CYTOLOGICAL AND MORPHOLOGICAL OBSERVATIONS IN CROSSES BETWEEN DIPLOID AND TETRAPLOID SORGHUM

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PREFACE

The binomial nomenclature of Johnsongrass is Sorghum halepense (L.) Pers. The morphological descriptions of S. halepense found in the literature are not consistent. This inconsistency is illustrated by the large amounts of variation observed in S. halepense along the highways of Oklahoma. This and the fact that crossing between S. halepense and other sorghums does occur has prompted this author to consider S. halepense as found in Oklahoma, a product of introgressive hybridization. For this reason, the material studied here will be referred to as Johnsongrass instead of S. halepense.

Throughout the course of this study the term diploid will be used to indicate the somatic (2n) chromosome complement of a plant, pollen grain, or microsporocyte; thus tetraploid will mean 4n or two times the somatic chromosome number. The term polyploid will mean chromosome numbers greater than the somatic chromosome complement.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDIX TABLES

CHAPTER I

INTRODUCTION

One characteristic that is widely used to study species relationships is the chromosome. An understanding of chromosomal characteristics such as meiotic behavior and degree of ploidy seems necessary for a clear knowledge of species relationships and their evolutionary divergence. These chromosomal characteristics have been studied in several sorghum species and their hybrids.

Another aspect of the study of species relationships is the nature or cause of failure of seed development in interspecific crosses. Interspecific crosses in Sorghum do result in frequent failure to form viable caryopses (McClure)[1].

This investigation was concerned with: 1) chromosomal associations of the interspecific hybrids of diploid (Sorghum vulgare Pers.) X tetraploid (Johnsongrass and Sorghum almum Parodi) sorghums and the influence of the parents upon the chromosomal associations; and 2) the embryogenesis of the non-viable caryopses resulting from interspecific crossing.

In addition, the possibility of using pollen grain size as an indication of chromosome number was investigated.

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[1]McClure, Walter Jay. 1962. Frequency of interspecific crossing between Sorghum vulgare Pers. and Sorghum halepense (L.) Pers., and between Sorghum vulgare Pers. and Sorghum almum Parodi. Unpublished M. S. Thesis. Oklahoma State University.

CHAPTER II

LITERATURE REVIEW

Chromosome Pairing of 2n X 4n Sorghum Crosses

Endrizzi (1957) studied eleven F_1 hybrids obtained from crosses between Sorghum vulgare Pers. (var. Texas Blackhull Kafir) and Sorghum halepense (L.) Pers. for chromosome number, meiotic behavior, and vegetative characteristics. Only one hybrid had 30 chromosomes and ten had 40 chromosomes. The 30-chromosome hybrid had an average of 4.34 I, 4.34 II, and 5.66 III chromosome associations with a maximum association of nine trivalents. The 40-chromosome hybrids averaged 0.97 I, 10.80 II, 0.31 III, and 3.72 IV chromosome associations with a maximum of eight tetravalent associations. Endrizzi interpreted these results to mean that regular pairing occurred between one genome of Sorghum vulgare and Sorghum halepense.

The type of female used in Sorghum vulgare X Sorghum halepense crosses influenced the chromosome number of the hybrids (Hadley, 1958). When the female was male-fertile and emasculated by the hot water technique, the predominate chromosome number was 30. Texas Blackhull Kafir was used as the female. The ratio of 30- to 40-chromosome hybrids of all the hybrids of this group was 24:2. However, when male-sterile lines, genetic or cytoplasmic were used, the predominating chromosome number of the hybrid was 40, the ratio of 30- to 40-chromosome hybrids

being 7:21. When cytoplasmic male-sterile Combine Kafir-60 was the female, the ratio of 30- to 40-chromosome hybrids was 4:9 and when genetic male-sterile stocks of Texas Blackhull Kafir were used, the ratio was 3:12. Either the source of sterility or the varietal difference was interpreted as the cause of the difference in ratios.

The chromosome number and meiotic association of S. vulgare (var. Texas Blackhull Kafir) X S. almum Parodi was determined by Endrizzi (1957). The chromosome number of twelve hybrids was determined: one hybrid had $2n = 30$, two had $2n = 39$, and nine had $2n = 40$. The average association of the 40-chromosome hybrids was 0.58 I, 11.69 II, 1.40 III, and 3.89 IV, with a maximum of ten tetravalents. The 30-chromosome hybrid had a maximum association of ten trivalents with an average of 1.65 I, 1.65 II, and 8.35 III. These results were interpreted to mean that most of the genetic complement of S. almum is composed of chromosome segments of S. vulgare.

Genetic Control of Chromosome Pairing

The concept that chromosome associations are controlled by the genetic system has been reported in both plants and animals. The formation of mostly bivalent associations in certain hybrids appears to be influenced by Mendelian characters as proposed by several theories. Muller, in 1922, proposed that chromosome pairing can be controlled by specific genes. Darlington (1937, 1958) explained mostly bivalent associations on the basis of differential affinity. The preferential pairing of chromosomes was suggested by Stebbins (1950).

The work of Riley, Unrau and Chapman (1958) and Riley, Kimber and Chapman (1961) on hexaploid wheat shows the control of chromosome pairing

to be influenced by one chromosome. Chromosome 5 of the B genome carries one or more genes which prevent homoeologous chromosome pairing at meiosis. When chromosome 5-B is absent from the genetic system, pairing between homoeologous chromosomes results in multivalent associations. The presence of a similar genetic control for pairing of homoeologous and non-homoeologous chromosomes in the D and B genomes of Bothriochloa was suggested by Chheda and Harlan (1962).

The action of a special genotypically controlled tendency for bivalent formation in Phleum was described by Münzing and Prakken (1940). Triploids of Phleum pratense of experimental origin known to contain three identical genomes, failed to form trivalent chromosome associations as would be expected. Instead of trivalent associations, these plants consistently formed bivalent pairs. When a particular set of homologous chromosomes was absent, the triploid plants formed the expected trivalent associations. The presence of genetic controlling factors on the chromosome was suggested. In the study of inbred lines of rye by Rees (1955), data were presented showing genetic control of chiasma formation and terminalisation of chiasma in chromosome pairing.

The diploid hybrid formed by a Primula verticillata and P. floribunda cross had a high number of bivalent associations. However, the tetraploid hybrid formed by such a cross seldom exhibited chromosome pairing (Darlington, 1958). This failure of pairing in the tetraploid was explained on the basis that, although there were four of each chromosome capable of pairing, only two of these chromosomes were quickly paired and the two less similar chromosomes became paired only occasionally. This failure of pairing in the tetraploid was the result of competition in pairing which gave rise to what Darlington (1937, 1958) called

differential affinity.

The influence of parental relation on chromosome pairing has been discussed by Rees (1961) and Sarvella (1958). Rees (1961) suggested that a high degree of structural divergence between parental chromosomes of barley may result in increased chiasma frequency or in the words of Rees "heterosis of chiasma frequency." On the other hand Sarvella (1958) indicated that hybrids of closely related species of Gossypium tended to form a high frequency of multivalents. Both workers, however, did show the genetic control of chromosome pairing. Enns (1960) found a mutant in x-ray irradiated plants of Hordeum vulgare (L.) (var. Huskey) that exhibited a reduced frequency of bivalent pairing in metaphase I. This expression was attributed to one gene.

Unreduced Gametes

The occurrence of unreduced gametes has been reported in many plants. The presence of functional unreduced gametes gives rise to F_1 hybrids having chromosome numbers greater than that normally expected in inter-generic or interspecific crosses. This phenomenon can occur as a result of irregular meiosis during gametogenesis or as a result of somatic apospory (Powers, 1945; Bergman, 1951; Harlan, et al., 1964; and Li, 1964).

The formation of unreduced embryo sacs in the genus Parthenium was described by Powers (1945). The unreduced embryo sacs were formed by failure in meiosis. Unreduced embryo sacs were found in 10.2 per cent of the ovules. The egg nuclei of the unreduced embryo sacs could not develop without pollination and fertilization. The offspring of the fertilized unreduced embryo sacs were triploids.

The formation of unreduced embryo sacs by irregularities in meiosis can occur in Antennaria carpatica (Bergman, 1951). The unreduced embryo sacs formed by faulty meiosis result when the dividing cells pass from prophase to telophase during the first division without cytokinesis. However, a functional fruit rarely develops from the achenes resulting from unreduced embryo sacs.

In the genus Datura, unreduced pollen grains were described by Satina (1950). In some lines of D. meteloides, up to 25 per cent of the pollen grains were unreduced. When this species was selfed, only diploid progeny were produced even though diploid pollen grains occurred frequently. In crosses between D. meteloides and D. stramonium, triploid hybrids were frequently produced as a result of the unreduced pollen grains taking part in pollination and fertilization. Thus, it seems that the unreduced pollen grains can become functional under certain conditions.

Li (1964) has described in detail the formation of unreduced gametes in Oryza. The formation of unreduced pollen grains was found to result from non-synchronization of mitosis and cytokinesis caused by an eccentrically placed nucleus. This eccentrically located nucleus caused delay in spindle formation and subsequently resulted in nuclear division without metakinesis. The following cell division resulted in a dyad, half of which contained all the chromosomes and half of which was anucleate. The nucleated half undergoes normal second meiotic division. Li indicated this process could occur in both micro- and megagametes.

The production of diploid eggs in Saccharum by failure in the mitotic division prior to the meiotic cycle was described by Price (1961). The F_1 hybrid of S. officinarum ($n = 40$) and S. spontaneum ($n = 40$) has $n = 120$ chromosomes. The formation of this hybrid was described as

being due to an unreduced egg that resulted from endomitosis prior to the meiotic divisions.

The abnormalities discussed above can also result in polyploid gametes. In 1961, Damon reported a low frequency of multiploid sporocytes in Sorghum. He described the presence of 4n microsporocytes on the basis of abnormalities in the last mitotic division prior to meiosis. The triploid and other polyploid microsporocytes were described as resulting from the digestion or destruction of the cell walls of adjacent sporocytes during meiosis. It was thought that this digestion or destruction began as early as pachytene. Smith (1942) described the destruction of cell walls of adjacent microsporocytes resulting in polyploid microsporocytes in barley.

The occurrence of multi-nucleate pollen mother cells in barley was described by Kamra (1960). He attributed the formation of the multinucleate condition to failure in cell wall formation in pre-meiotic mitosis. He described cells having synchronized and non-synchronized nuclei in meiotic division. Non-synchronized nuclei were also described by Damon (1961). The presence of triploid and tetraploid gametes due to pre-meiotic abnormalities in maize was described by Randolph and McClintock (1926).

The production of polyploid gametes in several species was described by Karpochenko (1927) as caused by failures in the reduction division, and at anaphase of the first meiotic division. By what he called non-conjunction plus non-reduction or simply absence of the first meiotic division, polyploid gametes were produced.

The aposporial development of chalazal cells can result in unreduced embryo sacs in Antennaria (Bergman, 1951). The embryo sacs produced by

this means seldom produced viable achenes even though fertilized. Parthenogenetic development of the unreduced embryo sacs produced by somatic apospory did not occur. The work of Harlan, Brooks, Borgaonkar, and De Wet (1964) pointed out that parthenogenetic development of unreduced embryo sacs occurred in Bothriochloa and resulted in viable seeds.

Ovary Development in Interspecific Hybrids

One of the first anxieties in making interspecific crosses is whether a viable F_1 hybrid plant can be obtained. Failure to obtain vigorous F_1 hybrid plants can be the result of genetic or cytoplasmic incompatibilities. The incompatibilities may be expressed by failure of fertilization or in death of the zygote at any stage between the early cell divisions and maturity (Stebbins, 1950). Stebbins (1958), and Kostoff (1930) described the following types of failures to obtain viable F_1 progeny from interspecific crosses: 1) crosses in which the pollen tube does not reach the ovary; 2) crosses in which the pollen tube upon entering the micropyle induces parthenocarpy; 3) crosses in which fertilization occurs, but the embryo usually dies in early stages of development; and 4) crosses in which seeds are developed, but the seedlings die.

In crosses of $3n$ X $2n$, $2n$ X $4n$, and n X $4n$ chromosomal types of Datura stramonium, the pollen tube swelled and burst before it reached the ovary (Buchloz and Blakeslee, 1929). They also reported failure of the "foreign" pollen to germinate. Janaki-Ammal (1941) reported the failure of Zea X Saccharum crosses as due to failure in Zea pollen germination due to the high sugar content of the Saccharum stigma.

In crosses of Nicotiana paniculata X N. rustica, Kostoff (1930) found that few pollen tubes were able to reach and fertilize the ovary. The reciprocal cross resulted in a high frequency of success. Crosses between Prunus avium and P. cantabrigiensis seldom resulted in mature seed (Raptopoules, 1942). The failure resulted when the pollen tube ceased to grow or became bent in the incompatible style.

The parthenogenetic development of the ovule of N. Langsdorfii X N. violacea was reported by Kostoff (1930). The presence of the pollen tube in the microyple induced the parthenogenetic development. The F_1 plant had the somatic chromosome number of the maternal parent and resembled it morphologically. The endosperm was also induced to develop parthenogenetically.

Crosses in Bothriochloa and Dichanthium often result in progeny of the maternal type (Harlan et al., 1964). The maternal types were the result of parthenogenetic development. In these crosses, contrary to the Nicotiana crosses described by Kostoff (1930), the polar nuclei are fertilized. The formation of sexual and asexual embryo sacs was observed, but the parthenogenetic development occurred in the asexual embryo sacs. This apomictic development was controlled by genetic factors.

Chemically treated ovaries of Datura and Melandrium developed proembryos (Van Overbeek, Conklin, and Blakeslee, 1941). The chemicals induced development of proembryos from endothelial or integument tissue. The embryos did not mature. These workers suggested, on the basis of their results, that pollen upon germination may release enzymes that can cause development of the ovary and ovule before fertilization.

The third type of failure to form viable seeds can result due to incompatibility, both genic and cytoplasmic, in chromosomal complements

of the parents. Crosses between species having the same chromosome number have been described by Laibach (1929), Satina and Blakeslee (1935), and by Lee and Cooper (1958). In crosses of diploid Solanum species, Lee and Cooper (1958) found that fertilization occurred, but mature viable seeds were not produced. The developing endosperm failed to differentiate properly and the endothelium became hyperplastic resulting in collapse of the endosperm. The collapse of the endosperm led to eventual degeneration of the embryo. The failure was attributed to failure of the differentiation of vascular tissue in the area between the chalazal pocket and the vascular strand of the ovule. In crosses of Linum perenne and L. astricum (Laibach, 1929), and Datura stramonium X D. metal (Satina and Blakeslee, 1935) similar failture to form viable seeds was noted. Fertilization was observed in both cases. Laibach (1929) attempted to culture excised embryos with partial success. Satina and Blakeslee (1935) determined that the failure occurred in the endosperm or proembryo following a few days of apparently normal development.

Crosses between related allopolyploids having different degrees of ploidy can also result in failure to form viable mature seeds. In crosses of this type embryo and endosperm development is usually better when the higher ploidy parent is the female.

In 6n X 2n Avena crosses, Kihara and Nishiyama (1932) reported that the growth and development of the embryo and endosperm was retarded, but otherwise rather normal. The reciprocal cross, however, resulted in abnormal mitosis in the endosperm causing degeneration of the endosperm and eventually deterioration of the embryo. In a cross of A. strigosa ($2n = 14$) X A. sativa ($2n = 42$), Brown (1964) reported that fertilization occurred normally, but about six days after pollination

the endosperm began to deteriorate. The embryo continued to increase in size, but failed to differentiate.

In 6n X 4n and 4n X 2n Triticum crosses, (Boyes and Thompson, 1937), the endosperm and embryo developed rather slowly. The endosperm nuclei divided normally, but the cell walls formed precociously causing an eventual deterioration of the endosperm. Hexaploid by diploid Solanum crosses failed due to a breakdown of the integumentary cells around the endothelium, chalaza, and micropyle (Beamish, 1955). There was cell formation in the endosperm, but not proper differentiation.

Crosses between diploids and autotetraploids also often result in shrunken, inviable seeds. In Lycopersicum pimpinellifolium the failure of seed development begins with a failure of the endosperm nuclei to divide properly (Cooper and Brink, 1945). The endothelium becomes meristematic and its excessive growth or hyplastia fills the endosperm cavity. This eventually causes starvation of the embryo.

Håkansson and Ellerström (1950) described a collapse of the seed in Secale cereale diploid X autotetraploid crosses. Following double fertilization the embryo and endosperm began to develop, but abnormal mitosis in the endosperm soon appeared. This resulted in degeneration of the endosperm and starvation of the embryo. In the reciprocal cross endosperm development was slow. However, prolonged meristematic activity of the endosperm resulted in degeneration of both endosperm and embryo.

Diploid by autotetraploid crosses of Hordeum vulgare (Håkansson, 1953) and Medicago sativa (Fredrikson and Bolton, 1963) resembled the crosses of Secale. Abnormal mitosis resulted in degeneration of the endosperm; and in the reciprocal crosses, prolonged meristematic activity and lack of starch formation on the part of the endosperm resulted in

"crowding out" the embryo.

In Zea mays (Cooper, 1951), the endosperm in diploid by autotetraploid crosses developed quickly and precociously; however no abnormal mitosis occurred. The endosperm continued to develop meristematically in the region of the embryo. This caused the embryo to become distorted, misshapen, and it often failed to differentiate a plumule-radical axis. The reciprocal cross resulted in faulty endosperm differentiation; thus eventual starvation of the embryo.

The final type of failure occurring in interspecific hybrids is plant death in seedling stages. The seeds produced in these cases matured and germinated, but did not produce mature plants. This type of failure can be illustrated by Crepis (Hollingshead, 1930) and by Triticum X Aegilops hybrids (Sears, 1944).

Reciprocal crosses made by Hollingshead (1930) between Crepis capillars and C. tectorum resulted in mature seeds. However, the seedlings often died prior to the cotyledon stage. He found that the frequency of death depended upon the strain of C. tectorum used in the cross. Genetic analysis of the occasional healthy segregates resulted in Hollingshead (1930) reaching the conclusion that C. tectorum was heterozygous for a lethal factor and modifiers.

Hybrids of Aegilops umbellulata and Triticum monococcum usually failed to produce mature plants (Sears, 1944). In this cross seeds were easy to obtain, but produced plants expressing two types of lethality. When T. monococcum var. flavescens was used as a parent, the hybrid seedlings usually died before the two leaf stage which Sears called "early-dying type." If T. monococcum var. vulgare-flavescens was one of the parents, the hybrids died after the three-leaf stage.

This "late-dying type" developed chlorosis and slowly withered away. Both of these types of lethal seedlings were caused by geneic allels and their modifiers.

Pollen Size as Related to Polyploidy

Determination of chromosome number is a time consuming procedure. However, the determination of degree of ploidy is important in cytogenetic study of interspecific hybrids and as a possible morphological classification for taxonomic identification (Erdtman, 1952, and Löve, 1951). The use of pollen grain size as such a tool to determine the chromosome number requires caution (Stebbins, 1950). The pollen grain size and chromosome number of the parents or species must be determined.

Numerous studies have been made that indicate that pollen grain size is a good indication of degree of ploidy. In a study of haploid, diploid, triploid, and tetraploid Datura, Löve (1951) found that the volume of pollen mother cells was nearly proportional to the number of haploid groups of chromosomes present. Chin (1946) in the study of diploid, triploid, tetraploid, and octaploid Hegari sorghum found that pollen grain size did indicate degree of ploidy. The pollen grain diameter fits a linear regression. Each increase by one haploid chromosome complement caused an increase in diameter by the square root of the haploid grain's diameter. Chin also calculated the influence on volume of pollen grains by chromosome ploidy. Each haploid complement caused an increase in volume by π /6Vi, Vi being the volume of the haploid pollen grain.

Schwanitz (1950) studied the correlation of pollen size and ploidy in diploid and tetraploid forms of several genera. In all species studied, the volume of diploid pollen grains was about two times that of the haploid pollen grain, and surface area was increased 50-60 per cent. The genera studied were Sinapis, Brassica, Raphanus, Rumex, Digitalis, and Cichorium. Investigation of Andropogon resulted in similar conclusions by Gould (1957).

A detailed analysis of pollen grain size as an indicator of chromosome number was conducted by Celarier and Mehra (1958). Using Bothriochloa and Dichanthium in their study, they concluded that 1) pollen grain size is usually a reliable indicator of polyploidy, 2) actual chromosome counts must be made from a few of the plants and pollen size from these plants used as a guide, and 3) data should be calculated in terms of range and means.

CHAPTER III

CHROMOSOME ASSOCIATIONS OF HYBRIDS BETWEEN DIPLOID AND TETRAPLOID SORGHUMS

Knowledge of chromosome associations of interspecific hybrids is necessary for adequate delineation of the relationship and ancestry of the species under study. Such studies in Sorghum were reported by Endrizzi (1957), Magoon and Shambulingappa (1961), and Magoon, Manchanda, and Ramanna (1964), to name only a few.

In a previous study, McClure[1] determined the frequency of crossing between five varieties of Sorghum vulgare Pers. and two species of tetraploid sorghum. A difference in frequency of crossing was observed depending on the parents used. The purpose of the present investigation was to study the chromosome associations of several of the crosses and to determine the influence of parents on such associations.

Materials and Methods

The parents used in this study consisted of three cytoplasmic male-sterile varieties of Sorghum vulgare, 2n = 20, (vars. Dwarf Redlan, Martin, and Combine Kafir-60), and two tetraploid species (2n = 40), Sorghum almum and Johnsongrass. The three varieties of S. vulgare were

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[1]McClure, Walter Jay. 1962. Frequency of interspecific crossing between Sorghum vulgare Pers. and Sorghum halepense (L.) Pers., and between Sorghum vulgare Pers. and Sorghum almum Parodi. Unpublished M. S. Thesis. Oklahoma State University.

chosen because they represented high, medium, and low frequency of crossing with the tetraploid species, respectively. The crosses were made in isolation blocks west of Lake Carl Blackwell in 1960 and 1961.

The plants for this study were obtained by selecting at random twenty-five seeds from each of the above crosses. The seeds were planted in the greenhouse in the fall of 1963. Microsporocyte collections were made and fixed in Carnoy's fluid (6:3:1). The plants that did not produce collectable microsporcyte material were transplanted to the field in 1964. Those plants that provided no microsporocyte material were again transplanted back to the greenhouse in the fall of 1964. Poor growth and death resulted in the loss of about ten plants of each cross.

Studies were made on the types and frequencies of chromosome associations at diakinesis and metaphase I of the hybrids and parents. The material was examined using the aceto-carmine smear technique modified from Smith (1947). Whenever possible, fifteen or more cells were examined to determine the types and frequencies of chromosome associations. Photomicrographs were made with a Minolta SR-7, 35 mm camera equipped with a microscope adapter, and Agfa IFF film with an ASA of 25.

Results

The summary of chromosome associations of the hybrids and parents is shown in Tables I through VII. From these tables it can be seen that many of the hybrids had a chromosome number of $2n=40$. The major association is in the form of bivalents. The $2n=40$ chromosome hybrids had a maximum of 20 bivalents, and the $2n=30$ hybrids had a maximum of 15 bivalents. Plates I and II show the chromosome associations of some of the hybrids and parents.

TABLE I

CHROMOSOME ASSOCIATION OF CYTOPLASMIC MALE-STERILE DWARF REDLAN X JOHNSONGRASS AT DIAKINESIS AND METAPHASE I

Plant Number	No. Cells Observed	2n Number	Range				Average per Cell				Remarks
			I	II	III	IV	I	II	III	IV	
2121	20	40	0-2	11-18	0-2	1-4	0.36	15.32	0.09	2.04	Occasional chain of 6 chrom.
2122	30	40	0-4	15-20	-	0-2	0.75	18.04	--	0.79	Lagging bivalents in A I
2123	30	40	-	16-20	-	0-2	--	18.75	--	1.00	Lagging bivalents in A I
2124	30	40	0-1	14-20	0-1	0-4	0.04	16.43	0.04	1.78	
2125	30	40	0-5	4-14	0-1	0-6	1.73	10.63	0.47	2.83	Lagging II, Rings of 5 & 6 chrom., and Chains of 3 & 4 chrom. in some cells
2126	30	40	0-4	14-20	-	0-3	0.02	17.89	--	1.00	Chain of 4 chrom. in some cells
2127	15	30	0-2	13-15	-	0-1	0.36	14.45	--	0.09	
2129	15	40	-	10-20	-	0-5	--	15.45	--	2.36	
2130	20	40	0-2	12-20	0-2	0-4	0.23	15.77	0.08	0.92	Chain of 3 chrom. in some cells

TABLE I (Continued)

Plant Number	No. Cells Observed	2n Number	Range I	Range II	Range III	Range IV	Average per Cell I	Average per Cell II	Average per Cell III	Average per Cell IV	Remarks
2131	10	40	0-3	10-17	0-2	1-4	1.44	14.75	0.94	1.81	2 nucleoli in pachytene in some cells
2133	30	20	-	10	-	-	--	10.00	--	--	Some 20 II cells
2134	18	40	0-5	5-15	0-4	1-6	1.33	9.94	1.00	3.00	
2135	15	40	0-6	7-20	0-2	0-5	0.87	14.87	0.20	2.13	Some 2n = ca 120
2136	11	40	-	11-18	0-2	1-3	--	14.36	0.18	2.09	Chain of 6 chrom. in some cells
2137	21	40	-	10-20	-	0-2	--	17.90	--	0.67	
2138	16	40	0-2	15-20	0-2	0-3	0.37	17.50	0.37	0.87	
2139	15	40	0-4	12-18	-	1-5	1.73	14.07	--	2.20	Some cells with chain of 4 chrom.

TABLE II

CHROMOSOME ASSOCIATIONS OF CYTOPLASMIC MALE-STERILE MARTIN X JOHNSONGRASS AT DIAKINESIS AND METAPHASE I

Plant Number	No. Cells Observed	2n Number	Range				Average per Cell				Remarks
			I	II	III	IV	I	II	III	IV	
2103	15	20	-	8-10	-	0-1	--	9.57	--	0.21	
2105	21	40	0-10	15-20	-	-	2.00	18.64	--	--	Some cells had 2 nucleoli; some cells 2n = 60 and 80
2107	15	20	-	10	-	-	--	10.00	--	--	
2108	15	20	-	8-10	-	0-1	--	8.62	--	0.69	Lagging bivalents in A I
2109	15	20	-	10	-	-	--	10.00	--	--	Lagging bivalents in A I
2110	15	20	-	6-10	-	0-2	--	9.29	--	0.35	
2112	27	40	-	16-20	-	0-2	--	19.91	--	0.45	Lagging bivalents in A I
2114	15	20	-	10	-	-	--	10	--	--	
2115	32	40	0-2	16-20	0-1	0-4	0.15	18.25	0.05	0.53	Some cells 2n = 20, 60 and 80
2116	30	40	0-4	14-20	-	0-3	0.29	17.54	--	1.15	Some cells 2n = 60
2117	15	20	-	10	-	-	--	10	--	--	

TABLE III

CHROMOSOME ASSOCIATIONS OF CYTOPLASMIC MALE-STERILE COMBINE KAFIR-60 X JOHNSONGRASS AT DIAKINESIS AND METAPHASE I

Plant Number	No. Cells Observed	2n Number	Range				Average per Cell				Remarks
			I	II	III	IV	I	II	III	IV	
2082	15	30	0-1	8-13	1-3	-	1.14	11.00	2.29	--	Several cells 2n = 60, 80, and 120
2083	15	20	-	10	-	-	--	10.00	--	--	
2085	30	30	0-7	7-14	0-4	-	2.71	11.57	1.14	--	Some cells 2n = ca 160
2093	15	20	-	10	-	-	--	10.00	--	--	
2094	15	20	-	10	-	-	--	10.00	--	--	
2096	27	30	0-9	7-15	0-2	0-2	3.31	10.61	0.54	0.61	
2097	15	20	-	10	-	-	--	10.00	--	--	
2100	60	[a]	-	-	-	-	--	--	--	--	2n = ca 80 and 120

[a]Plant 2100 had mostly 2n numbers of 80 and 120.

TABLE IV

CHROMOSOME ASSOCIATION OF CYTOPLASMIC MALE-STERILE DWARF REDLAN X *S. ALMUM* AT DIAKINESIS AND METAPHASE I

Plant Number	No. Cells Observed	2n Number	Range				Average per Cell				Remarks
			I	II	III	IV	I	II	III	IV	
2184	23	40	-	14-20	-	0-4	--	16.35	--	1.78	
2185	15	26	0-2	6-9	1-3	0-1	1.33	7.83	2.00	6.67	
2186	14	40	-	16-20	-	0-2	--	17.57	--	1.21	
2187	22	30	0-5	3-13	2-10	0-1	2.13	8.80	3.33	0.01	
2189	27	40	-	14-20	-	0-3	--	17.92	--	1.04	Lagging bivalents some cells 2n = 80
2193	30	30	0-2	8-15	0-2	0-5	0.85	12.27	0.54	1.15	
2194	30	40	-	10-20	-	0-5	--	14.75	--	2.60	Some cells with 2 nucleoli
2196	15	40	-	16-18	-	1-2	--	17.29	--	1.36	
2197	18	40	-	14-20	-	0-3	--	17.56	--	1.17	
2198	15	40	0-2	12-20	0-1	0-4	0.08	15.12	0.04	2.24	
2199	15	30	0-5	5-10	3-6	0-2	2.86	6.07	3.43	0.29	Lagging bivalents at A I
2200	15	20	0-1	4-10	0-1	0-3	0.08	8.5	0.08	0.58	

TABLE V

CHROMOSOME ASSOCIATIONS OF CYTOPLASMIC MALE-STERILE MARTIN X *S. ALMUM* AT DIAKINESIS AND METAPHASE I

Plant Number	No. Cells Observed	2n Number	Range				Average Per Cell				Remarks
			I	II	III	IV	I	II	III	IV	
2163	15	20	-	10	-	-	--	10.00	--	--	
2164	24	40	0-8	12-20	-	0-4	0.58	14.88	--	2.58	Some cells with chains of 4
2165	16	20	-	8-10	-	0-1	--	8.75	--	0.63	
2166	15	30	-	11-15	-	0-2	--	13.25	--	0.75	
2167	12	40	0-4	16-20	-	0-2	0.33	18.17	--	0.92	
2168	11	40	-	12-20	-	0-4	--	16.91	--	1.54	
2169	16	30	0-5	5-15	0-5	0-2	1.60	9.60	2.20	0.60	Lagging bivalents in all cells
2174	15	20	-	8-10	-	0-1	--	9.33	--	0.33	
2175	22	40	-	12-20	-	1-4	--	15.78	--	2.11	
2177	25	40	0-1	14-20	0-1	0-3	0.04	16.04	0.04	1.76	
2178	25	100	0-9	12-32	0-20	0-2	1.00	17.42	10.83	0.83	2 nucleoli in cells; some 2n = 40, 50, and 120 cells
2179	15	40	-	14-18	-	1-3	--	16.00	--	2.00	

TABLE VI

CHROMOSOME ASSOCIATIONS OF CYTOPLASMIC MALE-STERILE COMBINE KAFIR-60 X *S. ALMUM* AT DIAKINESIS AND METAPHASE I

Plant Number	No. Cells Observed	2n Number	Range				Average per Cell			
			I	II	III	IV	I	II	III	IV
2141	25	20	-	10	-	-	--	10.00	--	--
2143	16	20	0-1	7-10	0-1	0-1	0.06	8.69	0.06	0.62
2144	16	20	-	10	-	-	--	10.00	--	--
2146	15	20	-	8-10	-	0-1	--	9.67	--	0.17
2147	15	20	-	8-10	-	0-1	--	9.71	--	0.14
2148	16	20	-	10	-	-	--	10.00	--	--
2150	15	20	-	10	-	-	--	10.00	--	--
2151	16	20	-	7-10	-	0-2	--	9.37	--	0.31
2152	22	20	-	8-10	-	0-1	--	9.50	--	0.25
2153	15	20	-	10	-	-	--	10.00	--	--
2154	19	20	-	8-10	-	0-1	--	9.26	--	0.32
2158	16	20	-	10	-	-	--	10.00	--	--
2159	15	20	-	10	-	-	--	10.00	--	--
2160	12	20	-	8-10	-	0-1	--	9.33	--	0.33

Data from the hybrids of cytoplasmic male-sterile Dwarf Redlan and Johnsongrass are summarized in Table I. Seventeen plants were studied, and of this number 15 had a 2n number of 40, one had a 2n number of 20, and one had a 2n number of 30. The major association of chromosomes in the 2n = 40 hybrids was bivalent; however as many as six tetravalents were found. Many of the multivalents were in chains. Plants 2121, 2125, 2126, 2130, 2136, and 2139 had chains of 3 to 6 chromosomes. Plant 2125 had rings of 5 and 6 chromosomes.

The chromosome number of the cells in some of the plants varied. Plant 2135, a 2n = 40 hybrid, had as many as 2n = 120 chromosomes, and plant 2133, a 2n = 20 plant, had some cells with 20 bivalents. Lagging bivalents at anaphase I were observed in some of the plants.

The summary of the chromosome associations of cytoplasmic male-sterile Martin and Johnsongrass hybrids is shown in Table II. Among the eleven plants of Martin X Johnsongrass, four had a chromosome number of 2n = 40 and seven a 2n number of 20. All of the 2n = 40 chromosome plants had a maximum association of 20 bivalents. Lagging bivalents were observed in one of the 40-chromosome plants. Plant 2115 had chromosome numbers of 2n = 20, 40, 60, and 80. This plant will be referred to later.

Table III summarizes the results of the cytoplasmic male-sterile Combine Kafir-60 and Johnsongrass crosses. Of the eight plants studied, four plants had chromosome numbers of 2n = 20, three plants had 2n numbers of 30, and one had an extremely large 2n number. The 2n = 20 plants had regularly ten bivalents. One of the 2n = 30 chromosome plants, 2085, had cells with as many as 160 chromosomes. Plant 2100 was very irregular.

The results of the chromosome association of cytoplasmic male-sterile Dwarf Redlan X S. almum hybrids are summarized in Table IV. Twelve plants were studied. Among these plants, seven had 2n chromosome numbers of 40, three had 2n numbers of 30, one plant had a 2n number of 26, and one had a 2n number of 20. All of the 2n = 40 chromosome plants except plant 2196 had a maximum association of 20 bivalents. Plant 2196 consistently had one tetravalent. One of the 2n = 30 chromosome hybrids had a maximum association of 15 bivalents; another 2n = 30 plant had ten trivalents as a maximum association. Although the only 2n = 20 plant was rather irregular, several cells did have ten bivalents.

The summary of cytoplasmic male-sterile Martin X S. almum chromosome associations is presented in Table V. Among the twelve hybrids analyzed, six had 2n chromosome numbers of 40. Two plants were 2n = 30, three were 2n = 20, and one had a chromosome number of 2n = 100. The plants with 2n = 40 chromosomes had maximum associations of 20 bivalents, except plant 2179. The 2n = 30 plants had maximum associations of 15 bivalents. The one unusual plant was 2178. This plant had a 2n number of 100. In nearly every case 2178 had two nucleoli per cell. A few cells of 2178 were observed to have 2n = 40 chromosomes.

The results of the study of cytoplasmic male-sterile Combine Kafir-60 X S. almum are presented in Table VI. Among the fourteen plants studied no deviation from 2n = 20 was observed, see Plate II, Figure 14. Seven of the plants analyzed had regular ten bivalent associations.

The summary of the chromosome associations of the parents is shown in Table VII. The varieties of S. vulgare regularly had ten bivalent pairs. Cytoplasmic male-sterile Combine Kafir-60 was an exception to this in that it had an occasional cell with one tetravalent. These

TABLE VII

CHROMOSOME ASSOCIATIONS OF PARENTS AT DIAKINESIS AND METAPHASE I

Parent	No. Cells Observed	2n Number	No. Plants Observed	Range				Average Per Cell				Remarks
				I	II	III	IV	I	II	III	IV	
Dwarf Redlan	110	20	4	-	10	-	-	-	10	-	-	Several 2n = 40 cells
Martin	120	20	4	-	10	-	-	-	10	-	-	Several 2n = 40, 60, and 80 cells
Combine Kafir-60	50	20	4	-	8-10	-	0-1	-	9.88	-	0.06	
Johnson-grass	60	40	2	0-2	12-18	1-3	1-3	0.64	15.93	0.71	1.43	Some lagging bivalents. Ave. of .07 VI per cell
Sorghum almum	30	40	3	0-4	7-18	0-4	1-3	0.54	13.87	0.58	2.50	

tetravalents were so closely associated that they may have been confused with secondary association of bivalents, see Plate II, figure 17. Johnsongrass and S. almum had 2n chromosome numbers of 40. Their maximum chromosome association was 18 bivalents. Johnsongrass had an occasional cell that contained one hexavalent.

When Johnsongrass was one of the parents, 67 per cent of the plants produced had 2n = 30 or 2n = 40 as a chromosome number. On the other hand, when S. almum was the pollen parent less than 50 per cent of the plants produced had chromosome numbers greater than 2n = 20. When Combine Kafir-60 was crossed with S. almum only diploid, 2n = 20, plants were produced, but when it was the female with Johnsongrass nearly half of the plants produced were triploids.

The cytoplasmic male-sterile Dwarf Redlan X Johnsongrass and Dwarf Redlan X S. almum crosses resulted in a high frequency of tetraploid and relative few diploid plants. The occurrence of triploids i.e. 2n = 30, was more frequent when S. almum was the pollen parent.

The frequency of polyploid microsporocyte cells in the plants studied is shown in Table VIII. It was shown that the plants where S. almum was the pollen parent had fewer polyploid microspoyocyte cells than when Johnsongrass was the pollen parent, see Plate I, figures 6 and 7. Cytoplasmic male-sterile Martin X Johnsongrass had the highest frequency of polyploid microsporocyte cells; the only exception to this was plant 2178. Combine Kafir-60 X S. almum had no polyploid microsporocyte cells.

The frequency of polyploid microsporocyte cells in the parents are shown in Table IX. Polyploid microsporocyte cells were observed in Dwarf Redlan and Martin, see Plate II, figure 13. No polyploid

TABLE VIII

CHROMOSOME NUMBERS AND FREQUENCY OF MICROSPOROCYTES OTHER THAN 2N IN THE HYBRIDS BETWEEN DIPLOID AND TETRAPLOID SORGHUMS

Hybrid	2n Number	No. of Plants	No. Cells Observed	Frequency of Polyploid Cells						Total cells other than 2n
				n	3n	4n	5n	6n	8n	
						%				%
Dwarf Redlan X Johnsongrass	20	1	15	--	--	--	--	--	--	
-do-	30	1	15	--	--	--	--	--	--	
-do-	40	16	280	--	0.8	0.8	--	1.8	--	3.2
Martin X Johnsongrass	20	7	105	--	--	--	--	--	--	
-do-	40	4	133	1.5	4.5	10.5	--	--	--	17.3
Combine Kafir-60 X Johnsongrass	20	4	45	--	--	--	--	--	--	
-do-	30	3[a]	84	--	--	3.6	3.6	2.3	2.3	11.9
Dwarf Redlan X *Sorghum almum*	20	1	15	--	--	--	--	--	--	
-do-	26	1	15	--	--	--	--	--	--	
-do-	30	3	67	--	--	--	--	--	--	
-do-	40	7	142	--	--	2.1	--	--	--	2.1
Martin X *Sorghum almum*	20	3	46	--	--	--	--	--	--	
-do-	30	2	40	--	--	--	--	--	--	
-do-	40	6	109	--	--	--	--	--	--	
-do	100	1	25	40.0	8.0	--	--	--	--	56.0[b]

TABLE VIII (Continued)

Hybrid	2n Number	No. of Plants	Total No. Cells	Frequency of Polyploid Cells						Total cells other than 2n
				n	3n	4n	5n	6n	8n	
							%			%
Combine Kafir-60 X Sorghum almum	20	16	240	--	--	--	--	--	--	

[a]Plant 2100 not included in this table.

[b]This high frequency was due to plant 2178.

TABLE IX

CHROMOSOME NUMBERS AND FREQUENCY OF MICROSPOROCYTES
OTHER THAN 2N IN THE PARENTS

Parent	2n Number	No. of Plants	No. Cells Observed	Frequency of polyploid cells						Total cells other than 2n
				n	3n	4n	5n	6n	8n	
						%				%
Dwarf Redlan	20	3	110	--	--	.033	--	--	--	.033
Martin	20	5	120	--	--	.133	.008	.016	--	.175
Combine Kafir-60	20	2	50	--	--	--	--	--	--	--
Johnsongrass	40	2	60	--	--	--	--	--	--	--
Sorghum almum	40	3	30	--	--	--	--	--	--	--

LEGEND FOR PLATE I

Figure 1. Metaphase I of Dwarf Redlan X Johnsongrass, 2n = 60.

Figure 2. Metaphase I of Johnsongrass, 2n = 40.

Figure 3. Metaphase I of Dwarf Redlan X Johnsongrass, 2n = 40.

Figure 4. Metaphase I of Martin X Sorghum almum, 2n = 40, with chain of 6 chromosomes (arrow).

Figure 5. Metaphase I of Martin X Sorghum almum, 2n = 40.

Figure 6. Metaphase I of Dwarf Redlan X Johnsongrass, cell A has 10 II, cell B 20 II.

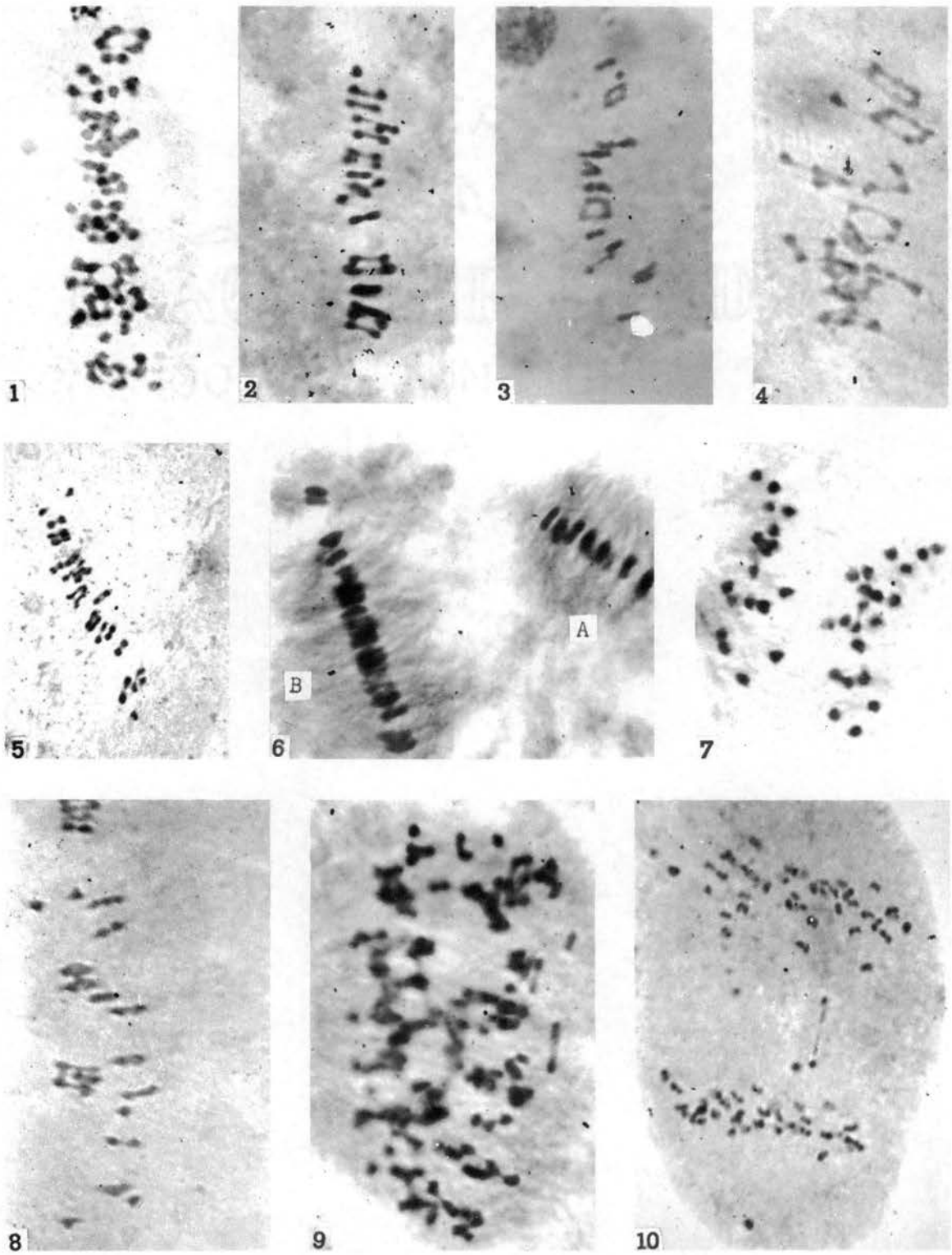
Figure 7. Anaphase I of Combine Kafir-60 X Johnsongrass with lagging bivalents, 2n = 40.

Figure 8. Metaphase I of Martin X Sorghum almum, 2n = 40.

Figure 9. Anaphase I of Martin X Johnsongrass, 2n = ca 180.

Figure 10. Anaphase I of Martin X Sorghum almum, with Bridge, 2n = 80.

PLATE I



LEGEND OF PLATE II

Figure 11. Anaphase I of Combine Kafir-60 X Johnsongrass, with 40 lagging univalents, 2n = ca. 120.

Figure 12. Anaphase I of Combine Kafir-60 X Johnsongrass, with lagging bivalents, 2n = 40.

Figure 13. Metaphase I of Martin, 2n = ca 160.

Figure 14. Metaphase I of Combine Kafir-60, 2n = 20.

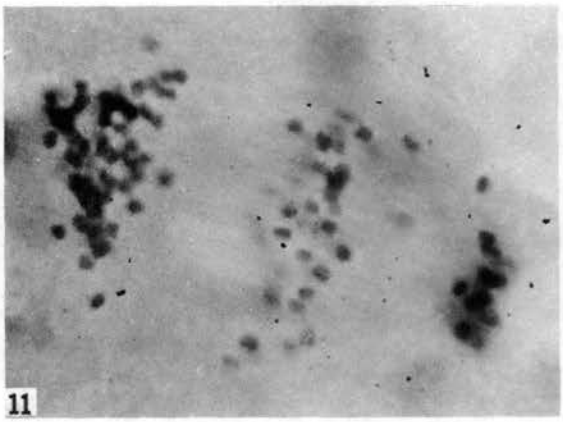
Figure 15. Metaphase I of Combine Kafir-60 X Johnsongrass, 2n = 30.

Figure 16. Anaphase I of Dwarf Redlan X Johnsongrass, 2n = 40.

Figure 17. Metaphase I of Dwarf Redlan X Johnsongrass, with secondary associations (arrow), 2n = 20.

Figure 18. Diakinesis of Combine Kafir-60 X Sorghum almum, 2n = 20.

PLATE II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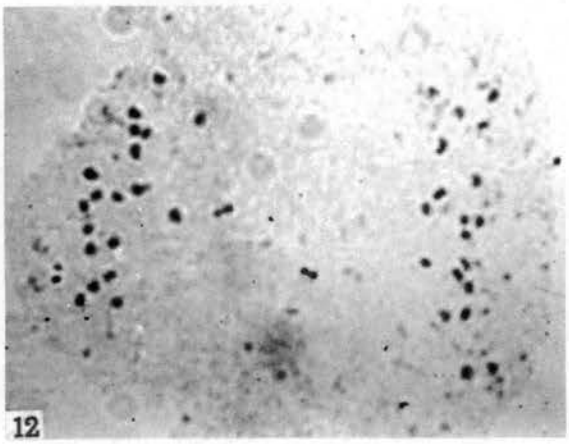
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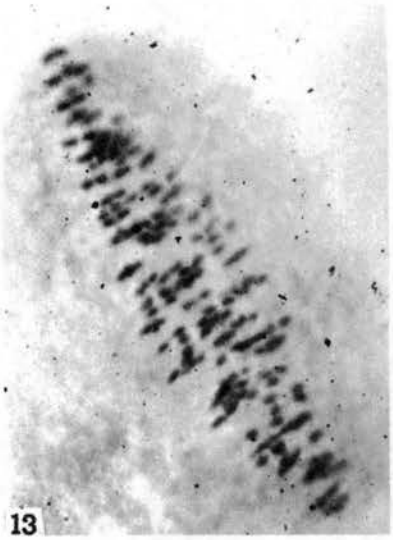
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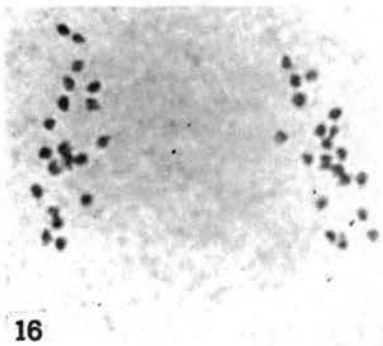
14


15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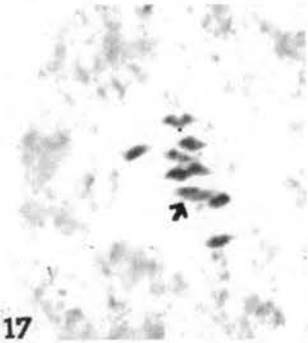
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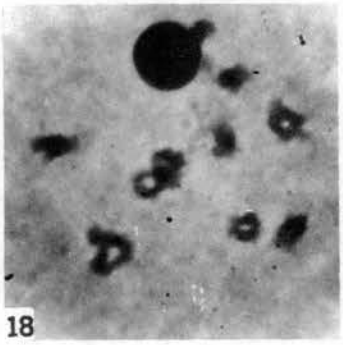
18

microsporocytes were observed in Combine Kafir-60, Johnsongrass, and Sorghum almum. The diploid parents that had polyploid microsporocytes were the females in all of the 2n = 40 hybrids. Plate II, figure 13 shows a polyploid microsporocyte cell of Martin.

Discussion

The meiotic chromosome configurations of Johnsongrass have been studied rather extensively (Huskins and Smith, 1932; Garber, 1941; Karper and Chisholm, 1936; Hadley, 1953; Endrizzi, 1957; and Celarier, 1958, 1959). All agree that Johnsongrass is 2n = 40. Their reports differ in the frequency of univalents, trivalents, tetravalents, and hexavalents. Celarier (1958) proposed the disagreements were the result of secondary associations of bivalents causing tetravalents and hexavalents. The reports agreed that in most instances anaphase and telophase I were regular with 20:20 distribution of the chromosomes.

The results of this study agree with the above reports. Good bivalent pairs were common, but univalent, trivalent, and hexavalent pairings were rare. The frequency of tetravalents averaged slightly more than one per cell. This indicated that if Johnsongrass were a segmental allopolyploid with the genome formula of 2 /(AB) (AC)/, as suggested by Hadley (1953), it had many segments homologous to S. vulgare. A more likely possibility was that Johnsongrass was an allopolyploid that had a gene controlling bivalent pairing. Riley, et al. (1961) described this phenomenon in hexaploid wheat. The absence of chromosome markers made the proof of such a hypothesis difficult in Sorghum. However, the presence of so many bivalent pairs in Johnsongrass tended to support this. If this hypothesis is true, the chromosomes of the genomes of

Johnsongrass may be homologous and not of the segmental allopolyploid relationship as set forth by Hadley (1953).

Sorghum almum had 2n = 40 chromosomes (Saez, 1949; Endrizzi, 1957; and Celarier, 1958). This species was more variable than Johnsongrass. All of the workers reported a high frequency of lagging bivalents at anaphase I. Univalents, trivalents, and tetravalents occurred more frequently than in Johnsongrass. They also agreed that the number of bivalents was usually less than in Johnsongrass.

The results of the present chromosome association study of S. almum tended to support the discussion set forth for Johnsongrass. The higher frequency of tetravalents may indicate that the chromosomes of S. almum were not as homologous as those of Johnsongrass, or that the bivalent pairing genetic control mechanism was not as effective in Johnsongrass

The cytological behavior of several varieties of S. vulgare have been described (Karper and Chisholm, 1936; Endrizzi, 1957; Magoon, et al., 1964). All agreed that meiosis of S. vulgare was regular and that there was little variation from ten bivalents. The varieties of S. vulgare used in this study had regular bivalent pairing. The only abnormality was the occurrence of polyploid microsporocytes and unreduced eggs, to be discussed later.

The hybrid of a cross between diploid (2n = 20) and tetraploid (2n = 40) sorghums would be expected to be 2n = 30. In this study, as well as in other reports (Endrizzi, 1957; and Hadley, 1957), 40-chromosome plants were common. The 40-chromosome plants were assumed to be the result of fertilization of unreduced eggs. The mechanism causing unreduced eggs is not known. The high frequency of polyploid pollen mother cells as shown in Table VIII indicated that something

was amiss, either in the premeiotic divisions or in the meiotic divisions.

The nature of the frequency of unreduced eggs in the hybrids indicated the presence of genetic control for this response. In the summary of results in Table VIII, the frequency of unreduced eggs was highest when cytoplasmic male-sterile Dwarf Redlan was the female, intermediate when Martin was the female, and absent when cytoplasmic male-sterile Combine Kafir-60 was the female. This showed a definite varietal difference in the ability to produce unreduced eggs. If this phenomenon is genetically controlled, Combine Kafir-60 could be homozygous for the absence of the character and Dwarf Redlan homozygous for the character. The character may be controlled by one or more genes and their modifiers.

The presence of polyploid microsporocytes appeared related to the presence of unreduced eggs. From Table VIII it can be seen that those hybrids resulting from unreduced eggs had polyploid microsporocytes. The only exceptions to this were the 100-chromosome plant of Martin X S. almum, and the 30-chromosome hybrids of Combine Kafir-60 X Johnsongrass. This relationship suggested the same type of mechanism that resulted in unreduced eggs, may also control the production of polyploid microsporocytes in the F_1 and parents. However, the breakdown of cell walls of the microsporocytes could also explain the presence of polyploid microsporocytes.

The 30-chromosome hybrids were what one would expect in a cross between 20- and 40-chromosome parents. The high frequency of bivalents supported the genetic controlling mechanism for pairing as suggested. The presence of at least one hybrid cell containing 10 III (plant 2187) indicated that the chromosomes could pair as an autopolyploid. This proved that there was much homology between the chromosomes of the

species crossed. Thus, one should expect multivalent association. The fact that such associations were rare suggested the presence of a genetic controlling mechanism. The mechanism could be genetic as already set forth or could be the result of preferential pairing as described by Darlington (1937).

The presence of the 20-chromosome plants could be explained by 1) the result of selfing, 2) crosses with other 20-chromosome sorghums, and 3) crosses with the tetraploid pollinators. Shedding of pollen by cytoplasmic male-sterile lines of S. vulgare occurred occasionally. The validity of calling these plants selfs was questioned when F_2 and F_3 progenies of these plants segregated for characters foreign to the female parents, see Appendix Table III.

The presence of pollen barriers such as trees and hills tended to reduce the possibility of foreign pollen entering the area. If foreign pollen did enter the crossing block area, the frequency of 20-chromosome plants in all crosses should be higher. This should be true due to similarities in blooming dates of the female parents.

The formation of 20-chromosome progeny as the result of crossing with the tetraploid pollinators is the third alternative. The 30-chromosome zygote during its development into a mature plant could undergo the loss of ten chromosomes. Since the 20 chromosomes of the hybrid are sufficiently homozygous to form ten bivalents, there must surely be some method by which the ten chromosomes showing the least homology to the others are the ones lost.

Plant 2185 had 26 chromosomes. The origin of this plant must be similar to that described above. The loss of at least four chromosomes

during early divisions of the zygote seemed a possibility.

Plant 2115 had cells containing microsporocytes of 20, 40, 60, and 80 chromosomes. This situation may be the result of cell wall breakdown of the microsporocytes. This was observed by Damon (1961) to occur in S. vulgare (var. Wheatland and Martin). However, if the formation of unreduced eggs were under the control of genetic factors, polyploid microsporocyte formation could conceivably be under genetic control. The presence of unusual numbers of polyploid microsporocytes in 2115 and 2178 may indicate a breakdown in the genetic control.

The chromosome number of the hybrids studied in this problem seemed to be influenced more by the female than the pollen parent. With both pollinators, Dwarf Redlan as the female resulted in the most 40-chromosome hybrids and the least number of 20-chromosome hybrids. The majority of the plants when Combine Kafir-60 was the female had 20-chromosomes, regardless of the pollinator. Martin was intermediate. The only difference observed between the hybrids produced by Johnsongrass and S. almum was that the number of bivalents was often slightly less when S. almum was the pollinator. However, the maximum associations of the hybrids of both pollinators were similar. This indicated that the two pollen parents used in this study had similar chromosome relationships.

The relationships of the Sorghum species used in this investigation are complex. The following steps seem necessary to further the understanding of their relationships: 1) determine chromosome associations of the reciprocal crosses; 2) clarify the origin of the 20-chromosome plants; 3) study further the mechanism involved in production of unreduced eggs; and 4) study megagametogenesis in sorghum thoroughly.

CHAPTER IV

CARYOPSIS COLLAPSE IN CROSSES BETWEEN DIPLOID AND TETRAPLOID SORGHUMS

The degeneration of endosperm and embryo results in non-viable seeds in many interspecific crosses. Stebbins (1958) indicated that such failure in obtaining viable seeds in interspecific crosses was common and is significant in an understanding of evolutionary divergences.

Several investigators have reported a low frequency of viable seeds in Sorghum vulgare Pers. X Johnsongrass crosses. This collapse of the caryopses of sorghum crosses was investigated by Panchal[1] and reported by Casady and Anderson (1952), Butany[2], Wilhite[3] and by McClure[4]. The purpose of this study was to determine the

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[1]Pnachal, Y. C. 1962. Study of frequency of seed setting and early embryogenesis in the interspecific cross Sorghum vulgare (Pers.) X Sorghum halepense (L.) Pers. Unpublished M. S. Thesis. Kansas State University.

[2]Butany, Washo Tikamdas. 1955. Chromosome behavior of five sorghum species and the use of embryo culture in growing their interspecific hybrids. Unpublished M. S. Thesis. A and M College of Texas.

[3]Wilhite, J. Ronald. 1959. Frequency of interspecific crossing utilizing cytoplasmic male-sterile lines of Sorghum vulgare exposed to pollen from Sorghum halepense. Unpublished M. S. Thesis. Oklahoma State University.

[4]McClure, Walter Jay. 1962. Frequency of interspecific crossing between Sorghum vulgare Pers. and Sorghum halepense (L.) Pers., and between Sorghum vulgare Pers. and Sorghum almum Parodi. Unpublished M. S. Thesis. Oklahoma State University.

cause and time of failure in caryopsis development. This study was divided into two segments: 1) the histology of embryogenesis, and 2) the gross rate of caryopsis development.

Materials and Methods

The parents used in this study consisted of two varieties of Sorghum vulgare (var. Wheatland and Redlan) and Johnsongrass. A and B lines[5] of Wheatland and Redlan were used to facilitate crossing. All plant material was grown in the greenhouse in the winter-spring of 1963-64 and 1964-65.

The material for the embryogenesis study was obtained from pollination of A Wheatland with B Wheatland and Johnsongrass. The A Wheatland X B Wheatland will frequently be referred to as "the check cross." The A Wheatland X Johnsongrass will occasionally be referred to as "the interspecific cross." Ten ovaries of each cross were collected and fixed in Craf III solution. The collections were made the day following pollination and at three-day intervals following the first collection. The fixed materials were dehydrated in a tertiary butyl alcohol series and infiltrated with paraffin according to a procedure modified from Sass (1945). Sections were cut 10-12 microns thick and stained in a hemalum, safranin-fast green series. See Appendix for details. Measurements of the dimensions of embryo, ovule, and ovary were made by means of an ocular micrometer. Photomicrographs were made of all stages using the equipment described in the previous chapter with Agfa IFF and Kodachrome II film.

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[5]A lines are cytoplasmic male-sterile, B lines are male-fertile but male-sterile producing.

The experiment on the gross rate of development, as indicated by weight of the caryopses, involved the following crosses:

A Wheatland X B Wheatland

A Wheatland X B Redlan

A Wheatland X Johnsongrass

A Redlan X B Wheatland

A Redlan X B Redlan

A Redlan X Johnsongrass

Ten ovaries of each cross were collected the day following pollination and on every second day thereafter. At least three crosses of each combination were made. The collections were fixed in standard Carnoy's fluid (6:3:1). The weights of the ovaries were determined by a model 5 B Mettler Analytical Balance. Statistical comparisons were made between crosses on each female for each collection date (Snedecor, 1956).

Results

The histology of embryogenesis involved measurements of embryo, endosperm, and ovule size of the crosses at the different stages (Table X). Figures 1 and 2 show the relative sizes of the caryopses of both crosses on each of the collection dates.

The length and width of the embryo of A Wheatland X B Wheatland increased rapidly and constantly throughout the collection period. The embryo of A Wheatland X Johnsongrass increased in size more slowly and became ovate instead of elongate as did the check cross. This is illustrated in Plate III, figure 23, and Plate IV, figure 29.

Although the embryo of the interspecific cross increased in size, it failed to differentiate into the plumule-radical axis. The check

TABLE X

REPRESENTATIVE MEASUREMENTS OF OVULES, ENDOSPERM AND EMBRYOS OF A WHEATLAND X B WHEATLAND AND A WHEATLAND X JOHNSONGRASS

Age of Embryo	Ovule		Endosperm				Embryo			
	Length		Width	Length	Width	Length	Width	Length	Width	Length
	Wheat X[a]	John X[b]	Wheat X[a]	Wheat X[a]	John X[b]	John X[b]	Wheat X[a]	Wheat X[a]	John X[b]	John X[b]
	μ	μ	μ	μ	μ	μ	μ	μ	μ	μ
14 hrs.	870.00	1200.00	-------	-------	-------	-------	-------	-------	-------	-------
2 days	1005.00	2100.00	-------	-------	-------	-------	42.00	108.50	35.00	61.25
5 days	1425.00	2587.50	232.50	630.00	292.50	532.25	87.50	115.50	54.25	122.50
8 days	3000.00	2775.00	750.00	1470.00	412.25	712.50	45.50	148.75	56.00	168.00
11 days	3525.00	3300.00	1275.00	2475.00	675.00	1162.50	115.50	300.00	148.75	253.75
14 days	2850.00	2985.00	1350.00	2737.50	787.50	1275.00	300.00	1125.00	105.00	227.50
17 days	3750.00	2700.00	2062.50	3750.00	1350.00	2475.00	525.00	2250.00	183.75	350.00
20 days	4200.00	3900.00	2475.00	4200.00	1200.00	1200.00	675.00	3150.00	218.75	455.00
23 days	5100.00	3150.00	2700.00	5100.00	1050.00	1125.00	787.50	3000.00	225.00	555.00

[a]Wheat X refers to A Wheatland X B Wheatland hybrid.

[b]John X refers to A Wheatland X Johnsongrass hybrid.

LEGEND FOR PLATE III

Figures 20 to 26 show median sagittal sections of the developing caryopses of A Wheatland X B Wheatland; X 80.

Figure 19. Ovule and embryo sac of unfertilized A Wheatland; X 100.

Figure 20. Ovule and embryo sac fourteen hours after pollination.

Figure 21. Ovule and embryo sac seventeen hours after pollination.

Figure 22. Embryo and endosperm seven days after pollination.

Figure 23. Embryo and endosperm eleven days after pollination.

Figure 24. Embryo and endosperm fourteen days after pollination.

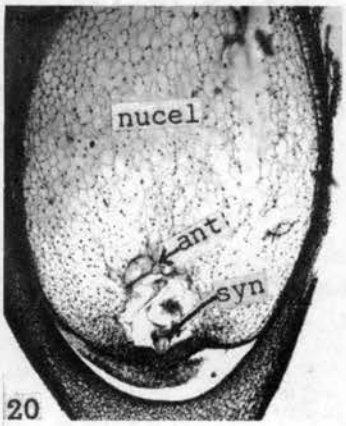
Figure 25. Embryo seventeen days after pollination.

Figure 26. Embryo twenty-three days after pollination.

nucel, nucellus; ant, antipodals; syn, synergids; endo, endosperm; emb, embryo; susp, suspensor; sct, scutellum; plum, plumule; col, coleoptile; ffl, first foliage leaf; gp, growing point; np, nodal plane; mx, metaxylem; pr, primary root; rc, root cap; coleor, coleorhiza.

PLATE III

19



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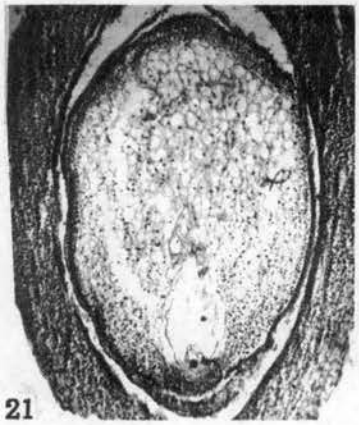
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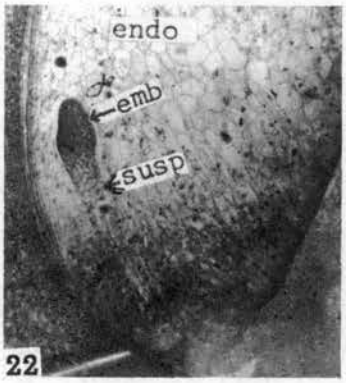

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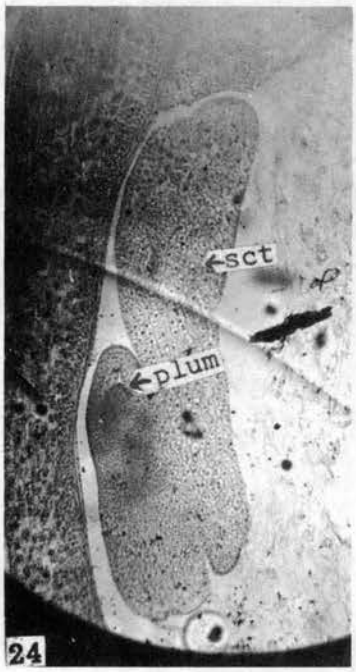

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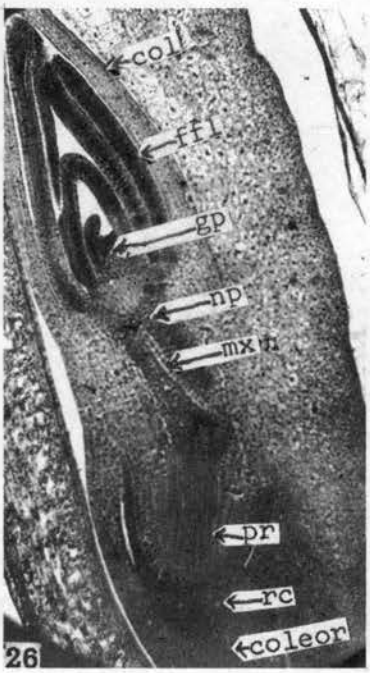


26

cross had developed a plumule-radical axis by the fourteenth day, see Plate IV, figure 30. The embryo of the check cross was well developed by the twentieth day and all the organs of a mature embryo were present by the twenty-third day, see Plate III, figure 26.

The endosperm of the check cross grew in length and width in rates that approached a geometric increase for the first fourteen days. The rate of increase in length and width of the A Wheatland X Johnsongrass cross was about one-half as fast as the check cross.

The endosperm of the check cross had filled the nucellar cavity by the eleventh day, whereas the endosperm of the interspecific cross never filled the nucellar cavity. By the eleventh day the endosperm of the interspecific cross occupied slightly more than one-third of the nucellar cavity and began to show indications of faulty cell divisions, see Plate IV, figure 29. The endosperm of the interspecific cross started disintegrating by the seventeenth day, see Plate IV, figure 31. The accumulation of starch was observed in the check cross by the eighth day, and by the twenty-third day the endosperm was completely filled with starch. Starch storage in the endosperm was not observed in the interspecific cross until the seventeenth day, and it was slight compared to the extensive starch storage of the check cross.

The length of the ovule of the interspecific cross increased faster than did that of the check cross for the first eight days. Following the eighth day the ovule of the check cross increased more rapidly than the interspecific cross. The size of the ovule of the interspecific cross changed very little after the eleventh day. Invaginations were observed in the ovary wall of the interspecific cross after the fourteenth day following pollination, see Figure 2.

LEGEND FOR PLATE IV

Figures 27 to 32 show median sagittal sections of the ovule of A Wheatland X Johnsongrass; X 80.

Figure 27. Ovule and embryo sac fourteen hours after pollination.

Figure 28. Ovule with embryo seven days after pollination.

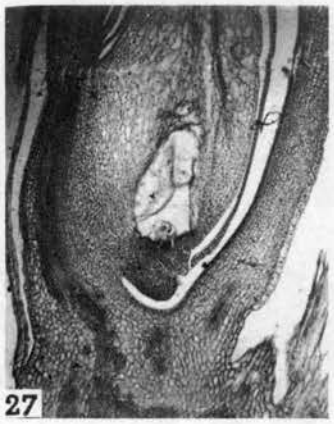
Figure 29. Embryo eleven days after pollination with long suspensor; Endosperm has anucleate cells (arrow a).

Figure 30. Embryo and endosperm fourteen days after pollination.

Figure 31. Embryo seventeen days after pollination, endosperm shows signs of disintegration (arrow b).

Figure 32. Embryo twenty-three days after pollination.

PLATE IV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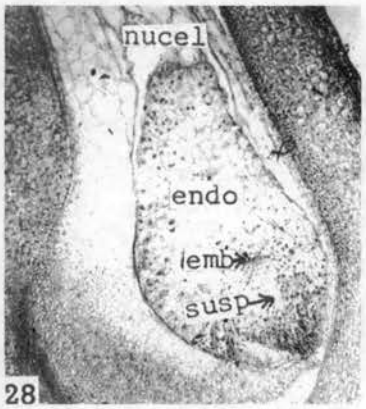

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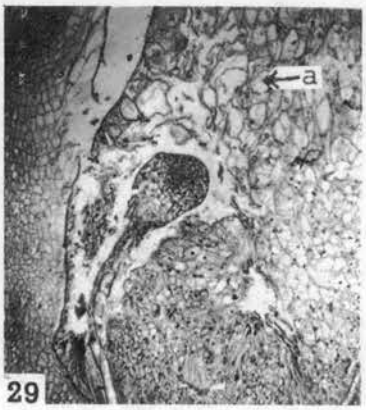

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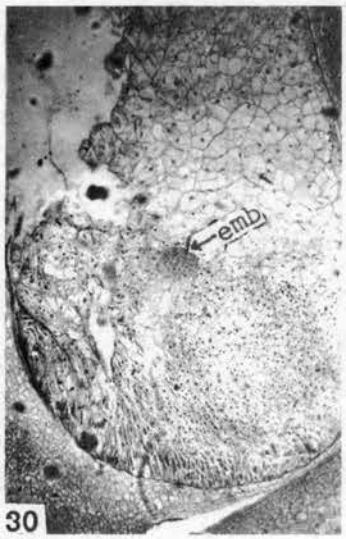

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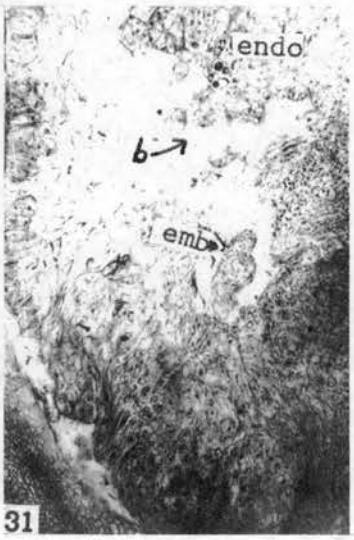

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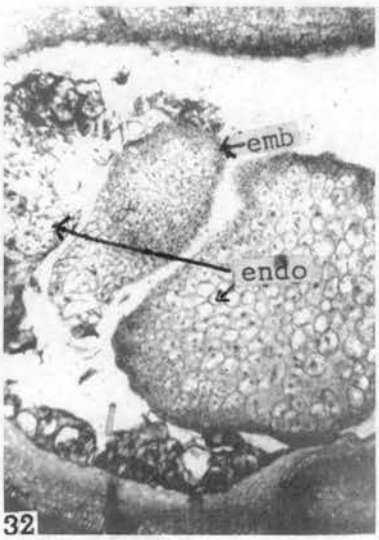

32

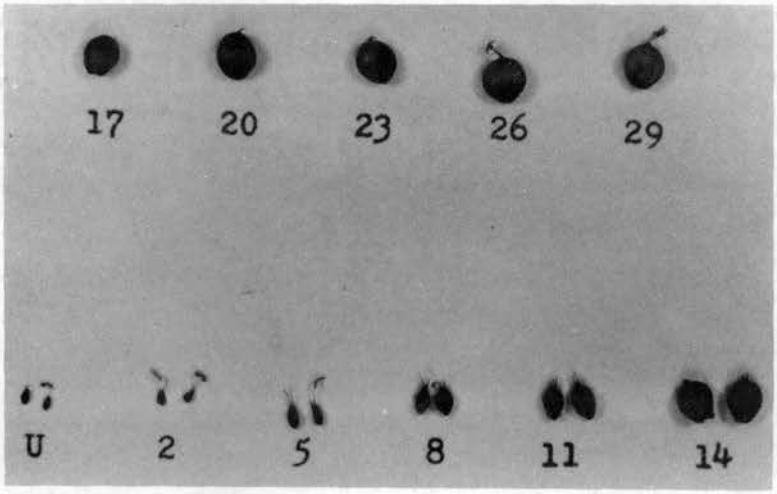

Figure 1. Relative size of developing caryopses on the collection dates of A Wheatland X B Wheatland.

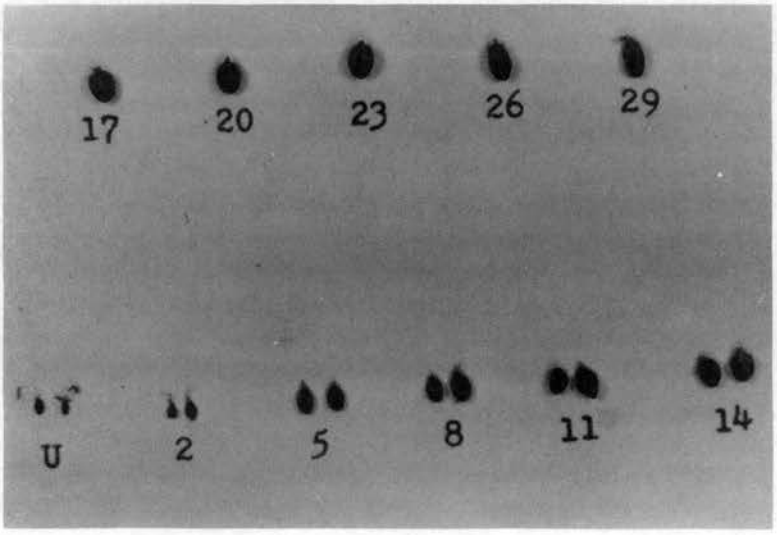

Figure 2. Relative size of developing caryopses on the collection dates of A Wheatland X Johnsongrass.

The gross rate of caryopsis development is illustrated by Figures 3 and 4 showing relative weights of caryopses of the crosses on each of the collection dates. In the crosses A Wheatland X B Redlan and A Wheatland X Johnsongrass (Figure 3), the initial ovary weight exceeded that of A Wheatland X B Wheatland. However, after the seventh day ovaries that resulted from Redlan and Johnsongrass pollination weighed less than those pollinated with Wheatland. After about the seventh day the Johnsongrass cross retarded in its development, and after about eleven days started to decline. Statistical analysis showed a significant difference in caryopsis weight between A Wheatland X B Wheatland and A Wheatland X Johnsongrass had developed by the thirteenth day. Mean squares of the ovary weight for each collection date and cross are shown in Appendix Table I.

The developing caryopses of A Redlan X B Wheatland paralleled those of A Redlan X B Redlan, see Figure 4. The A Redlan X Johnsongrass crosses also paralleled those of the A Redlan X B Redlan crosses until the seventh day. The developing caryopses of A Redlan X Johnsongrass then gained only slightly in weight reaching a peak on the thirteenth day. This was followed by a decrease in ovary weight during the remainder of the period. Statistical analysis indicated a significant difference in ovary weight between A Redlan X B Redlan and A Redlan X Johnsongrass had appeared by the eleventh day. The mean squares for each collection date and cross are shown in Appendix Table II.

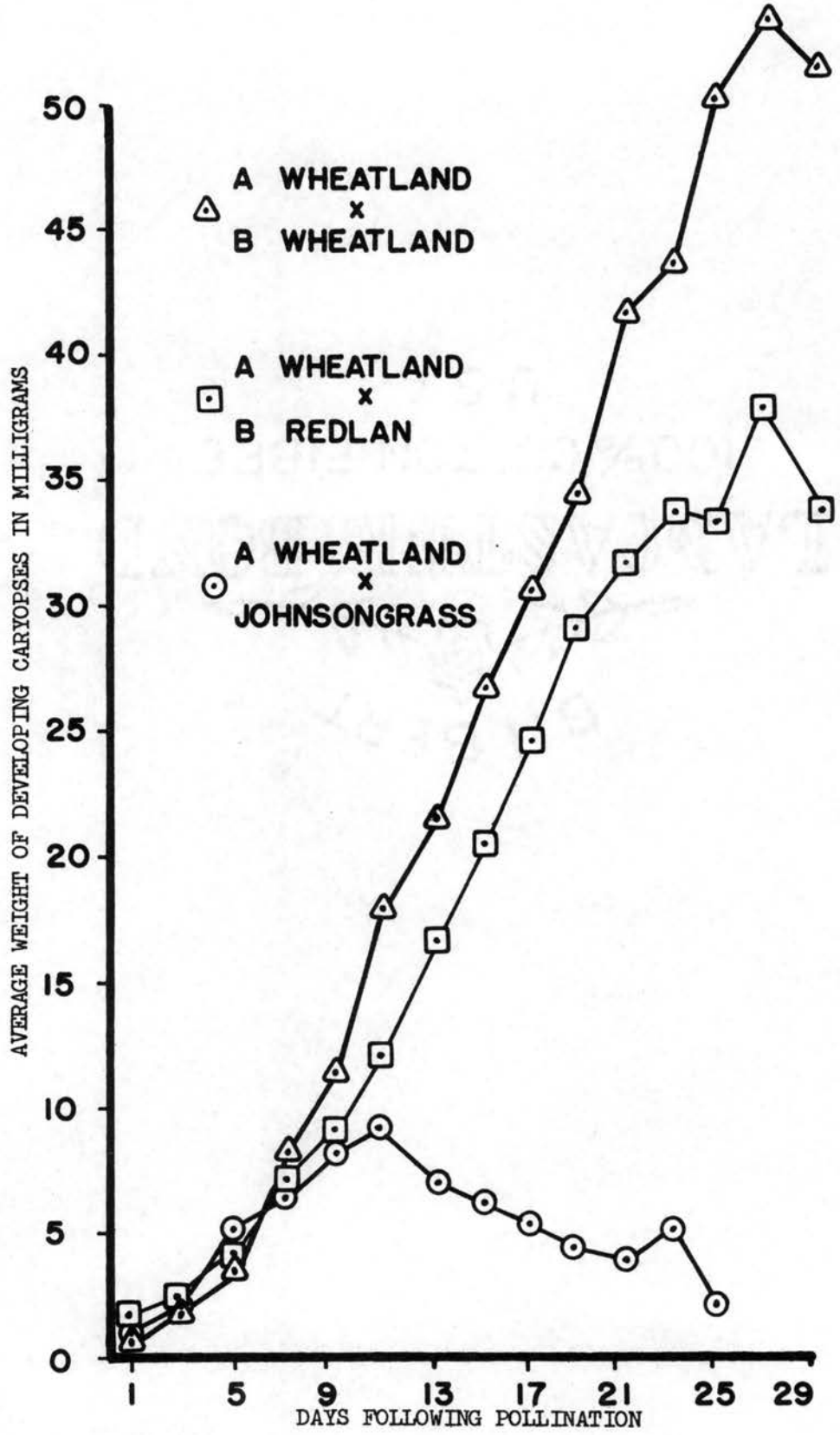

Figure 3. The average weight of developing caryopses on each collection date for each cross when A Wheatland was the female.

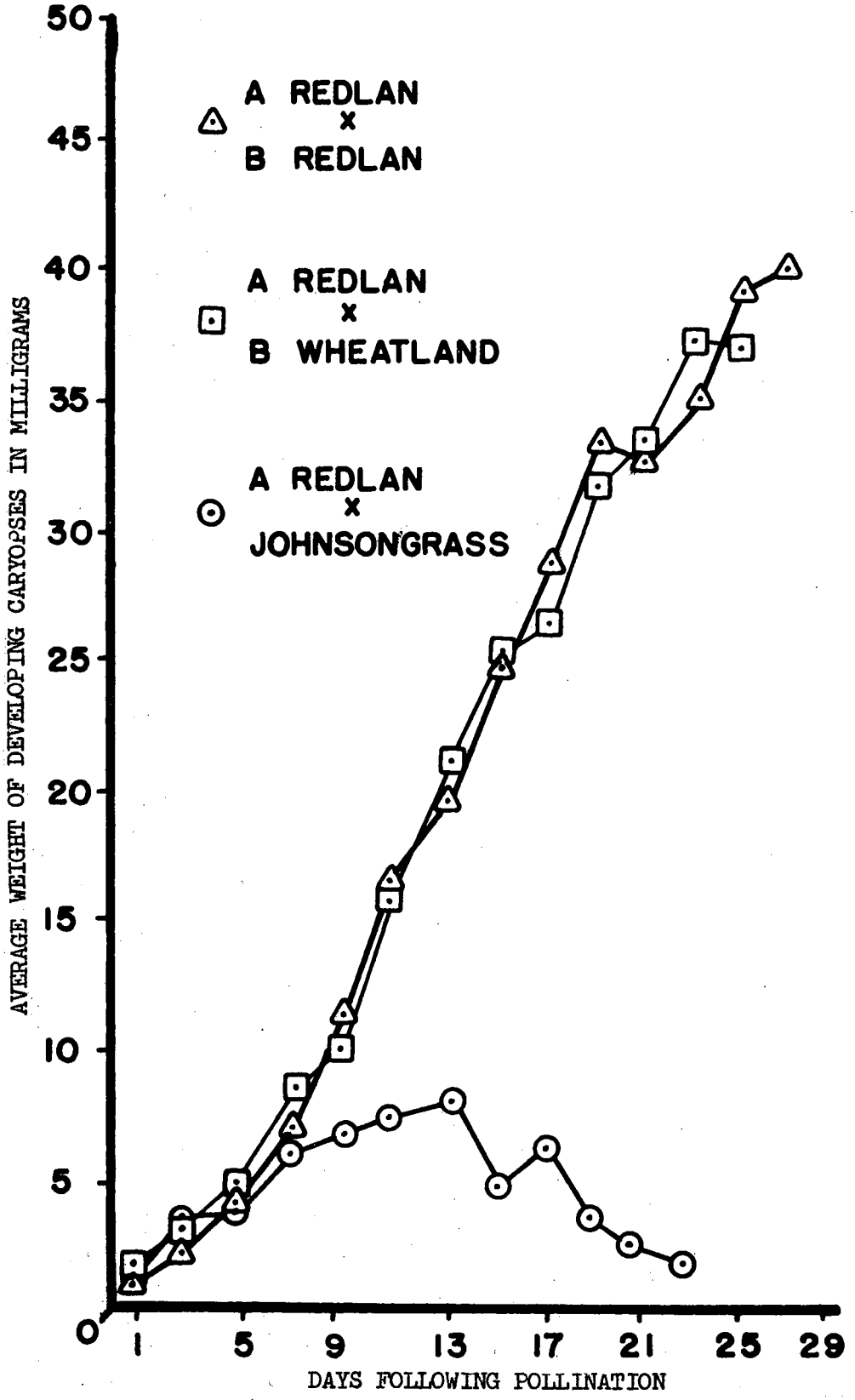

Figure 4. The average weight of developing caryopses on each collection date for each cross when A Redlan was the female.

Discussion

The early embryogenesis of Sorghum vulgare Pers. X Johnsongrass has been studied by Panchal[1]. He determined that the ovule length was doubled daily in Martin, Combine Kafir-60, and Redlan varieties of Sorghum vulgare. However, when Johnsongrass was used as a pollen parent in a cross with one of the varieties, the rate of increase in ovule length was only one-half that of the varieties. From the figures presented by Artschwager and McGuire (1949) in their study on embryogenesis of sorghum, the ovule length of S. vulgare was doubled during the first two days and changed little after this.

In the current study the development of the embryo of the interspecific cross and the check cross was similar for the first eleven days. Following this the embryo of the interspecific cross did not increase in size as extensively as did that of the check cross. The retardation in embryo development of the interspecific cross appeared related to the endosperm development.

The developing embryo obtains its nutrition from the endosperm; thus faulty endosperm development could result in inadequate nutrition for the developing embryo. The continuing growth of the embryo of this interspecific cross, but failure to differentiate properly seemed to indicate the lack of proper nutrition. The work of Van Oberbeck, et al, (1941) indicated that improper embryo development in interspecific crosses as described above could be the result of inadequate hormone production by the endosperm.

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[1]Panchal, Y. C. ibid.

During the early stages of development the endosperm derives much of its nutrition from the nucellus. As the endosperm of the interspecific cross utilized the nucellar tissue, no starch storage was observed and several anucleate cells were observed. This suggested that incompatibilities between the parental genes may have resulted in failure for starch formation and in abnormal mitosis. The cause of endosperm collapse in this study then appeared to be abnormal mitosis of the endosperm, the result of cytokinesis without nuclear division. A similar situation existed in Solanum species crosses (Beamish, 1955).

The results showed that immediately following pollination the interspecific cross had heavier ovaries and longer ovules than the check cross. This may be the result of interactions of the parental germ plasm. Early embryological development is dependent on maternal tissue and the presence of foreign germ plasm might act as a stimulus. However, as the incompatibilities, as suggested above, appeared and degeneration of the endosperm and retardation of the development of the embryo began, the ovaries of the interspecific cross decline in weight.

On the eleventh day after pollination the length of the endosperm and ovule were nearly identical in the check cross. This was due to the endosperm filling the nucellar cavity, and after this date any increase in size of the endosperm was reflected by a concurrent increase in size of the ovule.

On the basis of these observations one may expect some of the proembryos of the interspecific crosses to have the potential for development into mature plants. Stebbins (1950) stated that if maternal incompatibilities do exist one should be able to culture the excised embryos.

Butany[2] cultured excised embryos of interspecific crosses in sorghum; however, he did not try to culture embryos of S. vulgare X Johnsongrass. He found that the embryos should be at least seven days old before excising. Observations made during the current study indicated that embryos should be approximately seven to eleven days old. The embryo did not appear to have begun sufficient differentiation prior to seven days to survive excising and culturing. After eleven days, the embryo appeared too near starvation to live in culture.

In a previous study McClure[4] found a rather high frequency of seed development in S. vulgare X Johnsongrass and S. vulgare X S. almum. In chapter III, 30- and 40-chromosome hybrids were described. The hybrid of an interspecific cross between $2n = 20$ and $2n = 40$ parents should be $2n = 30$. The rare occurrence of such hybrids may indicate that they do not develop into mature viable caryopses. Culturing of excised embryos could overcome the maternal incompatibilities involved in such failures. Many of the cultured embryos should have the chromosome number of 30.

The mechanisms of caryopsis collapse have been discussed; however the origin of the incompatibilities has not been investigated. In order to determine the origin of the incompatibilities involved, culturing of excised embryos of both the crosses described in this study and reciprocal crosses should be attempted.

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[2]Butany, Washo Tikandas, ibid.

[4]McClure, Walter Jay. ibid.

CHAPTER V

POLLEN GRAIN SIZE OF *SORGHUM VULGARE* PERS., *SORGHUM ALMUM* PARODI, AND JOHNSONGRASS

The use of pollen grain size as a measure of chromosome number would be a fast and easy procedure if it proved feasible. Work in *Andropogoneae* (Gould 1937) indicated that the chromosome number could be determined by relative pollen size. The purpose of this study was to determine the reliability of such a tool in sorghums.

Materials and Methods

Pollen collections were made from the diploid species, *S. vulgare* (var. Redlan, Dwarf Redlan, Combine Kafir-60, and Wheatland) and two tetraploid species, *S. almum* and Johnsongrass. Heads were cut off the plants the day before anthesis and placed in a container of water. The following morning pollen collections were made. One slide of each variety or species was made on three different days.

The collections were made by shaking the pollen onto a slide covered with methyl green-glycerine jelly. The methyl green-glycerine jelly was made according to Wodehouse (1959).

The size of the pollen grains was measured at the widest diameter of one hundred and fifty grains with an ocular micrometer. Statistical analysis using average size of pollen grains was made. Each slide was considered a replication. The lack of sufficient pollen prevented

using Wheatland in the statistical analysis.

Results

The mean and range of size of the pollen grains of each variety and species are shown in Table XI. Dwarf Redlan had the smallest size pollen grains and Combine Kafir-60 the largest. The range in size of all overlap to such an extent that no differences were observed. Photomicrographs of the pollen of each variety are shown in Plate V.

Analysis of variance of means is shown in Appendix Table III. There were no significant differences in size between the varieties and species used in this study.

TABLE XI

POLLEN GRAIN SIZE OF SELECTED DIPLOID AND TETRAPLOID SORGHUMS

Variety	2n Chromosome Number	Mean Size	Range
		μ	μ
Combine Kafir-60	20	47.47	38.50 - 52.50
Dwarf Redlan	20	43.84	37.68 - 49.58
Redlan	20	44.53	39.67 - 51.33
Wheatland	20	44.83	36.75 - 52.50
Johnsongrass	40	44.54	32.08 - 51.92
Sorghum almum	40	44.68	35.58 - 51.92

LEGEND FOR PLATE V

Figures 33 to 36. 2n chromosome number is 20.

Figures 37 to 38. 2n chromosome number is 40.

Figure 33. Pollen grains of Dwarf Redlan.

Figure 34. Pollen grains of Combine Kafir-60.

Figure 35. Pollen grains of Redlan.

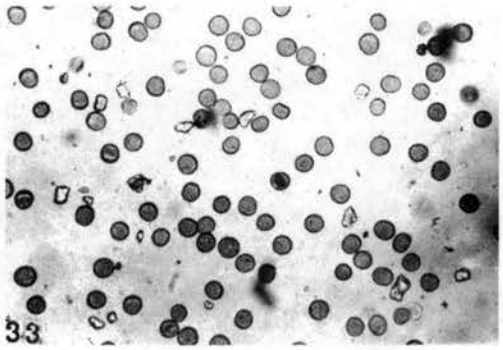
Figure 36. Pollen grains of Wheatland.

Figure 37. Pollen grains of Sorghum almum.

Figure 38. Pollen grains of Johnsongrass.

X ca 65

PLATE V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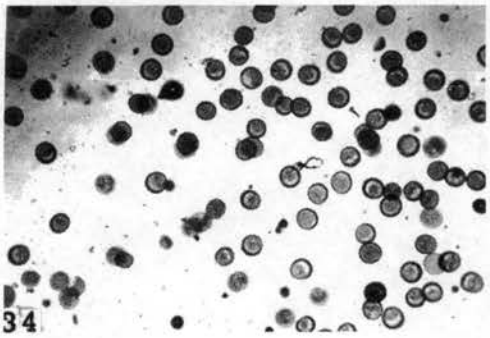
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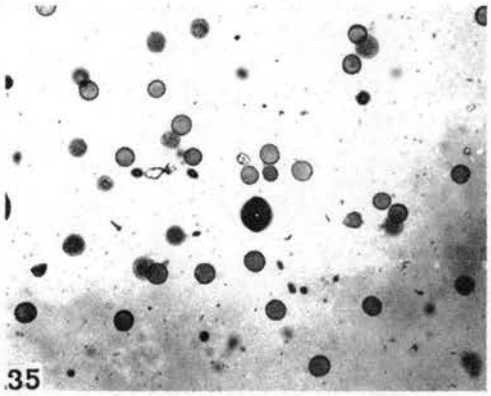
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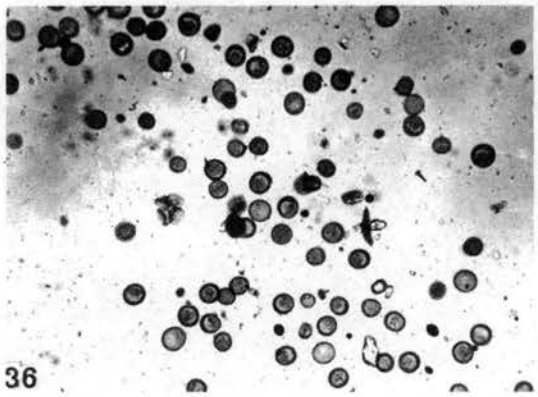
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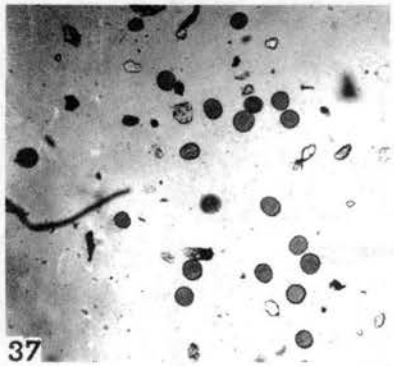
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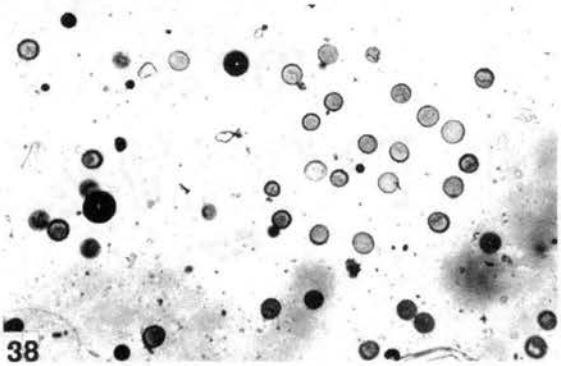
38

Discussion

The results obtained showed that the use of pollen grain size as a measure of chromosome number in sorghums was not a valid tool. The work of Chin (1946) suggested that the feasibility of using pollen size as an indication of chromosome number was of a different approach. He doubled the chromosome numbers of S. vulgare (var. Hegari) with colchicine. The mechanism of doubling chromosome number by colchicine does cause corresponding increase in cell size of the treated plant. This supports Erdtman's (1952) statement that tetraploid plants have larger cells than diploids of the same species.

Celarier and Mehra (1961) suggested pollen size and stomate size should be used together as a measure of chromosome number. In an experiment not reported here, stomate size of several parents and of F_1 hybrid plants was measured. The results were similar to those reported here on pollen size. Thus, the use of either pollen size or stomate size as an indication of chromosome size was not justified in Sorghum.

CHAPTER VI

SUMMARY AND CONCLUSIONS

A study was conducted to determine the nature of interspecific hybrids between Sorghum vulgare Pers., a diploid, and two tetraploid Sorghum species, Johnsongrass and Sorghum almum Parodi. The investigation was divided into three areas: 1) chromosomal associations of the interspecific hybrids and influence of the parents upon the chromosomal associations; 2) embryogenesis of the non-viable caryopses resulting from interspecific crossing; and 3) pollen grain size as an indication of ploidy.

1. Chromosomal associations investigation: The majority of hybrids had 40 chromosomes. Regular bivalent pairing was common. The number of bivalent pairs was highest when Johnsongrass was the pollen parent. The hybrids involving S. almum as a pollen parent had a higher frequency of multivalents than did those involving Johnsongrass. Genetic control of bivalent pairing was suggested.

The use of Johnsongrass as the pollen parent resulted in 67 per cent of the plants produced having 30- or 40-chromosomes. When S. almum was the pollen parent less than 50 per cent of the plants produced had chromosome numbers greater than $2n = 20$. The presence of 30-chromosome hybrids was more frequent when S. almum was the pollen parent.

The presence of polyploid microsporocytes was more frequent when Johnsongrass was the pollen parent. Cytoplasmic male-sterile Martin X

Johnsongrass had the highest frequency of polyploid microsporocytes; the only exception was plant 2178.

The 40-chromosome hybrids were considered to result from unreduced eggs. The mechanism involved in production of unreduced or polyploid eggs and microsporocytes appeared to be genetically controlled. The varieties of S. vulgare studied appeared to possess the characteristic to produce the unreduced gametes while Johnsongrass and S. almum did not.

2. Embryogenesis study: Fertilization and initial development of the ovary of the interspecific cross was similar to the check cross. The embryo and endosperm of the interspecific cross did not develop as rapidly as did the check cross. Following the eleventh day after pollination, the endosperm of the interspecific cross began to show signs of disintegration. The embryo of the interspecific cross did not differentiate into the plumule-radical axis; whereas the check cross had differentiated a plumule-radical axis by the fourteenth day.

The failure of the embryo of the interspecific cross to develop properly appeared to be related to endosperm development. The failure of proper endosperm development was attributed to abnormal mitosis of the endosperm. The abnormal mitosis was the result of cell division without nuclear division.

3. Pollen grain size: No significant differences in size of Sorghum vulgare (var. Martin, Redlan, Combine Kafir-60, and Wheatland), Johnsongrass, and S. almum pollen were observed. The use of pollen size was not a valid indication of chromosome number in the material used in this investigation.

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APPENDIX

SCHEDULE FOR SECTIONING DEVELOPING SORGHUM CARYOPSES IN PARAFFIN

Developing caryopses of sorghum were placed in Craf III killing fluid, and then evacuated with an aspirator. The caryopses were stored for at least twenty-four hours. Caryopses older than ten days were stored for at least two days; three or four days may be more desirable.

Dehydration in tertiary butyl alcohol was performed according to the following series.

Dehydration Schedule

Series No.	Water	95% Ethyl Alcohol	Absolute Alcohol	Tertiary Butyl Alcohol
1	95	5		
2	85	15		
3	75	25		
4	65	35		
5	55	45		
6	40	50		10
7	30	50		20
8	20	45		35
9	10	40		50
10			25	75
11				100
12				100
13				100

The caryopses were washed with water before placing in the first dehydration solution. The material was allowed to remain in each solution two hours, and overnight in the anhydrous tertiary butyl alcohol.

The addition of slightly less than 10% chloroform to the last change of tertiary butyl alcohol, increased the specific gravity sufficiently to float paraffin. If a 10% solution of chloroform was used, the younger ovaries would float.

The solvent was saturated and the caryopses infiltrated with a mixture consisting of 100 grams paraffin, 10 grams tissuemat, and 1 gram bees-wax. At intervals of 4 hours one half of the solvent-wax solution was decanted and replaced with wax. After four such changes all of the wax was decanted and three complete changes of wax were made; the material was then cast into paper molds.

The embedded caryopses were sectioned by using a rotary microtome. Single-edge razor blades with the blunt edge guard removed proved satisfactory for cutting the material. The sections were cut 10-12 microns in thickness. Occasionally the older material was soaked overnight in water at 35° C to soften the tissues.

The following staining schedule proved satisfactory for study and photography. This staining series was adequate for color and for black-and-white photography.

Staining Schedule

Series No.	Solution	Time	Series No.	Solution	Time
1	Xylene	10 min.	13	H_2O	rapidly
2	Xylene	10 min.	14	30% Alcohol	rapidly
3	Absolute alcohol	2 min.	15	50% Alcohol	rapidly
4	95% Alcohol	2 min.	16	70% Alcohol	rapidly
5	70% Alcohol	2 min.	17	95% Alcohol	rapidly
6	50% Alcohol	2 min.	18	Fast Green in 95% Alcohol	2-3 sec.
7	30% Alcohol	2 min.			
8	Dist. H_2O	2 min.	19	Absolute Alcohol	1 min.
9	Dist. H_2O	2 min.	20	Absolute Alcohol	1 min.
10	Mayer's Hemalum	10 min.	21	Absolute Alcohol	1 min.
11	Distilled H_2O		22	Carbol-Xylene	2 min.
12	Aqueous Safranin	10 hours	23	Xylene	2 min.
			24	Xylene	2 min.
			25	Xylene	2 min.

APPENDIX TABLE I

MEAN SQUARES FOR OVARY WEIGHT ON EACH COLLECTION DATE
WHEN A WHEATLAND WAS THE FEMALE

Source of Variation	df.	Days after pollination								
		1	3	5	7	9	11	13	15	17
Total	8	0.1946	0.1858	1.0933	1.9287	9.4834	24.0846	51.4558	84.8020	146.8183
Between	2	0.3424	0.0370	2.7317	0.9724	10.0834	53.9360	145.5722*	303.6867**	540.6060**
Within	6	0.1453	0.2354	0.5472	2.2474	9.2834	14.0035	20.0837	11.9447	15.5575

* indicates significance at the 0.05 level.
** indicates significance at the 0.01 level.

APPENDIX TABLE II

MEAN SQUARES FOR OVARY WEIGHT ON EACH COLLECTION DATE
WHEN A REDLAN WAS THE FEMALE

Source of Variation	df.	Days after pollination								
		1	3	5	7	9	11	13	15	17
Total	8	0.2520	0.6040	1.3182	2.0340	10.3697	28.2117	51.8032	119.8598	121.9117
Between	2	0.0975	0.5262	0.5095	4.4037	20.9222	73.0930*	156.1660*	415.5536**	448.7474**
Within	6	0.3035	0.6300	1.5878	1.2440	6.8522	13.2560	17.0156	21.2952	12.9664

* indicates significance at the 0.05 level.
** indicates significance at the 0.01 level.

APPENDIX TABLE III

SEGREGATION OF MORPHOLOGICAL CHARACTERISTIC OF PROGENY OF SOME OF THE 20-CHROMOSOME PLANTS

Pedigree	Generation	Coleoptile Color		Midrib Color		Seed Color	
		Red	Green	Dry	Juicy	Red	White
Martin X *S. almum*	F_1	-	Green	-	Juicy	Red	-
	F_2	0	228	0	172	133	33
	F_3	0	120	0	100	23	137
-do-	F_1	-	Green	-	Juicy	Red	-
	F_2	0	156	0	117	80	37
	F_3	8	210	0	156	68	88
-do-	F_1	Red	-	Dry	-	Red	-
	F_2	153	202	122	23	108	37
	F_3	107	25	70	0	70	0
-do-	F_1	Red	-	Dry	-	Red	-
	F_2	60	72	0	77	56	21
	F_3	132	187	0	226	140	86
Martin		0	108	0	108	108	0
Dwarf Redlan X *S. almum*	F_1	Red	-	Dry	-	Red	-
	F_2	31	21	39	3	24	8
	F_3	145	39	142	15	124	33
Dwarf Redlan		0	142	0	71	71	0
Combine Kafir-60 X *S. almum*	F_1	-	Green	Dry	-	-	White
	F_2	80	83	89	21	0	110
	F_3	148	264	197	99	0	296
Combine Kafir-60 X Johnsongrass	F_1	-	Green	Dry	-	-	White
	F_2	125	54	134	25	0	159
	F_3	52	43	46	25	0	71
Combine Kafir-60 X Johnsongrass	F_1	-	Green	Dry	-	-	White
	F_2	54	46	49	11	0	60
	F_3	30	144	59	66	0	155
Combine Kafir-60		0	121	0	56	0	56
Johnsongrass		Red	-	Dry	-	Brown	-
Sorghum almum		Red	-	Dry	-	Brown	-

APPENDIX TABLE IV

MEAN SQUARES FOR POLLEN SIZE OF THREE DIPLOID AND TWO TETRAPLOID SORGHUMS

Source of Variation	D.F.	S.S.	M.S.	F.
Total	14	63.2256	-	-
Replications	2	2.0455	1.0229	0.2220
Varieties	4	24.3226	6.0806	1.3198
Error	8	36.8526	4.6071	-

VITA

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