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Accomplishment of Microsporogenesis in a Garlic Clone

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Introduction

Garlic, *Allium sativum* L., has been regarded as a completely sterile species. Mann[7] stated, "There are many references to the true seed of garlic in the literature, but since no author who speaks of garlic seed states that viable seed has been seen first hand, it seems most probable that true seed of garlic is not known." Because of its sterility, garlic has never been crossbred during its long cultvated history since ancient Egypt, and transmission of virus by vegetative organs has become a more serious problem.

In many clones of garlic, pollen tetrads, microspores, or pollen grains usually degenerate between meiosis and the first pollen mitosis, though some other clones neither bolt nor differentiate any flower buds.

Degeneration of microspores after regular meiosis was observed in garlic by several investigators. Degeneration of microspores was also observed after irregular meiosis by a few investigators[4,10,11]. Furthermore, irregular meiosis and the succeeding degeneration of microspores were previously observed in 43 garlic clones by the author[1]. In these 43 clones, regular meiosis was never seen even in any pollen mother cell. One of the shortest ways to obtain the fertile pollen of garlic may lie in finding the clones showing regular meiosis, though regular meiosis followed by degeneration of microspores was reported previously. Therefore, the present investigation was undertaken for further observation of PMCs in many other clones to find out the clones showing regular meiosis.

Materials and Methods

Pollen mother cells of 28 garlic clones shown in Table 1 were observed to investigate the chromosome pairing at meiosis. Besides the clones of Table 1, PMCs or microspores of two other clones, No. 61 Italy and No. 146 Argentine, were observed, though their metaphase-I was unobservable. Clone No. 30 had been investigated previously[1], but its meiosis was observed here again, since its chromosome pairing had shown two different schemata, $1_{VIII} + 4_{II}$ and $1_{VI} + 5_{II}$, being almost equal in the number of PMCs. Both of the clones No. 112 and 117 are Ishū-wase clone, but No. 117 is a virus-free clone obtained through meristem culture in Nagasaki Prefecture. Some of the clones used here were purchased at the local markets.

The cloves of each clone used here were planted in Kagoshima in autumn of 1978 to 1981. The cloves of clones No. 12, 24, 26, 100, and 119 were stored in cold chamber before planting, because winter is too short in Kagoshima for these clones to produce long seed-stalks with flower buds in the

following spring. The flower buds were picked in the next spring or summer, and their anthers were smeared in iron-acetocarmine solution to observe PMCs, sometimes after fixed with Farmer's fluid.

Microsporogenesis after meiosis was observed in each clone. The pollen grains of Moscow clone survived beyond the first pollen mitosis, while those of all the other clones degenerated prior to it. Partial sterility was also noticed in Moscow clone. Therefore, viable, empty, or degenerating pollen grains of four anthers from Moscow clone were counted at different stages as shown in Table 2.

At anthesis, a large number of mature pollen grains were seen in Morcow clone, so that pollen-germination was examined on the agar medium. The agar medium (0.1 g/10 ml distilled water) was supplemented with 0.5, 1.0, 1.5, 2.0, 2.5, or 3.0 g of sucrose. Germinating pollen grains were counted about two hours after the pollen grains from a dehiscent anther were put on the media, and the germination was examined twice, using different anthers.

Results

Irregular meiosis was observed in 26 clones (Table 1). Their chromosome pairing showed mostly $1_{VIII} + 4_{II}$ or $1_{VI} + 5_{II}$, with the exception of clone No. 145. The typical configurations of their irregular meiosis are shown in Figs. 1 and 2. Clone No. 30 clearly showed $1_{VIII} + 4_{II}$. Clones No. 91 and 128 were newly obtained from China in 1979 and 1981, respectively, and both of them showed $1_{VIII} + 4_{II}$. Clones No. 110, 119, 125, 126, and 127 are the popular local clones in the northern part of Japan, and all of them showed $1_{VI} + 5_{II}$.

In clone No. 145, the chromosome behavior at metaphase-I was extremely irregular and too complicated to identify the pairing (Fig. 3). Also at anaphase-I, irregular behavior of chromosomes was observed in the same clone (Fig. 4). Chromosome bridges and fragments were always observed, and each pole infrequently received entire one-half the original chromosome number at telophase-I. Some chromosomes frequently formed micronuclei, without reaching the poles, at and after interkinesis (Fig. 5). Abnormal microspores such as pollen dyads instead of pollen tetrads, binucleate microspores, or malformed microspores as shown in Fig. 6, were observed everywhere. The microspores of clones No. 145, 61, and 146 were similar in abnormality, and all of them degenerated before pollen mitosis.

On the contrary, the regular pairing of chromosome (8_{II}) during meiosis was found in two clones, No. 130 and 148 (Figs. 7, 8). Normal pollen tetrads were formed after meiosis in the clone No. 130 (Fig. 9), while the pollen tetrads of clone No. 148 degenerated. The microspores of clone No. 130 were released in the anther loculi and developed into uninucleate pollen grains. After a while, small vacuoles occurred in the tapetum, which began to degenerate (Fig. 10). After the tapetum degenerated considerably, the first pollen mitosis began in a number of pollen grains (Fig. 11). The chromosome behavior was normal during the pollen mitosis (Figs. 12, 13). After the pollen mitosis, the pollen grains developed into normal binucleate ones, similar to those of other fertile *Alliums* (Fig. 14). At the early binucleate stage, the tapetum cells left only their scattered remnants. The nuclei of pollen moved and changed their shapes with pollen maturing (Figs. 15, 16). The whitish pollen grains were observed at dehiscent of anther. Thus, viable pollen grains as shown in Fig. 17 were obtained in clone No. 130.

The viable pollen of clone No. 130 was obtained from any inflorescences having normal flowers, whether the bulblets among the flowers had been removed out of the inflorescences or not. In clone No. 130, however, there was also a sterile plant which produced only malformed flowers bearing

Table 1. Chromosome pairings at meiosis

Clone	Number of pollen mother cells									
	Regular pairing (8_{II})	Irregular pairing								
		$1_{VIII}+4_{II}$	$1_{VI}+5_{II}$	$1_{V}+1_{III}+4_{II}$	$2_{IV}+4_{II}$	$1_{IV}+1_{III}+4_{II}+1_{I}$	$1_{IV}+6_{II}$	$1_{IV}+5_{II}+2_{I}$	$2_{III}+5_{II}$	Unidentified*
12. Matsumoto			10							
24. Saga-ooninniku			3							
26. Kagoshima-B			5							
30. Amami-2		6								
85. Sendai-A			2							
91. Bai Pi		3	1	1						
94. Mito-B		2	2				1			
95. Mito-C			4							
97. Utsunomiya-B		6	1		1	1				
99. Takasaki-B			3							
100. Takasaki-C		4								
101. Kooriyama			6							
103. Yamagata-A		4			1					
106. Sakata		13								
107. Kisakata			12							
110. Hachimantai			17							
112. Ishū-wase		3								
115. Matsuwo		4	2	1				1		
117. Ishū-wase (V.F.)		4								
119. Shichigahama-zairai	1		5							
121. Odāresu		14	3		2					
125. Manmosu			6						1	
126. Kitami-zairai			12							
127. Furano-zairai			6							
128. Peking		12								
130. Moscow	12									
145. Pag										20
148. Rumania	12									

*Not identified, though extremely irregular pairing (Fig. 3).

bulblets instead of ovaries. At anthesis of clone No. 130, violet anthers projected first, and it was rare that all the anthers of a flower projected simultaneously out of the perianths (Fig. 18). The styles projected a little later than dehiscence of anthers (Fig. 19). The flowers in an inflorescence opened irregularly, as compared with those of Japanese bunching onion which open successively from the top to the bottom of the inflorescence (Fig. 20). The bracts around the flowers in the inflorescence were short, though the spathe was long. Perianth lobes were whitish to pinkish or light violet at anthesis. Both of the outer and inner filaments were trifid with long lateral teeth (Fig. 21), and the teeth of the inner filaments were sometimes branched. Six dark violet stripes, like diamond shapes, were observed on the surface of the ovary. In the majority of flowers, all of six anthers exserted were violet, but in some flowers, a few of them were yellow, and occasionally all

Table 2. Frequency of viable pollen in Moscow clone

Anther*	Number of pollen grains			Frequency of viable pollen
	Viable	Empty or degenerating	Total	
A	3,031	583	3,614	83.9%
B	3,150	657	3,807	82.7
C	526	3,501	4,027	13.1
D	1	4,502	4,503	0.0

* A; An anther just after pollen mitosis.
B, D; An anther just before anthesis.
C; An anther at the binucleate stage shown in Fig. 22.

Table 3. Pollen-germination percentages of Moscow clone on the agar media supplemented with sucrose

Sucrose	Germination percentage	Number of pollen grains observed
0%	3.8%	793
5	30.7	353
10	61.5	278
15	41.8	193
20	50.4	247
25	2.6	408
30	3.8	264

of them were yellow. Almost all the pollen grains within the yellow anthers, such as "D" anther in Table 2, were empty or degenerating. Moreover, some of the violet anthers contained a conider-able number of empty or degenerating pollen grains (Fig. 22). These results are shown in Table 2.

To ascertain viability of pollen in clone No. 130, pollen-germination was examined on the agar medium. The results are shown in Table 3. The pollen grains rarely germinated on the medium supplemented with sucrose at 0, 25, or 30% (Figs. 24, 29, 30). On the medium of 5% sucrose, a number of pollen grains germinated, but their pollen tubes elongated infrequently (Fig. 25). On the medium of 15% sucrose, elongation of pollen tubes was observed in almost all the germinating pollen grains, but the frequency of germination was not so high (Fig. 27). A large number of pollen grains germinated on the medium of 10 or 20% sucrose. However, pollen tubes on the medium of 20% sucrose elongated more rapidly than those on the medium of 10% sucrose (Figs. 26, 28).

It took about three days from metaphase-I to the first pollen mitosis and about eleven days from the pollen mitosis to dehiscence of anther in the field.

Thus, the microsporogenesis in clone No. 130 was accomplished.

Discussion

In the previous investigation by the author[1], all of the garlic clones collected showed basically either $1_{VIII} + 4_{II}$ or $1_{VI} + 5_{II}$ as the chromosome pairing at meiosis, with an exception of $1_X + 3_{II}$. Most of these clones had been collected from the southern part of Japan, China, Taiwan, or Korea. In the present investigation, the clones were collected mainly from the northern part of Japan. Their chromosome pairings were similar to those in the previous investigation, and the pairing $1_{VI} + 5_{II}$ was observed in the clones from northern Japan more frequently than the pairing $1_{VIII} + 4_{II}$. Accordingly, it seems probable that, in spite of Katayama's report[4], all the clones in Japan show irregular pairing. Both of the two clones newly obtained from China also showed the irregular pairing

$1_{VIII} + 4_{II}$. The garlic clones originally cultivated in East Asia, namely, China, Japan, Korea, and Taiwan, may by presumed to show inherently irregular pairing of chromosomes.

Clone No. 145 was obtained from Yugoslavia through INRA of France, and its chromosome pairing was extremely irregular. In the clone No. 145, and also in clones No. 61 and 146, a large number of abnormal microspores or pollen grains were observed after meiosis, and all the pollen grains of these three clones were degenerated before pollen mitosis. Therefore, the chromosome pairings of clones No. 61 and 146 were presumed similar to that of clone No. 145. These three clones were also similar in appearance or growth habit. They must be originally cultivated in Mediterranean area, because the garlic clones of South America were probably introduced from Mediterranean area. The abnormal chromosome behavior at meiosis, the formation of micronuclei and malformed microspores in these three clones may be caused by desynapsis.

On the contrary, two clones from Rumania and USSR showed regular pairing at meiosis, and Moscow clone produced fertile pollen, while Rumania clone failed to produce. It may be necessary to grow Rumania clone again to examine the development of microspores, since only a few plants of the clone differentiated flower buds here, and they met a long rain after meiosis without spathes.

A few investigators attempted to obtain fertile pollen grains of garlic with the aid of antibiotics, and two attempts succeeded[5,6], and the others failed[2,8]. In the present investigation, the pollen grains of a virus-free clone also degenerated. However, clone No. 130 produced fertile pollen without any treatment, such as antibiotics, so the clone must be pollen-fertile, originally. And it seems preferable to obtain seeds from the pollen-fertile clones rather than obtaining seeds from the sterile ones by treatment of antibiotics, because antibiotics are toxic for the plants at high concentration.

According to the figure of a floret prior to anthesis in the report by Konvicka *et al.*[6] who succeeded in obtaining fertile pollen by the use of antibiotics, the style projected out of the floret earlier than the anthers. In the pollen-fertile clone observed here, styles projected always later. Garlic, as well as other cultivated *Alliums* such as common onion (*A. cepa* L.), or Japanese bunching onion (*A. fistulosum* L.), is presumed to be protandrous.

The outer filaments of garlic flowers usually bear no long teeth[3,9]. Those of the pollen-fertile clone observed here always bore the teeth. This pollen-fertile clone may possibly have an origin a little different from other common sterile clones.

In clone No. 130, some sterile anthers were observed, and the pollen grains within these sterile anthers degenerated at the uninucleate stage (Fig. 23) similarly to those of other sterile clones. Some anthers appeared partially fertile such as "C" anther in Table 2. These facts that the fertile, partially fertile, and sterile flowers were observed within an inflorescence may be of some use for analyzing the cause of sterility in garlic.

The pollen grains of clone No. 130 germinated well on the agar media, and their pollen tubes elongated most rapidly on 20% sucrose medium. The agar medium of 20% sucrose may be recommended for the germination test of garlic pollen. By the use of the fertile pollen grains in this clone, it may become feasible to obtain viable seed of garlic.

In conclusion, a pollen-fertile clone producing germinable pollen was found out here in this investigation for the first time in the long cultivated history of garlic.

Summary

The chromosome pairing at meiosis was observed in 28 clones of garlic, *Allium sativum* L. The irregular pairing, such as $1_{VIII} + 4_{II}$ or $1_{VI} + 5_{II}$, was observed in 25 clones, and in one clone,

the chromosome behavior was too complicated to identify the configuration of pairing. These clones showing irregular pairing at meiosis failed to produce fertile pollen grains.

On the other hand, the regular chromosome pairing (8_{II}) at meiosis was observed in two clones, and one of them succeeded in producing fertile pollen grains without any treatment, though another one failed. In the pollen-fertile clone, degree of pollen-fertility differed in different flowers or inflorescences. The frequent pollen germination and the rapid vigor elongation of pollen tubes, using the pollen grains from the pollen-fertile clone, were observed on the agar medium supplemented with 20% sucrose.

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Explanation of figures

Fig. 1. Metaphase-I ($1_{VI} + 5_{II}$) of clone No. 110, × ca. 1,100.

Fig. 2. Metaphase-I ($1_{VIII} + 4_{II}$) of clone No. 128, × ca. 620.

Figs. 3-6. Abnormal microsporogenesis in clone No. 145.
Fig. 3. Unidentified chromosome pairing at metaphase-I, × ca. 460. Fig. 4. Irregular chromosome behavior at anaphase-I to telophase-I, × ca. 620. Fig. 5. Micronuclei at interkinesis of meiosis, × ca. 620. Fig. 6. Malformed microspores, × ca. 250.

Fig. 7. Diakinesis (8_{II}) of clone No. 130, × ca. 620.

Fig. 8. Metaphase-I (8_{II}) of clone No. 148, × ca. 620.

Figs. 9–17. Microsporogenesis after meiosis in clone No. 130.
Fig. 9. Pollen tetrads and tapetum, × ca. 250. Fig. 10. Uninucleate pollen at the beginning of tapetal degeneration, × ca. 250. Fig. 11. Prophase and prometaphase of the first pollen mitosis, and degenerated tapetum, × ca. 250. Fig. 12. Metaphase of the first pollen mitosis, × ca. 620. Fig. 13. Telophase of the first pollen mitosis, × ca. 620. Fig. 14. Binucleate pollen just after pollen mitosis, × ca.460. Fig. 15. Binucleate pollen changing the shapes of nuclei, × ca. 360. Fig. 16. Maturing pollen just before anthesis, × ca. 620. Fig. 17. Matured pollen just before dehiscence of anther, × ca. 620.

Fig. 18. Flower with exserted anthers and teeth just before anthesis in clone No. 130.

Fig. 19. Flower with dehiscent anthers at anthesis in clone No. 130.

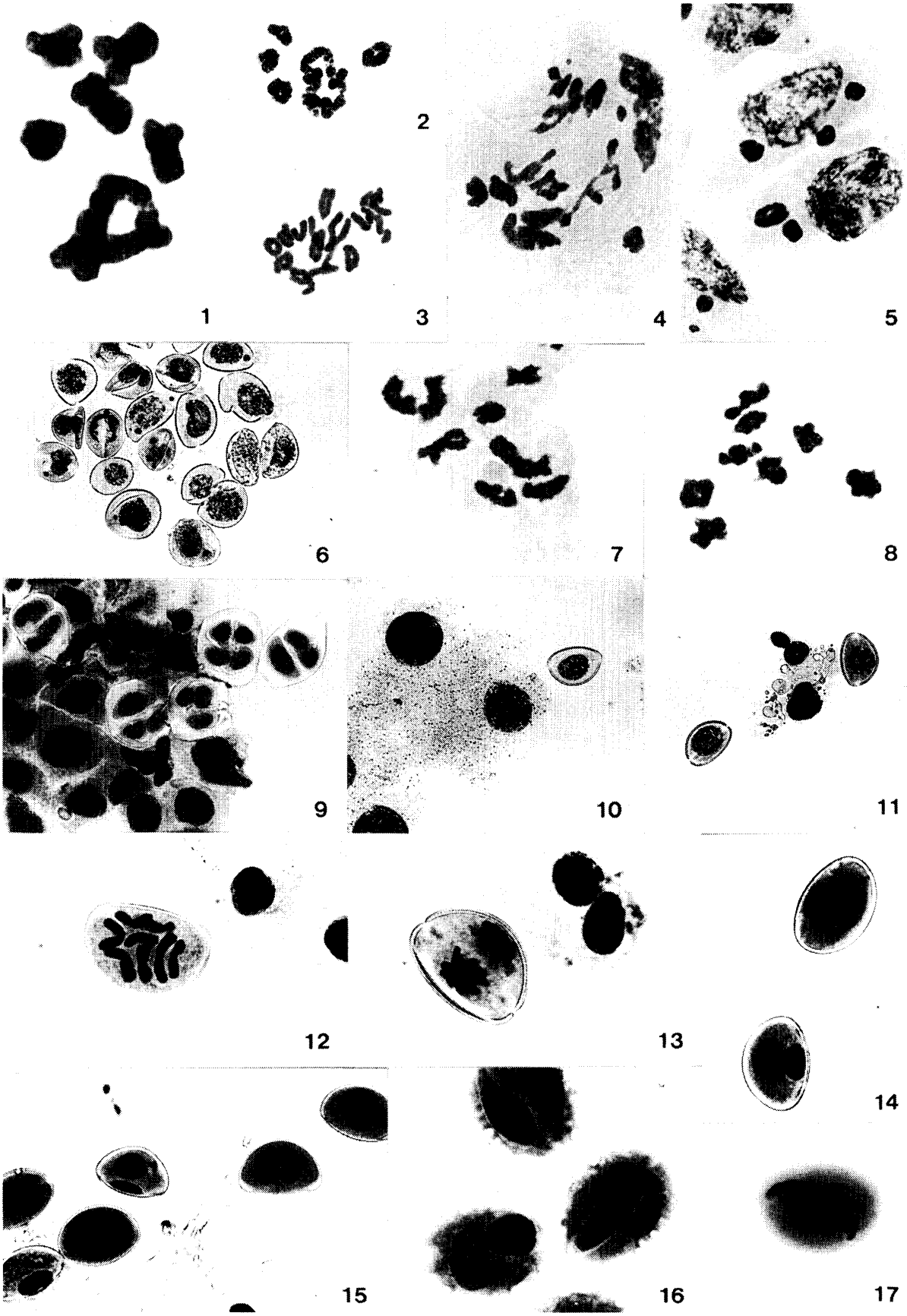
Fig. 20. Opened and unopened flowers in an inflorescence of clone No. 130.

Fig. 21. Dissected flower of clone No. 130. Both of inner and outer stamens bear long teeth.

Fig. 22. Viable, empty, or degenerating pollen grains at binucleate stage in a partially fertile anther of clone No. 130, × ca. 120.

Fig. 23. Degenerating uninucleate pollen in a sterile anther of clone No. 130, × ca. 250.

Figs. 24–30. Pollen-germination of clone No. 130 on the agar media supplemented with sucrose, × ca. 230.
Fig. 24. Medium of 0% sucrose. Fig. 25. Medium of 5% sucrose. Fig. 26. Medium of 10% sucrose. Fig. 27. Medium of 15% sucrose. Fig. 28. Medium of 20% sucrose. Fig. 29. Medium of 25% sucrose. Fig. 30. Medium of 30% sucrose.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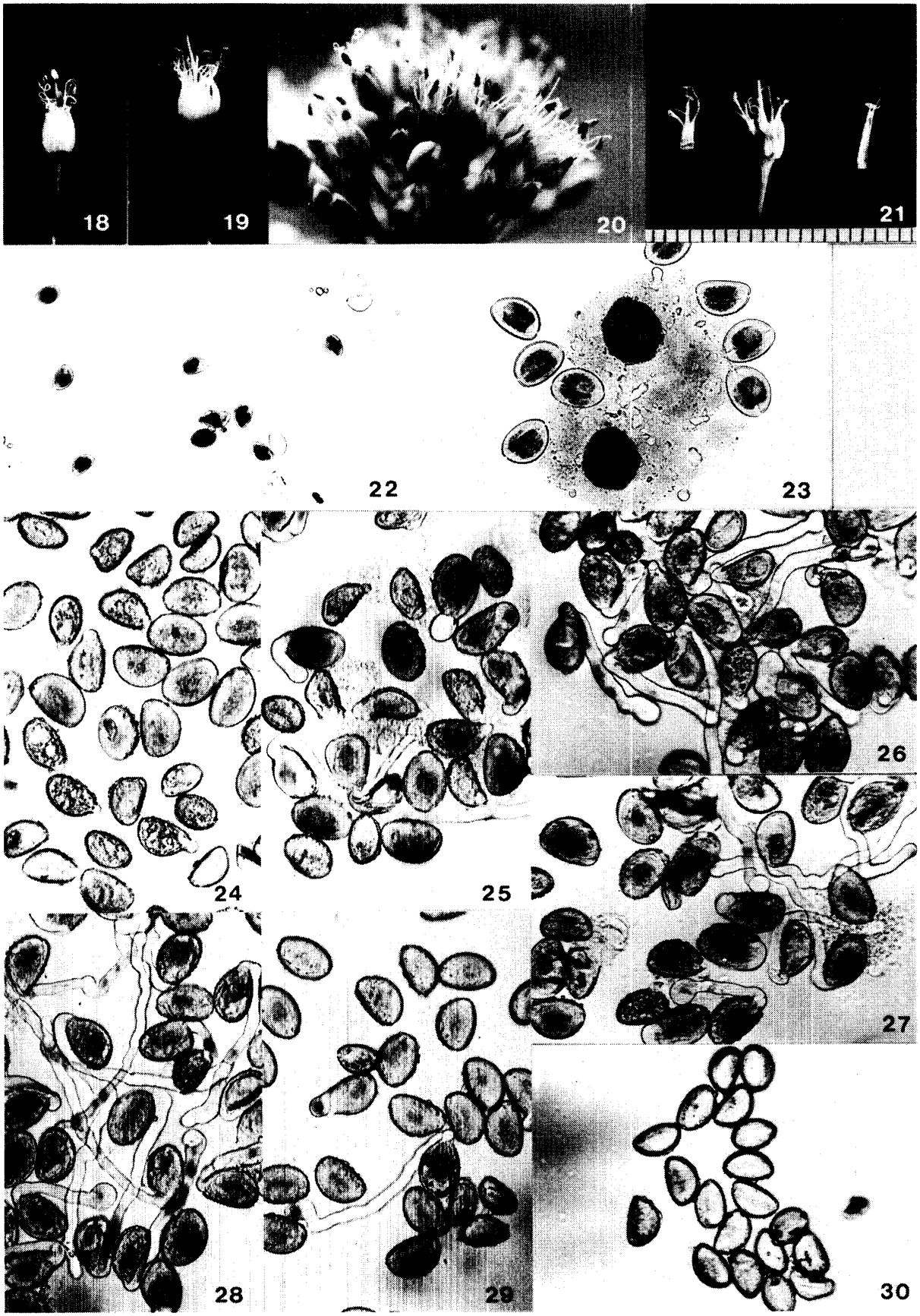
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