

Classification of embryo sacs in the *Eragrostis curvula* Complex

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ABSTRACT

At each of 17 collecting points between Johannesburg and Brits in the Transvaal, three plants which belong to the *Eragrostis curvula* Complex were collected and studied. A total of 3 902 embryo sacs was examined in this sample. Of the embryo sacs examined, 3 306 were apomictic by means of diplospory, whereas 99 were sexual monosporic *Polygonum*-type embryo sacs. One hundred and nineteen embryo sacs were abnormal or divergent, and 378 were degenerated. There are indications that seasonal climatic fluctuations may be responsible for embryo sacs developing abnormally or degenerating. Simple and multiple correlations confirmed that sexual embryo sacs usually do not develop abnormally or degenerate during the later developmental stages. This finding lends credence to both the system of classification of individual embryo sacs and to the validity of the estimate of the proportion of sexuality of the plants sampled at each sampling point.

INTRODUCTION

The *Eragrostis curvula* Complex produces two main embryo sac types, namely apomictic 4-nucleate diplosporic embryo sacs (Gustafsson, 1946) and 8-nucleate sexual monosporic *Polygonum*-type embryo sacs (Liebenberg, 1961; Brix, 1974, Voigt & Bashaw, 1972; Vorster & Liebenberg, 1977). Apart from these, abnormal and degenerated embryo sacs may also be found.

In the literature there seems to be some doubt about the validity of allocating individual embryo sacs to any one of the four types (sexual, diplosporic, degenerate or abnormal). There is also uncertainty about the factors governing the development of sexual embryo sacs, which has in turn resulted in doubts about the validity of estimates of sexuality in individual plants. A study was therefore undertaken to evaluate the variation in embryo sac development of plants growing in a restricted area under natural conditions (Vorster & Liebenberg, 1977).

MATERIAL AND METHODS

The plant material examined in this study corresponds with that discussed by Vorster & Liebenberg (1977), and material was collected from different individuals occurring in 17 natural populations in a restricted area in the Transvaal (South Africa). The inflorescences were fixed in Navashin's fixative (Stockholm modification — Maheshwari, 1939). Embedding and staining techniques outlined in Johansen (1940) were followed, using Heidenhain's Haematoxylin as the nuclear stain and Orange G as the outer stain. A minimum of 50 embryo sacs from every plant was investigated.

RESULTS

A total of 3 902 embryo sacs was examined the results being summarized in Table 1. Of these, 3 306 embryo sacs were apomictic by means of diplospory, whereas 99 were sexual monosporic *Polygonum*-type (Maheshwari, 1950) embryo sacs. One hundred and nineteen embryo sacs were abnormal or divergent, and 378 were degenerated (Table 1).

1 Sexual embryo sac development

The archesporial mother cell differentiates from a hypodermal cell of the nucellar tissue (Fig. 1) and enlarges somewhat to undergo a transverse first meiotic division. The second meiotic division follows directly, with both the chalazal and the micropylar cells dividing transversally to form a linear tetrad, or the micropylar cell dividing perpendicularly to the chalazal cell to form a T-shaped tetrad. The three micropylar megaspores degenerate soon after the formation of the tetrad and the chalazal megaspore cell forms the embryo sac in the majority of cases (Fig. 2).

This process proceeds as follows:

(a) the chalazal megaspore enlarges and undergoes the first mitotic division. A vacuole develops between the two nuclei and enlarges so that the nuclei are forced to the opposite poles of the embryo sac (Fig. 3). The enlargement of the vacuole eventually also causes the embryo sac to be forced against the nucellus so that the remnants of the degenerated megaspores eventually disappear (Fig. 4);

(b) the second mitotic division only follows after the embryo sac reaches this size. With the continued enlargement of the vacuole the embryo sac is forced deeper into the nucellus (Fig. 5);

(c) the third mitotic division gives rise to an embryo sac with four nuclei at the micropylar pole and four nuclei at the chalazal pole. Cell walls then form around three of the micropylar nuclei to form the egg cell and two synergids. One chalazal nucleus migrates to the micropylar pole to form, together

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TABLE 1. — Classification of the embryo sac types

Plant number	Chromosome number	Diplosporic					Sexual								Ab-normal	Degen-erated	% diplo-sporic	% sexual	% abnormal	% degen-erated	Collection date	Total
		M	4N	2N	OD	YD	Di	Te	1N	2N	4N	8N	M									
A-1		18	5	3	8	9							2	10	30	50,59	2,35	11,76	35,29	Y	85	
A-2		41	2	1	2									4	5	83,64		7,27	9,09	X ₁	55	
A-3		35	1	2	2									8	8	71,43		14,29	14,29	X ₁	56	
B-1		22		8	9	12		1				1		3	28	60,71	2,38	3,57	33,33	Z ₁	84	
B-2		12	6	5	22	14				2				3	2	89,39	3,03	4,55	3,03	Y	66	
B-3		17	4		3	6								2	3	85,71		5,71	8,57	X ₁	35	
C-1		34	2	2	1	4								7	14	67,19		10,94	21,88	Z ₁	64	
C-2	60	4	2	14	34	18									3	96,00			4,00	W ₃	75	
C-3	60	2	5	7	32	11								1	4	91,94		1,61	6,45	W ₃	62	
D-1		3	4	5	33	17								3	5	88,57		4,29	7,14	Z ₁	70	
D-2	60	12	9	9	41	9			1					1	3	94,12	1,18	1,18	3,53	W ₃	85	
D-3	60	13	13	10	50	25					3			4	13	84,73	2,29	3,05	9,92	W ₃	131	
E-1		46	1											1	1	95,92		2,04	2,04	Z ₁	49	
E-2	60		3	4	58	16								1	9	89,01		1,10	9,89	W ₃	91	
E-3	60		2	7	39	12									8	88,24			11,76	W ₃	68	
F-1		7	5	5	22	17								1	4	91,80		1,64	6,56	Z ₂	61	
F-2		8	1	6	19	13									3	94,00			6,00	Z ₂	50	
F-3	60	13	9	12	35	11					2		1	3	10	82,47	4,12	3,09	10,31	W ₂	97	
G-1		41	4	5	4	7									1	2	95,31	1,56		3,13	Z ₂	64
G-2	60	20	5	5	28	9					1			1	12	81,71	2,44	1,22	14,63	W ₂	82	
G-3	60	31	5	11	36	5					1			9	4	86,28	0,98	8,82	3,92	W ₂	102	
H-1	60	21	7	1	24	11									12	84,21			15,79	W ₂	76	
H-2	60	14	2	10	28	8								4	10	81,58		5,26	13,16	W ₂	76	
H-3	80	1	3	24	63	14									15	87,50			12,50	W ₃	120	
I-1		8	5	1	20	12					1			3	10	75,41	3,28	4,92	16,39	Z ₂	61	
I-2			2	1	32	27								2	9	84,93		2,74	12,33	W ₁	73	
I-3	50	1	4	12	33	17					1			2	10	83,75	1,25	2,50	12,50	W ₁	80	
J-1	60	17	14	12	40	4								4	4	91,58		4,21	4,21	W ₃	95	
J-2	60	3	7	9	52	13					2				14	84,00	2,00		14,00	W ₃	100	
J-3	60	25	15	8	50	18					8			1	7	87,88	6,06	0,76	5,30	W ₃	132	
K-1		5	4	5	29	15					1	1		2	2	93,55	3,23		3,23	Z ₂	62	
K-2					31	16	1			5	1			2	14	67,14	10,00	2,86	20,00	X ₂	70	
K-3			1	9	23	15								4	6	82,76		6,90	10,34	X ₂	58	
L-1	60	23	10	15	39	16		1	2			1		4	10	85,12	3,31	3,31	8,26	W ₃	121	
L-2		8	6	7	66	11					2			4	6	89,09	1,82	3,64	5,45	W ₃	110	
L-3	40	6	6	19	31	10	1	1	13	3	3			3	6	70,59	20,59	2,94	5,88	W ₃	102	
M-1				1	37	20									7	89,23			10,77	Z ₃	65	
M-2					27	18	1	8	3					3	9	65,22	17,39	4,35	13,04	X ₂	69	
M-3	40		10	19	44	13					2	2		4	13	74,14	11,21	3,45	11,21	V	116	
N-1		12	4	4	22	9							1	3	5	85,00	1,67	5,00	8,33	W ₁	60	
N-2		3	2	2	24	19									3	94,34			5,66	W ₁	53	
N-3	60	2		2	27	17									3	94,12			5,88	W ₁	51	
O-1		2	1	2	30	31								2	1	95,65		2,90	1,45	Z ₃	69	
O-2		7	3	3	23	9						1		3	5	83,33	1,85	5,56	9,26	W ₁	54	
O-3		16	7	5	26	15									8	89,61			10,39	W ₁	77	
P-1		1	1	3	41	22								4	1	93,15		5,48	1,37	Z ₃	73	
P-2		29	3	5	17	2								2	9	83,58		2,99	13,43	X ₂	67	
P-3	60	46	13	13	31	8				1		1	2	3	1	93,28	3,36	2,52	0,84	W ₂	119	
Q-1		1	1	2	32	10					1				3	92,00	2,00		6,00	Z ₃	50	
Q-2				1	23	28									3	94,55			5,45	W ₁	55	
Q-3		5		2	23	25									1	98,21			1,79	W ₁	56	

Di, diad; Te, tetrad; OD, old diplosporic mother cell; YD, young diplosporic mother cell; 1N, one-nucleate stage; 2N, two-nucleate stage; 4N, four-nucleate stage; 8N, eight-nucleate stage; M, mature embryo sac.
Collection dates: Z₁ = 22.12.71; Z₂ = 23.12.71; Z₃ = 24.12.73; Y = 3.1.73; X₁ = 2.3.73; X₂ = 3.3.73; W₁ = 11.10.73; W₂ = 22.10.73; W₃ = 6.11.73; V = 1.12.73.

with the remaining micropylar nucleus, the two polar nuclei. The two polar nuclei do not fuse before fertilization (Fig. 6) and

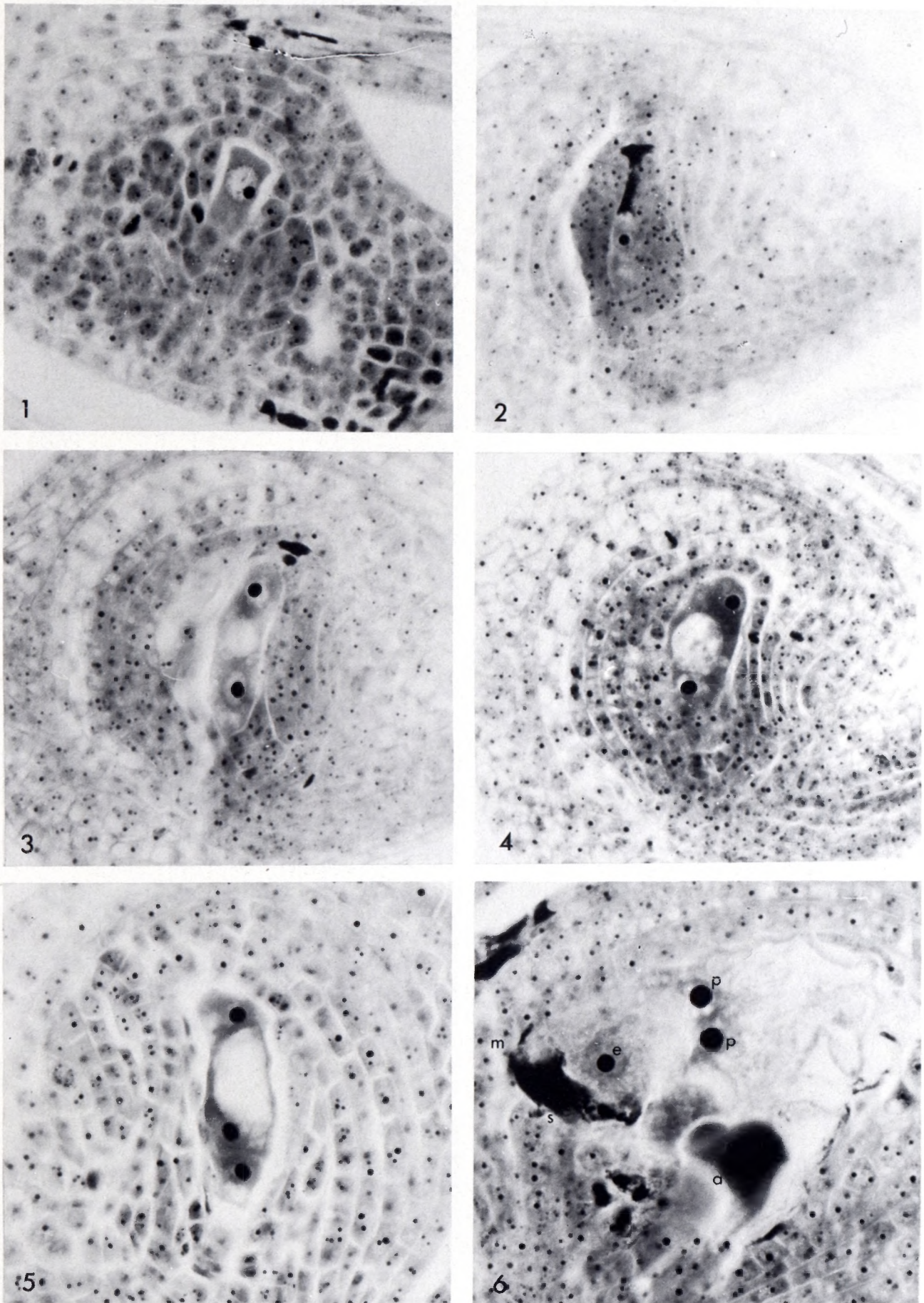
(d) cell walls are formed around the remaining three chalazal nuclei to form the antipodal cells. These cells then divide until an average of 15 cells are formed. Degeneration now takes place so that only degenerated remnants can be seen in the mature embryo sac, but these degenerated cells always remain visible in sexual embryo sacs. (Fig. 6).

2 Diplosporic embryo sac development

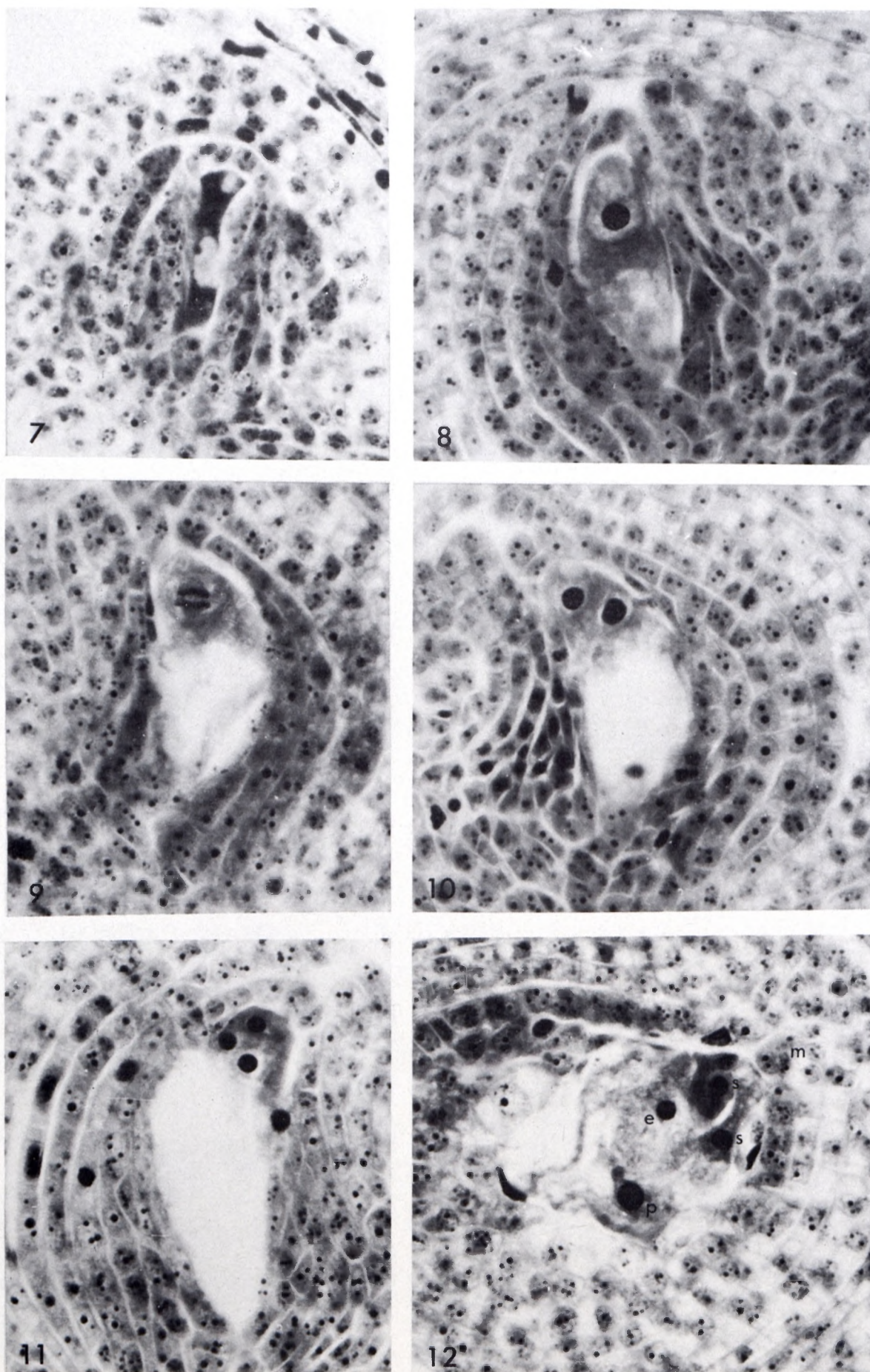
As in sexual embryo sac development, the archesporial mother cell also differentiates from a hypodermal cell of the nucellar tissue (Fig. 1), but does not undergo a meiotic division. It immediately begins to enlarge to form the diplosporic mother cell.

Vacuoles develop on either side of the nucleus and slowly enlarge. The chalazal vacuole enlarges faster and becomes dominant (Fig. 7) so that the nucleus is forced to the micropylar pole. The micropylar vacuole eventually disappears. The enlargement of the chalazal vacuole continues to a stage where the size of the embryo sac roughly equals that of a two- to four-nucleate sexual embryo sac (Fig. 8).

The first apparent mitotic division then occurs (Fig. 9) forming the 2-nucleate stage with both nuclei at the micropylar pole (Fig. 10). The second mitotic division follows, with the vacuole still enlarging, to form the 4-nucleate stage (Fig. 11). The 4-nucleate stage is short-lived, because cell walls are almost immediately formed around three of the nuclei to give rise to an egg cell and two synergids. The fourth nucleus remains free in the cytoplasm and functions as the polar nucleus (Fig. 12).



FIGS 1-6.—Sexual monosporic *Polygonum*-type embryo sac development, original magnification ca. $\times 600$. 1, archesporial mother cell; 2, degenerated micropylar megaspores with the functional chalazal megaspore; 3, young 2-nucleate stage; 4, old 2-nucleate stage; 5, 4-nucleate stage — one of the micropylar nuclei appears on another section; 6, mature embryo sac; a, antipodal cells; e, egg cell; m, micropylum; p, polar nuclei; s, degenerated synergids.



FIGS 7-12.—Diplosporic embryo sac development, original magnification ca. $\times 650$. 7, young diplosporic mother cell; 8, old diplosporic mother cell; 9, the first apparent mitotic division; 10, 2-nucleate stage; 11, 4-nucleate stage; 12, mature embryo sac; e, egg cell; m, micropylum; p, polar nucleus, s, synergids.

From the preceding, it is clear that it is only during the archesporial mother cell stage that the two embryo sac development types are not distinguishable from one another. Directly following the archesporial mother cell stage the nucleus either undergoes meiosis, or else vacuoles develop on either side of the nucleus, to initiate diplosporic embryo sac development.

DISCUSSION

1 *Degeneration of young sexual embryo sac stages*

A study of the literature leads to the conclusion that doubt still exists as to what extent the percentage of sexuality, found in a specific plant, is influenced by degeneration. It is therefore possible that due to the degeneration of young sexual embryo

TABLE 2. — Percentage sexual embryo sacs in young and old ovules

Plant number	Number diplosporic embryo sacs up to 2N	Number sexual embryo sacs up to 2N	Percentage sexual embryo sacs up to 2N	Number diplosporic embryo sacs from 4N to M	Number sexual embryo sacs from 4N to M	Percentage sexual embryo sacs from 4N to M
A-1	20			23	2	8,00
A-2	3			43		
A-3	4			36		
B-1	29	1	3,33	22	1	4,35
B-2	41	2	4,65	18		
B-3	9			21		
C-1	7			36		
C-2	66			6		
C-3	50			7		
D-1	55			7		
D-2	59	1	1,67	21		
D-3	85	3	3,41	26		
E-1				47		
E-2	78			3		
E-3	58			2		
F-1	44			12		
F-2	38			9		
F-3	58	2	3,33	22	2	8,33
G-1	16			45	1	2,17
G-2	42	1	2,33	25	1	3,85
G-3	52	1	1,89	36		
H-1	36			28		
H-2	46			16		
H-3	101			4		
I-1	33	1	2,94	13	1	7,14
I-2	60			2		
I-3	62	1	1,59	5		
J-1	56			31		
J-2	74	2	2,63	10		
J-3	76	8	9,52	40		
K-1	49	1	2,00	9	1	10,00
K-2	47	7	12,96			
K-3	47			1		
L-1	70	3	4,11	33	1	2,94
L-2	84	2	2,33	14		
L-3	60	18	23,08	12	3	20,00
M-1	58					
M-2	45	12	21,05			
M-3	76	11	12,64	10	2	16,67
N-1	35			16	1	5,88
N-2	45			5		
N-3	46			2		
O-1	63			3		
O-2	35			10	1	9,09
O-3	46			23		
P-1	66			2		
P-2	24			32		
P-3	52	1	1,89	59	3	4,84
Q-1	44	1	2,22	2		
Q-2	52					
Q-3	50			5		
Total	2452	79		854	20	
Sexual embryo sacs as a percentage of the totals			3,12	2,29		

2N, two-nucleate stage; 4N, four-nucleate stage; M, mature embryo sac

sacs the calculation of the percentage sexuality at earlier stages could be subject to a certain amount of error. For this reason, the collected data were examined to determine whether such a discrepancy could be detected.

This examination was based on the following consideration: it is important to know what the percentage of young sexual embryo sacs are during the early stages, in relation to the percentage of sexual embryo sacs present during the older stages. It would then be possible to determine if young sexual stages could be studied to give a reliable estimate of the percentage of functional sexual embryo sacs formed.

Table 2 was drawn up to determine these proportions. The end of the 2-nucleate sexual and diplosporic stages approximates the intermediate point in both types of embryo sac development (Liebenberg, 1961), and was therefore used as a dividing point in this Table.

Comparisons could not be drawn within single collections, because often either young or only advanced stages were found (Table 2). Average percentages of 3,12% and 2,29% (Table 2) were found for young sexual and mature sexual stages, respectively, all the collections examined having been taken into account.

If the young sexual ovules developed abnormally or degenerated before reaching maturity, then the average percentage sexuality in young ovules would be expected to be higher than the average percentage sexuality in mature ovules. Table 2 shows that these percentages are very much the same, one can therefore conclude that sexual embryo sacs are not inclined to degenerate or to develop abnormally, after the 2-nucleate stage.

2 Classification of embryo sacs

A further problem, similar to the one discussed above, was that young stages, classified as sexual, could just as well have been divergent diplosporic or abnormal embryo sacs.

At first, considerable difficulty was experienced with the classification of the 2- and 4-nucleate stages in sexual embryo sacs. These difficulties have also been reported by other researchers. Remnants of the three degenerated megaspores still showed in the two- as well as in some of the 4-nucleate sexual stages of diploid *Eragrostis curvula* var. *conferta* (Voigt & Bashaw, 1972) and of *E. plana* (Liebenberg, 1961), whereas Streetman (1963) recorded remnants only in 2-nucleate stages of *E. superba*.

Of 99 sexual ovules investigated in this study, 29 were 2-nucleate stages (Table 1). Only one of these showed remnants of the three degenerated micropylar megaspores and even in this instance these had nearly disappeared. (Fig. 3). None of the ten 4-nucleate stages examined in this study showed any such remnants.

If all the abovementioned 2-nucleate sexual stages had been classified as abnormal or divergent, this would have resulted in the average percentage of young sexual stages being lower than the percentage

of mature sexual stages. This would imply that a larger number of mature sexual and diplosporic stages were observed in ovules of plants where sexuality was found, whereas younger stages were observed mainly in plants with a very low percentage sexuality or without sexuality at all. The results show that the opposite is in fact true (Table 1). The sexual 2- and 4-nucleate stages were furthermore usually accompanied by younger and/or older sexual stages which were no problem to classify (Table 1).

The classification of 2- and 4-nucleate stages as being sexual, even when remnants of the degenerated megaspores are no longer visible, therefore seems to be justified.

3 Abnormal embryo sacs

Only 3,5% of all the embryo sacs investigated, were abnormal or divergent. Taking into consideration that sexual embryo sacs show no tendency to develop abnormally after the 2-nucleate stage, the question now arises as to what causes the degeneration and abnormal development of embryo sacs in the earlier stages of development of the sexual embryo sacs and of the non-sexual embryo sacs.

The collections of this study were made during three different growing seasons. The number of diplosporic, sexual, abnormal and degenerated embryo sacs were grouped according to the seasons in which they had been collected so that comparison between the different embryo sac types and the collecting periods was possible (Table 3).

From Table 3 it is obvious that the number of abnormal and degenerated embryo sacs fluctuated seasonally, especially when correlated against the amount of rainfall received. The rainfall for December 1971 was characteristic for that month. Table 3 shows that average percentages of abnormal and degenerated embryo sacs, from plants collected during that month to be much lower than those collected during the earlier months of 1973, when a much lower than average rainfall was measured. On the other hand, the end of 1973 formed part of a particularly good rainy season with the result that the percentages of abnormal and degenerated embryo sacs are lower (Table 3).

The average percentages of sexual embryo sacs also show this tendency but it is not as clearly defined as in the case of the other two embryo sac types.

It seems as if climatological changes may, therefore, be a factor responsible for inducing abnormal embryo sac development as well as embryo sac degeneration in the plants studied.

4 Correlations amongst embryo sac types

If the above statement is erroneous and it is in fact sexual embryo sacs which developed abnormally or degenerate, then one would expect a positive correlation between the number of sexual embryo sacs and the number of abnormal or degenerated embryo sacs investigated for a specific collection. With this in mind, simple and multiple correlations were made between the four different embryo sac

TABLE 3. — Comparative table between the period of collection and the different embryo sac types

Number of collections	Collection period	Number abnormal embryo sacs	Percentage abnormal embryo sacs	Number sexual embryo sacs	Percentage sexual embryo sacs	Number degenerated embryo sacs	Percentage degenerated embryo sacs	Number diplosporic embryo sacs	Total
13	22/23/24.12.1971	24	2,92	8	0,97	81	9,85	709	151
2	03.01.1973	13	8,61	4	2,65	32	21,19	102	410
7	02/03.03.1973	25	6,10	19	4,63	54	13,17	312	
9	11.10.1973	10	1,79	3	0,55	47	8,41	499	559
6	22.01.1973	20	3,62	11	1,99	49	8,88	472	552
13	06.11.1973	23	1,78	41	3,17	102	7,89	1126	1292
1	01.12.1973	4	3,45	13	11,21	13	11,21	86	116
Total		119		99		378		3306	3902

types according to the standard method and the results are summarized in Tables 4 and 5.

The percentages of diplosporic embryo sacs are in all cases significantly negatively correlated with the other embryo sacs predominate in all collections.

The percentages of sexual, abnormal and degenerated embryo sacs when compared show no significant correlation (Table 4).

In order to find any possible, more complex multiple relationships (correlations), a multiple

regression analysis was done. No further relationships could be found (Table 5), so that it seems as if no significant connections exist amongst the different embryo sac types. The percentage of abnormal and degenerated embryo sacs do not, therefore, seem to depend on the percentage of sexual embryo sacs.

CONCLUSIONS

Seen as a whole, one has to conclude from this study, that the sexual embryo sacs showed no tendency to degenerate or to develop abnormally during the later stages of their development. This finding lends credence to both the system of classification used for individual embryo sacs, and to the estimation of the proportion of sexuality in each collection.

It seems, furthermore, that abnormal development and degeneration of embryo sacs may be caused by climatological changes.

TABLE 4. — Simple correlations between the different embryo sac types

	Abnormal embryo sacs	Degenerated embryo sacs	Diplosporic embryo sacs
Sexual embryo sacs	0,01	0,05	-0,49*
Abnormal embryo sacs		0,13	-0,45*
Degenerated embryo sacs			-0,57*

* Significant at a $p = 0,05$ level

TABLE 5. — Multiple correlations between the different embryo sac types

Independent variables	Dependent variables	
	Abnormal embryo sacs	Degenerated embryo sacs
Diplosporic embryo sacs	-0,6064* -0,3677•	-0,5644* -0,3186•
Diplosporic- and sexual embryo sacs	0,6822* 0,4654•	0,6191* 0,3832•
Diplosporic- sexual- and abnormal embryo sacs	not calculated	0,6875* 0,4726•

* Correlation (becomes positive in the case of the multiple correlations)

• R-squared

UITTREKSEL

Drie plante versamel by elk van 17 versamelpunte tussen Johannesburg en Brits in die Transvaal, behorende tot die Eragrostis curvula Kompleks is bestudeer. Uit 'n totaal van 3 902 kiemsakke wat bestudeer is, was 3 306 apomikties deur middel van diplosporie terwyl 99 geslagtelike monosporiese Polygonum-tipe kiemsakke was. Honderd en negentien kiemsakke was abnormaal of afwykend terwyl 378 kiemsakke gedegenerereerd was. Aanduidings is gevind dat klimaatskommeling hoofsaaklik daarvoor verantwoordelik was dat kiemsakke neig om abnormaal te ontwikkel of te degenereer. Enkelvoudige en meervoudige korrelasies het verder bevestig dat geslagtelike kiemsakke nie geneig het om abnormaal te raak of later te degenereer nie. Dit impliseer nie net dat die bepaling van die persentasies vir geslagtelike kiemsakke wat by die verskillende versamelings verkry is, betroubaar is vir daardie spesifieke versameling nie, maar ook dat die beslissing of 'n kiemsak geslagtelik is of nie, betroubaar was.

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