CYTOLOGICAL AND REPRODUCTIVE CHARACTERISTICS OF DIPLOID AND TETRAPLOID EASTERN GAMAGRASS PLANTS AND THEIR F_1 HYBRIDS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Eastern gamagrass, Tripsacum dactyloides (L.) L., is a very palatable, highly productive forage grass (Rechenthin, 1951). This monoecious, warm-season, native, perennial grass once flourished over extensive areas from Texas to Kansas and eastward to the Atlantic Ocean (Hitchcock and Chase 1951, Polk and Adcock, 1964). It has been eliminated from much of its former range by overgrazing (Rechenthin, 1951).

Eastern gamagrass grows in clumps reaching four feet in diameter (Polk and Adcock, 1964). It is usually found growing in isolated colonies along streams and in swales, in open areas of pine-oak forest, in the North American prairies, and the eastern coastal plains (de Wet et al., 1982).

Eastern gamagrass can be developed into a significant component of forage and beef production ecosystems (Vogel et al., 1985). It is highly productive where annual precipitation is 80 cm or greater, or when grown under irrigation. Adequate forage production can also be achieved in semi-arid regions that receive only 40 cm of annual precipitation. Eastern gamagrass produces forage earlier in the spring than most other warm-season grasses. It will also flourish in frequently flooded areas that are

not suitable for most other crops. This not only increases the productivity of such areas, but also stabilizes the sites and reduces erosion. The perenniality of gamagrass offers advantages over annual forages. These advantages are lower cultural energy requirements, less soil erosion potential, and lower production costs (Vogel et al., 1985).

The recent discovery of a diploid gynomonoecious sex form (GSF) (Dewald and Dayton, 1985) has contributed to expanded breeding efforts to improve eastern gamagrass as a forage. This newly discovered germplasm is expected to greatly enhance seed production, and seed quality of the species. A major goal in eastern gamagrass improvement is to transfer the GSF trait to the tetraploid level. A potential method of accomplishing this is by making diploid X tetraploid crosses and selecting F_1 hybrid plants resulting from fertilization of an unreduced egg. Before the GSF can be fully utilized in a breeding program, it must be investigated taxonomically, morphologically, and cytologically. Little information is available on the cytological behavior of diploid X tetraploid hybrids of Tripsacum dactyloides.

The objectives of this study were to: 1) confirm the chromosome number and study the meiotic behavior of selected parental diploid GSF plants and tetraploid normal monoecious accessions of eastern gamagrass, 2) cross GSF females X normal tetraploid monoecious males, 3) evaluate selected F_1 hybrids for chromosome number, pairing behavior

and occurrence of meiotic irregularities, and 4) determine seed germination, female fertility, and pollen stainability of the F_1 hybrids. This research is part of a larger effort to transfer the gynomonoecious characteristic to higher ploidy levels.

SELECTED LITERATURE REVIEW

The genus Tripsacum extends from the northcentral and northeastern United States south to Mexico and central America, the West Indies, and Bolivia and Paraguay in South America (Newell and de Wet, 1974b). Brink and de Wet (1983) recognized 16 species of Tripsacum, with T. dactyloides being the most widespread and morphologically variable species. Gamagrass has been studied extensively by taxonomists and geneticists for morphological and cytological clues to the evolution of maize (Mangelsdorf, 1974) and for its relationship to other Tripsacum species (Newell and de Wet, 1974a).

Wright et al. (1983) demonstrated a considerable range in agronomic traits in gamagrass and provided evidence of an abundance of variability available for genetic manipulation of the species. Kenna (1984) showed that variation for yield, forage quality, and basic fertility (percent of florets setting a live seed) is heritable and theoretically responsive to selection. Coyne and Bradford (1985) found significant differences in stomatal and chloroplast functions between strains of gamagrass in relation to water use efficiency, which suggests the feasibility of selection and breeding for improvement of the physiological parameters of gamagrass.

Cytologically, Tripsacum dactyloides is a polyploid complex and has some races with 18 pairs of chromosomes and others with 36 pairs (Anderson, 1944). "The species is characterized by functional diploid (2n=2x=36), triploid (2n=3x=54) and tetraploid (2n=4x=72) races" (de Wet, Harlan, and Brink, 1982). Farquharson (1954a) investigated chromosome numbers of twin tetraploid seedlings. She obtained 2n numbers of 36, 45, 54, 72, 90, and 108. "Diploids reproduce sexually, while polyploids are facultative gametophytic apomicts" (de Wet et al., 1983). Farquharson (1955) reported polyembryony and facultative apomixis in tetraploid forms. Farquharson (1955) wrote that Tripsacum demonstrates apomixis by frequently developing embryos without the occurrence of fertilization or pollination. However, pollination is necessary for endosperm development and complete seed formation (pseudogamous development) (Farquharson, 1955). de Wet et al. (1973) reported an equilibrium between sexual and apomictic mechanisms occurring in gamagrass polyploids.

Gutierrez (1974) wrote about some specific cytological findings. He stated that "diploid T. dactyloides (2n=2x=36) shows bivalent formation at prophase I and regularly produces cytologically reduced gametes". He also stated that triploids (2n=3x=54) possess irregular meiosis. Univalents and multivalents can be seen at prophase I and there may be missing chromosomes in the daughter nuclei at telophase I (Gutierrez, 1974). A contrasting observation

was reported by Anand and Leng (1964). They observed the formation of 27 bivalents in microsporocytes of a triploid clone of T. dactyloides that was obtained from Farquharson.

There are two basic cytological types of tetraploid T. dactyloides. One race exhibits normal chromosomal behavior during meiosis of microsporogenesis (de Wet, 1979). These plants reproduce sexually. In this type, chromosomes synapse into pairs and regularly produce cytologically reduced gametes (de Wet et al., 1972). The other race of tetraploid T. dactyloides is characterized cytogenetically as an autotetraploid (de Wet, 1979). Tetravalents are usually produced during meiosis in microsporogenesis (Newel and de Wet, 1974a), and they reproduce as facultative gametophytic apomicts (de Wet et al., 1973).

Tetraploids are usually distinguishable from diploids because the former has larger leaves, pollen grains, and rachis segments (Farquharson, 1954a). In most instances, tetraploids are slightly more robust than their neighboring diploids (Newell and de Wet, 1974b; Dunfield, 1986). In at least one location, triploids were found to be more vigorous in appearance than the tetraploids (Farquharson, 1955).

Successful hybridization between diploid and tetraploid forms of T. dactyloides was reported by Farquharson (1954b) and Randolph (1970). However, they did not report additional information about the characteristics

of the F_1 hybrids. The expected result of a diploid X tetraploid cross is a triploid, which is usually sterile (Burns, 1983). Dujardin and Hanna, (1988) described the cytology and breeding behavior of a partially fertile triploid pearl millet, Pennisetum glaucum (L.). The triploid pearl millet "was partially male fertile, with 67% stainable pollen, and partially female fertile". de Wet et al. (1972) reported Tripsacum triploids to be "very irregular in meiotic behavior. The triploids are partially sterile, and only cytologically unreduced gametes are functional. They may function sexually or develop parthenogenetically." Butler (1973) investigated megasporogenesis and embryo-sac development of gamagrass. He postulated that in some cases apomictic embryo sacs may develop from unreduced megaspores.

The normal inflorescence of T. dactyloides is monoecious. The upper two-thirds to three-fourths of the raceme is composed of male spikelets, and the lower portion of the raceme is composed of one to several female spikelets which are individually sunken into hardened rachis internodes (Hictchcock and Chase, 1951). The female spikelets are composed of a single fertile floret, while the male spikelets are composed of two functional florets, each containing three anthers.

The variant sex form, first discovered in 1981, is gynomonoecious with perfect spikelets in the distal 2-3 cm of each raceme and the pistillate spikelets below (Dewald

and Dayton, 1985). Spikelets, throughout the inflorescence, have well-developed ovaries. The basal solitary spikelets contain two fertile pistillate florets, as do each of the paired spikelets that are normally male. At the base of the paired spikelet section stamens are rudimentary, the glumes are indurated and the lowermost paired spikelets are set into cupules that become progressively more shallow in the distal portion. Toward the distal end, cupules are flat and inconspicuous, glumes are more chartaceous, and stamens are functional resulting in perfect flowers (Dewald, et al., 1987). When compared to the normal monoecious sex form (MSF), the prolific GSF has the potential to increase seed production 20 to 25 fold (Dewald and Dayton, 1985; Dewald and Jackson 1986). The GSF is entirely fertile. Dewald et al. (1987) crossed GSF I and GSF II with normal MSF diploids during 1982-1984. Hybrids were produced, selfed, intermated and backcrossed to GSF I and GSF II. The first generation hybrids were monoecious and fertile. "The progeny of selfed and intermated F_1 hybrids segregated 3 MSF to 1 GSF" (Dewald et al., 1987). The researchers concluded that the gynomonoecious trait was under recessive monogenic control.

MATERIALS AND METHODS

Plant Materials

The plant material used in this study consisted of two GSF and four MSF plants (Table 1). The two GSF plants were selected as female parents because of their potential to increase the seed production of eastern gamagrass. Both MSF plants had previously been reported to be diploid having 36 chromosomes. Three of the four MSF plants were selected to be used as the male parents because they had previously been reported as tetraploids, and also due to their desirable agronomic characteristics. The other MSF plant was also a tetraploid and was selected because it was thought to be highly apomictic.

Breeding Methods

During spring 1985, the two diploid GSF selections were used as females in crosses with four tetraploid MSF selections used as male parents. The crosses were made in the greenhouse at the OSU Agronomy Farm. Immature inflorescences were covered with glassine pollinating bags before stigma emergence. To prevent selfing, the bisexual portion of the GSF inflorescence was removed by clipping off the top 2.5 to 5 cm of the inflorescence before

anthesis. After stigma emergence, pollen was collected from the male donors and applied to the exposed stigmas of the females via a small paint brush. The heads were re-bagged to prevent contamination, and to contain the resulting seed. The inflorescences were harvested approximately one month following pollination. After allowing ample time to dry, caryopses were manually dissected from the cupulate fruitcases.

Germination Procedures

Seeds produced by GSF X MSF F_1 hybrids were evaluated for percent seed germination and hybrid seedlings were obtained using uniform germination procedures at Woodward, Oklahoma during the fall and winter of 1985. Caryopses were surface sterilized by soaking five minutes in a 1:1 solution of chlorox and distilled water followed by rinsing in distilled water. They were then placed on sterile agar media in individual 16 x 100 mm culture tubes which were capped to maintain an aseptic condition. The culture tubes were maintained at 21-27 °C for 21 days to permit germination. Seeds which did not germinate during this interval were placed in a vernalization chamber at 5-10 °C for 42 days, and returned to the 21-27 °C temperature for an additional 21 day period. Seedlings were transplanted from the culture tubes into individual 10 cm pots containing soil when they reached a height of 2.5 - 5.0 cm.

During May 1986, approximately 1500 F_1 hybrid plants were field transplanted on 1.12 m centers in a uniform nursery at the USDA-ARS, Southern Plains Range Research Station, Woodward, Oklahoma.

Agronomic Evaluation

During the summer of 1987, two year old F_1 plants were evaluated in the field for desirable agronomic traits and for the presence of the GSF characteristic. The two year old plants were scored for leaf width in comparison to the narrow leafed diploid female parents and wider leafed tetraploid male parents. The plants were independently scored by two experienced agronomists. All plants with leaf widths of 1.17 cm or less were grouped in the narrow category and all plants with leaf widths of 2.54 cm or greater were grouped in the wide category. All plants with leaf measurements between 1.17 cm and 2.54 cm were grouped in the intermediate category. The F_1 plants were also observed for the presence of the mutant GSF character expression. This F_1 hybrid population was also screened for female fertility by calculating the percentage of florets setting seed. This was done by collecting mature seed heads, and determining both the number of florets, and the number of mature open-pollinated (OP) caryopses. The mature OP caryopses were evaluated for percent germination during fall 1987 using the procedure outlined previously.

Male fertility of the F_1 hybrids was estimated by calculating the percentage of starch-filled pollen grains via a pollen grain stainability test. A sample of pollen grains was collected from each plant at anthesis. A small sample of pollen was placed on a glass slide and a drop of iodine-potassium-iodide solution was added. A cover slip was dropped into place to insure a mono-layer of cells. A few minutes were allowed to elapse, to permit the absorption of the stain, before viewing the slide. Three representative microscope fields were counted and averaged to estimate the percent pollen stainability of each plant evaluated.

Based on the results of the pollen stainability test, a representative sample of the selected hybrid population was collected for cytological evaluation.

Cytological Evaluation

Chromosome numbers of the parent plants were confirmed and their pairing relationships were recorded during 1985. During the summer of 1987, immature staminate inflorescences were collected from the selected F_1 hybrids for chromosomal evaluation. The immature inflorescences were fixed in a 5:2:1 modified Carnoy's solution made from 95% alcohol, glacial acetic acid, and chloroform. Anthers were squashed in acetocarmine stain. Chromosomes were counted by observing pollen mother cells at diakinesis to

early first metaphase. A detailed account of the cytological evaluation procedure that was used is outlined in the author's Masters Thesis (Dunfield, 1984).

RESULTS AND DISCUSSION

Crossing Results

The two GSF female plants were respectively crossed with each of the four MSF male plants, producing approximately 9,000 seeds (Table 2). Selected seeds were germinated, and they produced 1,550 hybrid F_1 plants.

Seed Germination

F_1

Mean germination of F_1 seed produced by GSF X MSF crosses was 28.6%. Significant differences ($P < 0.05$) were observed due to both male and female parents. However, the male by female interaction was not statistically significant ($P > 0.05$). GSF I and MSF 1008 transmitted higher percent seed germination to their F_1 progeny (Table 3).

Open-Pollinated

The mean germination of seed obtained from the open-pollinated F_1 plants was 17.9%. Only slight differences were observed, and they were not statistically significant ($P > 0.05$).

Agronomic Evaluation

The F_1 population was highly variable in regard to degree of vigor, leaf width, growth habit, and leaf blister susceptibility caused by larvae of the leaf beetle, Anisostena sp. Leaf widths of the F_1 hybrids were variable within all parental combinations tested. Over half (53.6%) of all F_1 hybrids had leaf width ratings intermediate between the narrow leafed (1.17 cm) diploid female parent and the wider leafed (2.54 cm) tetraploid male parent. Plants with narrow leaf width scores made up 18.4% of the F_1 hybrids; whereas 28.0% were scored in the wide leaf category (Figure 1). This variation was anticipated because the diploid females reproduce sexually (Burson et al., 1990) and genetic recombination promotes variation. The higher percentage of F_1 hybrids with wide leaf scores, compared to narrow leaf scores, may reflect the double complement of chromosomes received from the wide leaf tetraploid parent. All F_1 hybrids exhibited the monoecious sex form.

Many more F_1 plants produced seed and shed pollen than anticipated on the basis of cytological analysis. Seed set of 294 F_1 hybrid plants ranged from 0 to 53%, with a mean of 6.9% (Figure 2, Table 4). Most of the F_1 plants had less than 10%, and only one plant had more than 50%, seed set. However, the differences were not statistically significant ($P > 0.05$).

Stainable pollen in 477 F_1 plants ranged from 0 to 67% with a mean of 30%. Figure 3 illustrates 67% stainable pollen and Figure 4 illustrates 15% stainable pollen. Pollen stainability, indicated by the presence of starch, has been positively correlated with male fertility (Knox and Williams, 1986). Therefore, determining pollen stainability provides a quick method for screening large numbers of individual plants (Janssen and Hermsen, 1976). Figure 5 and Table 3 illustrate the frequency distribution of the percent stainable pollen of the F_1 population. Pollen stainability varied from 10 to 50% in most of the F_1 plants. Significant differences ($P < 0.05$) attributable to both male and female parents were found in the pollen stainability tests of the F_1 hybrids. However, male by female interaction was not statistically significant ($P > 0.05$) (Table 3). GSF I and MSF 55 transmitted higher percent pollen stainability to the F_1 progeny. There was no correlation between percent pollen stainability and percent seed set.

Cytological Evaluation

The chromosome numbers and the meiotic behavior of the two GSF plants and the four MSF plants used as parents were determined (Table 5). Both GSF I and GSF II were diploids (2n=2x=36) (Figures 6 and 7). Chromosome pairing in the diploids was strictly as bivalents. All four of the MSF plants used as males were tetraploids (2n=4x=72) (Figures

8, 9, 10, and 11). The chromosome configurations for the tetraploids ranged from univalents to quadravalents with the frequency of different associations varying among plants. One of the male parents, MSF 1008, had a greater number of univalents and more laggards at telophase indicating meiotic instability.

Chromosome counts were made for 47 selected F_1 hybrids, all of which were triploids (2n=3x=54) (Figures 12 and 13). This was the expected result. Meiosis tended to be more irregular in the triploids as compared to the tetraploids, and had more univalent and trivalent associations (Table 6). Trivalent configurations were found in some of the F_1 hybrids from crosses between GSF I x MSF 42, GSF I x MSF 55, and GSF I x MSF 1008.

SUMMARY AND CONCLUSIONS

During the preliminary stages of this research, we anticipated the F_1 hybrids to be triploid and highly sterile. We also anticipated there would be little or no pollen shed from the F_1 hybrids. We further hypothesized that a plant with high pollen stainability would also have high seed set. We also thought that if we found a plant with high seed set, it would be a tetraploid. However, these assumptions were not the case. The unusually high female fertility in the triploid F_1 population can best be explained by apomixis.

Researchers have recently described the actual mechanism of apomixis in eastern gamagrass (Burson et al., 1990). The researchers investigated megasporogenesis and embryo sac development in ovules of eight eastern gamagrass genotypes: five diploid GSF plants (2n=2x=36), two triploid (2n=3x=54) accessions, and one tetraploid (2n=4x=72). The diploids investigated resulted from MSF accessions backcrossed with GSF I. The tetraploid investigated was MSF 1008. Their research demonstrated that the five GSF plants reproduce sexually and the three polyploid eastern gamagrass accessions reproduce by diplosporous apomixis. They were the first to report apomixis in triploid (2n=3x=54) eastern gamagrass. In the polyploids, the

embryo developed parthenogenetically while pollination or fertilization was necessary for endosperm development (pseudogamy). The researchers concluded that the "apomictic mechanism in eastern gamagrass is diplospory of the Antennaria type followed by pseudogamy".

Sherman and others (1990) recently crossed a sexual diploid (2n=3x=36) with an apomictic triploid (2n=3x=54). "Half of the hybrids were either completely sexual or apomictic, but the other half had both..." (Sherman et al., 1990), which demonstrates facultative apomixis. They stated that "it seems possible that apomixis can be transferred from the polyploid to the diploid level of eastern gamagrass".

This research project demonstrated that the progeny of diploid X tetraploid crosses of eastern gamagrass are triploid. It also showed that some triploid eastern gamagrass hybrids produce stainable pollen and viable seed. This study also supports the recent findings of Burson et al. (1990) and Sherman et al. (1990) regarding apomixis in triploid T. dactyloides.

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APPENDICES

Table 1. Plants used as Parents and Previously Reported Ploidy.

Parent	Reported Ploidy	Origin
GSF I	diploid	Ottawa Co., Kansas
GSF II	diploid	Ottawa Co., Kansas
MSF 40	tetraploid	Bowie Co., Texas
MSF 42	tetraploid	Upshur Co., Texas
MSF 55	tetraploid	Navarro Co., Texas
MSF 1008	tetraploid	Callahan Co., Texas

Table 2. Eastern Gamagrass Crosses Made and Seed Produced in 1985.

Female Parent	Male Parent	Seed Produced
GSF I	MSF 40	1,689
GSF I	MSF 42	1,408
GSF I	MSF 55	1,041
GSF I	MSF 1008	261
GSF II	MSF 40	2,270
GSF II	MSF 42	1,079
GSF II	MSF 55	982
GSF II	MSF 1008	234

Table 3. Percent Pollen Grain Stainability and Percent Seed Germination of F_1 Hybrids.

Parent	% Pollen Stainability	Rank	% F_1 Seed Germination	Rank
Male				
MSF 40	32.9*	2	24.9	3
MSF 42	28.6	3	29.3	2
MSF 55	37.6	1	22.4	4
MSF 1008	19.3	4	37.3	1
Female				
GSF I	33.6	1	31.6	1
GSF II	25.3	2	25.3	2
$LSD_{0.05}$	10.2		6.3	

* Means should be compared within male parents and within female parents.

Table 4. F_1 Hybrid Frequency Class, Mean, Standard Deviation, and CVs for Percent Pollen Stainability and Seed Set.

	Percent										
	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	Mean	Deviation	CVs
Stainability	41	104	104	96	80	41	10	1	12.8	5.6	43.7
Seed Set	222	24	23	18	16	1	0	0	6.9	11.5	166.6

Table 5. Chromosome Number and Pairing Relationships in Parental Gamagrass Lines.

Parent	Number of Cells	(2n) Number	Mean Diakinesis Pairing		
			I	II	IV
GSF I	5	36	0.0	18.0	0.0
GSF II	5	36	0.0	18.0	0.0
MSF 40	20	72	4.5	22.1	5.8
MSF 42	20	72	4.6	19.3	7.2
MSF 55	20	72	5.3	21.7	5.8
MSF 1008	20	72	13.0	19.0	5.3

Table 6. Chromosome Number and Pairing Relationships of Triploid (2n=3x=54) F_1 Hybrids.

Crosses	Number of Plants	Number of Cells	Mean Diakinesis Pairing I	II	III	IV
GSF I X MSF 40	7	22	9.2	16.8	0.0	2.8
GSF II X MSF 40	4	11	8.2	14.0	0.0	4.5
GSF I X MSF 42	6	26	7.9	16.1	0.0	3.5
GSF II X MSF 42	5	13	8.3	16.4	0.0	3.2
GSF I X MSF 55	8	30	5.8	15.6	0.0	4.2
GSF II X MSF 55	7	13	7.4	17.3	0.0	3.0
GSF I X MSF 1008	7	17	6.8	17.3	0.1	3.1
GSF II X MSF 1008	3	7	7.1	17.1	0.0	3.1

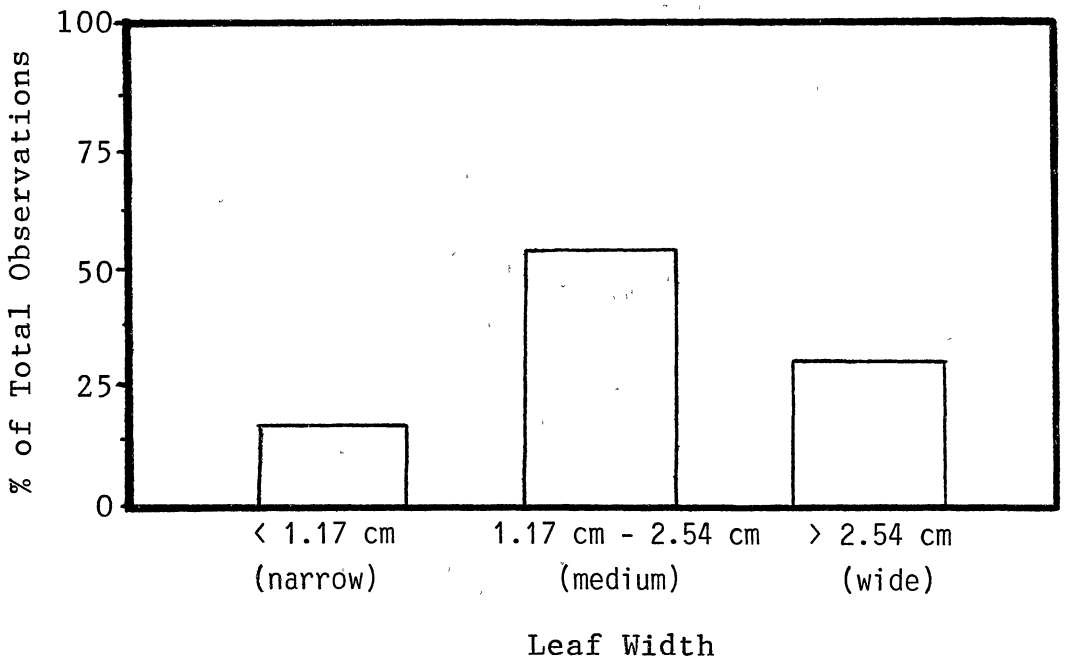

Figure 1. Percentage Distribution of Leaf Width of 1,463 F_1 Hybrid Plants.

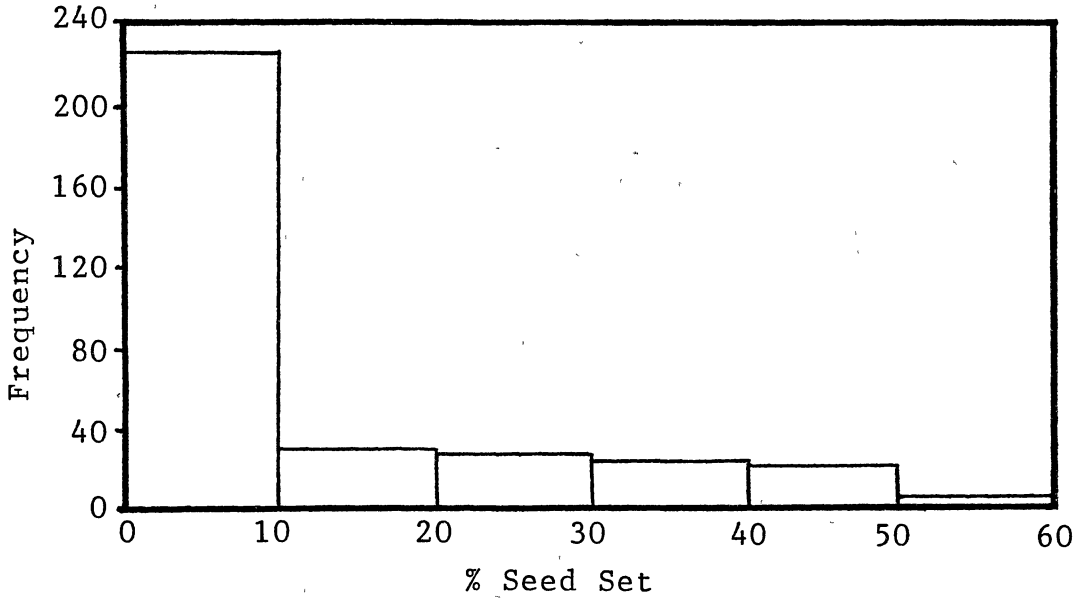

Figure 2. Frequency Distribution Observed in F_1 Hybrids for Percent Seed Set.

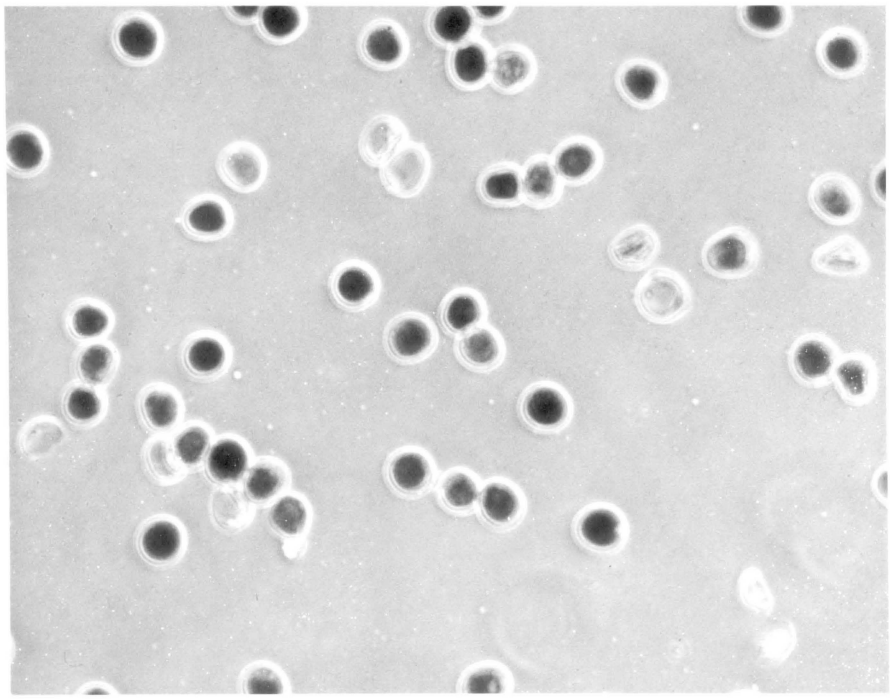

Figure 3. Pollen Grains from a F_1 Hybrid Indicating 67% Stainable Pollen.

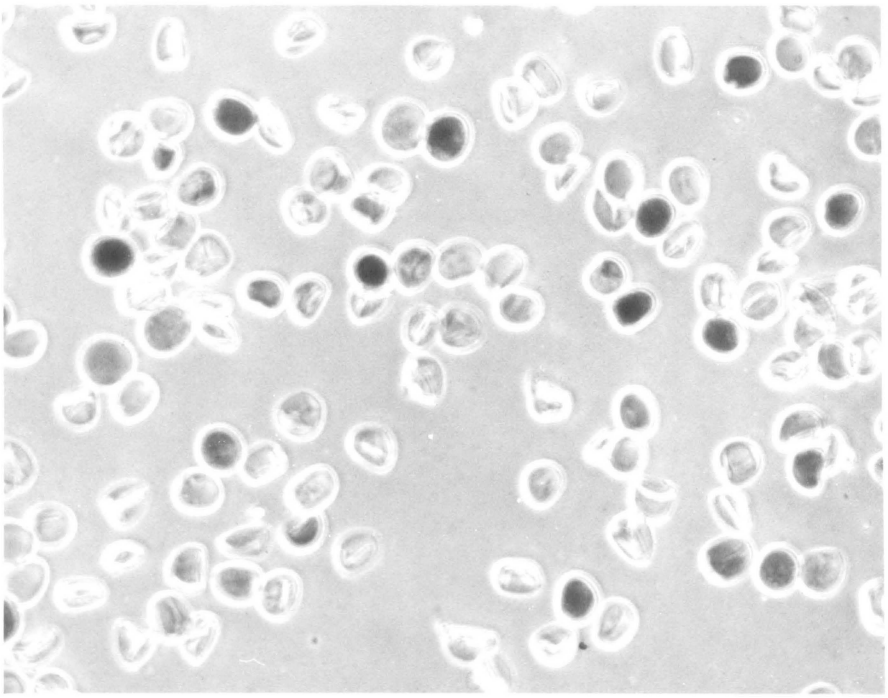

Figure 4. Pollen Grains from a F_1 Hybrid Indicating 15% Stainable Pollen.

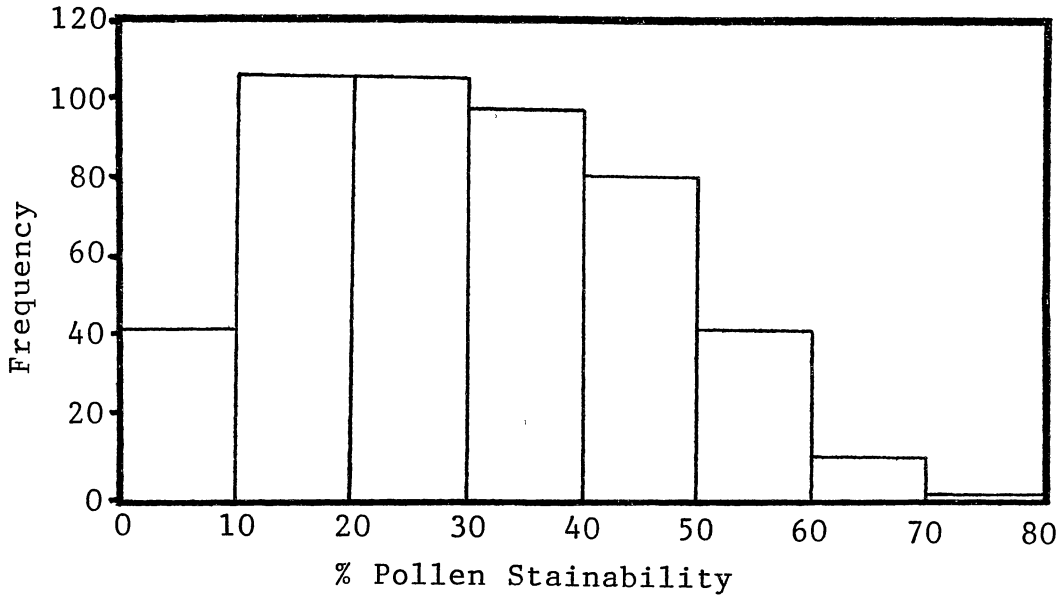

Figure 5. Frequency Distribution Observed in F_1 Hybrids for Percent Pollen Stainability.

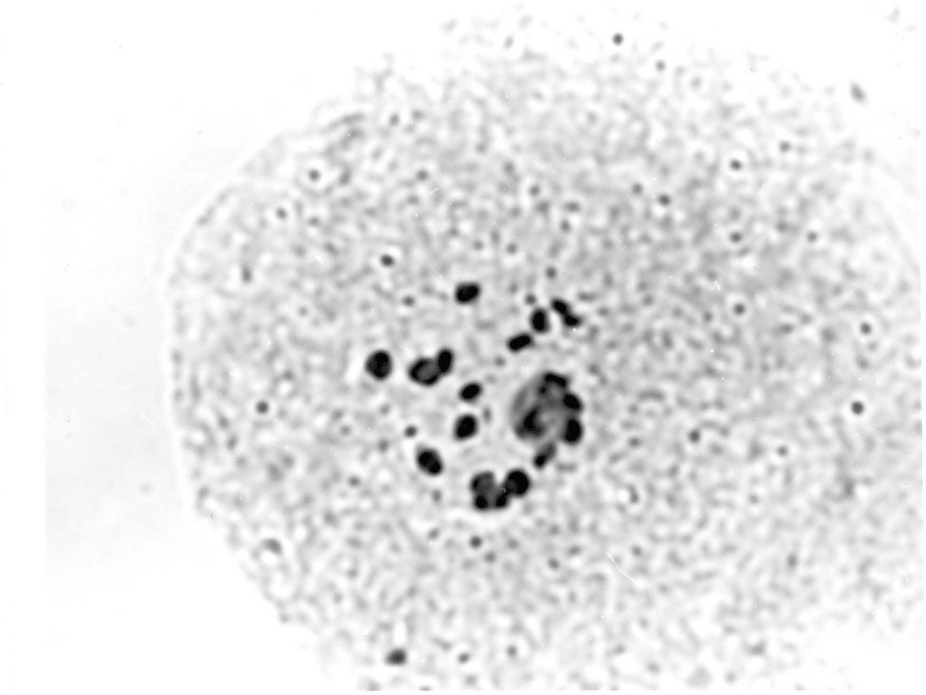

Figure 6. GSF I, a Diploid with 2n=2x=36 Chromosomes.

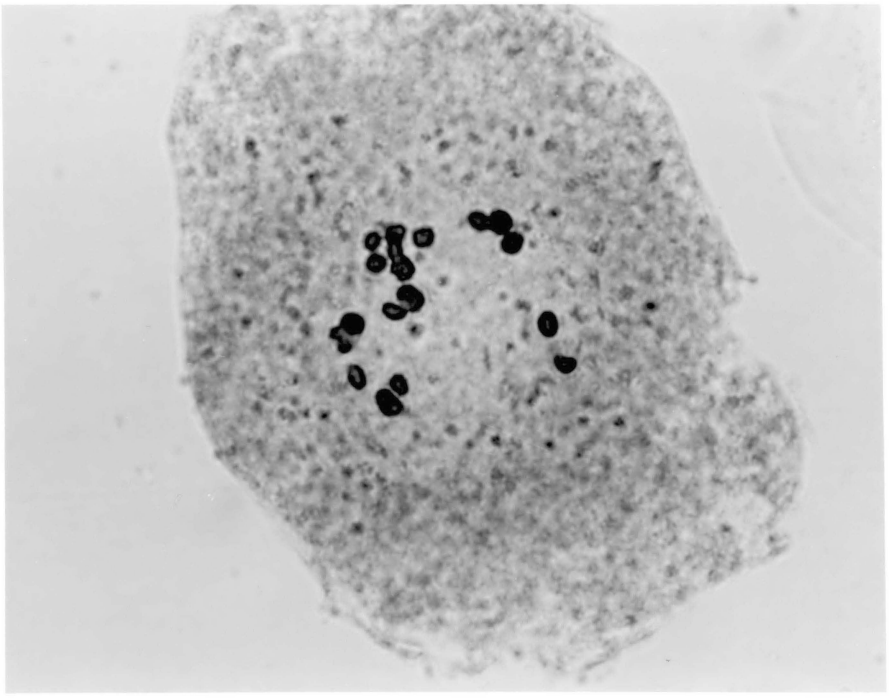

Figure 7. GSF II, a Diploid with 2n=2x=36 Chromosomes.

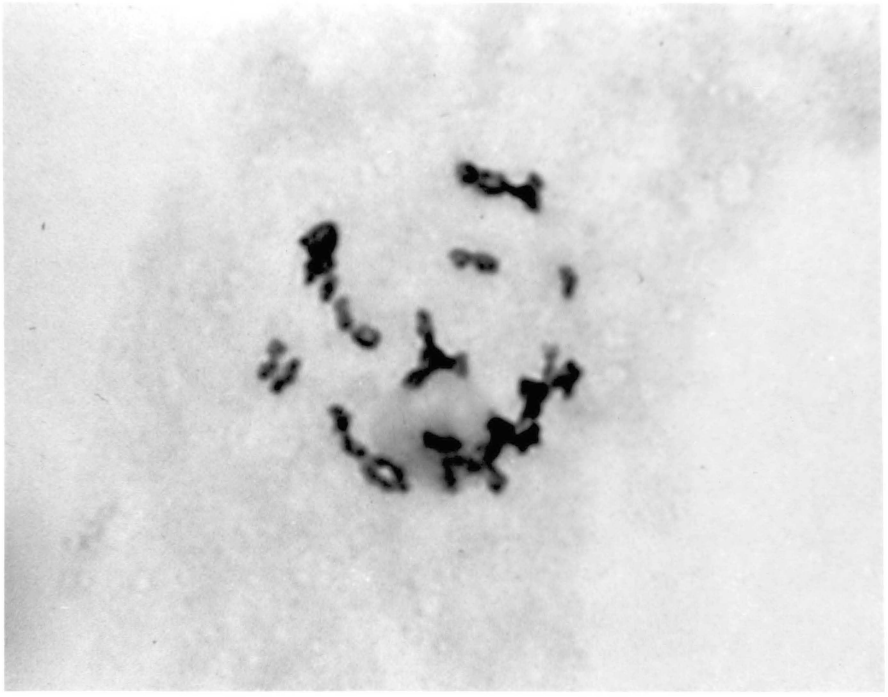

Figure 8. MSF 40, a Tetraploid with 2n=4x=72 Chromosomes.

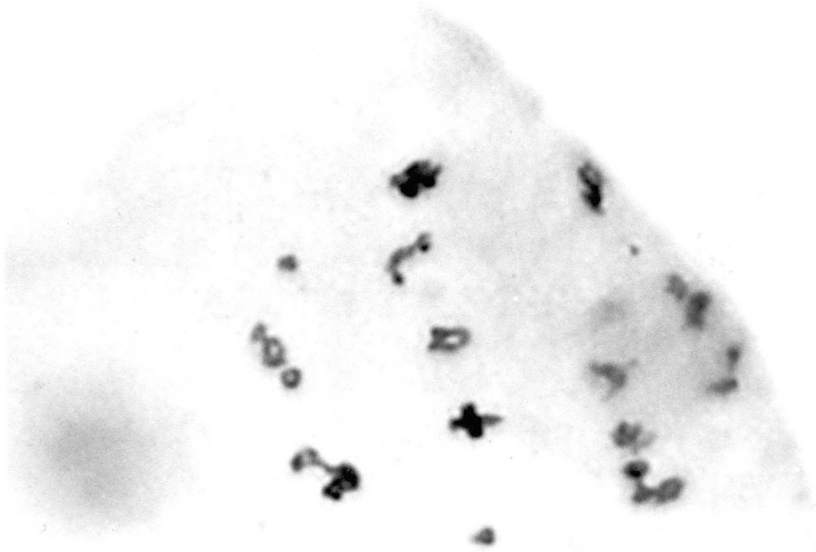

Figure 9. MSF 42, a Tetraploid with 2n=4x=72 Chromosomes.

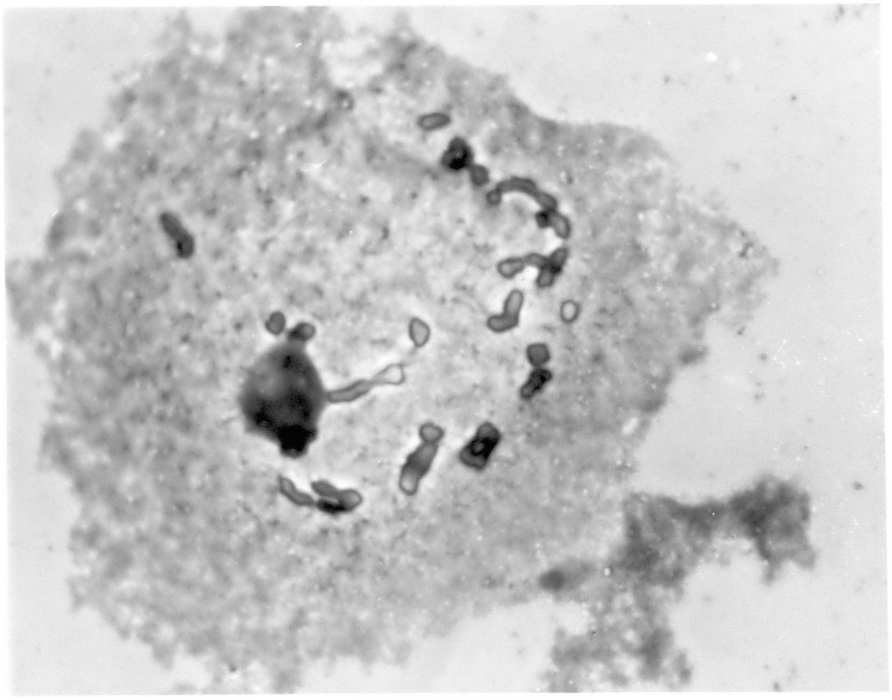

Figure 10. MSF 55, a Tetraploid with 2n=4x=72 Chromosomes.

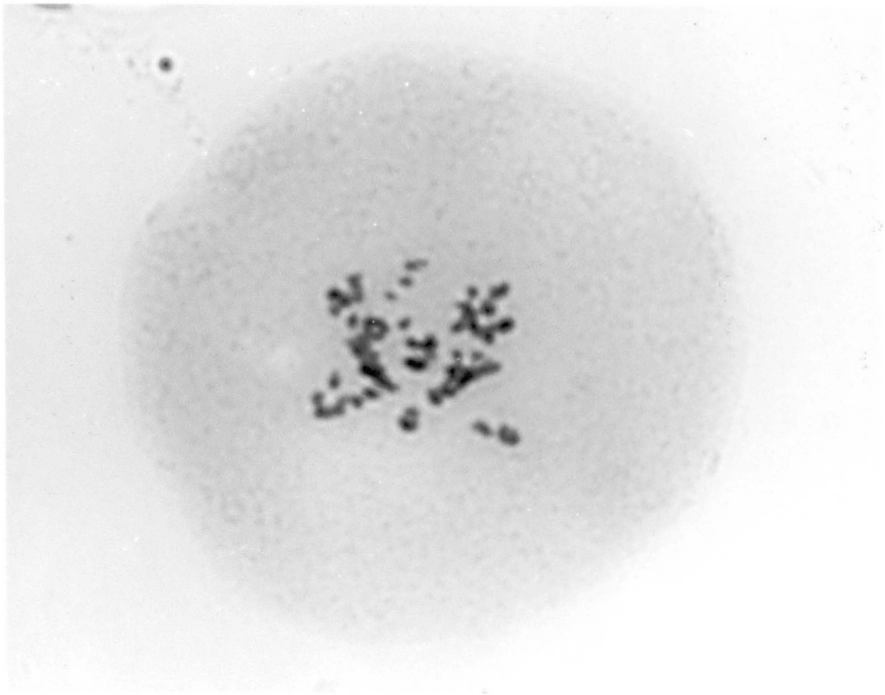

Figure 11. MSF 1008, a Tetraploid with 2n=4x=72 Chromosomes.

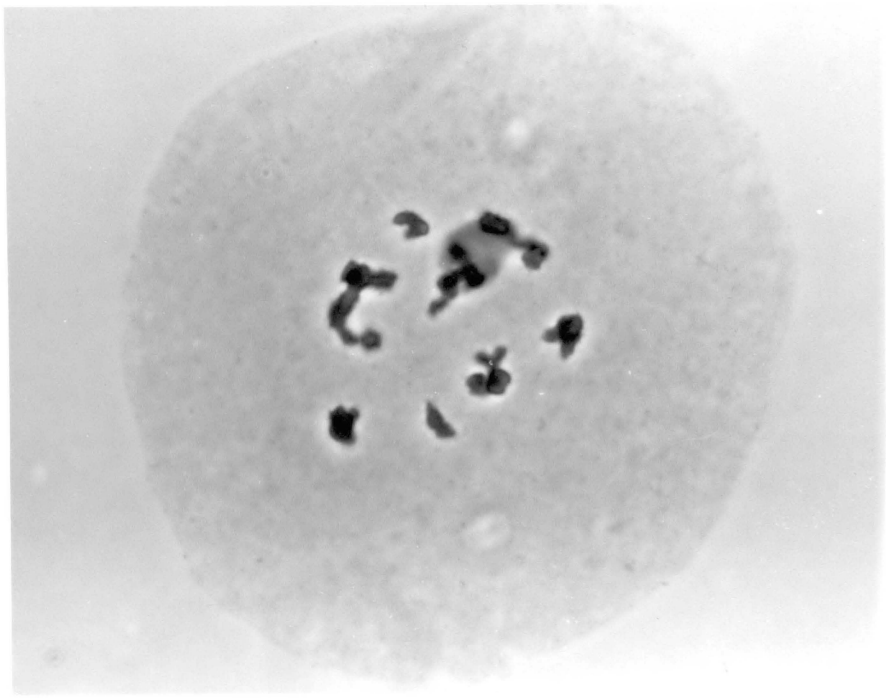

Figure 12. F_1 Hybrid, a Triploid with 2n=3x=54 Chromosomes.

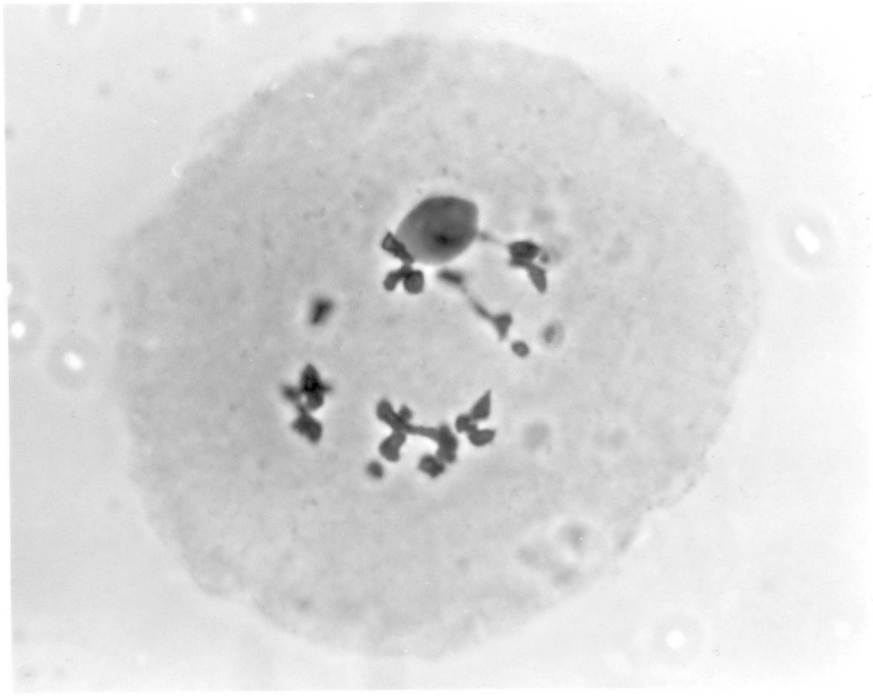

Figure 13. F_1 Hybrid, a Triploid with 2n=3x=54 Chromosomes.

VITA

Patti Curry Dunfield

Candidate for the Degree of

Doctor of Philosophy

Thesis: CYTOLOGICAL AND REPRODUCTIVE CHARACTERISTICS OF DIPLOID AND TETRAPLOID EASTERN GAMAGRASS PLANTS AND THEIR F_1 HYBRIDS.

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