AN ABSTRACT OF THE THESIS OF

Paul Richard Beuselinck for the degree of Doctor of Philosophy

in Crop Science presented on January 17, 1980

Title: Ovule Characteristics of Two Tall Fescue (Festuca arundinacea Schreb.) Genotypes and Their Sterile Hybrid

Abstract approved: Redacted for privacy

Dr. Rod V. Frakes

Single-cross progeny derived from two genetically diverse tall fescue (Festuca arundinacea Schreb.) genotypes was male sterile and also highly barren of seed. A study was conducted to determine if the barrenness was an expression of true sterility of the female gametophyte or sterility caused by other complicating factors.

Megasporogenesis and megagametogenesis were studied in four, fertile control genotypes. A single archesporial cell formed the megaspore mother cell. Two meiotic divisions resulted in the formation of a linear tetrad of megaspores. The megaspore closest to the chalaza became the functional megaspore, after the degeneration of the other three. Three mitotic divisions formed an eight-nucleated Polygonum-type megagametophyte. Further development resulted in a seven-celled embryo sac at maturity with three densely staining multinucleated antipodals, an egg apparatus of a highly vacuolated egg and two synergids, and fused polars (2) within the central cell.

The parent genotypes, P_1 and P_9, consistently produced fertile ovules containing normal embryo sacs. In contrast, sterile, non-functional ovules of the five different hybrid genotypes examined were devoid of embryo sacs. A cytomorphological examination indicated that the sterile ovules completed a normal megasporogenesis, but showed a failure of the post-meiotic mitotic processes leading to megagametophyte formation. A strand of compressed tissues of nucellar origin was found to occupy the area in the sterile ovules where the megagametophyte normally develops. Nucellar compression accompanying meiotic or early post-meiotic failures appears to be a characteristic feature observed in sterile ovules, as evidenced by the literature.

The combined observations of a small number of hybrid ovaries in stages of normal megagametogenesis, and backcross progenies showing differences in growth and vigor, indicate that fertile florets are produced and fertilized. The rate of production, as estimated from counts of germinable seed for total florets produced, is extremely low.

The causal factor(s) responsible for the observed sterility were not determined, however, B-chromosomes, abnormal chromosome behavior or cytoplasmic inheritance, do not appear to be involved. Genetic or genetic-cytoplasmic interactions are hypothesized as factors which may account for the sterility of the F_1 hybrid. The determination of the cause(s) of sterility in this unique hybrid may provide a manageable source of complete sterility in tall fescue.

Ovule Characteristics of Two Tall Fescue (*Festuca arundinacea* Schreb.) Genotypes and Their Sterile Hybrid

by

Paul Richard Beuselinck

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Commencement June 1980

APPROVED:

Redacted for privacy

Professor of Plant Breeding in charge of major

Redacted for privacy

Head of Department, Crop Science

Redacted for privacy

Dean of Graduate School

Date thesis is presented January 17, 1980

Typed by Sharon Mosley for Paul Richard Beuselinck

ACKNOWLEDGEMENTS

The author would like to express his sincere thanks to his major professor, Dr. Rod. V. Frakes, for the interest, guidance, and support given throughout the duration of this study. Appreciation is expressed to Drs. D. I. Mills, K. L. Chambers, C. J. Weiser, and D. O. Chilcote for their interest as advisors on the graduate committee. For their technical advice and the use of equipment and facilities, the author also expresses his gratitude to Drs. A. Haunold, and F. R. Rickson.

The author would also like to express his thanks to Mrs. Doris Aponte for her help in the preparation of this manuscript.

Finally, I would like to thank Charlotte, my wife, for her invaluable confidence and support.

Table of Contents

	Page
Introduction	1
Materials and Methods	4
Results	7
Discussion	12
References	20
Appendix - Literature Review	38
Appendix Bibliography	56
Appendix - Tables	61
Addendum I	68
Addendum II	73

List of Figures

Figures		Page
1	Archesporial cell.	18
2	Megaspore mother cell.	18
3	Linear tetrad of megaspores.	19
4	Tetrad after start of degeneration of the three megaspores closest to micropyle.	19
5	Tetrad after degeneration of the three megaspores closest to the micropyle.	20
6	Three megaspores have degenerated leaving a functional chalazal megaspore.	20
7	First mitotic division.	21
8	Two-nucleate stage of megagametophyte.	21
9	Four-nucleate stage of megagametophyte development.	22
10	Ovule with third mitotic division in progress.	23
11	Eight-nucleate stage of megagametophyte development.	24
12	Eight-nucleate stage of megagametophyte development during polar migration to central cell.	25
13	Megagametophyte with polar nuclei in the central cell, partially differentiated egg and synergids, and proliferating antipodal nuclei.	25
14	Three multinucleated antipodals, differentiated egg apparatus and polar nuclei in central cell near antipodals.	26
15	Entire ovary showing megagametophyte orientation .	27
16	Mature megagametophyte with highly vacuolated egg, two synergids, three multinucleated antipodals and two unfused polar nuclei with fused cytoplasm .	28

List of Figures

Figures		Page
17	Entire ovary showing megagametophyte orientation.	29
18	Remaining megaspore after degeneration of the rest of the tetrad as seen in the hybrid genotypes.	30
19	Degeneration of remaining megaspore as seen in the hybrid genotypes.	31
20	Degeneration of remaining megaspore as seen in the hybrid genotypes.	32
21	Darkly staining strand of compressed tissues of nucellar origin as seen in the hybrid genotypes.	33
22	Entire ovule of hybrid genotypes.	34

List of Tables

Tables		Page
1	Summarized data of seed production and estimated floret production as observed on the hybrid genotypes used in attempted backcrosses, and the pollination block experiment.	37

List of Appendix Tables

Appendix Tables		Page
1	Number of seeds obtained and germinated from the selfings, outcrosses, and backcrosses conducted in the greenhouse.	61
2	Ratio (x/y) of seeds germinated (x) to seeds obtained (y) for each of the hybrid genotypes studied in the pollination block experiment.	65
3	Various anther characteristics of the hybrid, parent, and control genotypes observed in both field or laboratory examinations.	66

Ovule Characteristics of Two Tall Fescue (Festuca arundinacea Schreb.) Genotypes and Their Sterile Hybrid

Introduction

By crossing an early anthesis tall fescue genotype (P_1) from Switzerland to a late anthesis genotype (P_g) from Turkey, Moutray and Frakes (10) generated hybrid progeny showing high dry matter production. Vasquez (15) examined five different genotypes from this single-cross progeny and identified them as probable male steriles. He found the expressed sterility to be caused by a failure of the post-meiotic mitotic processes in the microsporocytes. The failure of anther dehiscence was a character observed to accompany the expression of sterility.

Occasional seeds were found on the hybird genotypes grown in both field and greenhouse conditions by Moutray (9) and Vasquez (15), respectively, however, viability of the seeds was not ascertained. Repeated attempts to obtain more than a few seeds from the hybrid genotypes have failed (Frakes, unpublished).

To account for the observed low seed set, an expression of post-meiotic mitotic failure similar to that seen in the microsporocytes was suspected in the case of female sterility. However, incompatability, a failure of fertilization, or failure of post-fertilization development were possible since some seed has been produced.

The objective of this study was to identify if the low seed set on the hybrid genotypes was due to gametophytic sterility or other complicating factors such as incompatability, a failure of fertilization or post-fertilization failures. This required a study of megasporogenesis and megagametogenesis as it normally occurs in tall fescue for comparative purposes.

Embryo sac development in tall fescue, Festuca arundinacea Schreb. has not been previously described. This species, however, is distantly related to other well described members of the Graminae. Similar descriptions of megasporogenesis are reported for Bromus (11), Agrostis (6), Oryzopsis (7), and Stipa (7, 8). In these grasses a linear or modified T-shaped tetrad develops following meiotic divisions and cytokinesis. The functional megaspore develops from the chalazal end of the tetrad and megagametogenesis results in the Polygonum-type embryo sac (5). The seven celled (eight-nucleate) embryo sac consists of an egg apparatus, antipodals, and polar nuclei within a central cell.

Sterility has been extensively studied in many plants (2). Comparatively few studies examine sterility as expressed in ovule development. Nielsen (12) catagorized the development of the female gametophyte as affected by sterility into two general classes: (a) those attributable to meiotic disturbances and early failure during development and (b) those dealing with post-fertilization development. Within his first classification Nielsen reported a case in Bromus inermis where early digestion of the nutrients of the antipodals and degeneration of the egg apparatus preceeded the

eventual collapse of the gametophyte. In another study (13) of B. inermis, Nielsen alluded to the ovary sterility in an F_1 progeny as being due to post-meiotic mitotic failure. Four abnormal classes of ovaries of varied behavior were observed by Landes (4), in selfed and crossed rye, Secale cereale. Three classes of those ovaries ranged from types where the embryo sac failed to form, to those that failed to become fertilized although normal in appearance. Gametophyte failure or degeneration prior to fertilization was indicated as having been due to meiotic or post-meiotic failure. Mitotic failures anytime after megasporogenesis are reported to account for the high level of ovule abortion and subsequent low seed set in Hilaria (1).

These studies indicate that the time of development at which the disturbance occurs is important to the observed morphological changes since they can characterize the gametophytically expressed sterility as being pre-meiotic, meiotic, or post-meiotic.

Materials and Methods

Four propagules each of P_1 (PI 234-906) and P_9 (PI 174-209) were established in half-gallon pots. Eight propagules each of five hybrid genotypes and four control genotypes were also established in a manner identical to the parent genotypes. The two parents and five hybrid genotypes were the same as those examined by Vasquez (15). The four control genotypes were selected from among the eight genotypes used as parents for the variety 'Fawn'. When well established, the plants were transferred to a controlled environment of short days and long nights (ten hours of illumination) at 5°C for a period of seven weeks to induce reproductive development. At the end of this inductive schedule the plants were transferred to a greenhouse environment of 20 hours daylength and 20°C to stimulate reproductive tiller development. The P_9 propagules did not develop reproductive tillers under this schedule. Propagules of this genotype that had been induced in a field environment were substituted. They successfully produced fertile tillers in the greenhouse environment described.

After panicle emergence from the sheath, whole inflorescences were collected at all stages of panicle development before and after pollination for the megagametophyte development study. These were divided and fixed in FPA_{50} (90 cc 50% ethanol, 5 cc proprionic acid, 5 cc formalin) or FAA_{50} (90 cc 50% ethanol, 5 cc acetic acid, 5 cc formalin). Tissues fixed in FPA_{50} were transferred to 70% ethanol for storage. Ovaries were disected from the florets, dehydrated in tertiarybutyl alcohol and embedded in Paraplast. Sections were cut

on a rotary microtome at 7 - 18 microns (μ) depending on ovary size, and were stained with saffranin and fast green. Figures were drawn with the aid of a camera-lucida. They are arranged so that the base of the gynoeceium is toward the bottom of the page.

All possible crosses were made between the two parent genotypes, P_1 and P_9, and the control genotypes. Crosses were not made among the control genotypes themselves. Individual panicles of two plants to be crossed were bagged together. Those used as the female recipient of pollen were emasculated prior to bagging. Panicles used as pollen donors were removed two weeks after anthesis. Selfing was accomplished by bagging single panicles. Backcrosses of the hybrid genotypes of P_1 and P_9 were attempted using the hybrid genotypes as female members in the crosses.

Surface disinfested seeds obtained from each cross were imbibed with 10% KNO_3 in darkness for seven days at $5^{o}C$. The treated seeds were transferred to a germination environment of 8/16 hours of day/night corresponding with $25/15^{o}C$. Each seedling was individually planted in peat pellets. After establishment these were transplanted into half-gallon pots where frequent clippings were practiced to promote tillering. Conditions the same as previously cited were used to promote floral induction and reproductive development except that constant illumination was used after the induction period to hasten flowering. All plants were tested for characters associated with the expression of sterility in the hybrid genotypes.

Twenty-five different hybrid genotypes derived from the $P_1 \times P_9$ single-cross were examined for anther indehiscence. The 25 hybrid genotypes were also established in ten spaced-plant crossing blocks in the fall of 1978. Each block contained rows of one pollen-producing genotype alternated with rows of the 25 hybrid genotypes. The ten pollen-producing genotypes were selected for the production of viable pollen and for anthesis date to correspond with that of the hybrids. Following pollination, but before seed shattering, all of the panicles on each of the hybrid genotypes were harvested. Total seed set and floret number per plant were measured on those five hybrid genotypes used in the developmental study.

Results

I. Normal Ovule Development

The ovule in tall fescue is hemianatropous with the micropyle pointed toward the base of the locule, bitegmetic, and pseudocrassinucellate. It is initiated on the ventral side of the developing gynoecium. Early in development the ovule is anatropous, but as development continues the ovule gradually bends downward to become hemianatropous. Development follows the Polygonum-type megagametogenesis.

Megasporogenesis and Megagametogenesis

A single archesporial cell (Fig. 1) becomes the megaspore mother cell (Fig. 2) which undergoes two meiotic divisions. These divisions result in a linear tetrad of megaspores (Fig. 3). Walls are formed between all four megaspores. The megaspore at the chalazal end of the tetrad is the one which continues to develop (Figs. 4 and 5).

The other three megaspores degenerate and are replaced by intruding nucellar cells (Figs. 4 and 5). The functional megaspore then begins to elongate both toward the micropylar and chalazal ends (Fig. 6). This cell is somewhat oblong and widened at the chalazal end prior to the first mitotic division. The enlarging megagametophyte appears to digest the immediately surrounding nucellar cells with its advancing maturity. The nucleus of the functional megaspore undergoes three successive divisions (Figs. 7, 8, 9, and 10). The third division (Fig. 10) produces eight nuclei, each with its own cell wall (Fig. 11).

Polar Nuclei

One polar migrates from both the chalazal and micropylar ends of the megagametophyte leaving three nuclei at each of the two poles (Fig. 12). The two polars meet in the central cell, median between the two poles (Fig. 13). During early maturity the polars were often seen to be located near the antipodals (Figs. 14 and 15), but prior to fertilization they migrate toward the egg apparatus (Figs. 16 and 17). By the time they reach a position above the egg, their cytoplasms have fused but the nuclei remain unfused until fertilization (Fig. 16). The polars are fertilized before the egg.

Antipodals

Before both polar nuclei migrate, there are three single nucleated antipodals (Fig. 12). When the polars are nearing their position in the center of the megagametophyte the antipodals are multinucleated (Fig. 13). Actual nuclear divisions occur, but are held within a common cytoplasm. Four or more nuclear divisions probably occur before fertilization (Fig. 14). The antipodals become the largest cells of the megagametophyte due to these multiple divisions (Figs. 15, 16 and 17). Their large size is attained before complete maturity of the embryo sac but it is not known if nuclear divisions occur after fertilization. In the mature megagametophyte, the antipodals are oriented as a group in a lateral position against the inner boundary closest to the vascular trace (Figs. 15, 16 and 17). At maturity the antipodals are highly vacuolate and densely staining.

Egg Apparatus

A highly vacuolate egg and two synergids are found at the micropylar end of the megagametophyte (Figs. 14 and 16). In the final mitotic division forming the 8-nucleate megagametophyte the egg and polar appear to be derived from one micropylar nucleus, as are both synergids from the second nucleus. When the polars meet in the central cell, the egg can usually be distinguished from the synergids by its increased vacuolation and larger size (Fig. 16). A filiform apparatus is formed on both synergids. One of the synergids appears to degenerate prior to fertilization.

II. Ovule Development in the Sterile Hybrid Genotypes

The hybrid genotypes complete a meiotic process identical to the normal megasporogenesis as already described. The megaspore closest to the chalaza remains after the degeneration of the rest of the tetrad (Fig. 18). This megaspore, however, does not continue to enlarge and enter into the formation of the megagametophyte. Rather, this megaspore starts to degenerate until it is replaced by invading nucellar cells (Figs. 19 and 20). No embryo sac is formed. Normal, continued ovule growth results in an accumulation of cell walls and contents of nucellar origin where the embryo sac is normally formed (Figs. 21 and 22). A strand of compressed tissues extending from near the center of the ovule, below the chalaza to the micropylar end, increases in width with increased ovule age. All of the ovaries from the hybrid genotypes were identical in this respect, except

three ovaries which were found to be in some state of normal megagametogenesis. One of these ovaries had completed a normal megagametogenesis as evidenced by its normal embryo sac.

The hybrid genotypes successfully produced a limited amount of seed from the attempted backcrosses and from the pollination block experiment. The number of germinable seed produced to total florets produced, used as an estimate of fertile floret production, as seen in Table 1, revealed that fertile florets on the hybrid panicles were extremely rare. Hybrid genotype panicles from the backcrosses showed this number of fertile florets to be on the average, one in approximately 14,000 florets. Panicles of the same hybrid genotypes taken from the pollination block experiment showed a lower fertile floret production rate of one in approximately 37,000 florets.

Genotypic differences were observed in the number of germinable seed produced by the hybrid genotypes. The hybrid genotypes, 8 and 14, failed to produce germinable seed in the attempted backcrosses, but all of the hybrid genotypes successfully produced germinable seed in the pollination block experiment. Of the 26 germinable seeds obtained in the field expeiment, 18 were produced by the F_1 genotype, 10, with an approximate average of one germinable seed per 6,600 florets produced.

Many of the germinated seeds died before seeding establishment. Of the surviving seedlings, differences were observed in their vigor and growth characteristics.

III. Anther Characteristics

The character, non-dehiscence of anthers, was observed in all of the 25 different hybrid genotypes examined. There was a complete absence of pollen release.

An examination of the progenies generated to examine the transmission of the anther character associated with the expressed sterility was incomplete. Some observations were made, but the induction schedule used on the progenies to promote reproductive development was only partially successful. Many of the progenies failed to produce fertile tillers. Different inductive conditions and further examinations are necessary to properly identify the factors responsible for the sterility observed in the hybrid genotypes.

Discussion

Normal megasporogenesis and megagametogenesis in tall fescue as determined from studying the ovaries of the four control genotypes, are similar to that described for taxonomically related members of the Graminae (6, 7, 8, 11). P_1 and P_9 consistently formed normal megagametophytes. In contrast, all but three of the hybrid ovaries examined revealed that the hybrid genotypes produced sterile, non-functional ovaries, devoid of embryo sacs.

The evidence indicates that the failure of the female gametophytic stages in the sterile ovaries of the hybrid genotype was due to post-meiotic mitotic failure after megasporogenesis. These findings are consistent with those of Vasquez (15) who concluded that the absence of microgametes in these same hybrid genotypes was due to post-meiotic mitotic failure after microsporogenesis.

Similar observations of sterility expressed in the androecium and gynoecium of the same sporophyte have been reported. Nielsen (13) found the F_1 progeny of two inbred B. inermis parents to be vigorous and uniform, but characteristically low in seed set. His examination of ten different F_1 genotypes found them to all be highly sterile and most of them to produce degenerated pollen. Post-meiotic mitotic failures were observed to precede pollen degeneration. Such mitotic failures were alluded to as the cause of ovary sterility and low seed set, since the F_1 was highly self- and cross-sterile. The presence of accessory chromosomes observed in one parent and other cytological evidence, led Nielsen to hypothesize the action of B-chromosomes,

chromosome abnormalities, or the interaction between these to account for the sterility observed in the F_1.

Sheth et al (14) described the appearance of sterile ovaries in Pangolagrass (Digitaria decumbens Stent). Meiotic irregularities as indicated by scattered univalents and lagging chromosomes observed in microsporcyte examinations were concluded to account for the high degree of pollen sterility. The unbalanced chromosome complement of 27 chromosomes, instead of the expected 30, was concluded to result in the observed ovary sterility.

In contrast to the reports by Nielsen (13) and Sheth et al (14), both of the tall fescue parent genotypes and the hybrid genotypes used in this study have been previously observed by Vasquez (15) to have normal meiotic behavior. Some limited abnormal chromosome behavior expressed as univalents, pseudobivalents, occasional multivalents, and fragments at diakinesis was observed. The average number of bivalents per microsporocyte was observed to be 20.70, 20.44, and 20.13 (2N = 42) for P_1, P_9, and the hybrid, respectively. Although micronuclei were observed in some of the quartets, their probable origin was thought to be from univalents and fragments observed at diakinesis, not B-chromosomes. Vasquez concluded that chromosome abnormalities could account for only 30% of the complete sterility observed in the microsporocytes of the hybrid. Therefore, descriptions of the sterility expressions found in the reported studies are similar to what has been observed in the tall fescue genotypes studied. However, the types of causal factors concluded in

those studies are not sufficient to explain the sterility observed in the hybrid genotypes. A review of the literature indicates that this case of complete sterility in an intraspecific hybrid of tall fescue, is the first in this species, that cannot be explained by chromosome abnormalities, karyotypic differences, or irregular chromosome pairing.

A series of crosses was made, involving the parental, hybrid, and control genotypes, to examine the mode of inheritance of the sterility trait. Indehiscence of the anthers was to be used as the criterion for identifying post-meiotic mitotic failures in the progeny. However, the attempts to induce flowering in the limited number of progeny obtained were only partially successful and the data on anther dehiscence are very limited. Nonetheless, important genetic information can be inferred by the success in generating those progenies. Both parent genotypes successfully produced viable seed when either self- or cross-pollinated. Their ovaries were observed in the developmental study to be normal. The combined findings, therefore, indicate that the transmission of sterility is not maternal or exclusively cytoplasmic.

Although limited genotypic differences were noted in the numbers of germinable seeds produced in the pollination block experiment, the level of production is so low that ovule sterility must be nearly complete. Complete pollen sterility is indicated by the uniform expression of anther indehiscence in the 25 different genotypes of the hybrid examined. Vasquez (15) postulated a nuclear-cytoplasmic interaction expressed through the sporophyte or affecting the

gametophyte itself, to account for the inhibition of the post-meiotic mitotic divisions. This hypothesis remains a possible explanation; however, genetic factors alone provide another possible hypothesis, based on the information obtained in studies to date.

Features observed in the sterile ovules of the hybrid genotype, and sterile ovules that have been examined and reported by others, are similar. Landes (4) found a small percentage of ovaries in selfed and crossed rye where no embryo sacs had formed. In some of these there was a group of small cells at the center of the nucellus, and in others a narrow strip of deeply staining material from the center of the ovule to the micropylar end. The latter of her two descriptions can apply to the condition found in the ovaries of the hybrid genotypes. In these hybrids, as Figs. 21 and 22 show, compressed tissues of nucellar origin form a strand extending from the micropylar region toward the chalaza. Landes, too, found that no megaspore enlarged to form the embryo sac, and she presumed that the breakdown occurred at or immediately after meiosis.

The similarity between the sterile ovaries described by Landes and those of the hybrid fescue genotypes indicates that nucellar compression is probably characteristic when early degeneration of the grass meagagametophyte occurs. If the maternal tissues continue to grow normally, the observed dark strand will be formed in the center of the ovule where the embryo sac normally would be expected to develop. The sterile ovaries reported in Pangolagrass by Sheth et al (14) resulted from the degeneration of the megaspore-mother-cell

before megasporogenesis. They described a region of crushed nucellar cells extending from the center of the nucellus to the micropyle in the sterile ovaries.

Two characters have been observed, that are associated with the particular expression of sterility in the tall fescue hybrid. Florets of the hybrid genotypes were consistent in their expression of both characteristics. Anther indehiscence was seen to accompany microsporocyte failure and megasporocyte failure was accompanied by ovules with a strand of compressed nucellar tissues, indicative of complete sterility.

However, three hybrid genotype ovules were observed in stages of development comparable to those seen in normal megagametogenesis in the control genotypes. One of these was in an eight-nucleate stage. Also, backcross progenies were successfully obtained, and they showed definite differences in vigor and growth characteristics and, therefore, do not appear to be apomictic in origin. These observations, when viewed as a whole, indicate that normal ovaries are produced at a low frequency, and that they can be fertilized, to yield viable offspring.

Actual fertilization was not observed, but because the maternal ovary tissues appeared normal, the germination and growth of viable pollen on the hybrid genotype ovaries, both sterile and fertile is probable. The orientation of pollen tube growth through the tissues of the sterile ovaries may not be normal, however, since no embryo sacs are formed. The synergids which are absent in these hybrid ovaries, are thought to be involved in the directional orientation of

pollen tube growth to the micropyle (2). Verification of pollen germination or pollen tube growth was not attempted in this study.

A continued effort is being made to determine the genetic factor(s) responsible for the sterility observed in the hybrid genotypes. The final determination may lead to the identification of a manageable source of complete sterility in tall fescue.

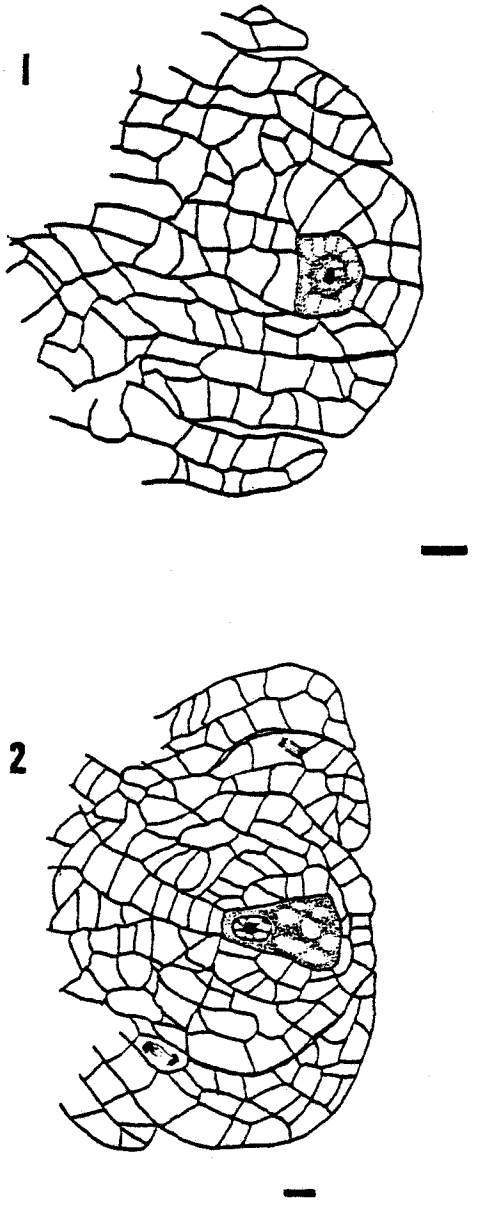


Figure 1. Archesporial cell.

Figure 2. Megaspore mother cell.

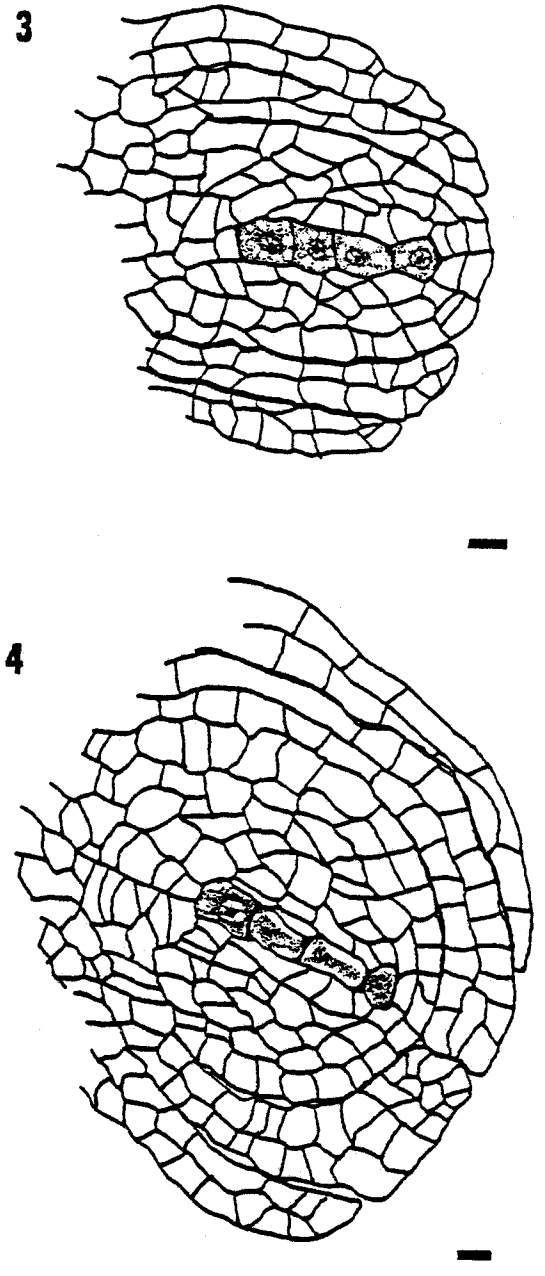


Figure 3. Linear tetrad of megaspores.

Figure 4. Tetrad after start of degeneration of the three megaspores closest to micropyle.

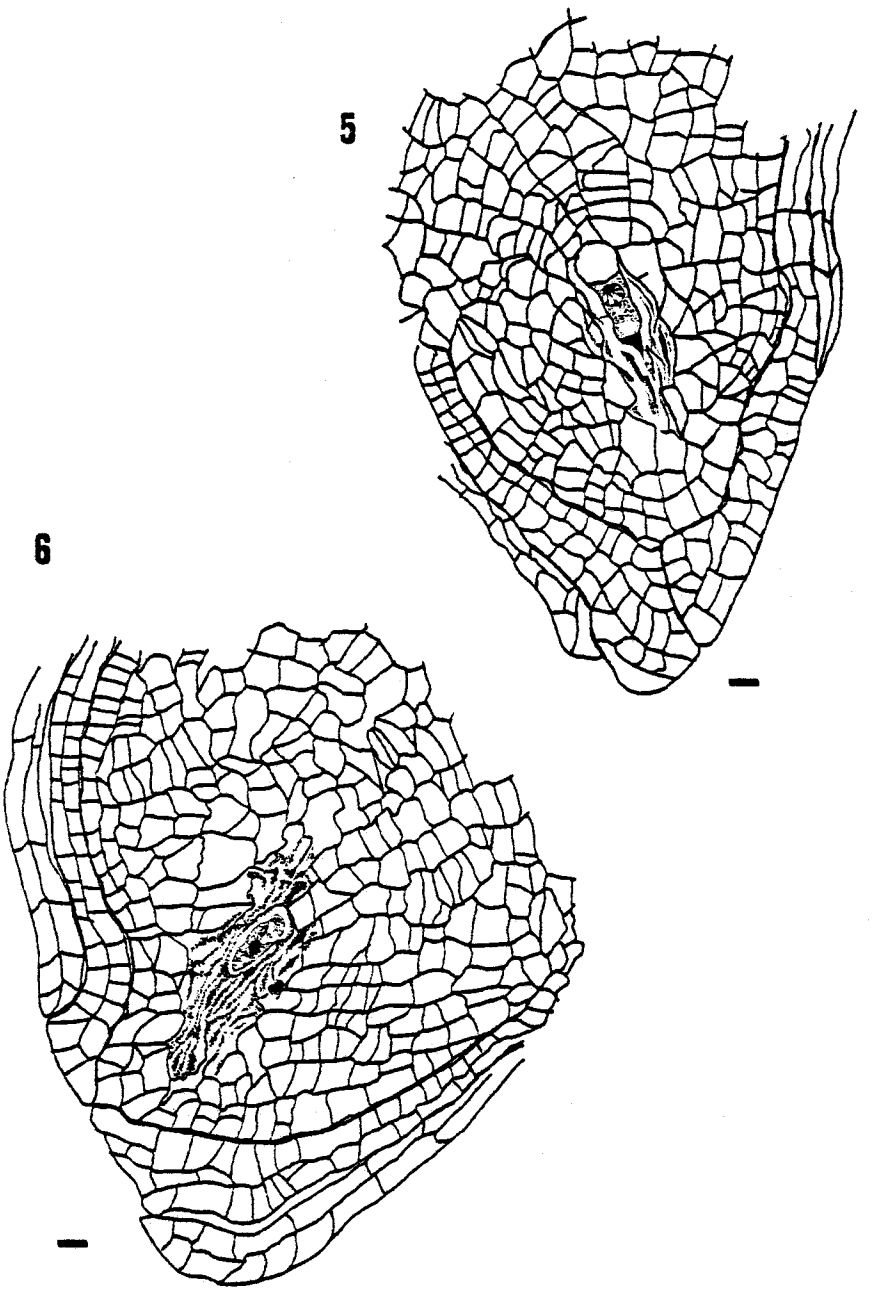


Figure 5. Tetrad after degeneration of the three megaspores closest to the micropyle.

Figure 6. Three megaspores have degenerated leaving a functional chalazal megaspore.

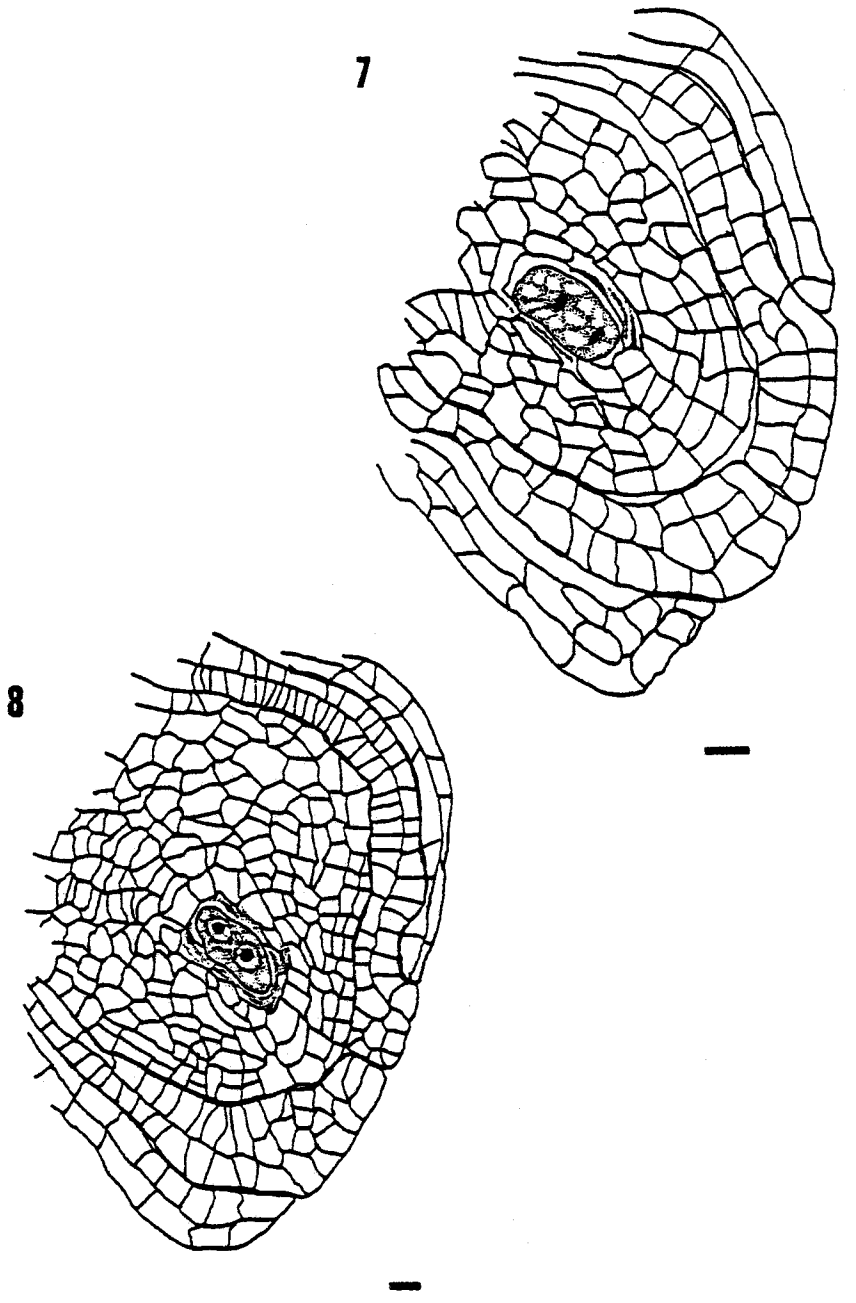


Figure 7. First mitotic division.

Figure 8. Two-nucleate stage of megagametophyte.

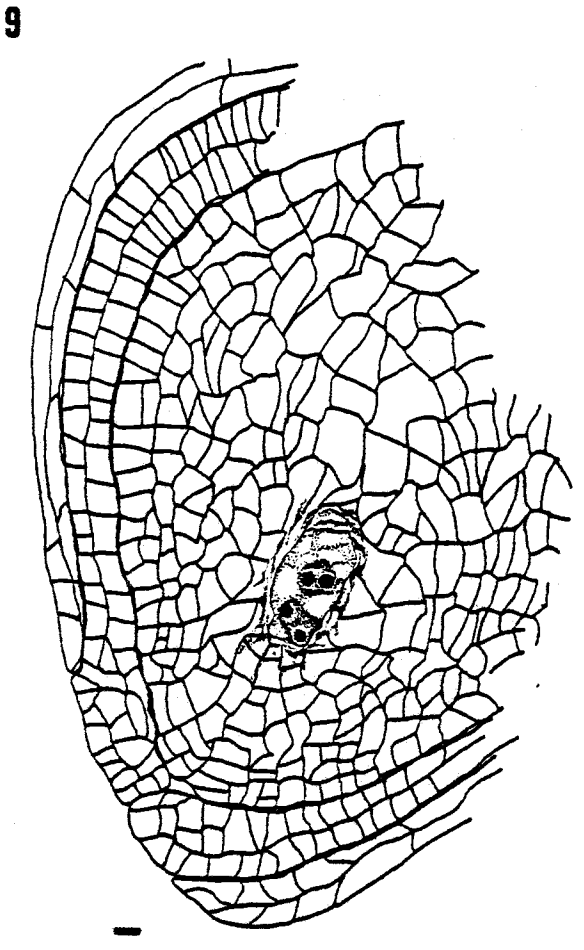


Figure 9. Four-nucleate stage of megagametophyte development.

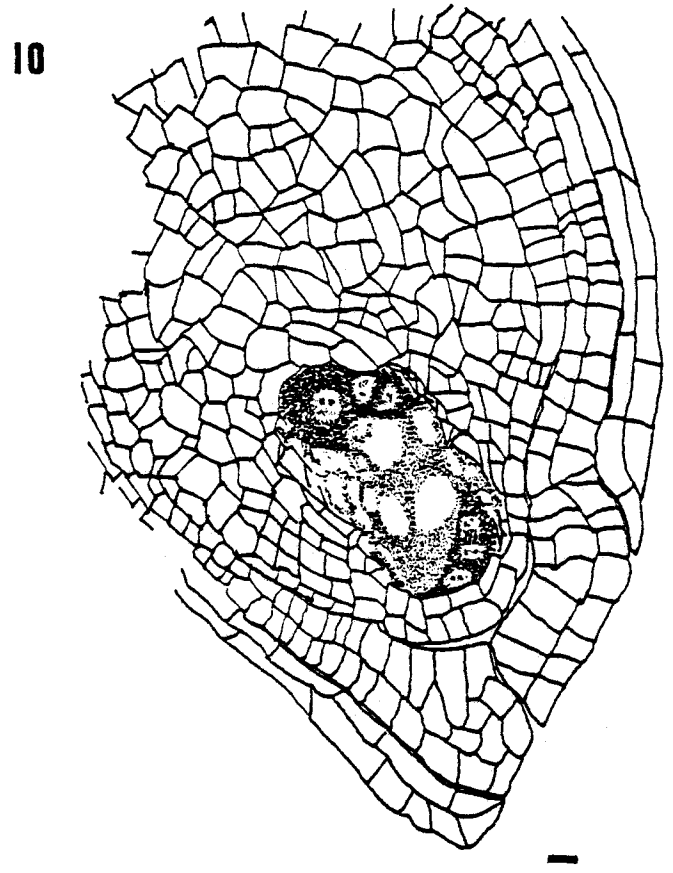


Figure 10. Ovule with third mitotic division in progress.

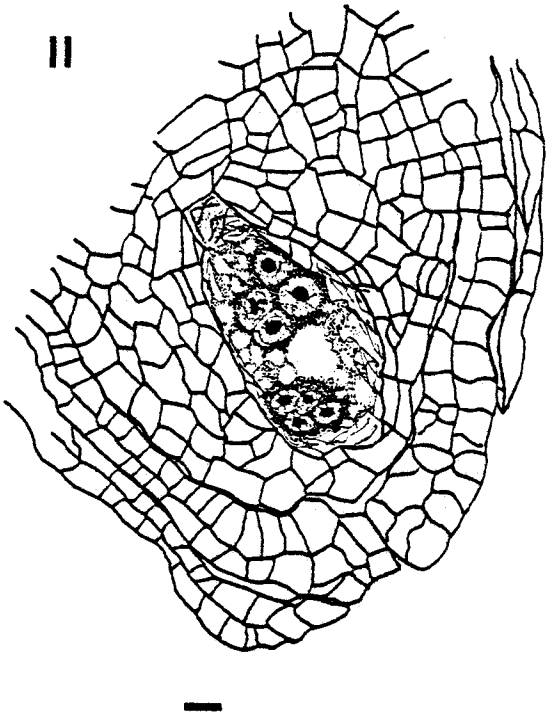
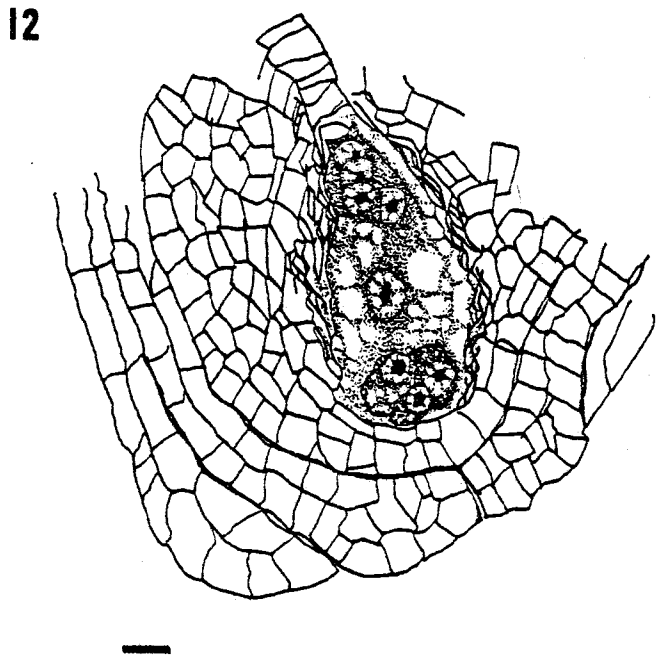


Figure 11. Eight-nucleate stage of megagametophyte development.



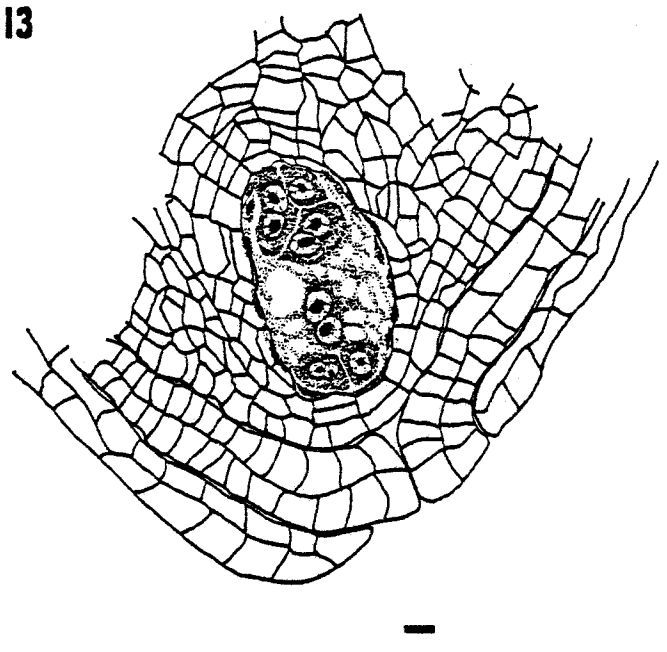


Figure 12. Eight-nucleate stage of megagametophyte development during polar migration to central cell.

Figure 13. Megagametophyte with polar nuclei in the central cell, partially differentiated egg and synergids, and proliferating antipodal nuclei.

14

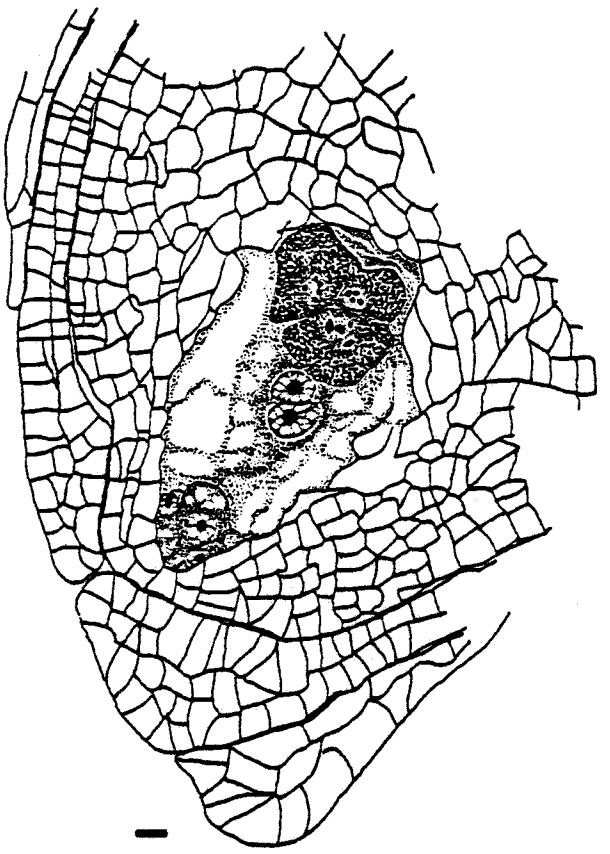


Figure 14. Three multinucleated antipodals, differentiated egg apparatus and polar nuclei in central cell near antipodals.

15

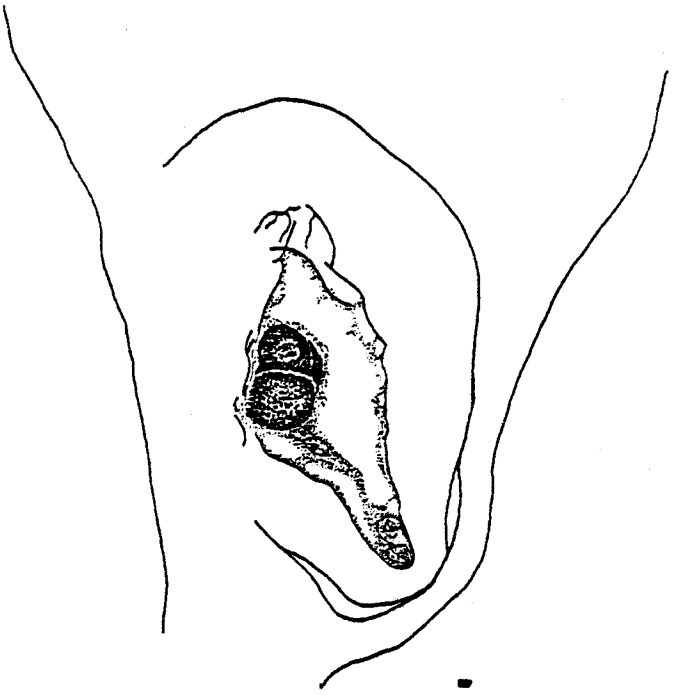


Figure 15. Entire ovary showing megagametophyte orientation.

16

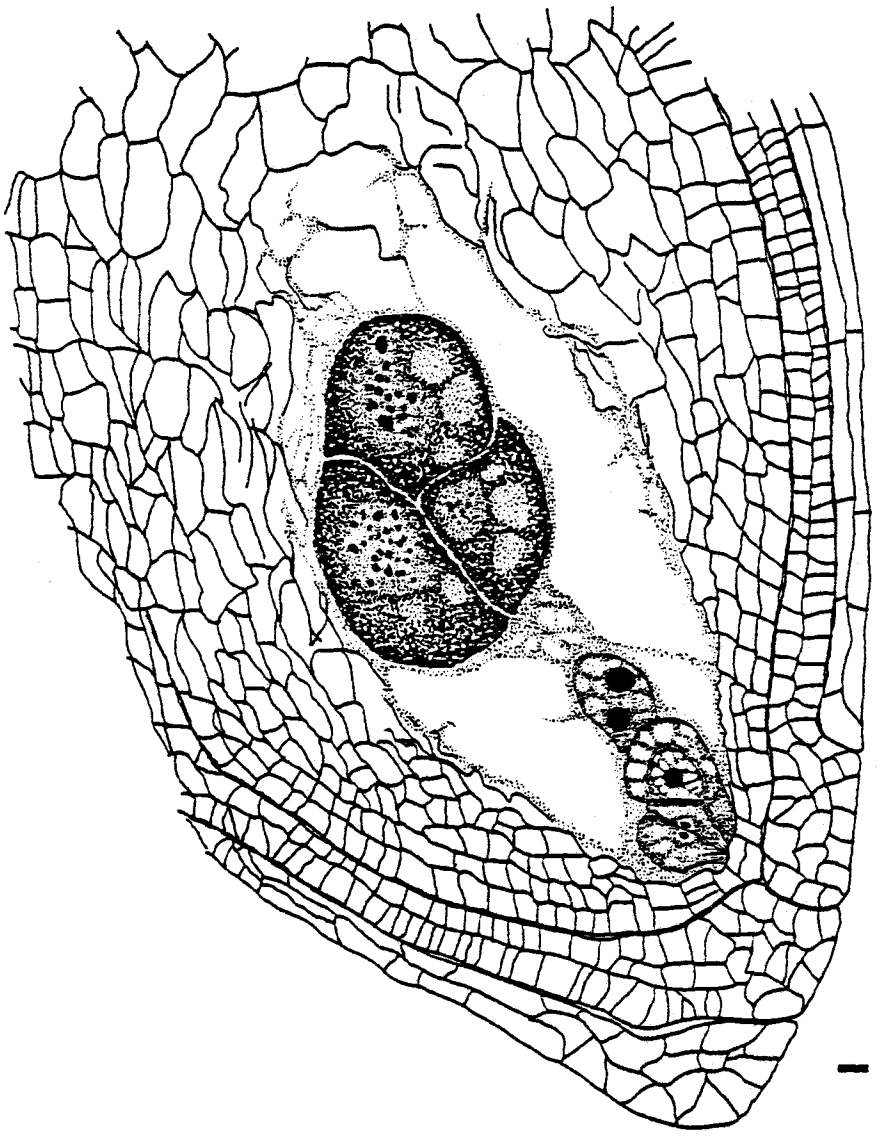


Figure 16. Mature megagametophyte with highly vacuolated egg, two synergids, three multinucleated antipodals and two unfused polar nuclei with fused cytoplasm.

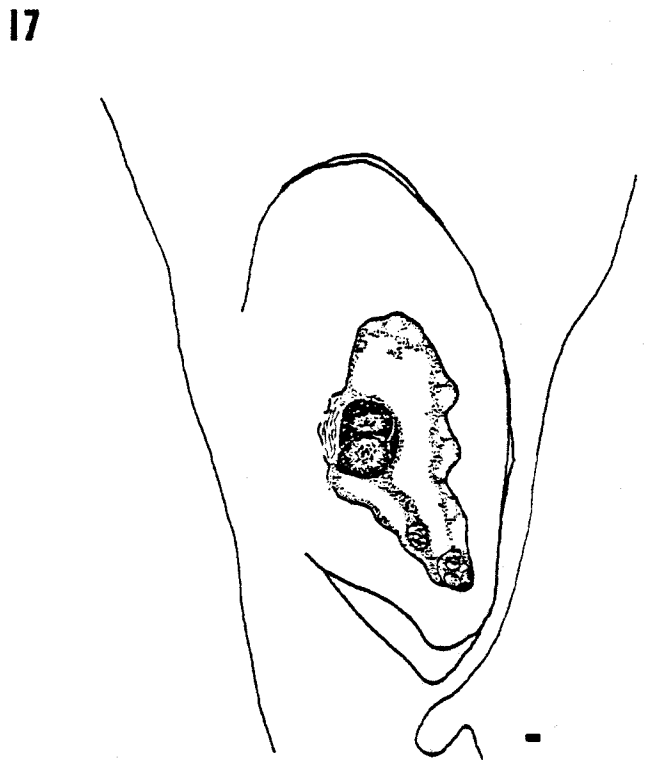


Figure 17. Entire ovary showing megagametophyte orientation.

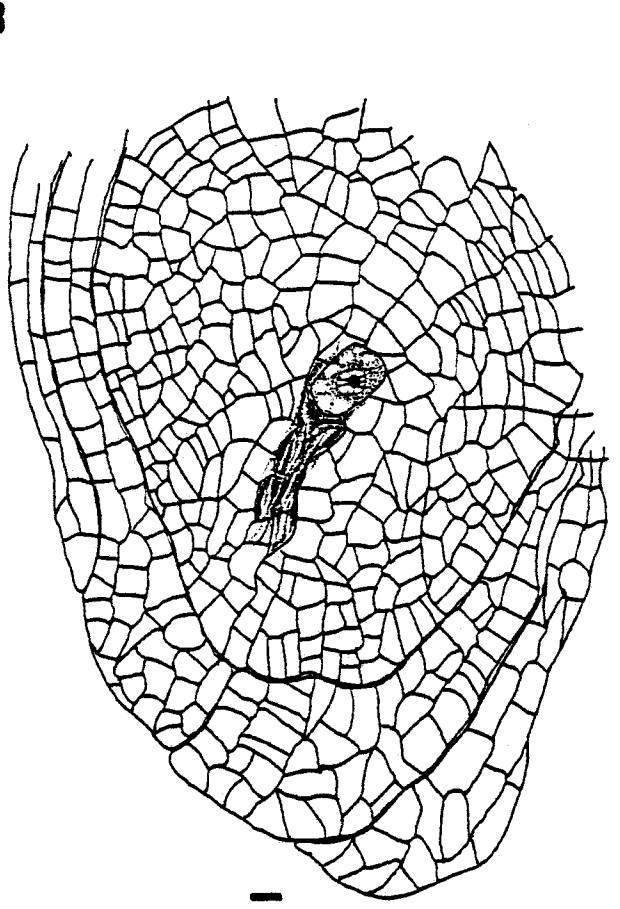


Figure 18. Remaining megaspore after degeneration of the rest of the tetrad as seen in the hybrid genotypes.

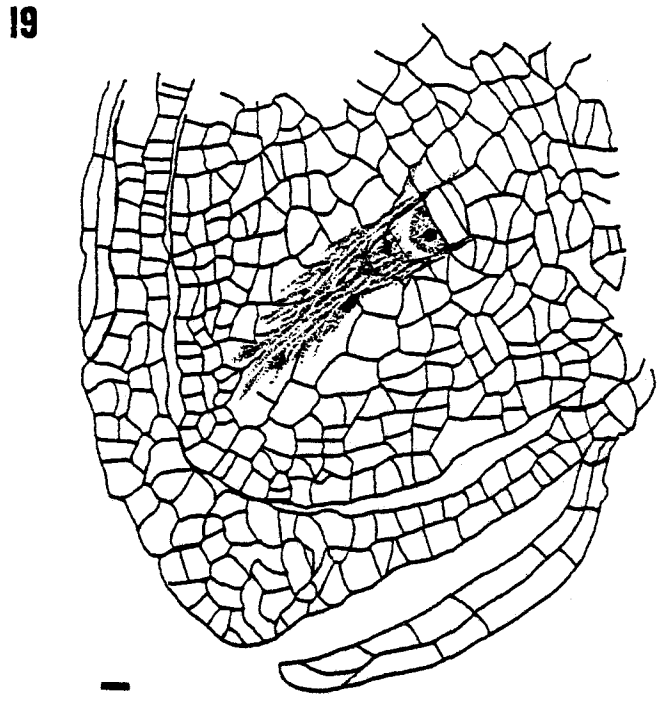


Figure 19. Degeneration of remaining megaspore as seen in the hybrid genotypes.

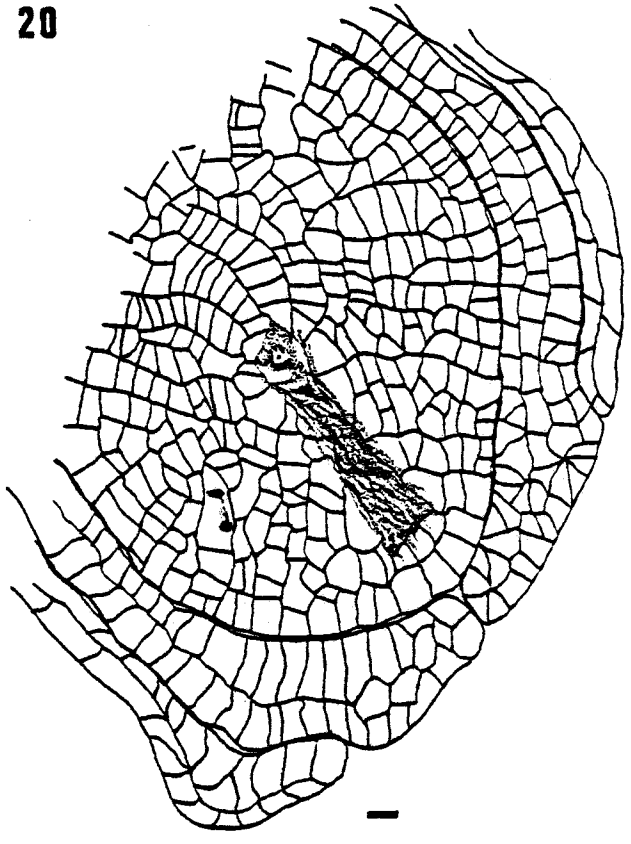


Figure 20. Degeneration of remaining megaspore as seen in the hybrid genotypes.

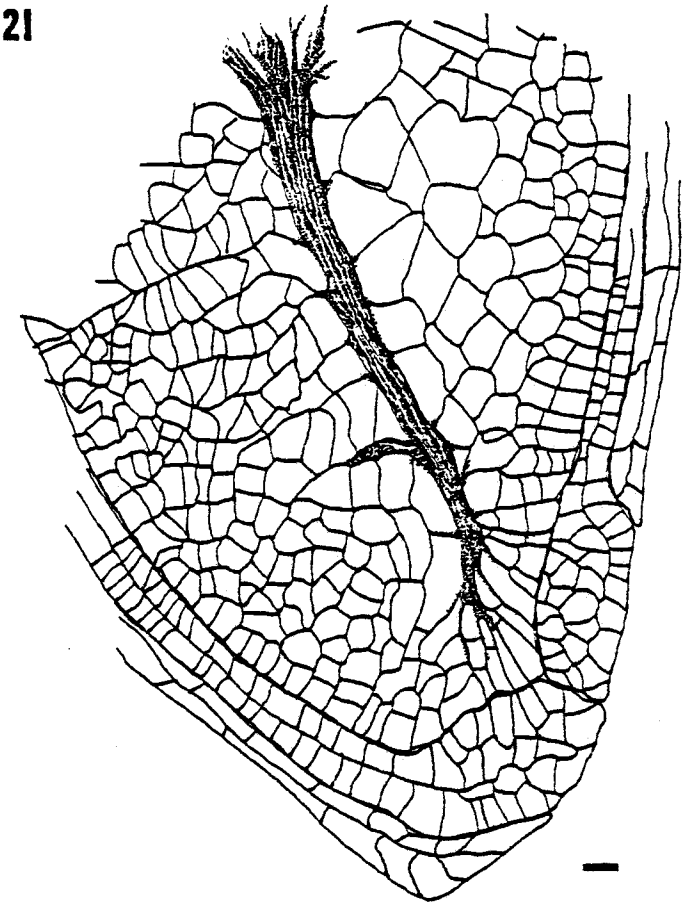


Figure 21. Darkly staining strand of compressed tissues of nucellar origin as seen in the hybrid genotypes.

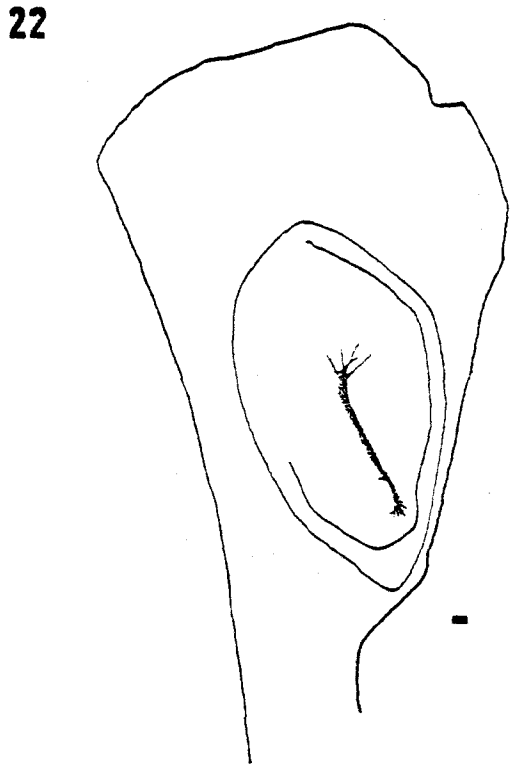


Figure 22. Entire ovule of hybrid genotypes.

Table 1. Summarized data of seed production and estimated floret production as observed on the hybrid genotypes grown in field and greenhouse conditions.

Genotype		Total # of Panicles	Total # of Spikelets	$\bar{x}$ Florets/ Spikelet	# of Seeds Obtained	Frequency of Seeds Obtained for Estimated Florets Produced	# of Seeds Germinated	Frequency of Germinated Seeds for Estimated Florets Produced
F_1 - 2	a	c	1132	c	5	5.387×10^{-4} d	1	1.077×10^{-4} d
	b	193	27441	8.20	7	3.279×10^{-5}	2	1.640×10^{-5}
F_1 - 6	a	c	1444	c	3	2.377×10^{-4} d	2	1.585×10^{-4} d
	b	285	27259	8.74	4	1.719×10^{-5}	2	8.595×10^{-6}
F_1 - 8	a	c	2032	c	0	0 d	0	0 d
	b	243	22163	8.76	3	5.847×10^{-5}	3	1.949×10^{-5}
F_1 - 10	a	c	2127	c	2	1.051×10^{-4} d	2	1.051×10^{-4} d
	b	170	13397	8.95	24	2.014×10^{-4}	18	1.119×10^{-5}
F_1 - 14	a	c	1212	c	4	3.152×10^{-4} d	0	0 d
	b	230	17662	10.47	1	5.439×10^{-6}	1	5.439×10^{-6}
Total	a	c	7947	c	16	2.240×10^{-4} d	5	7.000×10^{-5} d
	b	1121	107922	9.05	39	4.131×10^{-5}	26	1.589×10^{-6}

a . . . . greenhouse backcross with data summarized from results using P_1 of P_9 as pollinators
b . . . . field outcrossing with data summarized from results using ten different pollinators
c . . . . data not available
d . . . . estimates based on $\bar{x}$ florets/ spikelet based on 10 spikelets observed in field study

References

1. Brown, W. V. and G. E. Coe. 1951. A study of sterility in Hilaria belangeri (Steud.) Nash and Hilaria mutica (Buckl.) Benth. Am. Jour. Bot. 38: 823-830.

2. Carnahan, H.L. and H. D. Hill. 1961. Cytology and genetics of forage grasses. Bot. Rev. 27: 1-162.

3. Jensen, W. A. 1973. Fertilization in flowering plants. Bio Science 23: 21-27.

4. Landes, M. 1939. The causes of self-sterility in rye. Am. Jour. Bot. 26: 567-571.

5. Maheshwari, P. 1950. An introduction to the embryology of angiosperms. McGraw-Hill, New York.

6. Maze, J. and L. R. Bohm. 1974. Embryology of Agrostis interrupta (Graminae). Can. Jour. Bot. 52: 365-379.

7. Maze, J., L. R. Bohm, and L. E. Mehlenbacher, Jr. 1970. embryo sac and early ovule development in Oryzopsis milacea and Stipa tortilis. Can. Jour. Bot. 48: 27-41.

8. Maze, J. and Shu-Chang Lin. 1975. A study of the mature megagametophyte of Stipa elmeri. Can. Jour. Bot. 53: 2958-2977.

9. Moutray, J. B. 1971. An examination of seedling vigor and the effects of genetic diversity on response to heterosis in tall fescue (Festuca arundinacea, Schreb. L.) Ph.D. Thesis. Oregon State University.

10. Moutray, J. B. and R. V. Frakes. 1973. Effects of genetic diversity on heterosis in tall fescue. Crop Sci. 13: 1-4.

11. Nielsen, E. L. 1947. Macrosporogenesis and fertilization in Bromus inermis. Am. Jour. Bot. 34: 431-433.

12. Nielsen, E. L. 1953. Some cytological behaviors related in sterility in Bromus inermis. Am. Jour. Bot. 40: 824-827.

13. Nielsen, E. L. 1955. Cytological disturbances influencing fertility in Bromus inermis. Bot. Gaz. 116: 293-305.

14. Sheth, A. A., L. Yu, and J. Edwardson. 1956. Sterility in Pangolagrass (Digitaria decumbens Stent). Agron. Jour. 48: 505-507.

15. Vasquez, R. M. 1978. Meiotic chromosome behavior in two tall fescue (Festuca arundinacea Schreb.) genotypes and their sterile hybrid. Ph.D. Thesis. Oregon State University.

APPENDIX

Appendix

Introduction

Tall fescue, Festuca arundinacea, Schreb., is an allohexaploid grass species with 2n = 42. Based on meiotic analysis of intergeneric and interspecific crosses between Lolium perenne, L. multiflorum, Festuca pratensis,and F. arundinacea, it has been postulated that F. arundinacea is composed of three genomes of three related diploid species of the Lolium-Festuca complex (Jauhar, 1975). The origin of F. arundinacea is a matter of continued controversy (Malik and Thomas 1966a,b, 1973; Malik and Mary, 1970).

Tall fescue is a forage grass characterized by its good forage production capabilities, drought resistance, persistance,and potential for high seed yield. The management of tall fescue stands can be diverse since it can be utilized as permanent pasture, for hay production, or exclusively for seed production.

Background

Moutray and Frakes (1973) selected tall fescue genotypes which differed in morphology, origin, and anthesis date. The selected plants were grouped as early, intermediate, and late with respect to anthesis date, and single crosses were made between and within groups. Of special interest was one single-cross between an early parent (P_1) from Switzerland (PI 234-906) and a late parent (P_9) from Turkey (PI 174-209). The single-cross progeny showed considerable heterosis expressed as forage yield. The hybrids resulting from this cross

appeared sterile and would not set seed. Vasquez (1978) performed a cytological study of the parent and hybrid genotypes to determine if the apparent sterility was due to irregular chromosome behavior.

Meiotic irregularities are frequently found in natural populations, particularly in plants with high levels of polyploidy. In such populations irregularities such as univalents, trivalents, chromosome stickiness, laggards at anaphase I and II, numerical non-disjunctions and the presence of micronuclei are common. Such abnormalities usually result in some degree of reduced fertility.

The chromosome number in both parent genotypes and the hybrids was observed by Vasquez to be 2n = 42. No differences were detected among the parental and hybrid genotypes in the shape or relative length of the chromosomes. The meiotic process was similar in the parental and the hybrid genotypes.

In both parents the anthers dehisced normally and produced large amounts of pollen. In all hybrid genotypes examined, the anthers failed to dehisce, thus releasing no pollen. Pollen grains teased from the hybrid anthers exhibited a very high percentage of empty and shriveled pollen. A high frequency of this pollen contained only one nucleus, and indicated that the post-meiotic mitotic division that should generate the gametic nuclei had failed to occur. One-hundred percent of the pollen was infertile.

Literature Review

The ensuing review of the pertinent literature reflects the various stages of megagametophyte development, pre-fertilization processes, and post-fertilization relationships in the grass family where information is available. Following this review, is a presentation of the literature pertaining to the expression of sterility as observed from studies of the gynoceium.

General Description

Depending on the number of megaspore nuclei taking part in the development, the female gametophyte of angiosperms may be classified into three main types: monosporic, bisporic, and tetrasporic (see Maheshwari, 1950). The monosporic female gametophyte embryo sac falls under two types: 8-nucleate or 4-nucleate. In the development of the 8-nucleate embryo sacs, the first division of the functioning megaspore gives rise to two nuclei: the primary micropylar and primary chalazal. The second division produces one pair of nuclei at the micropylar and chalazal ends. The third results in two groups of four nuclei lying at opposite poles of the elongated embryo sac. The micropylar quartet differentiates into a three-celled egg apparatus and the lower polar nucleus. The chalazal quarter differentiates into a group of three antipodal cells and the upper polar nucleus. The two polar nuclei fuse to give rise to a secondary nucleus. This type of embryo sac is the most common and is designated the Polygonum-type. It occurs in at least 70% of the angiosperms known (Maheshwari, 1950).

The first sporogenous cell (archesporial cell) occurs beneath the protoderm at the apex of the ovule primordium. Slightly below the apex, the inner integument is initiated by periclinal divisions in the protoderm. With the appearance of the integument the nucellus of the primordium becomes delimited as the part enveloped by the integument.

Usually a narrow micropyle remains at the top of the integument. The outer integument arises in the protoderm and develops in a manner similar to the inner integument (Esau, 1965).

Megasporogenesis

No descriptions of megasporogenesis or megagametogenesis for Festuca have been found, but several related genera have been studied and reported. Descriptions of megasporogenesis for Bromus (Nielsen, 1947), Agrostis (Maze and Bohm, 1974), Oryzopsis and Stipa (Maze et al., 1970) are similar. In general, a linear or modified T-shaped tetrad results following meiotic divisions and cytokinesis. The three megaspores nearest the micropylar region show evidence of collapse and eventual disintegration of the cytoplasm after the second meiotic division. The chalazal megaspore begins its enlargement shortly after its formation. This functional megaspore eventually develops into the embryo sac. When the three microylar megaspores have degenerated, the surrounding nucellar cells intrude the vacant space. Similar descriptions of megasporogenesis in other grasses have been reported for Eragrostis (Mengesha and Guard, 1966; Stover, 1937), Setaria (Narayanaswami, 1956), Pennisetum (Snyder et al., 1955) and Zea (Weatherwax, 1919).

Megagametogenesis

Egg Apparatus-Description

The functional megaspore mitotically divides three times to produce a seven-celled (8-nucleate) embryo sac consisting of an egg apparatus,

antipodals, and central cell. The egg apparatus consists of an egg and two synergids. The first step in the differentiation of the egg and synergids is that the egg becomes vacuolate and then outgrows the synergids (Maze et al., 1970). As the synergids differentiate a filiform apparatus develops in them at their micropylar tip. Then one synergid begins to decrease in size in preparation for fertilization. In Agrostis interrupta (Maze and Bohm, 1974) the fully differentiated egg apparatus consists of a slightly vacuolated egg, a degenerated synergid lacking a nucleus, but with a filiform apparatus, and a vacuolated, non-degenerated synergid lacking a filiform apparatus. The time of synergid degeneration varies from species to species. In Stipa elmeri (Maze and Lin, 1975) the two synergids are similar in size and appearance, before the pollen tube arrives at the megagametophyte. Before fertilization, both synergids then undergo cytological change and one decreases in size (Maze and Bohm, 1973). Narayanaswami (1956) indicates that both synergids degenerate prior to fertilization in Setaria italica. In Eragrostis cilianensis only one synergid is formed (Stover, 1937).

Antipodals-Description

At the stage when the egg and the synergids have been differentiated, there may be three to many antipodals. A review of the literature by Stover in 1937 revealed the number of antipodals in the grass tribes to vary from three to sixty. Maze and Bohm (1973) indicate that in the Graminae rarely only three antipodals are found.

By the time the egg apparatus in Agrostis interrupta has begun development, the antipodals begin to proliferate (Maze and Bohm, 1974). Some of these early proliferated antipodals are binucleate. In Eragrostis tef, the antipodals proliferate until fertilization when 16 to 19 are present (Mengesha and Guard, 1966). After fertilization the antipodals increase considerably in size. At or just prior to fertilization, an increase in size of the five to eight, a maximum of ten antipodals in three species of Bromus is reported by Beck and Horton (1932). Large multinuleolate nuclei may be found in the proliferated mass of cells and may be the result of abnormal cell division or fusion of several nuclei. The fate of the antipodals is one of degeneration and eventual absorbtion as evidenced by Beck and Horton (1932) who found the antipodals to be absorbed by the developing endosperm.

Polar Nuclei-Description

Polar nuclei normally migrate to the middle portion of the megagametophyte, in close proximity to the egg, held in position by cytoplasmic strands running from the micropylar to the chalazal ends. The two polar nuclei are generally provided from opposite ends of the embryo sac, however, Stover (1937) reported in E. cilianensis both polar nuclei being of micropylar origin. Maze et al. (1970) found the polar nuclei in S. elmeri, even before they migrate, to be differentiated and distinguishable from the other nuclei in the embryo sac by a vacuole in their nucleoli. The polars of O. milaceae contained no vacuoles.

The polar nuclei can (a) fuse prior to fertilization or (b) one can fuse with a sperm nucleus and this diploid nucleus can then fuse with the other polar nucleus. In S. elmeri the polar nuclei remain unfused (Maze and Bohm, 1973) while in A. interrupta polar nuclei fuse before fertilization (Maze and Bohm, 1974). Actual triple fusion is obscured or missed in most embryo sac studies.

Fertilization General Description

The course of the pollen tube from stigma hair through the nucellar layer covering the micropylar end of the ovule has been described in Hordeum (Pope, 1946). A general description of pollen style reaction and pollen tube growth is given by Frankel and Galun (1977). After pollination the pollen germinates on the stigma and puts forth a pollen tube which makes its way down the style into the ovarian cavity and along the ovary wall into the micropylar region of the ovule. The time to complete this process varies in grass species from minutes to hours. The entrance and discharge of the pollen tube into the embryo sac and the role of the synergids is a source of controversy among angiosperm embryologists (Maheshwari, 1950; Jensen, 1973). There appear to be three distinct sites of pollen tube entry into the embryo sac: (1) between the egg and one synergid; (2) between the embryo sac wall and one synergid; (3) directly into one of the synergids. Pollen tube discharge into one synergid has long been thought to destroy it.

Jensen and Fisher (1968) questioned the destruction of the synergid by the pollen tube and showed that one of the synergids of cotton begins to degenerate after pollination but before the pollen tube reaches the embryo sac. The pollen tube enters and discharges its contents into the degenerate synergid. Cass and Jensen (1970) note similar discharge in Hordeum. Degeneration of the synergid is suggested by Jensen and Fisher (1968) as being caused by an earlier physiological change possibly initiated by pollination. If the cotton flower goes unpollinated, synergid degeneration does not occur. In the pollinated flower the synergids begin to degenerate within six to eight hours after pollination. Similar descriptions of synergid degeneration are found in Agrostis (Maze and Bohm, 1974), Stipa (Maze and Bohm, 1973) and Hordeum (Cass and Jensen, 1970).

Synergid and Polar Nuclei - Function

The synergids are thought to function in the production of substances which attract the pollen tube to the megagametophyte (van der Pluijm, 1964; Diboll and Larson, 1966). In some species they may also participate in nourishing the young embryo (Jensen, 1965). Maheshwari (1950) suggests that the filiform apparatus might repel foreign pollen tubes by an antibody-antigen reaction.

In S. elmeri, as in other plants, the filiform apparatus is an elaboration of the micropylar portion of the synergid wall. Maze and Lin (1975) hypothesized the filiform apparatuses of the two synergids to perform different functions. In the degenerated synergid, the filiform apparatus serves to increase the surface area of the plasma membrane and, thereby, offering a larger area for pollen tube growth-directing compounds to diffuse out of the synergid. In the persistent synergid the filiform apparatus is one of many features which indicate that the persistent synergid is involved in the transference of materials into the megagametophyte. Jensen and Fisher (1968) have speculated that synergid degeneration is instrumental in the cessation of pollen tube growth and may offer a mechanism whereby the plasma membrane of the egg and the sperm cells can come into contact and thereby effect fertilization.

Double fertilization is the rule in these normal embryo sacs with one sperm nuclei fusing with the egg and the other sperm nuclei fusing with the polar nuclei for triple fusion and the formation of the primary endosperm nucleus. Synygamy and triple fusion can occur simultaneously as in Setaria italica (Narayanaswami, 1956), with synygamy before triple fusion as in Bromus inermis (Nielsen, 1947) or vice-versa as in Hordeum (Cass and Jensen, 1970). Following triple fusion endosperm formation in grasses is initially free nuclear, changing to cellular within several days post-fertilization (Maheshwari, 1950; Stover, 1937). Esau (1977) has described normal grass embryo formation following synygamy.

Antipodal-Function

The antipodals are generally the most metabolically active cells in the megagametophyte. Proliferation of the antipodals, as discussed previously, can occur pre- or post-fertilization, or both. One possible function of grass antipodals is the production of growth controlling substances. Based on endosperm and antipodal responses in hybrids, of Hordeum jubatum x Secale cereale, Brink and Cooper (1944) came to the conclusion that grass antipodals are involved in the control of early endosperm growth. Another possible function of grass antipodals is the transference of some materials into the megagametophyte (Maze and Lin, 1975). The proposed direction of metabolite flow across the cell walls into the megagametophyte is from nucellus to antipodal to central cell (List and Steward, 1965). The development of papillate walls in maize antipodals adjacent to the nucellus and in the central cell adjacent to the antipodals is interpreted as supporting this hypothesis (Diboll and Larsen, 1966). Papillate cell walls are found to be present in cells from which secretory products emerge. If the antipodals rapidly accumulate (or synthesize) and release hormones, then one might expect hormonal imbalance as reflected in the changes in antipodal cytology, specifically the number of nuclei per cell, as discussed previously (Maze and Bohm, 1974).

Sterility

Sterility has been extensively studied in many plants. Much of the data that have been accumulated are primarily of a genetic nature, but some come from cytological studies of the developing microsporocytes. A review of this literature was made by Vasquez (1978). Cytology and genetic reviews of sterility give evidence as to the number of studies using the microsporocyte in meiotic studies (Carnahan and Hill, 1961; Bosemark, 1957; Myers, 1947; Crowder, 1953; Church, 1929). Meiotic irregularities seen in the microsporocytes, however, do not necessarily reflect the condition of female fertility. Examples of female sterility with normal pollen fertility have been reported for sorghum (Casady, 1959), red clover (Povilaitis, 1959), and grape (Pratt, 1961). It should be remembered that only one of four potential products function as the female gamete after Polygonum-type megasporogenesis. Because the megagametophyte is better protected from the hazards of the environment, defective gametes may be transmitted through the female gamete (Briggs and Knowles, 1967).

The genic expression of female sterility can be classified into two basic types (1) those affecting the exterior structure of the ovary and (2) those affecting the development of the female gametophyte. Within this first classification the deformation of floral parts, or their complete absence has been reported as responsible for sterility in Capsicum annuum, (Bergh, 1965), sudangrass (Townsend, 1960), sorghum (Casady, 1959), and tobacco (Wan, 1967).

Sterility as affected by the development of the female gametophyte can be categorized into two general classes: (a) those attributable to meiotic disturbances and early failure during development and (b) those dealing with post-fertilization development with considerable emphasis being placed on embryo-endosperm relationships (Nielsen, 1953).

Landes (1939) observed varied behavior in four abnormal classes of ovaries in selfed and crossed rye. The first of those that she reports include those wherein

> ". . . .no embryo sac has been formed. In some of these there is a small group of cells at the center of the nucellus, and in others a narrow strip of deeply staining material extends from the center toward the micropylar end. Presumably the breakdown occurs at or immediately after meiosis ----no megaspore enlarges to form the embryo sac . . . . In the second group the embryo sac appears normal in every respect but remains unfertilized. At five days after pollination signs of degeneration appears . . . In the third group the embryo sac is apparently formed but soon aborts . . . . In the last group some degree of development occurs . . . (those) in which the irregularities are extreme may abort in later stages. The abnormalities affect the embryo less than the endosperm."

The embryo sac disturbances that Landes described indicates the degree of complexity involved in the expression of female sterility or abortion. She found the selfed and crossed rye ovaries to differ only in the degree of occurrence of the abnormal types, and discounted chromosome irregularities as accounting for the abnormalities found.

Sheth et al. (1956) described the appearance of sterile ovaries examined in Pangolagrass. Their description of the region of crushed nucellus is like that of the first class of abnormal ovaries discussed by Landes, but Sheth et al. documented the time of developmental breakdown to occur at meiosis. The megaspore-mother-cells were never seen to progress past early meiosis, probably leptotene.

Ovule sterility was concluded to result from an unbalanced chromosome complement. An analysis of somatic cells revealed only 27 chromosomes, whereas 30 were expected. Univalents, bivalents, and multivalents were observed at diakinesis. Scattered univalents and lagging chromosomes during microsporogenesis were concluded to account for a high degree of pollen-sterility. Pollen viability as estimated by KI_2 staining was 0.4% viable.

Hakansson and Ellerstrom (1951) in a study of seed development following crosses between diploid and tetraploid rye, also reported that some ovules lacked embryo sacs while in others organization was atypical. Among those ovaries that lacked embryo sacs, they found only darkly staining, amorphous remains of the megaspore-mother-cell or chalazal megaspore were observed within the ovary tissues.

In Hilaria sp., Brown and Coe (1951), reported that the low seed setting capabilities of two different species they examined were due to ovule abortion. Abortion was found to occur at any time after megasporogenesis but usually before pollination. As the gametophyte degenerated, they noted no changes in the nucellar tissues, or any changes in the ovary tissues. It was concluded that the collapse of the gametophyte before pollination must be inherent in the gametophyte or sporophyte which bears it. They did not elaborate to the cause of ovule abortion.

In tomato, Lycopersicon esculentum, Rick (1946) reported of three classes of ovule types he found in diploid, triploid, and tetraploid plants. The ovule type he designated as the "substitution" type was found when abnormal development occurred before meiosis. Abnormal post-meiotic development resulted in "collapsed" type ovules. He found these two types of ovules to be as large and well formed as normal ones, the third ovule type, but functionless. Triploids and tetraploids were characterized by the "collapsed" type ovule and was reasoned to be conditioned by an adverse chromosomal imbalance of the gametophyte itself. Such an unbalanced complement could not account for all examples of the "collapsed" type since it was also found in low frequencies in fruitful diploids and mutant diploids.

The "collapsed" type ovules showed hypertrophy of the inner layer of the integumentary cells, being maternal tissue. This is in contrast to the sterility seen in Hilaria, where no change in the maternal tissues was seen.

In Bromus inermis cytological disturbances are reported to have an influence on fertility. The female gametophytes of some sterile plants described by Nielsen (1953) survive beyond the critical reduction division but commence to degenerate prior to fertilization. Eventual gametophyte collapse was preceeded by the early digestion of the nutrients of the antipodals and degeneration of the egg apparatus. He further presented the suggestion that this type of sterility may be the manifestation of certain gene combinations, possibly related to the formation of certain necessary growth substances. Such combinations may usually go undetected in a highly heterozygous condition that usually exists in B. inermis.

Nielsen (1955) also reported some of his observations made of microsporocyte fertility as he found them in the F_1 progeny of two inbred parents. The F_1 progeny were vigorous and uniform but all were characteristically low in seed set. The results were similar when selfed or outcrossed. An examination of ten different F_1 genotypes revealed to all be highly sterile with half of them producing degenerated pollen. Pollen degeneration occurred after meiosis, but before mitosis. Examination of the parents showed that one had normal fertile pollen, while the other showed pollen degeneration like that of the F_1 progeny. Further cytological examination led Nielsen to hypothesize the action of B-chromosomes, chromosome abnormalities, and (or) interaction between these to account for the sterility observed in the F_1. Although an examination

of the megagametes was not conducted, Nielsen alluded to the failure of the F_1 to set seed upon crossing, as being evidence for similar sterility of the gynoecium.

Brink and Cooper (1947) emphasize that fertilization of the egg and central cell are parallel events. This results in stimulation of the entire ovule to rapid growth. Further, endosperm and embryo grow in intimate association with each other and the maternal tissues. Thus all three are affected in aborting seeds. Their observations of embryo, endosperm and maternal tissue relations in angiosperms may be summarized as follows:

(a) "The endosperm is primarily responsble for the maintenance of continuity in the life cycle at the early seed stage. Although the embryo is the cardinal component of the seed . . . this structure is initially incapable of performing certain essential growth functions."

(b) "The double complement of inheritance received by the endosperm from the maternal plant is an adaptation which facilitates functioning of the endosperm in its intercalary position between mother plant and embryo."

(c) "The secondary fertilization in angiosperms is interpreted as a device tending to offset the disadvantage in reproduction associated with extreme reduction of the female gametophyte, and to enhance the aggressiveness of the endosperm through conferring upon this tissue the physiological advantages of hybridity."

They further summarize several kinds of genetic alterations in the balance between the endosperm, embryo and maternal tissues which are known to lead to seed abortion. These are listed below without exemplification (Brink and Cooper, 1947): (a) enforced self-fertilization in a normally cross-fertilized species, (b) crossing of species not differing in chromosome number: seed failure in this class results from combining qualitatively diverse genomes in the seed, (c) crossing races differing essentially only in degree of polyploidy: purely quantitative changes in the 2:3:2 chromosomal ratio of maternal tissue, endosperm and embryo is an important cause of seed collapse, (d) crossing species differing in both degree of polyploidy and genic complement, (e) aneuploidy, (f) maternal genotype causes arrested growth of the seed regardless of the source of sperm participating in fertilization; seedless fruits; parthenocarpic fruits.

This review of the literature pertaining to the expression of sterility associated with the female gametophyte reveals a variety of types. Of interest to this study are those reports where female sterility was caused by meiotic disturbance and failure of the gametophyte. The number of such studies is relatively few in comparison to the number of studies examining expressions of sterility in plants (7). Among this low number of reports, there is variation in the causes of sterility. Similarities occur, in the observed morphological changes resulting from these causes, however, indicating that the time of disturbance can be important in characterizing the sterility expression as being pre- or post meiotic.

Bibliography

1. Beck, P. and J. S. Horton. 1932. Microsporogenesis and embryology in certain species of Bromus. Bot. Gaz. 93: 42-54.

2. Bergh, B. D. and L. F. Lippert. 1965. A gene difference that affects fertility in Capsicum annuum L. Am. Nat. 99: 159-166.

3. Bösemark, N. O. 1957. On accessory chromosomes in Festuca pratensis V. Influence of accessory chromosomes on fertility and vegetative development. Hereditas 43: 211-235.

4. Briggs, F. N. and P. F. Knowles. 1977. Introduction to plant breeding. Reinhold Publishing Corporation.

5. Brink, R. A. and D. C. Cooper. 1947. The endosperm in seed development. Bot. Rev. 13: 423-541.

6. Brown, W. V. and G. E. Coe. 1951. A study of sterility in Hilaria belangeri (Steud.) Nash and Hilaria mutica (Buckl.) Benth. Am. Jour. Bot. 38: 823-830.

7. Carnahan, H. L. and H. D. Hill. 1961. Cytology and genetics of forage grasses. Bot. Review 27: 1-162.

8. Casady, A. J., E. G. Heyne, and D. E. Weibel. Inheritance of female sterility in sorghum. Jour. Heredity 51:35-38.

9. Cass, D. D. and W. A. Jensen. 1970. Fertilization in barley. Am. Jour. Bot. 57: 62-70.

10. Church, G. L. 1929. Phenomena in certain Graminae. I. Festuceae, Aveneae, Agrostideae, Chlorideae, and Phalarideae. Bot. Gaz. 87: 608-629.

11. Crowder, L. V. 1953. A survey of meiotic behavior in tall fescue grass. Am. Jour. Bot. 40: 348-354.

12. Diboll, A. G. and D. A. Larson. 1966. An electron microscope study of the mature megagametophyte in Zea mays. Am. Jour. Bot. 53: 391-402.

13. Esau, K. 1965. Plant anatomy - 2nd Ed. John Wiley and Sons, Inc., New York.

14. Esau, K. 1977. Anatomy of seed plants - 2nd Ed. John Wiley and Sons, Inc., New York.

15. Evans, G. M., H. K. Asay, and R. G. Jenkins. 1973. Meiotic irregularities in hybrids between diverse genotypes of tall fescue (F. arundinacea, Schreb.). Crop Sci. 13: 376-379.

16. Frankel, R. and E. Galun. 1977. Pollination mechanisms, reproduction and plant breeding. Springer-Verlag. New York.

17. Häkansson, A. and S. Ellerstrom. 1951. Seed development after reciprocal crosses between diploid and tetraploid rye. Hereditas 26: 286-296.

18. Jauhar, P. P. 1975. Genetic regulation of diploid-like chromosome pairing in the hexaploid species, Festuca arundinacea Schreb. and F. rubra L. (Graminae). Chromosoma (Berl.) 52: 363-382.

19. Jensen, W. A. 1965. The ultrastructure and histochemistry of the synergids in cotton. Am. Jour. Bot. 52: 238-256.

20. Jensen, W. A. 1973. Fertilization in flowering plants. Bio Science 23: 21-27.

21. Jensen, W. A. and D. B. Fisher. 1968. Cotton embryogenesis: the entrance and discharge of the pollen tube in the embryo sac. Planta (Berl.) 78: 158-183.

22. Landes, M. 1939. The causes of self-sterility in rye. Am. Jour. Bot. 26: 567-571.

23. Lewis, E. J. 1963. Hybrids between geographic races of F. arundinacea Schreb.) Rep. Welsh Plt. Breed. Sta. for 1962: 26-27.

24. List, A., Jr. and F. C. Steward. 1965. The nucellus, embryo sac, endosperm and embryo of Aesculus and their interdependence during growth. Ann. Bot. 29: 1-15.

25. Maheshwari, P. 1950. An introduction to the embryology of angiosperms. McGraw-Hill Book Co., Inc., New York.

26. Malik, C. P. 1970. Pachytene studies in the intraspecific hybrid of Festuca arundinacea Schreb. (2n = 42). Genetica Polonica 11 (3-4): 379-383.

27. Malik, C. P. and P. T. Thomas. 1966a. Chromosomal polymorphisms in Festuca arundinacea. Chromosoma (Berl.) 18: 1-18.

28. Malik, C. P. and P. T. Thomas. 1966b. Meiosis in the intergeneric hybrid between Lolium multiflorum(2n = 14) x F. arundinacea (2n = 70) and its amphiploid (2n = 84). Z.Pflanzenzucht 55: 81-94.

29. Malik, C. P. and T. N. Mary. 1970. Probable genotype of BB' genome in Festuca arundinacea Schreb. (2n = 42). Zeits. Biol. 116: 501-506.

30. Maze, J. and L. R. Bohm. 1973. Comparative embryology of Stipa elmeri. Can. Jour. Bot. 51: 235-247.

31. Maze, J. and L. R. Bohm. 1974. Embryology of Agrostis interrupta (Graminae). Can. Jour. Bot. 52: 365-379.

32. Maze, J., L. R. Bohm, and L. E. Mehlenbacher, Jr. 1970. Embryo sac and early ovule development in Oryzopsis milacea and Stipa tortilis. Can. Jour. Bot. 48: 27-41.

33. Maze, J. and Shu-Chang Lin. 1975. A study of the mature megagametophyte of Stipa elmeri. Can. Jour. Bot. 53: 2958-2977.

34. Mengesha, M. H. and A. T. Guard. 1966. Development of the embryo sac and embryo of Teff, Eragrostis tef. Can. Jour. Bot. 44: 1071-1075.

35. Moutray, J. B. 1971. An examination of seedling vigor and the effects of genetic diversity on response to heterosis in tall fescue (Festuca arundinacea, Schreb. L.) Ph.D. Thesis. Oregon State University.

36. Moutray, J. B. and R. V. Frakes. 1973. Effects of genetic diversity on heterosis in tall fescue. Crop Sci. 13: 1-4.

37. Myers, W. M. 1947. Cytology and genetics of forage grasses. Bot. Rev. 13: 319-421.

38. Narayanaswami, S. 1956. Structure and development of the caryopsis in some indian millets. VI. Setaria italica. Bot. Gaz. 112-122.

39. Neilsen, E. L. 1947. Macrosporogenesis and fertilization in Bromus inermis. Am. Jour. Bot. 34: 431-433.

40. Neilsen, E. L. 1953. Some cytological behaviors related to sterility in Bromus inermis. Am. Jour. Bot. 40: 824-827.

41. Neilsen, E. L. 1955. Cytological disturbances influencing fertility in Bromus inermis. Bot. Gaz. 116: 293-305.

42. Pope, M. N. 1946. The course of the pollen tube in cultivated barley. Jour. Am. Soc. Agron. 38: 432-440.

43. Povilaitis, B. and J. W. Boyes. 1959. Embryo-sac production in relation to seed yields of diploid Dollard red clover. Can. Jour. Plt. Sci. 39: 364-374.

44. Pratt, C. and J. Einset. 1961. Sterility due to premeiotic ovule abortion in small-clustered and normal Concord grapes. Am. Jour. Hort. Sci. Proc. 78: 230-238.

45. Rick, C. M. 1946. The development of sterile ovules in Lycopersicon esculentum Mill. Am. Jour. Bot. 33: 250-256.

46. Sheth, A. A., L. Yu and J. Edwardson. 1956. Sterility in Pangolagrass (Digitaria decumbens Stent). Agron. Jour. 48: 505-507.

47. Snyder, L. A., A. R. Hernandez, and H. E. Warmke. 1955. The mechanism of apomixis in Pennisetum ciliare. Bot. Gaz. 209-221.

48. Stover, E. L. 1937. The embryo sac of Eragrostis cilianensis (All.) Link. Ohio Jour. Sci. 37: 172-184.

49. Thomas, P. T. and H. Thomas. 1972. Problems of chromosome manipulation in plant breeding. Rep. Welsh Plt. Bred. Sta. for 1971, 117-122.

50. Thomas, P. T. and H. Thomas. 1973. Chromosome manipulation and plant breeding. Proc. IAEA Seminar, Italy, p. 377-378.

51. Townsend, C. E. 1960. Sterility in sudangrass: I. Barren and chimeral partial-sterility. Agron. Jour. 52: 153-155.

52. van de Pluijm, J. E. 1964. An electron microscopic investigation of the filiform apparatus in the embryo sac of Torenia fournieri, p. 6-16. In H. F. Linskins (ed.) Pollen physiology and fertilization. North Holland Publishing Co., Amsterdam.

53. Vasquez, R. M. 1978. Meiotic chromosome behavior in two tall fescue (Festuca arundinacea Schreb.)genotypes and their sterile hybrid. Ph. D. Thesis. Oregon State University.

54. Wan, H. and T. J. Mann. 1967. Inheritance of a female sterility in tobacco. Jour. Heredity 58: 85-87.

55. Weatherwax, P. 1919. Gametogenesis and fecundation in Zea mays as the basis of xenia and heredity in the endosperm. Bull. Torrey Bot. Club 46: 73-90.

Appendix Table 1. The number of seeds obtained and germinated from selfings, outcrosses, and backcrosses made between the parent, hybrid, and control genotypes conducted in the greenhouse.

Parents-Crosses	Number of Seeds Obtained	Number of Seeds Germinated	% Germination
$P_1 \times P_9$	157	119	d
$P_9 \times P_1$	208	105	d
Parents-Selfings			
$P_1 \times P_1$	144	95	66
$P_9 \times P_9$	55	26	47
Parents-Open Pollinated			
$P_1 \times OP$	85	76	89
$P_9 \times OP$	89	86	97
Hybrid Backcrosses to P_1			
$F_1 - 2 \times P_1$	5	1	20
$F_1 - 6 \times P_1$	3	2	66
$F_1 - 8 \times P_1$	a	-	--
$F_1 - 10 \times P_1$	2	2	100
$F_1 - 14 \times P_1$	3	0	0

Appendix Table 1. (Continued) The number of seeds obtained and germinated from selfings, outcrosses, and backcrosses made between the parent, hybrid, and control genotypes conducted in the greenhouse.

Hybrid Backcrosses to P_9	Number of Seeds Obtained	Number of Seeds Germinated	% Germination
F_1 - 2 x P_9	4	3	75
F_1 - 6 x P_9	a	-	--
F_1 - 8 x P_9	a	-	--
F_1 - 10 x P_9	6	6	100
F_1 - 14 x P_9	1	0	0
P_1 Outcrosses to Control			
P_1 x FP_{16}	11	7	64
P_1 x FP_{18}	a	-	--
P_1 x FP_{20}	a	-	--
P_1 x FP_{22}	3	1	33
FP_{16} x P_1	a	-	--
FP_{18} x P_1	a	-	--
FP_{20} x P_1	a	-	--
FP_{22} x P_1	b	b	b

Appendix Table 1. (Continued) The number of seeds obtained and germinated from selfings, outcrosses, and backcrosses made between the parent, hybrid, and control genotypes conducted in the greenhouse.

P_9 Outcrosses to Control	Number of Seeds Obtained	Number of Seeds Germinated	% Germination
$P_9 \times FP_{16}$	72	0	0
$P_9 \times FP_{18}$	43	23	53
$P_9 \times FP_{20}$	a	--	--
$P_9 \times FP_{22}$	b	--	--
$FP_{16} \times P_9$	72	6	8
$FP_{18} \times P_9$	101	101	100
$FP_{20} \times P_9$	158	113	d
$FP_{22} \times P_9$	b	b	b
Controls-Selfings			
$FP_{16} \times FP_{16}$	c	c	c
$FP_{18} \times FP_{18}$	137	101	74
$FP_{20} \times FP_{20}$	c	c	c
$FP_{22} \times FP_{22}$	c	c	c

Appendix Table 1. (Continued) The number of seeds obtained and germinated from selfings, outcrosses, and backcrosses made between the parent, hybrid, and control genotypes conducted in the greenhouse.

Controls-Open Pollinated	Number of Seeds Obtained	Number of Seeds Germinated	% Germination
FP_{16} - OP	102	102	100
FP_{18} - OP	115	115	100
FP_{20} - OP	20	9	45
FP_{22} - OP	21	20	95

a . . . . . No seed obtained
b . . . . . Crosses not made
c . . . . . Selfings not made
d . . . . . Estimate no applicable

Appendix Table 2. Ratio (x/y) of seeds germinated (x) to seeds obtained (y) for each of the hybrid genotypes in each of the pollination blocks.

Pollinator Block	Pollinator Identification	Origin[1]	F_1 - 2	F_1 - 6	F_1 - 8	F_1 - 10	F_1 - 14
1	PI 315-434	USSR	0/0	0/0	0/0	0/0	0/0
2	PI 265-361	Holland	0/0	1/1	0/0	9/11	0/0
3	Ore L. 309	Oregon	0/0	0/0	0/0	0/0	0/0
4	PI 265-359	Netherlands	0/0	0/0	0/0	1/2	0/0
5	PI 289-004	Hungary	0/0	0/0	0/0	0/0	0/0
6	Kentucky 31	Missouri	0/0	1/3	1/1	2/2	0/0
7	FEI - 68 (Missouri Designation)	Missouri	a	0/0	2/2	0/0	0/0
8	Kenhy 26G1 - 298 (Missouri Designation)	Missouri	1/1	0/0	0/0	1/2	0/0
9	PI 315-434	USSR	1/4	0/0	0/0	5/7	1/1
10	PI 292-851	East Germany	0/2	0/0	a	0/0	0/0

1 . . . . state or country
a . . . . data not available

Appendix Table 3. Various anther characteristics of the hybrid, parent, and control genotypes observed in both field or laboratory examinations.

	Field	Laboratory	$\bar{x}$ Anther Length (mm)[a]	Anther Color
F_1 - 1	NP	NP	5.58	Yellow
F_1 - 2	NP	NP	5.23	Yellow
F_1 - 3	NP	NP	b	b
F_1 - 4	NP	NP	5.50	Yellow
F_1 - 5	NP	NP	5.34	Yellow
F_1 - 6	NP	NP	3.90	Yellow
F_1 - 7	NP	NP	b	b
F_1 - 8	NP	NP	5.08	Yellow
F_1 - 9	NP	NP	b	b
F_1 - 10	NP	NP	4.90	Yellow
F_1 - 11	NP	NP	b	b
F_1 - 12	NP	NP	b	b
F_1 - 13	NP	NP	b	b
F_1 - 14	NP	NP	4.48	Yellow
F_1 - 15	NP	NP	3.87	Yellow
F_1 - 16	NP	NP	3.86	Yellow
F_1 - 17	NP	NP	b	b
F_1 - 18	NP	NP	5.50	Yellow
F_1 - 19	NP	NP	b	b
F_1 - 20	NP	NP	b	b

Appendix Table 3. (Continued) Various anther characteristics of the hybrid, parent, and control genotypes observed in both field or laboratory examinations.

	Field	Laboratory	$\bar{x}$ Anther Length (mm)[a]	Anther Color
F_1 - 21	NP	NP	4.81	Yellow
F_1 - 22	NP	NP	4.70	Yellow
F_1 - 23	NP	NP	4.50	Yellow
F_1 - 24	NP	NP	4.44	Yellow
F_1 - 25	NP	NP	5.63	Yellow
P_1	P	b	4.26	Yellow
P_9	P	b	b	Yellow
314 FP_{16}	P	b	4.13	Yellow
339 FP_{18}	P	b	4.71	Yellow
342 FP_{20}	P	b	5.38	Yellow
352 FP_{22}	P	b	5.66	Yellow

a . . . . . based on ten measurements
b . . . . . no data available
NP . . . . no pollen shed; indehiscent anther

Addendum I

Some of the techniques available for ovule examination that were tried in this study proved to be unacceptable. Although unsatisfactory in this study they have valuable qualities and as such they are presented here with reasons for not being used, as reference for future studies. Reasons favoring the use of the technique employed are also given.

Varied types of techniques have been employed for ovule and female gametophyte examination. These include ovule squash methods, ovule clearing techniques, and various fixing and staining solutions used in the paraffin-embedding procedure. A fast, easily employed, and accurate technique is desirable for ovule examinations.

The ovule clearing technique described by Herr (1971) initially looked promising for use in this study because of its reported simplicity and efficiency. Ovaries that had been previously fixed in FPA_{50}, and stored in 70% ethanol, were placed in a clearing fluid for 24 hours. The clearing solution was composed of lactic acid (85%), chloral hydrate, phenol, clove oil, and xylene (2:2:2:2:1, by weight). After 24 hours whole ovaries were transferred with some of the fluid to a slide prepared according to the description by Herr. When covered with a plastic coverslip, slight pressure on the coverslip forced the ovule from the ovary without damage. The transparent ovules were examined by a phase oil objective.

I have indicated the technique appeared to be simple and efficient. Indeed, this was found to be true of the clearing procedure. Identification of the various components of the ovule, however, was not. Identification requires a previous knowledge of the structure and appearance of the megagamete and the stages leading to its formation. Without that previous concept of what to look for, the technique proved to be undesirable because of the difficulties in identifying the structures of interest.

In an effort to alleviate the identification problems, it seemed conceivable that a nuclear stain might assist in identifying the nuclear components of the megagamete. Feulgen stain is a nuclear stain in which the mordant molecule is thought to position itself within the actual DNA molecule. It has a distinctive color which would be easily recognized if the surrounding area was unstained or transparent.

The ovules of ovaries fixed in FAA_{50} were disected from the ovary tissues and prepared for staining with 1N HCL for 15 minutes at 58 - 60°C. Three different staining schedules of 1, 3, or 5 hours in the stain were used. After staining, each received three 10 minute washes in 10% K_2SO_3. These ovules were then placed into the clearing solution, as already described.

In all treatments the feulgen stain bled out of the ovule tissues into the clearing solution. Each component of the clearing solution was tested separately and found to cause the same result. It became apparent that the clearing technique, with or without the modifications attempted, was not satisfactory for use in this study.

A second attempt to find a method for efficient screening of a large number of ovaries yielded similar results. Ovule squash methods employing either feulgen (Hillary, 1940) or acetocarmine (Bradley, 1948) stains were tested for their feasibility. These techniques gave stained nuclei of variable quality, and because of the squashing procedure, gave no indication of the structure of the megagamete. The fixing solutions are thought to account for the variability seen in the quality of the staining. It is also emphasized here, again, that a previous concept of megagamete structure and the orientation of the nuclei are prerequisites for using these techniques.

The paraffin-embedding technique (Johannsen, 1940) is tedious and time-consuming, but provides a reasonably good, permanent record of the stage of development at the time of fixation. Tissues and cell components are positioned as they would be expected to be found if they could be examined in living tissues. With proper staining technique the sectioned tissues are presented in an easily identifiable manner, and as such this technique was determined to be the most useful for this study.

A variety of fixing and staining procedures can be used with the paraffin-embedding technique, depending upon the requirements of the observer. Ovary tissues, in this study, were fixed in both FPA_{50} and FAA_{50}. Those tissues fixed in the FPA_{50} were less brittle and provided sections of better quality than those tissues fixed in the FAA_{50}.

A standard staining procedure, saffranin followed by fast-green, was determined to be sufficient for the megagamete examination. Nuclei are stained red by the saffranin and the fast-green stains the cell walls green, providing for maximum contrast.

Although the first several techniques proved to be inappropriate for this study, it does not preclude their use in future studies. A concept of the components or structures wanted may prove to be valuable when using these techniques, specifically ovule clearing or squashing. They both appear to be simple, fast, and relatively efficient. The ovule clearing technique has the advantage that it can provide a view of the stages involved in the development of the megagamete, or its components, unavailable through any other technique now used. Since the tissues are intact they can be viewed from any angle, and may give the observer the opportunity to investigate beyond the limits imposed by either the squash or section techniques.

Addendum Bibliography

1. Bradley, M. V. 1948. An acetocarmine squash technique for mature embryosacs. Stain Technol. 23: 29-40.

2. Herr, J. M., Jr. 1971. A new clearing-squash technique for the study of ovule development in angiosperms. Am. Jour. of Botany 58: 785-790.

3. Hillary, B. B. 1940. Uses of the feulgen reaction in cytology II. New techniques and special applications. Bot. Gaz. 102: 225-235.

4. Johannsen, D. A. 1940. Plant microtechnique. McGraw-Hill. New York.

Addendum II

Emasculation was practiced on many of the parent and control genotypes used as female parents in crosses between the genotypes. In this study the techniques found to be most satisfactory are as follows.

After identifying the genotypes to be used in the cross, panicles from each, which are near the same stage of development were selected. To emasculate the female parent panicle, only one spikelet was worked with at a time. Two or four of the uppermost florets were pulled off since these were the last to mature, and harder to emasculate. The florets were cut about one-third of the way back from the end of each floret, with scissors. This cut the anthers,but did not harm the ovary tissues. A fine tipped pair of forceps were used to force the lemma and palea apart and extract the anthers. When the entire spikelet was emasculated, the next spikelet was treated in an identical manner until the entire panicle was emasculated. Emasculated panicles were bagged with the pollen parent panicle, and tagged accordingly.

There is some indication that severing the anthers by cutting, as previously described, may be sufficient enough to result in emasculation, without actual removal of the anthers. The severed anthers will quickly dry and shrivel, disrupting normal development. This is a practice effectively used in barley and wheat(1) and should be evaluated on tall fescue.

Addendum Bibliography

1. Wells, D. G. and H. R. Caffey. 1956. Scissor emasculation of wheat and barley. Agron. Jour. 48: 496-499.