. This would give a 35:1 ratio in the F_2 generation. Practically all tetraploid data fit this ratio. Method 4. Random assortment of eight chromatids. This is the result of crossing over. If any gene locus is far enough removed from the kinetochore, a crossover may affect every such gene locus among the eight chromatids. This would result in a random interchange among the eight chromatids, the maximum state being a wholly random assortment of the eight. If the gene locus is near the kinetochore, there would be less chance of an interchange separating sister chromatids, and the assortment would be like Method 3. If the random assortment of chromatids was at a maximum, the proportion of diploid gametes would be 3AA + 8Aa + 1aa and would give a 20.8:1 F_2 ratio. With less crossing over, such as would hold for genes near the kinetochore, an approach to the 35:1 F_2 ratio as a limit would result. In other words, the percentage of recessive gametes from an AAaa individual would vary between 16.7 and 21.4 percent, depending upon the amount of crossing over between the kinetochore and the gene in question. Hayes and Immer (17, p. 18) have pointed out that random chromatid segregation occurs only when the gene concerned is 50 or more crossover units from the kinetochore, and that for closer distances the ratios are intermediate between those expected for chromosome and chromatid segrega-

tion, approaching chromosome segregation as the gene becomes closer to the kinetochore. Sansome (33) stated that random chromatid segregation cannot occur in autotetraploids without approximately 100 percent quadrivalent formation.

The inheritance of characters controlled by linked genes is different in autotetraploids than in diploids, since the genes are present in quadruplicate. Sansome (33) has listed the gametic output expected for the various phases of linkage in autotetraploids.

MATERIALS AND METHODS

General

All the work was carried on in the greenhouse at Kansas State College, with the exception of the observations made of three F_2 progenies which were grown in the field at the Ft. Hays Branch Experiment Station, Hays, Kansas. The plants in the greenhouse were grown in six-inch clay pots to allow for maneuverability. Once the original stalks of the plants had matured and served their usefulness they were cut away, allowing the secondary stalks to develop. By this means it was possible to extend the usefulness of individual plants over the entire course of the experiments. Due to the limited amount of soil in the pots and the frequent applications of water, a nutrient deficiency was almost always present. To alleviate this situation in so far as possible, reasonable applications of a 4-12-4 commercial fertilizer were made. The plants also became pot-bound from time to time; therefore, they were occasionally removed from the pot, trimmed of their old, decaying roots, and repotted in fresh soil.

Parental Material

Johnsongrass x 4n sudangrass parents. Seven johnsongrass x 4n sudangrass selections were used as parental material. Three of these selections, designated as greenhouse numbers 2, 3, and 4, originated respectively from seed harvested from rows 24, 28, and 33 in the 1946 sudangrass nursery at Kansas State College. One selection, designated as greenhouse number 51, originated from the lot of seed used to plant row 51 in the 1947 sudangrass nursery. The selections with the greenhouse numbers 70, 73, and 77 originated respectively from the lots of seed used to plant rows 70, 73, and 77 in the 1948 sudangrass nursery. Each of the selections was very uniform; therefore, the individual plants within the respective selections were used indiscriminately in the crosses. However, each plant in a selection was given a letter designation in order to distinguish individual plants in cytological and hybridization studies.

All the johnsongrass x 4n sudangrass selections used were dry-stalked as indicated by the white midribs of the leaves, and it was ascertained by previous breeding records that they were homozygous for this character. All the selections also had the blackish purple plant color and blackish purple glumes. As previously reviewed, plant color and glume color are both controlled by two pairs of genes (P and Q) in diploid Sorghum. Since the blackish purple plant color and blackish purple glumes are present only when the Q gene is in the homozygous recessive condition, these selections were obviously homozygous for the Q gene, and previous breeding records showed them to be homozygous for P. Therefore, the genotype of the johnsongrass x 4n sudangrass selections for the characters studied specifically was DDDD PPPP qqqq. All the selections used as parents had retained the rhizomatous type of perennial habit of growth from the johnsongrass. There was some variation between the

selections in the size and vigor of the rhizomes under greenhouse conditions. Greenhouse No. 3 had the largest and most vigorous rhizomes, and greenhouse No. 2 had the smallest and least vigorous. In no case, however, were the rhizomes of any selection found to be so large and vigorous as those produced by the johnsongrass plants grown in the greenhouse.

Sudangrass parents. Two groups of sudangrass were used for colchicine treatment in an attempt to produce the autotetraploid sudangrass parents. The first group originated from seed from the 1947 sudangrass nursery at Kansas State College. The various lines used were given the greenhouse numbers 26a, 32, 57, 69, 70, and 80 which corresponded to the 1947 nursery row number from which they originated.[1] Three concentrations of colchicine in aqueous solutions were used to treat these plants, and individual plant numbers were accompanied by a fraction designating the percent of colchicine in the solution with which they were treated. The percentage was expressed as a fraction of a percent. Also each plant of a given greenhouse number and a given colchicine percentage was assigned a letter for identification in cytological and hybridization studies. No plants with tetraploid tissue were found in greenhouse Nos. 26a and 80; consequently, no F_1 seed was obtained in crosses involving these lines; therefore, they need no further consideration. Greenhouse No. 32 is known as K. S. 1044 in the Kansas State College sudangrass breeding program and originated from the following cross: (Leoti sorgo x common sudangrass$_4$) x (Leoti sorgo x common sudangrass$_2$). In this line tetraploidy was observed in two plants, 32 1/10 B and 32 1/10 D.

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[1] The greenhouse number 70 in the sudangrass parents should not be confused with the greenhouse number 70 in the johnsongrass x 4n sudangrass parents. In the case of the sudangrass parents, the 70 will always be accompanied by a fraction, the percent of colchicine, and a letter. In the johnsongrass x 4n sudangrass parents, the 70 will be accompanied only by a letter.

As far as could be determined 32 1/10 B was entirely tetraploid, but 32 1/10 D produced both diploid and tetraploid stalks. Plant 32 1/10 D was juicy-stalked and had brown plant color and mahogany glumes; therefore, its tetraploid stalks were of the genotype dddd pppp gggg. Plant 32 1/10 B was dry-stalked and had blackish purple plant color and blackish purple glumes. This plant was assumed to be the result of an outcross, since the line of sudangrass to which it belonged had been selected for the genotype of 32 1/10 D. Greenhouse No. 57 is designated as Georgia 3-1 common sudangrass in the Kansas State College breeding program. Only one plant, 57 1/10 C, in this line was found to have tetraploid tissue; this plant was dry-stalked and had blackish purple plant color and blackish purple glumes, but its genotype for these characters was not definitely determined. The line to which greenhouse No. 69 belonged is a selection of Tift sudangrass. Two plants, 69 2/10 C and 69 2/10 X, produced tetraploid stalks, but both also produced diploid stalks. The tetraploid parts of these plants were of the genotype dddd pppp gggg. Greenhouse No. 70 also belonged to a line coming from a selection of Tift sudangrass. Only one plant, 70 2/10 B, was observed to have tetraploid tissue, and it seemed to be entirely tetraploid. It was of the genotype dddd pppp gggg. Another plant, 70 1/10 X, bore one seed in a cross with a johnsongrass x 4n sudangrass selection, but at the time it was studied cytologically no tetraploid tissue could be found. The F_1 plant grown from the one seed was observed cytologically and was found to have 40 chromosomes (Fig. 7). Since the johnsongrass x 4n sudangrass plant used in this cross was known to have 40 chromosomes, two explanations are possible. The successful cross was made with the original stalk of the sudangrass, but the cytological observation was made from the stalks that were produced after the original stalk was cut away; therefore, it is possible that the only

tetraploid tissue in this plant was in the original stalk. Another explanation is that an unreduced gamete was produced by a diploid plant. The diploid stalks of plant 70 1/10 X was of the genotype dd rr oo.

The other group of sudangrass was treated with colchicine by soaking the ungerminated seed in an aqueous solution. The lines used were grown from seed used to plant the 1948 sudangrass nursery. The various lines were given the greenhouse numbers 22, 24, 27, 28, 31, and 65. These numbers corresponded to the rows of the same numbers in the 1948 sudangrass nursery. Individual plants in this group of sudangrass are also accompanied by the percentage of aqueous colchicine solution used and by a letter designating individual plants. Greenhouse Nos. 22 and 24 originated from a selection out of Sweet sudangrass, and 27, 28, 31, and 65 represented various lines of K. S. 1044. No autotetraploids were found in any of this material; consequently, no viable seed was produced from the matings with the johnsongrass x 4n sudangrass selections. Such being the case, a discussion of the genetics of these plants is not warranted.

Method of Inducing Autotetraploidy in the Sudangrass Parents

The autotetraploid sudangrass parents were obtained by the use of colchicine. The seed was germinated on blotter paper in a seed germinator. When the seedlings were approximately one inch long, the young shoots were laid over the edge of a petri dish and entirely submerged in an aqueous solution of colchicine where they were allowed to remain six hours. The seedlings were then removed from the blotter paper and placed in soil in six-inch clay pots. Three concentrations of the solution were used, 1/20, 1/10, and 2/10 percent of colchicine by weight.

Method of Artificial Cross-pollination

The crosses were made in the following manner. The plants to serve as the pistillate parents were observed carefully until the anthers in the apical florets showed yellow and appeared just ready to burst out of the glumes. The panicle was then trimmed from the apex and base, leaving 15-30 central florets. The pedicellate, staminate florets were removed, and the remaining florets emasculated by slightly spreading the glumes and gently forcing out the anthers. The emasculated florets were immediately covered with a parchment paper bag approximately 4 x 18 inches in size. A panicle from the plant to serve as the staminate parent was chosen that was almost ready to flower, or had just started anthesis at the apex. This panicle was placed in the parchment bag with the emasculated florets of the pistillate parent. Care was taken to place the panicle of the staminate parent slightly above the emasculated florets so that the pollen would fall onto the stigmas of the emasculated florets. This method of cross-pollination was easily carried out because the plants were in pots which could be moved about at will. Furthermore, it would seem that this method would assure pollination, since it was almost certain that, because of the progressive blossoming downward from the apex of the inflorescence exhibited by Sorghum, viable pollen would be shed from the staminate parent during the period when the stigmas of the emasculated florets were receptive.

Methods Used in Cytological Studies

All cytological studies were made of the meiotic chromosomes in microsporocytes. The acetocarmine smear technic described by Smith (36) was used in preparing the slides. The material for study was taken when the developing

panicle could be felt through the boot but still exhibited resiliency. Either the entire panicle was removed or only a few branches, depending on the need for the panicle in the hybridization studies. If only a few branches were taken, the boot was slit with a razor blade only sufficiently to permit the removal of the desired material after which the opening was sealed with a piece of Scotch tape to prevent desiccation.

RESULTS

Morphological Characters of the Autotetraploid Sudangrass

The autotetraploid sudangrass plants were compared to the diploid plants to determine if differences could be observed that might serve as preliminary criteria in detecting the autotetraploid condition. The following characters were studied: (1) general appearance of the plant, (2) size of stomata, (3) size of microsporocytes at diakinesis, (4) size of pollen grains from freshly exposed anthers, and (5) size of mature seed. In making the comparisons, care was taken to use autotetraploid and diploid material as closely related as possible in order to hold genetical differences to a minimum. In some cases autotetraploid and diploid tillers occurred on the same plant; in such cases the comparisons were made between these tillers from the same plant. In cases when autotetraploid and diploid tillers did not occur on the same plant, the comparisons were made between sister plants.

Observations were made of the general appearance of the autotetraploid and diploid plants, and also of the autotetraploid and diploid tillers of the same plants. In general, there was no striking difference between the autotetraploids and diploids. However, slight differences were observed. The autotetraploids were slightly shorter and stockier. The leaves were somewhat wider and longer in the autotetraploids; this difference was especially

noticable in the flag leaf. Figure 1 shows the contrast between autotetraploid and diploid sister plants. Two characters often differing between autotetraploids and diploid plants, namely, deeper green color and later maturity, were not observable in the greenhouse.

Stomata were observed and measured under the microscope. The average length and width of 100 stomata from each source are given in Table 1. The first two comparisons were between sister plants, whereas the third comparison was made between tetraploid and diploid tillers of the same plant.

Table 1. Comparison of stomata size from lower leaf surfaces of autotetraploid and diploid sudangrass plants.

Plant designation	Haploid chromosomes	Average width of 100 stomata	Average length of 100 stomata	Increase in width	Increase in length
	number	microns	microns	percent	percent
70 1/10 X	10	22.41	32.20		
70 2/10 B	20	31.87	43.33	42.21	34.57
69 1/10 A	10	16.10	24.90		
69 2/10 X	20	20.42	31.04	26.83	24.66
32 1/10 D	10	17.10	23.90		
32 1/10 D	20	23.57	34.20	37.84	43.10

Table 2 gives the comparison of the average diameter of 100 microsporocytes at diakinesis for three different lines of sudangrass. All comparisons were made between sister plants. The autotetraploids gave the larger size.

The comparison of the average diameter of 100 pollen grains is given in Table 3. The first two comparisons were between sister plants, but the third comparison was between autotetraploid and diploid tillers from the same plant. In all cases the autotetraploids had the larger pollen grains.

Fig. 1. Colchicine-induced autotetraploid sudangrass plant and normal diploid sudangrass plant. Right: diploid. Left: autotetraploid.

Table 2. Comparison of the diameter of microsporocytes at diakinesis from autotetraploid and diploid sudangrass.

Plant designation	Haploid chromosomes	Average diameter of 100 microsporocytes	Increase
	number	microns	percent
69 2/10 D	10	24.65	
69 2/10 C	20	33.20	34.69
70 1/20 E	10	26.06	
70 2/10 B	20	34.94	34.08
57 2/10 B	10	27.56	
57 1/10 C	20	36.55	32.26

Table 3. Comparison of the diameter of pollen grains from autotetraploid and diploid sudangrass.

Plant designation	Haploid chromosomes	Average diameter of 100 pollen grains	Increase
	number	microns	percent
70 1/10 E	10	49.22	
70 2/10 B	20	58.52	18.90
69 1/10 C	10	40.75	
69 2/10 X	20	49.18	20.69
32 1/10 D	10	54.76	
32 1/10 D	20	69.47	26.86

The comparison of seed size is presented in Table 4. The first, second, and third comparisons were made between sister plants. In the fourth comparison the seed from the autotetraploid was compared with the seed from a diploid tiller of the same plant. In all cases the seeds from the autotetraploids were larger.

Table 4. Comparison of seed size in autotetraploid and diploid sudangrass.

Plant designation	Haploid chromosomes	Seeds used	Total weight	Weight per 100 seeds	Increase
	number	number	grams	grams	percent
70 1/10 X	10	375	5.38	1.43	
70 2/10 B	20	375	6.91	1.84	28.67
32 1/10 D	10	400	4.08	1.02	
32 1/10 D	20	400	5.25	1.31	28.43
69 1/20 D	10	200	2.69	1.35	
*69 2/10 X	20	190	3.21	1.69	25.18
69 2/10 X	10	100	1.34	1.34	
*69 2/10 X	20	190	3.21	1.69	26.12

*These data are from the same plant.

Table 5. Comparison of the fertility of autotetraploid and diploid sudangrass.

Plant designation	Haploid chromosomes	Fertile florets	Infertile florets	Total florets	Fertility
	number	number	number	number	percent
*32 1/10 D	10	592	162	754	78.51
32 1/10 D	20	424	527	951	44.58
*32 1/10 D	10	592	162	754	78.51
32 1/10 B	20	939	642	1581	59.39
69 1/10 C	10	197	97	294	67.00
69 1/20 D	10	586	69	655	89.46
69 2/10 X	20	157	61	218	72.02
70 1/10 X	10	516	53	569	90.68
70 2/10 B	20	405	740	1145	35.37

*These data from the same plant.

Table 5 gives the comparative fertility of the autotetraploids and their diploid counterparts. The first comparison is between tetraploid and diploid tillers of the same plant, whereas the remainder are between sister plants. All the diploids were more fertile. However, the differences between the autotetraploids and the diploids were not consistent.

The Hybridization of Sudangrass and the Johnsongrass x 4n Sudangrass Selections

Table 6 presents data evaluating the hybridization exhibited between the colchicine-induced autotetraploid sudangrass and the johnsongrass x 4n sudangrass selections. Certain attempted crosses failed to produce seed and have been omitted, the justifications for these omissions are hereby given. Crosses 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 15, 16, 18, 19, 22, 25, 44, 45, and 74 were attempted before the colchicine treated sudangrass was examined cytologically, and, since the sudangrass plant or tiller involved proved to be diploid, these crosses have been disregarded. Two crosses, 35 and 48, involving johnsongrass have been omitted because, for some undetermined reason, the strain of johnsongrass used proved to be almost completely sterile. The johnsongrass x 4n sudangrass selection, No. 4, proved to be highly sterile due to asynapsis or desynapsis; therefore, the two crosses, 31 and 39, involving this selection have been omitted. During the latter part of December, 1948 to the latter part of January, 1949, considerable sterility was encountered in the johnsongrass x 4n sudangrass selections, Nos. 70, 73, and 77. The cause was not determined, but the fact that some of the most highly sterile plants during this period gave a good seed set later indicated that some environmental factor was responsible. In so far as possible, plants showing sterility during this period were not used in the crosses, but in three crosses, 38, 42, and

46, no selfed seed was set in the panicles of the johnsongrass x 4n sudangrass plants used as the intended staminate parent. For this reason, it seems justifiable to omit these three crosses. It is questionable if cross 33 should be considered a 4n x 4n mating, since the tiller used in cross 34 was the only tetraploid tiller found in the sudangrass plant involved. However, since the tiller used in cross 33 was not examined cytologically, the cross will be included as a 4n x 4n mating. Of the 53 crosses considered in Table 6, 39 were made using the autotetraploid sudangrass lines as the pistillate parent, and the remaining 14 crosses were made using the johnsongrass x 4n sudangrass selections as the pistillate parent. Of the 39 cross-pollinations made using the autotetraploid sudangrass as the pistillate parent, 32 (82.05 percent) were successful, whereas only four (28.57 percent) of the reciprocal cross-pollinations proved successful.

Table 6. Frequency of hybridization between colchicine-induced autotetraploid sudangrass and (johnsongrass x 4n sudangrass).

Cross no.	Pistillate parent	Staminate parent	Florets emasculated	Seeds obtained
			number	
* 1	70 1/10 X	3C	18	1
* 2	69 2/10 X	3E	37	18
*11	69 2/10 C	51K	25	8
*14	69 2/10 C	3E	22	0
*17	69 2/10 X	3E	21	19
*20	70 2/10 B	3C	27	4
*21	70 2/10 B	3C	24	22
*23	69 2/10 X	2E	18	0
*24	69 2/10 C	3F	25	1
*26	70 2/10 B	51D	24	6
*27	70 2/10 B	2E	19	0
*28	69 2/10 C	70A	26	0
*29	32 1/10 B	3C	28	19
*30	69 2/10 C	51K	12	3
*32	70 2/10 B	73C	17	9
*33	57 1/10 C	3C	15	0

Table 6 (concl.).

Cross no.	Pistillate parent	Staminate parent	Florets emasculated	Seeds obtained	
			number		
*34	57 1/10 C	51I	16	7	
*36	70 2/10 B	2E	18	11	
*37	32 1/10 B	51K	17	4	
*40	69 2/10 X	51K	20	0	
**41	51B	70 2/10 B	24	0	
*43	32 1/10 B	2E	35	5	
*47	32 1/10 B	77A	32	18	
*49	69 2/10 X	70H	16	7	
*50	69 2/10 X	70D	29	13	
*51	32 1/10 B	2E	33	16	
*52	70 2/10 B	70F	25	6	
*53	70 2/10 B	77H	24	13	
*54	69 2/10 X	73A	20	1	
*55	32 1/10 B	73J	27	20	
**56	2E	70 2/10 B	25	0	
*57	32 1/10 B	70L	24	7	
*58	69 2/10 X	51I	29	12	
**59	77A	32 1/10 B	24	0	
*60	69 2/10 X	73G	26	16	
**61	2E	70 2/10 B	27	0	
*62	70 2/10 B	70G	29	0	
**63	51K	32 1/10 B	19	0	
**64	77E	32 1/10 B	36	0	
**65	77G	70 2/10 B	28	1	
*66	69 2/10 X	2E	19	13	
*67	69 2/10 X	77A	25	14	
**68	77B	32 1/10 B	29	0	
**69	73J	32 1/10 B	24	0	
**70	2F	32 1/10 B	26	10	
*71	32 1/10 D	3A	34	14	
*72	32 1/10 D	51K	31	11	
**73	70D	69 2/10 X	29	1	
*75	32 1/10 D	70E	28	21	
**76	70B	32 1/10 B	27	0	
**77	77K	69 2/10 X	28	0	
*78	32 1/10 D	2H	28	11	
**79	77H	32 1/10 B	19	3	
*Total			943	350	37.12 percent fertility
**Total			365	15	04.11 percent fertility
Total for all crosses			1308	365	27.91 percent fertility

*Autotetraploid sudangrass used as pistillate parent.
**Johnsongrass x 4n sudangrass selections used as pistillate parent.

Results of another group of attempted crosses are given in Table 7. The sudangrass plants involved in this case represent a group of plants treated with colchicine by soaking the seed in an aqueous solution. These crosses were all attempted before any of the treated plants were examined cytologically. Although cytological studies showed none of the sudangrass to be doubled in chromosome number, some interesting data were obtained concerning the hybridization of tetraploid and diploid sorghums. None of these crosses resulted in mature seed, but 12 of these crosses produced aborted seed which indicated that fertilization had taken place. This phenomenon was not observed until some time after the early crosses in the group of crosses presented in Table 6 were made, but no doubt it occurred in some of those crosses in which diploid sudangrass was used. Later this phenomenon was also observed in three 2n x 4n matings in this group of crosses; these three crosses are also included in Table 7. In all the 2n x 4n matings the development of the seed appeared to progress normally until approximately the fifteenth day after pollination, but shortly thereafter the young seed began to show shriveling and discoloration, a condition which continued until the seed was entirely shriveled and discolored. Figure 2 shows the contrast between seed produced from a 4n x 4n mating and a 2n x 4n mating. In these two matings the parent plants were identical, but the group of well developed seeds at the left (cross 78, Table 6) was borne on a tetraploid tiller, whereas the group of aborted seeds at the right (cross 74, Table 7) was borne on a diploid tiller of the same plant. The one aborted seed occurring in the 4n x 4n mating may be assumed to be the result of a fertilization involving one, or perhaps two, aneuploid gametes.

Table 7. Frequency of fertilization between sudangrass and (johnsongrass x 4n sudangrass).[1]

Cross no.	Pistillate parent	Staminate parent	Florets emasculated	Aborted seed obtained
	n = 10	n = 20	number	
1	27 1/10 B	73A	22	0
2	65 2/10 B	73B	15	0
3	31 2/10 A	70K	18	9
4	28 3/10 A	73D	23	1
5	27 1/10 A	77F	22	0
6	24 3/10 A	73J	18	10
7	27 2/10 F	73E	20	0
8	28 3/10 C	73C	17	14
9	31 2/10 B	73D	19	0
10	22 3/10 A	77J	21	10
11	27 3/10 C	70E	22	5
12	31 2/10 E	77A	21	0
13	24 3/10 B	70B	20	0
14	28 2/10 D	70A	22	0
15	28 1/10 C	73O	20	10
16	24 3/10 C	77I	19	12
17	24 2/10 E	70C	17	0
18	31 2/10 D	70B	12	8
19	65 2/10 A	77G	20	15
20	27 3/10 B	73L	21	0
21	24 2/10 C	73G	23	20
22	27 2/10 D	70H	22	0
23	65 2/10 C	70D	24	7
*44	57 1/10 C	2F	28	9
*45	70 1/10 X	77C	31	12
*74	32 1/10 D	2H	26	12
Total			543	154
Percent of fertilization				28.36

[1] Diploid sudangrass used as pistillate parent in all cases.
*These matings are from the group of crosses presented in Table 6.

Fig. 2. Seed from 4n x 4n (left) and 2n x 4n (right) sorghum matings. The same plants were used as parents in both matings. The well formed seed to the left is from an autotetraploid tiller of the pistillate parent. The aborted seed to the right is from a diploid tiller of the pistillate parent. x 3.

Cytological Studies

Meiotic chromosome studies were made of the diploid sudangrass, the autotetraploid sudangrass, the johnsongrass x 4n sudangrass selections, and the F_1 plants of crosses 1 and 17 in Table 6.

All the microsporocytes of the diploid sudangrass studied showed normal meiosis. The chromosomes paired normally as bivalents, and in no case was there even a suggestion of a multivalent association of any of the chromosomes (Fig. 3).

The forty chromosomes of the autotetraploid sudangrass usually synapsed in some combination of bivalents and quadrivalents. Univalents and trivalents were observed occasionally, but in no case were 20 bivalents found. In the bivalent and quadrivalent combinations 14_{II} and 3_{IV}, 12_{II} and 4_{IV}, and 10_{II} and 5_{IV} were found. The 12_{II} and 4_{IV} association was found to predominate (Fig. 8, Plate I). Observations revealed the fact that an equal distribution of chromosomes at anaphase I was the most common occurrence, but an unequal distribution was not uncommon. Figure 4 shows an even distribution of the 40 chromosomes at anaphase I. Figure 9, Plate I shows a lagging chromosome at late anaphase I; the lagging chromosome would doubtless be lost in such cases, giving an unequal distribution of 19 and 20 chromosomes. Another type of unequal distribution is shown in a microsporocyte at metaphase II (Fig. 10, Plate I). In this instance the distribution is 19 and 21, which undoubtedly is the result of an extra chromosome migrating to one pole at anaphase I, leaving the group that migrated to the other pole short one chromosome.

The chromosomes of the johnsongrass x 4n sudangrass selections usually associated in some sort of a bivalent and quadrivalent combination (Fig. 11,

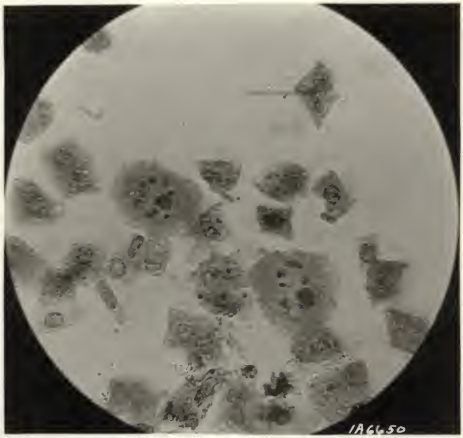

Fig. 3. Microsporocyte of diploid sudangrass showing ten bivalents. x 600.

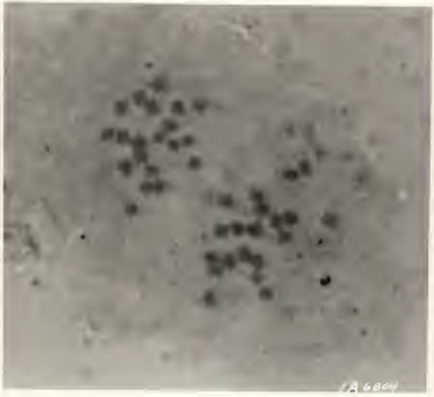

Fig. 4. Microsporocyte of colchicine-induced auto-tetraploid sudangrass at anaphase I, showing an even distribution of the 40 chromosomes. x 2100.

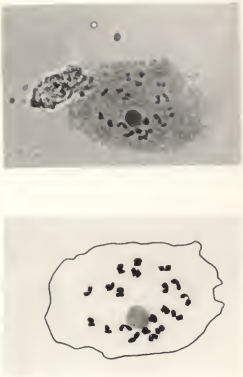

Fig. 5. Microsporocyte at diakinesis from a tetraploid sorghum (Sorghum halepense x autotetraploid S. vulgare var. sudanense), showing 20 bivalents. Photomicrograph x 690. Camera lucida drawing x 870.

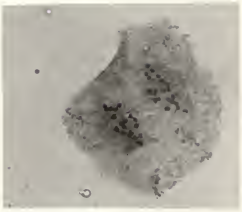

Fig. 6. Microsporocyte of a tetraploid sorghum (Sorghum halepense x S. vulgare var. sudanense) at anaphase I, showing even distribution of the chromosomes. Photomicrograph x 680. Camera lucida drawing x 910.

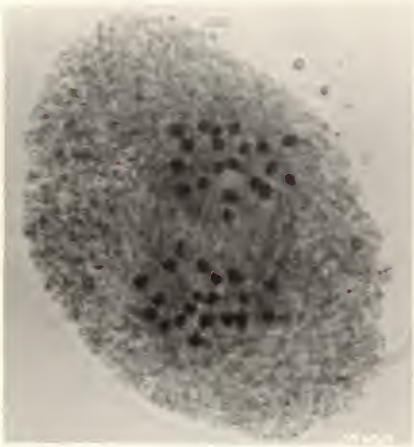

Fig. 7. Microsporocyte at anaphase I from an F_1 plant from the cross, autotetraploid sudangrass x (johnsongrass x 4n sudangrass), showing an equal distribution of the 40 chromosomes. x 2100.

Fig. 8

Fig. 9

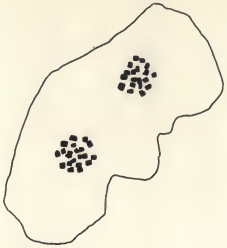

Fig. 10

Fig. 11

PLATE I

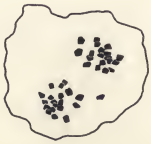

Fig. 12

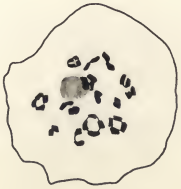

Fig. 13

Fig. 14

Plate I), but 20 bivalents at late diakinesis were found occasionally (Fig. 5). These selections gave a very even distribution of chromosomes (Fig. 6). In fact no unequal distribution was encountered in the cytological studies; however, it is evident that such do occur, since plant 73L was found to have only 39 chromosomes (Fig. 12, Plate I).

In the observations made of the F_1 plants, it was found that the chromosomes in all the cells studied associated in some combination of bivalents and quadrivalents (Fig. 13, Plate I). If univalents and trivalents occurred, they were not observed. The distribution of the chromosomes appeared to be equal (Fig. 7). It was revealed in the studies of the F_1 plants that at least some of the cross-fertilized seed produced had aneuploid gametes involved (Fig. 14, Plate I).

Inheritance of Certain Characters in F_1 and F_2 Progenies

F_1 Progenies. The F_1 progenies of the crosses 1, 17, 20 & 21 (considered as one cross, since their parentage was identical in all respects), and 30 (Table 6) were grown in the greenhouse and studied for certain characters. In general appearance all the F_1 plants resembled the johnsongrass x 4n sudangrass parent somewhat more closely than they did the autotetraploid sudangrass parent. However, in all cases the leaves were noticeably wider than the johnsongrass x 4n sudangrass parent, and the characteristics of the panicle could be considered intermediate to the two parents. It was also observed that the F_1 plants all produced rhizomes, as did the johnsongrass x 4n sudangrass parents. In no case were the rhizomes so numerous nor so vigorous as those produced by the parent, but they were capable of producing new aerial stems. As for the two characters studied specifically, all the F_1 plants were dry-stalked and had the purple plant color. This was as expected, since both of

these characters are dominant; furthermore, this proved the authenticity of these four crosses, since the dry-stalked, blackish purple johnsongrass x 4n sudangrass was used as the staminate parent.

The F_1 plants showed good fertility under greenhouse conditions. The fertility of the 16 euploid F_1 plants obtained from cross 17 was studied in detail. From 4,285 florets counted, 3,567 well matured kernels were obtained, giving a fertility percentage of 83.29. This was slightly higher than the 80.27 fertility percentage observed for the johnsongrass x 4n sudangrass parent under the same conditions. The fertility of these F_1 plants was found to compare rather favorably with the 85.45 fertility percentage observed in heads taken at random from six diploid sudangrass plants.

F_2 Progenies. The F_2 progeny of cross 1 was grown in the greenhouse at Manhattan, Kansas. Crosses 17, 20 & 21, and 30 were grown in the field at Hays, Kansas. In general appearance, the F_2 plants ranged from those types which resembled the autotetraploid sudangrass parent to those types which resembled the johnsongrass x 4n sudangrass parent.

The F_2 plants grown in the greenhouse were not examined for the production of rhizomes. The F_2 plants grown in the field were examined rather closely for rhizome formation. Rather well developed rhizomes were observed in the johnsongrass x 4n sudangrass parental stock grown in the field. Nearly all the F_2 plants also produced rhizomes, but there were a few plants which apparently failed to produce rhizomes. The rhizomes produced by the F_2 plants ranged from those that were fairly well developed to those that were very short and stubby. In no case did the size and the number of the rhizomes produced by the F_2 plants equal those produced by their johnsongrass x 4n sudangrass parent. A number of the rhizomes, both the fairly well developed type and the short, stubby type, were removed from the F_2 plants and planted

in clay pots; they all proved perfectly capable of perpetuating the life of the plant from which they originated.

In general, the fertility of the F_2 progenies was good both in the greenhouse and the field. Certain plants, however, exhibited fertility considerably below normal, while a few plants were almost completely sterile. The cause of this partial to almost complete sterility was not determined.

The two characters, dry vs. juicy-stalk and purple plant color vs. brown plant color, were studied for their mode of inheritance in the F_2 progenies. Since either or both of these pairs of characters could have followed either random chromosome segregation or random chromatid segregation, chi-squares were calculated for both possibilities. Tables 8 and 9, respectively, present the calculations assuming random chromosome segregation and random chromatid segregation for the dry vs. juicy-stalk character. Table 10 gives the random chromosome segregation calculations for the plant color characters, and Table 11 gives the random chromatid segregation calculations for these characters. The chi-square calculations for the double coupling phase of the 30 percent linkage between the dry vs. juicy-stalk gene and the P gene for plant color are given in Table 12. These linkage calculations were made on the assumption that both pairs of characters, when considered independently, followed random chromosome segregation.

Table 8. Chi-square calculations for the segregation of the dry vs. juicy-stalk character in the F_2 progenies of autotetraploid sudangrass x (johnsongrass x 4n sudangrass) crosses, assuming a random chromosome segregation (35:1).

Cross no.	Total plants	Dry		Juicy		Chi-squares	Approx. P-values
		Observed	Calculated	Observed	Calculated		
1	210	205	204.17	5	5.83	0.121	0.73
17	273	263	265.42	10	7.58	0.795	0.39
20 & 21	372	359	361.67	13	10.33	0.710	0.42
30	371	360	360.70	11	10.30	0.049	0.83
Sum of 4 chi-squares						1.675	0.80
Total	1226	1187	1191.94	39	34.06	0.736	0.41
Interaction chi-square						0.939	0.82

Table 9. Chi-square calculations for the segregation of the dry vs. juicy-stalk character in the F_2 progenies of autotetraploid sudangrass x (johnsongrass x 4n sudangrass) crosses, assuming a random chromatid segregation (20.8:1).

Cross no.	Total plants	Dry		Juicy		Chi-squares	Approx. P-values
		Observed	Calculated	Observed	Calculated		
1	210	205	200.37	5	9.63	2.333	0.13
17	273	263	260.48	10	12.52	0.531	0.48
20 & 21	372	359	354.94	13	17.06	1.012	0.32
30	371	360	353.98	11	17.02	2.231	0.14
Sum of 4 chi-squares						6.107	0.19
Total	1226	1187	1169.76	39	56.24	5.539	0.019
Interaction chi-square						0.568	0.90

Table 10. Chi-square calculations for the segregation of the purple vs. brown plant color character in the F_2 progenies of autotetraploid sudangrass x (johnsongrass x 4n sudangrass) crosses, assuming random chromosome segregation (35:1).

Cross no.	Total plants	Purple		Brown		Chi-squares	Approx. P-values
		Observed	Calculated	Observed	Calculated		
1	210	204	204.17	6	5.83	0.005	0.95
17	273	262	265.42	11	7.58	1.587	0.21
20 & 21	372	364	361.67	8	10.33	0.541	0.47
30	371	362	360.70	9	10.30	0.169	0.69
Sum of 4 chi-squares						2.302	0.68
Total	1226	1192	1191.94	34	34.06	0.0001	0.98+
Interaction chi-square						2.3019	0.51

Table 11. Chi-square calculations for the segregation of the purple vs. brown plant color character in the F_2 progenies of autotetraploid sudangrass x (johnsongrass x 4n sudangrass) crosses, assuming random chromatid segregation (20.8:1).

Cross no.	Total plants	Purple		Brown		Chi-squares	Approx. P-values
		Observed	Calculated	Observed	Calculated		
1	210	204	200.37	6	9.63	1.434	0.24
17	273	262	260.48	11	12.52	0.194	0.67
20 & 21	372	364	354.94	8	17.06	5.042	0.03
30	371	362	353.98	9	17.02	3.961	0.05
Sum of 4 chi-squares						10.631	0.034
Total	1226	1192	1169.76	34	56.24	9.218	< 0.01
Interaction chi-square						1.413	0.70

Table 12. Chi-square calculations for the linkage of the dry vs. juicy-stalk character and the purple vs. brown plant color in the F_2 progenies of autotetraploid sudangrass x (johnsongrass x 4n sudangrass) crosses, assuming the linkage to be 30 percent.[1]

Cross no.	Total plants	Dry				Juicy				Chi-squares	Approx. P-values
		Purple		Brown		Purple		Brown			
		Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated		
1	210	201	199.73	4	4.43	3	4.43	2	1.40	0.769	0.86
17	273	257	259.65	6	5.76	5	5.76	5	1.82	5.693	0.13
20 & 21	372	335	353.81	4	7.85	9	7.85	4	2.48	2.992	0.40
30	371	354	352.86	6	7.83	8	7.83	3	2.47	0.551	0.91
Sum of 12 chi-squares										10.005	0.60
Total	1226	1167	1166.06	20	25.88	25	25.88	14	8.17	5.527	0.16
Interaction chi-square										4.478	0.87

[1] These calculations were made on the assumption that the two pairs of characters considered separately fit a random chromosome segregation.

DISCUSSION

The Characteristics of the Autotetraploid Sudangrass

The observations made of the autotetraploid and the diploid sudangrass plants indicated that the general appearance of the plants would furnish little aid in detecting the autotetraploids. Although the autotetraploids appeared somewhat shorter and stockier and had somewhat wider and longer leaves than their diploid counterparts, these differences were so slight that the autotetraploids could have been easily mistaken for a deviation from type in a normal diploid population.

As observed in the greenhouse, depth of green color and later maturity were of no aid in distinguishing the autotetraploid sudangrass plants, but a final evaluation of these characteristics is probably not justified, considering the conditions under which all the plants grew. It is believed that both of these characteristics might have been expressed in the autotetraploids had the plants been grown under those environmental conditions considered to be optimum for sorghum culture. This probably would be especially true for the depth of green color, inasmuch as it was noted that all the plants were considerably below normal in the depth of green color, despite an attempt to supply the required nutrients in the form of commercial fertilizer.

Certain other characteristics exhibited by the autotetraploid plants appeared to be of value as preliminary criteria in distinguishing the diploid sudangrass plants from their autotetraploid derivatives. In all cases studied the autotetraploids had larger stomata, pollen grains, microsporocytes, and mature seeds. However, observations made in these respects indicated that the comparisons to be of value should be made between autotetraploid and diploid plants of very close relationship, since it was found that the diploids

of one line of sudangrass may have larger stomata, pollen grains, microsporocytes, and mature seed than the autotetraploids of another line (Tables 1, 2, 3, and 4).

It is questionable if fertility comparisons can be used too reliably in distinguishing autotetraploid sudangrass plants. It has been found that the fertility of artificially induced autotetraploids may range from almost complete sterility to almost complete fertility. On the other hand, the entire process of sexual reproduction is considered to be one of the most critical periods in the life history of any plant, and it is dependent on a large number of genetic and environmental factors, all of which must be favorable, if seed set is to be at a maximum. It is quite conceivable, then, that a rather infertile diploid might be mistaken for an autotetraploid, or a rather fertile autotetraploid for a diploid. An instance in which a fertility comparison could possibly have given an erroneous indication occurred in greenhouse No. 69. Plant 69 1/10 C, a diploid, had a fertility of 67.00 percent, whereas plant 69 2/10 X, an autotetraploid, had a fertility of 72.02 percent (Table 5). Nevertheless, the results of the observations made would seem to indicate that fertility comparisons should not be overlooked as a preliminary means of detecting autotetraploid sudangrass plants.

The reason for the different levels of fertility found in the autotetraploid plants was not determined. The difference in the rate of aneuploid gamete production cannot be offered as an explanation, because cytological studies showed the frequency of unequal chromosome distributions to be approximately the same in all the autotetraploid plants. The difference in the fertility displayed by the two sister plants, 32 1/10 B and 32 1/10 D, can be explained on the assumption that 32 1/10 B, since it did not conform to the characters of the sudangrass line from which it originated, was the result of an outcross

and was, therefore, relatively heterozygous. On the other hand, 32 1/10 D was probably relatively homozygous, since it conformed very closely to the sudangrass line from which it originated. Heterozygosity does not offer an explanation for the relatively high degree of fertility exhibited by the plant 69 2/10 X. In the first place, its characteristics showed it to be a true representative of the sudangrass line to which it belonged. Secondly, plants grown at Hays, Kansas from seed produced by this plant were very uniform, indicating that 69 2/10 X was relatively homozygous.

The Hybridization of the Ten- and Twenty-chromosome Sorghums

The results of the hybridization experiments showed no difficulty in obtaining cross-fertilized seed from matings involving the johnsongrass x 4n sudangrass selections and autotetraploid sudangrass. This was especially true when the autotetraploid sudangrass was used as the pistillate parent, in view of the fact that 82.05 percent of the matings and 37.12 percent of the florets emasculated produced mature seed. However, when the johnsongrass x 4n sudangrass selections were used as the pistillate parents, the frequency of hybridization was considerably lower, evidenced by the fact that only 28.57 percent of the matings and 4.11 percent of the total number of florets produced mature seed.

Of the 26 johnsongrass x 4n sudangrass and diploid sudangrass matings observed, 15 produced aborted seed. Of the total number of florets emasculated in these matings, 28.36 percent produced aborted seed, which indicated that fertilization took place at least 28.36 percent of the time. Therefore, it is evident that it was not the failure of fertilization, but rather a failure of post-fertilization processes that prevented hybridization in these 2n and 4n sorghum matings. These results may also throw some light on the failure

of Sorghum halepense and S. vulgare to hybridize freely. The original cross, from which the johnsongrass x 4n sudangrass selections used in these studies originated, revealed the fact that the difference in chromosome number accounted for at least a part of the difficulty encountered in attempts to hybridize these two Sorghum species, but it was not revealed whether the difficulty was due to pre- or post-fertilization processes. It is probable that the failure of post-fertilization processes due to the difference in chromosome numbers serves as a barrier to hybridization between S. halepense and S. vulgare. However, the results presented in Table 6 of the crosses in which the johnsongrass x 4n sudangrass served as the pistillate parents indicate that some other factor may be involved when S. halepense is pollinated by S. vulgare. This also finds support in the fact that Winall (46) was successful in hybridizing S. halepense and S. vulgare only when S. vulgare was used as the pistillate parent. No attempt was made to determine the cause of the poor results obtained when the johnsongrass x 4n sudangrass selections were used as the pistillate parents, but it is suggested that some mechanical difficulty, such as the length of stigma in relation to pollen tube growth, or some physiological incompatability between the tissues of the stigma and pollen tube as a result of the johnsongrass parentage was responsible.

Cytological Observations

The cytological studies of the autotetraploid sudangrass plants showed that the behavior of their chromosomes at meiosis was typical of autotetraploids, giving univalents, bivalents, trivalents, and quadrivalents at prophase I. Likewise, cytological studies of the johnsongrass x 4n sudangrass selections and the F_1 plants of crosses 1 and 17 showed chromosome behavior typical of autotetraploids. In fact, the meiotic chromosome behavior of the

johnsongrass x 4n sudangrass selections and the F_1 plants appeared to be similar to the behavior displayed by the autotetraploids, with the exception that the autotetraploids appeared to give univalent, trivalents, and unequal distributions of chromosomes more frequently. Also previous investigations have shown the meiotic chromosome behavior of Sorghum halepense to be similar to that of colchicine-induced autotetraploids of S. vulgare. If, then, the presence of quadrivalents can be taken as a measure of autotetraploidy, it would seem that S. halepense is as much an autotetraploid as S. vulgare in which the chromosome number has been doubled by the use of colchicine.

Further evidence that S. halepense is an autotetraploid was furnished by the regular behavior of the chromosomes observed in the F_1 progeny of S. halepense and autotetraploid S. vulgare var. sudanense matings (32). Had S. halepense been an allotetraploid a certain amount of irregularity would have occurred. Although the regularity of the chromosomes in these F_1 plants indicated the autotetraploidy of S. halepense, it did not reveal whether or not S. halepense resulted from the doubling of chromosomes in some variety of S. vulgare. In other words, it did not show that the chromosomes of the two genomes furnished by the S. halepense parent were capable of synapsing with the chromosomes of the two genomes furnished by the autotetraploid S. vulgare var. sudanense parent. There were two possible ways in which the chromosomes of the F_1 plants could have synapsed, neither of which would discount the hypothesis that S. halepense is an autotetraploid. Either the four genomes could have synapsed in a typical autotetraploid fashion, HHVV, or they could have synapsed in a typical amphidiploid fashion, HH and VV.[1] It was fairly evident from the cytological observations made of the johnsongrass x 4n sudangrass selections

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[1] H = genome from S. halepense. V = genome from S. vulgare.

used in these studies that certain chromosomes of the two halepense genomes and the two vulgare genomes are homologous, since quadrivalents were regularly formed, but these observations did not exclude the possibility that some, if not all, of the remaining bivalents were pairing in a typical amphidiploid fashion. However, the behavior of the chromosomes at diakinesis in the F_1 progenies of crosses 1 and 17 showed that all the halepense chromosomes are capable of synapsing with vulgare chromosomes. If any chromosome of the johnsongrass x 4n sudangrass selections had been pairing in typical amphidiploid fashion to give A_hA_h and A_vA_v, these parents would have produced only A_h-A_v gametes. Therefore, since the autotetraploid sudangrass parent produced A_v-A_v gametes, the F_1 plants would have been of the constitution A_h-A_v-A_v-A_v, and at prophase I the probable association of chromosomes would have been A_h and $A_vA_vA_v$. Cytological studies of these F_1 plants certainly would have permitted ready detection of the regular prevalence of these univalents and trivalents, but no univalents or trivalents were observed in these plants, although there is little doubt they did occur occasionally. If, then, the homology of chromosomes, as evidenced by their ability to synapse, can be used to establish the relationship of two species of plants, it would appear evident that S. halepense is the result of the doubling of the chromosome number in some variety of S. vulgare. If such a hypothesis is accepted, the only phase of the evolution of S. halepense left unexplained is the manner in which the production of rhizomes came into being. This hypothesis does not fully explain the evolution of S. halepense because there is apparently no variety of S. vulgare which produces rhizomes, nor has it been found that the mere doubling of the chromosome number in S. vulgare brings about the rhizomatous habit of growth. The cytological evidence being such as it is, it does not seem illogical to postulate that S. halepense is actually an autotetraploid

of some variety of S. vulgare, and that certain of the extra sets of genes, no longer needed for the functions normal to the plant, have changed their functions to bring about a new function in the plant, namely, the production of rhizomes.

The regular chromosome behavior of the F_1 progenies of crosses 1 and 17 indicated that the johnsongrass x 4n sudangrass and autotetraploid sudangrass matings, in respect to the breeding problem involved, should give reasonably satisfactory results.

Characters of the F_1 and F_2 Progenies

F_1 Progenies. The general appearance of the F_1 progenies of crosses 1, 17, 20 & 21, and 30 was intermediate between the two parental types. This was as might be expected, since the various characters, such as length, width, size, shape, etc., that together make up the general appearance of an organism are usually considered to be inherited quantitatively. The rhizome development in these F_1 progenies also could be considered to be intermediate. All the F_1 plants produced rhizomes, but all the rhizomes were considerably smaller and less vigorous than those produced by their johnsongrass x 4n sudangrass parents. It was not surprising that rhizome production appeared to be intermediate in the F_1 plants, because it had been previously suggested that the production of rhizomes in Sorghum halepense is controlled by several factors.

All the F_1 plants of the crosses observed were dry-stalked and had the purple plant and glume color. This was to be expected because both these characters show complete dominance in diploid sorghums.

The relatively high fertility of the F_1 progeny of cross 17 agreed with the regular behavior of the chromosomes observed in these plants.

F_2 Progenies. The general appearance of the F_2 progenies, with a range

from those plants that closely resembled the johnsongrass x 4n sudangrass parents to those that closely resembled the autotetraploid sudangrass parents, conformed to that expected in cases of quantitative inheritance.

The observations made of the rhizome development in the F_2 progenies of crosses 17, 20 & 21, and 30 again suggested that the rhizome development was a multiple factor inheritance. There were a few plants found in the F_2 population which had no underground development at the time examined that was suggestive of rhizomes. However, nearly all of the plants had some sort of underground growth which suggested rhizomes, but the size and appearance of these growths presented considerable range. Some were very short and stubby, whereas others were relatively large and extended some distance from the central axis of the plant, but in no case would any of them compare in size with those produced by the johnsongrass x 4n sudangrass parent. This range in size and vigor of the rhizomes produced by the F_2 plants would seem to suggest a multiple factor inheritance.

In general, the fertility of the F_2 progenies was good. There were plants, however, that showed considerable sterility, and some were almost completely sterile. No attempt was made to determine the cause of this sterility, but it is not improbable that these plants represented varying degrees of aneuploidy.

The results obtained from the segregating F_2 progenies of crosses 1, 17, 20 & 21, and 30 indicated a random chromosome segregation for the dry vs. juicy-stalk character. A review of Table 8 will show that the P-values for the individual F_2 progenies, assuming a 35:1 segregation, indicated a very close fit to the expected. Table 9 shows, however, that none of the chi-squares for the individual crosses, assuming a 20.8:1 segregation, are significant; in fact, it is indicated that they all fit the expected well, but, with the exception of cross 17, none of the crosses fit the 20.8:1 hypothesis

so well as they did the 35:1 hypothesis. Likewise the sum of the chi-squares in both cases is not significant, but the larger P-value for the 35:1 hypothesis indicated a much closer fit. The critical chi-square in both cases is the total chi-square. In the case of the 35:1 hypothesis, a very close fit to the expected was denoted by P = 0.41, whereas in the case of the 20.8:1 hypothesis, the P-value of 0.019 indicated a highly significant chi-square. The value of the total chi-square for each hypothesis is supported by the low interaction chi-square obtained in each case (37). The fact that the individual chi-squares and the sum of the chi-squares were not significant under the 20.8:1 hypothesis probably indicates that the segregation was intermediate between random chromosome and random chromatid segregation, but, being somewhat closer to 35:1 than 20.8:1.

In the case of the purple vs. brown plant color, the segregation of the F_2 progenies apparently fit a random chromosome segregation very closely. All the chi-squares for this type of segregation (Table 10) indicated a very close fit to the expected. In the case of chi-square calculations for the 20.8:1 (Table 11), two of the individual chi-squares and the sum of chi-squares were significant, and the total chi-square was highly significant. The value of the latter chi-square was confirmed by a low interaction chi-square.

The chi-square calculations (Table 12) for the linkage of the dry vs. juicy-stalk gene (D) and the purple vs. brown plant color gene (P) reasonably confirmed that these genes were linked 30 percent in these autotetraploid sorghums as they are in diploid sorghums.

The respective segregations observed for the D and P genes together with their 30 percent linkage would seem to give some indication of the relative position of these two genes in the chromosome they both occupy. Since these

two genes are seperated by 30 crossover units, it is obvious that, if they are in the same relative position in respect to the kinetochore, one of them must necessarily be at least 30 crossover units from the kinetochore. If such were the case, the one 30 crossover units from the kinetochore should give a somewhat closer fit to a random chromatid segregation than a random chromosome segregation, since it would be by 5 crossover units closer to the point where full random chromatid segregation comes into play (50 crossover units from the kinetochore). Although the segregation for the D gene suggested a type of segregation intermediate to 35:1 and 20.8:1, neither gene showed a segregation closer to random chromatid segregation than random chromosome segregation. It would seem, then, that neither of the two genes could be 30 crossover units from the kinetochore. A possible explanation offered is that the two genes lie in opposite arms of the chromosomes, the dry vs. juicy-stalk gene (D) being somewhat less than 30 crossover units from the kinetochore in one arm, and the purple vs. brown plant color gene (P) being somewhat more than 5 crossover units from the kinetochore in the other arm. It is realized that the validity of such a postulation is certainly dependent on a great deal more research.

SUMMARY

1. In general appearance, the autotetraploid sudangrass plants were similar to their diploid counterparts, but were shorter and stockier and had somewhat longer and wider leaves.

2. The autotetraploid sudangrass plants had larger stomata, pollen grains, microsporocytes, and mature seed than the diploids. It appears that the larger size of these plant parts may be used as preliminary criteria in detecting autotetraploid sudangrass plants, but to be reliable the comparisons should be made between diploids and autotetraploids of very close relationship.

3. Except for one instance, the autotetraploid sudangrass plants were less fertile than the diploid plants.

4. No difficulty was encountered in obtaining cross-fertilized seed from the matings between the johnsongrass x 4n sudangrass selections (n = 20) and the autotetraploid sudangrass (n = 20). The frequency of hybridization was considerably higher when the autotetraploid sudangrass served as the pistillate parent.

5. No mature cross-fertilized seed was obtained from the johnsongrass x 4n sudangrass and diploid sudangrass matings, but the number of aborted seed obtained indicated that fertilization took place at least 28.36 percent of the time. These results showed that the failure of hybridization in these 2n x 4n sorghum matings was not due to the failure of fertilization, but rather to a failure of post-fertilization processes as a result of the difference in chromosome number.

6. The results of the hybridization studies made of the johnsongrass x 4n sudangrass and diploid sudangrass matings may be applicable to the failure of hybridization between Sorghum halepense and diploid S. vulgare. However, the fact that the frequency of hybridization was considerably lower in johnsongrass x 4n sudangrass and autotetraploid sudangrass matings, when the johnsongrass x 4n sudangrass served as the pistillate parent, indicated that some factor other than the difference in chromosome number may be involved.

7. The meiotic chromosome behavior of the autotetraploid sudangrass was typical of autotetraploids.

8. Quadrivalents and bivalents were usually present at diakinesis in the johnsongrass x 4n sudangrass selections, but occasionally only bivalents were found. Trivalents and univalents appeared to be rare in these selections.

9. Cytological studies of the F_1 progenies of two johnsongrass x 4n

sudangrass and autotetraploid sudangrass matings revealed a combination of bivalents and quadrivalents. No univalents or trivalents were observed. The distribution of the chromosomes appeared to be equal.

10. The cytological observations reviewed above, together with previous investigations of the chromosome behavior of Sorghum halepense, autotetraploid S. vulgare var. hegari, and the F_1 progeny of a S. halepense and autotetraploid S. vulgare var. sudanense mating, advanced the hypothesis that S. halepense is probably an autotetraploid of some variety of S. vulgare.

11. The observations made of rhizome development in the F_1 and F_2 progenies of johnsongrass x 4n sudangrass and autotetraploid sudangrass matings further indicated that rhizome development in Sorghum is controlled by multiple factors.

12. In respect to the dry vs. juicy-stalk character (D vs. d), the F_2 progenies of crosses 1, 17, 20 & 21, and 30 gave a segregation that appeared to be intermediate to random chromosome and random chromatid segregation. However, the ratio obtained appeared to be somewhat closer to 35:1 than 20.8:1.

13. The F_2 progenies of crosses 1, 17, 20 & 21, and 30 apparently segregated very closely to random chromosome segregation for the purple vs. brown plant color (P vs. p).

14. It appeared from the observations made of the F_2 progenies of crosses 1, 17, 20 & 21, and 30 that the D and P genes were linked 30 percent in these 4n sorghums as they are in Sorghum vulgare.

15. A final analysis of the facts presented in (12), (13), and (14) above suggested the possibility that the D gene is somewhat less than 25 crossover units from the kinetochore in one arm and that the P gene is somewhat more than 5 crossover units from the kinetochore in the other arm of the same chromosome.

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