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EMBRYOGENIC RESPONSES OF THE POLLEN TO VARIED SUCROSE CONCENTRATIONS IN ANTHER CULTURE*

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Abstract

Anthers of the species as given below were cultured on media each containing sucrose at 2, 5, 10 and 15% concentrations, with the aim of assessing the inductive effect of sucrose on embryogenesis of pollen grains in anther cultures. Assessment of pollen embryogenic responses was made, in the anther squashes stained with FEULGEN reagent on the 10th and 20th days of culture, by scoring frequencies not only of anthers with dividing pollen grains but of dividing grains per anther and the number of dividing nuclei per grain.

Pollen responses to varied sucrose concentrations differed widely with different species ; 1) species making greater response at lower sucrose levels (*Nicotiana tabacum*), 2) ones making it at higher suerose levels (*Brassica oleracea, Hordeum distichum, Oryza sativa, Solanum verrucosum*), 3) ones responding at any level of sucrose (*Lycopersicon esculentum*), and 4) ones with no response at any sucrose level (*Glycine max, Petunia hybrida, Zea mays*). Among these responsive species there was found also a marked difference in frequency of anthers with dividing pollen grains. When anthers were cultured at the most effective level of sucrose, *Hordevm* and *Nicotiana* gave dividing grains in 50.9 and 73.7% of anthers, respectively, whereas *Brassica, Lycopersicon, Oryza* and *Solanum* had them only in 8.0, 16.7, 3.9 and 11.4%, respectively. A similar situation was also seen in the number of divided nuclei per pollen grain as well as in the frequency of dividing grains per anther. In the instances more highly responsive to higher sucrose concentrations, sucrose at increased levels appeared to be effective only for initiating pollen divisions but to give little or no effect on further development of divided grains

The initial behaviors of pollen grains observed fell into the two main patterns ; one with no response to anther culture and the other with greater or less response. The latter, furthermore, comprised one being regarded as non-embryogenic in which multinucleate grains formed by free nuclear divisions either remain quiescent or enlarge with starch deposition, and the other as embryogenic in which multicellular grains formed with cell-wall formation result either from the vegetative or from the generative nuclei, or from both. Of these embryogenic patterns, the last seemed to be the most favorable pathway for leading to plantlet formation. The marked effect of sucrose was not recognizable on the above embryogenic patterns.

Since GUHA & MAHESHWARI[3,4] had achieved success in induction of pollen plants from *Datura* anthers cultured *in vitro*, a number of plant genera have been studied in this way, with or without success, by many workers[7,11]. Despite much work, however, anther culture has not reached the stage at which generalization can be made about culture conditions for inducing constantly successful pollen embryogenesis in any species. For making this substantial, first of all the following two problems have

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to be brought about a satisfactory solution ; one of how pollen grains can be triggered into embryogenic initiation and what factors do its control, and another of how multicellular grains formed can be made to continue their further morphogenetic development and what factors control it. From such points of view, attempts have already been done by a few workers[1,2,8,9] to assess inductive effects of some exogenous factors on pollen embryogenesis. Among the factors assessed, chemical ones were such as iron, nitrogen, phytohormones, sucrose and vitamins, and as the consequence suggestions have been made that iron may be effective for *Nicotiana* and sucrose for *Hordeum*, *Lolium* and *Lycopersicon*. Of this respect, however, our knowledge is as yet very incomplete.

The present study has been undertaken to assess severally exogenous factors, particularly of chemicals for their effects on the initiation and cotinuation of pollen embryogenesis in anther cultures. In this paper are given the results obtained, on the effect of sucrose, by comparing several species for their pollen response to sucrose at varied concentrations.

Materials and Methods

The materials used in this study, as listed in Table 1, comprise 13 species involving nine genera and four families. Floral buds for culture were collected from plants grown vigorously at an early stage of flowering.

Culture : In all cases, except *Nicotiana*, the culture media used were prepared on the MURASHIGE & SKOOG's formula[6], with the supplement of 2 mg/l of 2,4-dichlorophenoxyacetic acid and kinetin each, and grouped into four series by adding sucrose at the concentrations of 2, 5, 10 and 15% (w/v). For *Nicotiana*, the medium containing sucrose only at the same levels as above was used. These media all were solidified with 0.8% agar and adjusted to pH 5.3-6.0. Anthers with microspores at sporad to late uninucleate stages were planted in 21×120 mm test-tubes, each containing 8ml of the medium, with 6 to 10 anthers per tube. Each treatment consisted of 10 replicate cultures. Cultures all were incubated under darkness at 25±2°C.

Table 1. Materials used in this study

Species	Cultivar	2n
Hordeum distichum	Svanhals	14
Oryza sativa	Asahi	24
Zea mays var. saccharata	Golden Cross	20
Lycopersicon esculentum	Fukuju No. 2	24
Nicotiana tabacum	Bright Yellow	48
Petunia hybrida	—	28
Solanum berthaultii	PI 218215*	24
S. chacoense	H 82**	24
S. phureja (formerly *S. rybinii*)	PI 197761*	24
S. pinnatisectum	WAC 3241***	24
S. verrucosum	WAC 3375***	24
Brassica oleracea var. aciphala	Chirimen Habotan-Ginsei	18
Glycine max	Harosoy	40

*, ** and *** : Supplied from the Inter-regional Potato Introduction Station, USA ; from the Botany Department, University of Birmingham, England; and from the Institute of Plant Breeding, Wageningen, The Netherlands. These species all are tuber-bearing.

Observations : In each experiment, one or two out of the anthers cultured were sampled from each tube both 10 and 20 days after inoculation and fixed with FARMER's fluid for 3 h. The fixed anthers were hydrolysed in 1 N·HCl at 60°C for 10 min. or so and squashed in a drop of 45% acetic acid after staining with FEULGEN reagent for 1 to 3 h. Cultures thus prepared were examined for the developmental state of pollen grains, the presence of pollen grains with four or more divided nuclei being employed as a criterion for appraising successful development of the pollen. In this case there were included both multicellular grains with cell-wall formation and multinucleate ones without it, though the former was distinguished as being embryogenic from the latter in considering further development of these grains. Assessment of the sucrose effect to pollen development was made by scoring, in each case, 1) the frequency of anthers containing pollen

grains with divided nuclei more than four, 2) the frequency of such grains per anther, and 3) the number of divided nuclei per grain.

Results

Embryogenic Response of the Pollen to Sucrose Concentrations

Embryogenic responses of pollen grains in the cultured anthers to sucrose varied widely at different levels of sucrose concentration as well as in different species. This situation is given in Table 2, here being summarized the percentage frequencies of the anthers with dividing pollen grains as affected by varied sucrose concentrations. Among the species used, *Hordeum*, *Oryza*, *Lycopersicon*, *Nicotiana*, *Solanum* (two species only) and *Brassica* were responsive in a more or less extent, and the others did not respond at any level of sucrose concentrations. In Figures 1 and 2 are graphed comparisons made among several species for the changes in pollen response with increasing sucrose levels. The results obtained in these respects will be outlined in the following.

Hordeum distichum : This species was the most responsive of all the species used, there being seen a marked inductive effect of sucrose on the formation of either multicellular or multinucleate pollen grains. Frequency of anthers with dividing pollen grains was increased with increasing levels of sucrose content in medium. In Figure 2, there are given the frequencies of anthers that were scored under the four classes set up according to the numbers of divided nuclei per grain, 4-7, 8-15, 16-29 and 30<. On the 10th day of culture, no pollen grains responded at the 2% sucrose level, but a few were indicative of response at the 5%. At the 10% level, the anthers in the 4-7 and 8-15 classes occurred in 6.5 and 4.0% of the anthers cultured, respectively, and at the 15% level, the anthers in the 4-7, 8-15, 16-29 and 30< classes were found in 7.5, 15.4, 12.9 and 6.0%, respectively. After 20 days of culture, the frequencies of such anthers became higher, responsive anthers being seen even at 2% sucrose level, though occasional, and at the 15% level, as many as half of the anthers inoculated had pollen grains with divided nuclei in the different classes. This probably implies that pollen development proceeds as the duration of cnlture increases for up to 20 days of culture.

Pollen grains undergoing nuclear divisions in each anther became more frequent with increasing levels of sucrose concentrations, their maximum frequency being found at the 15% sucrose level on both the 10th and 20th days of culture (Fig. 3) This situation

Table 2. Percentage frequency of anthers with dividing pollen grains*

Material	Days after inoculation	Sucrose concentrations (%)			
		2	5	10	15
Hordeum distichum	10	0	1.8	13.5	41.5
	20	3.9	12.5	31.8	50.9
Oryza sativa	10	0	0	2.2	3.5
	20	0	0	0	3.9
Zea mays	10	0	0	0	0
	20	0	0	0	0
Lycopersicon esculentum	10	0	12.5	6.3	11.8
	20	0	15.8	5.9	16.7
Nicotiana tabacum	10	36.8	47.4	55.0	30.0
	20	65.0	73.7	36.8	15.8
Petunia hybrida	10	0	0	0	0
	20	0	0	0	0
Solanum berthaultii	10	0	0	0	0
	20	0	0	0	0
Solanum chacoense	10	0	0	0	0
	20	0	0	0	0
Solanum phureja	10	0	0	0	0
	20	0	0	0.02	0.08
Solanum pinnatisectum	10	0	0	0	0
	20	0	0	0	0
Solanum verrucosum	10	0	0	3.2	7.2
	20	0	2.8	11.4	10.8
Brassica oleracea	10	0	0	5.6	0
	20	4.1	1.9	8.0	7.8
Glycine max	10	0	0	0	0
	20	0	0	0	0

*Confined to the grains with four or more nuclei.

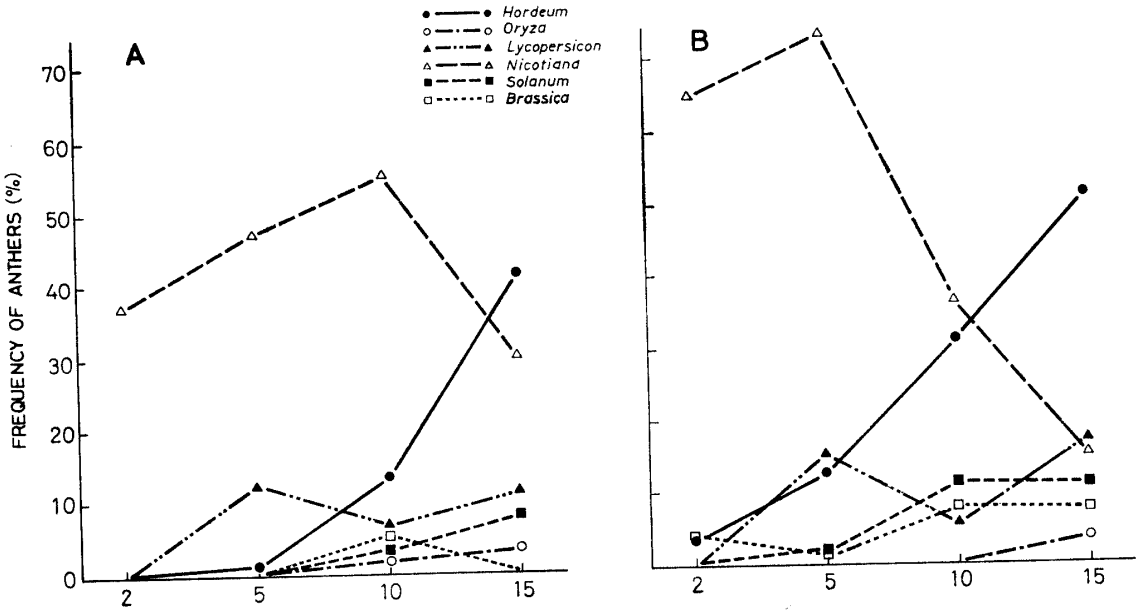


Fig. 1. Comparison between the species used for the frequency of anthers with dividing pollen grains at different sucrose concentrations. Observations were made on the 10th (A) and 20th (B) days of culture.

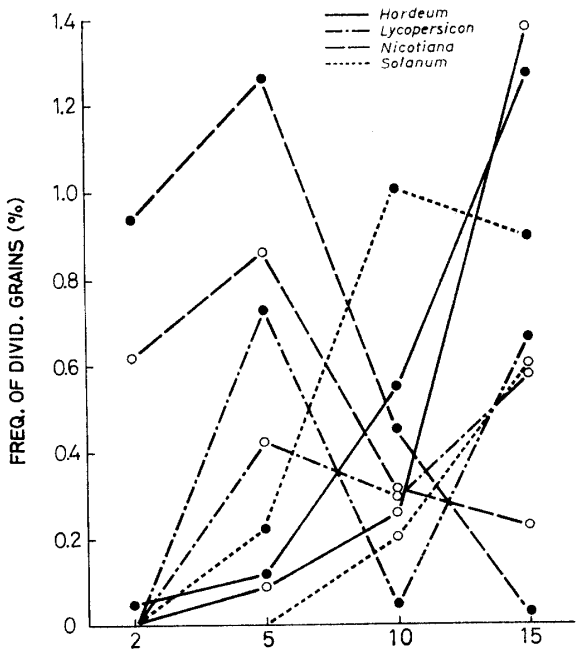


Fig. 2. Comparison between the species used for the frequency of dividing pollen grains per anther at different sucrose concentrations. Observations were made on the 10th (○) and 20th (●) days of culture.

was similar to that in the anther frequency mentioned above. The frequency of dividing grains per anther, however, did always not correlate with increasing duration of culture. The frequencies at the 15% sucrose level counted on the 20th day were not appreciably different from those on the 10th, though slightly increased frequencies were found at the other levels of sucrose. This fact suggests that pollen grains capable for embryogenesis within an anther may be limited numerically by certain causes.

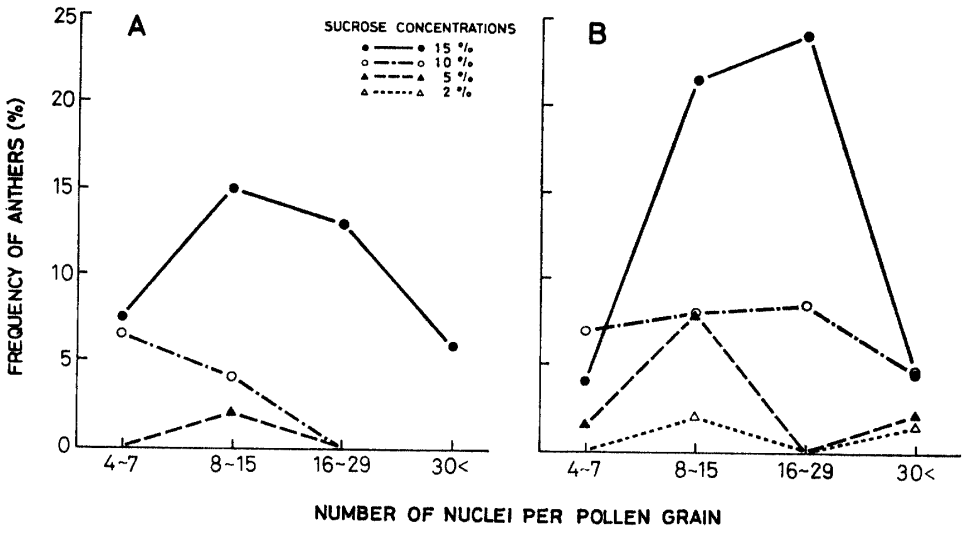


Fig. 3. Frequency of anthers with dividing pollen grains as affected by varied sucrose concentrations in *Hordeum distichum*. For the letters A and B, see Fig. 1.

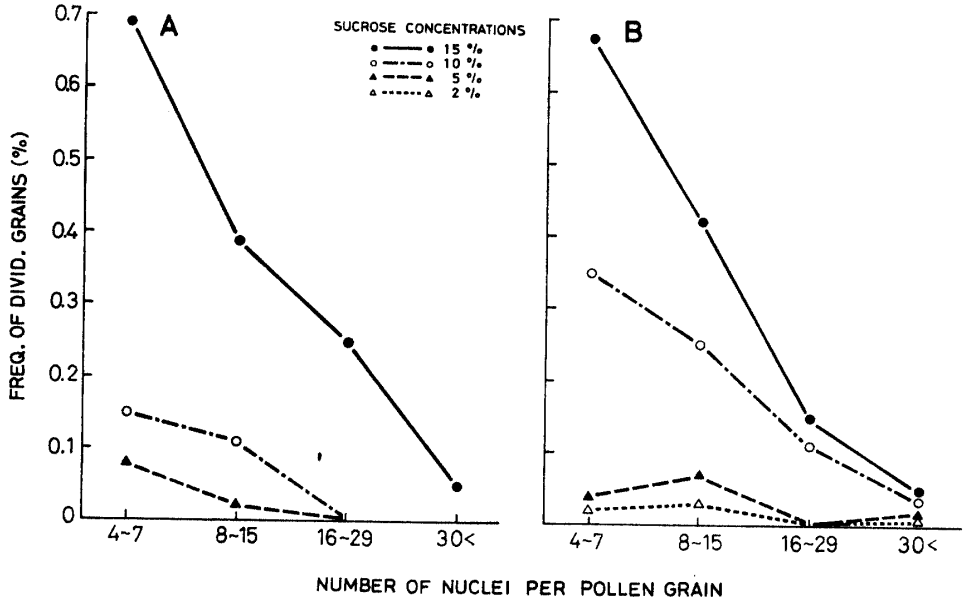


Fig. 4. Frequency of dividing pollen grains per anther as affected by varied sucrose concentrations in *Hordeum distichum*. For the letters A and B, see Fig. 1.

A similar feature was also observed in the numder of divided nuclei per grain. On the 10th day of culture, grains with nuclei in the 16-29 and 30< classes were present at the 15% sucrose level but not at lower levels. At sucrose levels other than 15%, the number of nuclei per grain increased with increasing duration of culture, but such a increase was not found at the 15% level, showing little difference between the frequencies counted both on the 10th and on the 20th days.

In *Hordeum*, of the pollen grains assessed to be dividing, many were in a multinucleate state (Fig. 10-j) but some had multicellular structure (Fig. 12-c). The latter grains, as mentioned already, are those which should be marked as being capable of further embryogenic development.

Oryza sativa : *Oryza* showed a less pollen

response to sucrose than did *Hordeum*. Dividing grains, on the 10th day, were scored only in as few as 3% of anthers cultured at the 10 and 15% sucrose levels and any of them had not more than four nuclei. After the next 10 days, they occurred in 3.9% of anthers at the 15% level only and consisted of eight nuclei. Although higher sucrose concentrations appeared to be available for development of *Oryza* pollen, a significant difference was not recognizable in frequency of the anthers with dividing grains not only between increased and decreased levels of sucrose but also between 10 and 20 day's durations of culture. Compared with *Hordeum*, a noticeable point was that almost all of dividing pollen grains had nuclei in a free state and multicellular grains occurring rarely were made up of cells extremely variable in their size (Fig. 13-c). The sucrose effect was not appreciable also in this case.

Zea mays : Any response was not obtained in this material. At higher sucrose levels there were occasionally seen anomalously enlarged grains that were filled with starch. Apart from the present study, many attempts have so far been done to induce pollen embryogenesis but the results all were the same as above.

Lycopersicon esculentum : Anthers cultured at the 2% sucrose level did not yield dividing pollen grains but at the 5% or higher levels produced them with a frequency around 10% (Table 2). A noticeable difference, however, was not found between each of the sucrose levels tried. The dividing grains per anther was somewhat higher at the 15% sucrose level than at the others. Cultures at the 5% level had rarely pollen grains consisting of 10 nuclei or so (Fig. 4). With *Lycopersicon*, thus, sucrose appeared to be not so effective for inducing pollen embryogenesis as with *Hordeum*.

Nicotiana tabacum : Anthers were cultured on the agar medium containing only sucrose at the same levels as in the other species. A high pollen response was obtained in spite of lacking all other ingredients. As seen from Table 1 and Figure 5, dividing grains occurred frequently in anthers cultured at any level of sucrose concentrations, but their frequency was higher at lower levels of sucrose rather than at higher levels, particularly in the anthers observed on the 20th day of culture. Also, with increasing duration of culture, anthers cultured at lower sucrose levels had dividing grains in considerably increased frequency, whereas those at higher levels did always not so. Likewise, the frequency

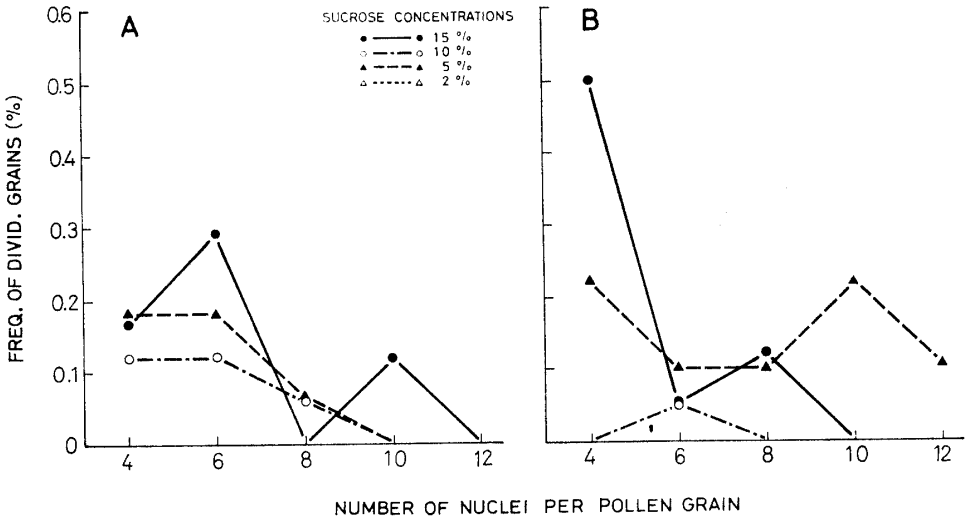


Fig. 5. Frequency of dividing pollen grains per anther as affected by varied sucrose concentrations in *Lycopersicon esculentum*. For the letters A and B, see Fig. 1.

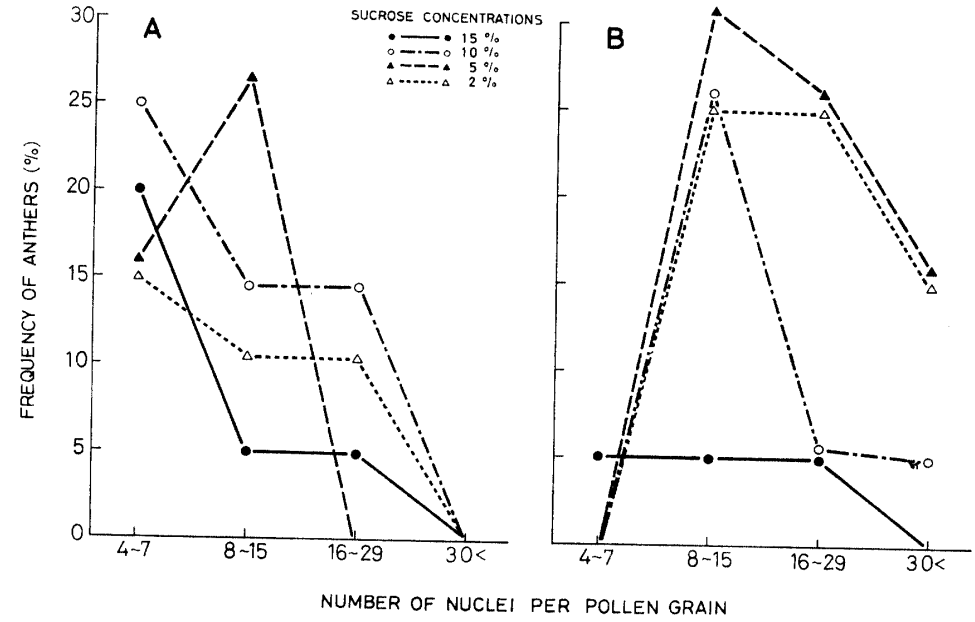


Fig. 6. Frequency of anthers with dividing pollen grains as affected by varied sucrose concentrations in *Nicotiana tabacum*. For the letters A and B, see Fig. 1.

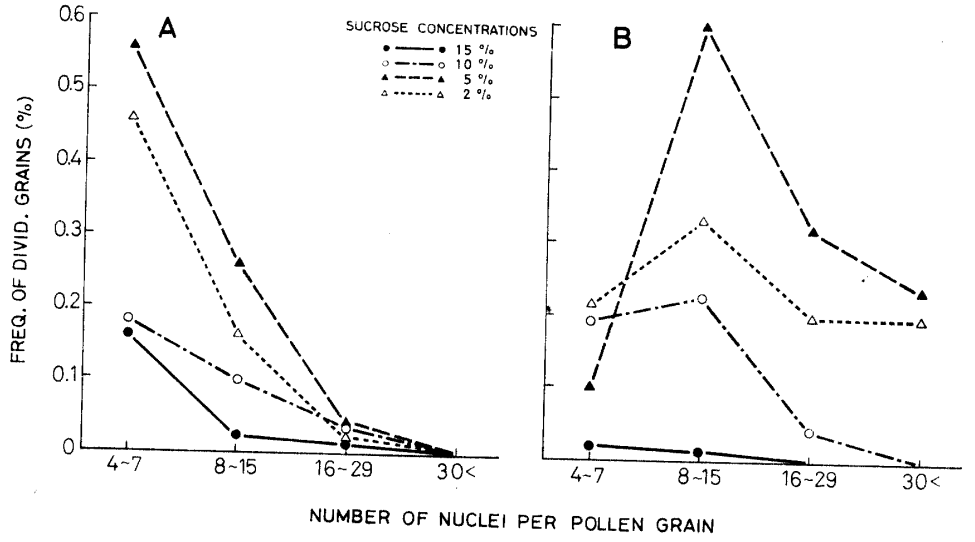


Fig. 7. Frequency of dividing pollen grains per anther as affected by varied sucrose concentrations in *Nicotiana tabacum*. For the letters A and B, see Fig. 1.

of dividing grains per anther tended also to raise in cultures at lower sucrose levels, the lowest being at the 15% level (Fig. 6). At the 10 and 15% sucrose levels, pollen grains with 30 or more nuclei were never detected in any case, and ceased their further development with up to a maximum of 12 nuclei, while even grains consisting of cells too numerous to count were occasionally found at lower sucrose levels (Fig. 13-e). From anthers cultured at the 2% sucrose level, thereafter, plantlets emerged with the frequency of 6.7% of anthers after as long as three months of culture. Such features are in marked contrast to those of *Hordeum*.

Petunia hybrida : No embryogenic development of the pollen was found in any level of sucrose concentrations. Even after 20 days of culture pollon grains remained

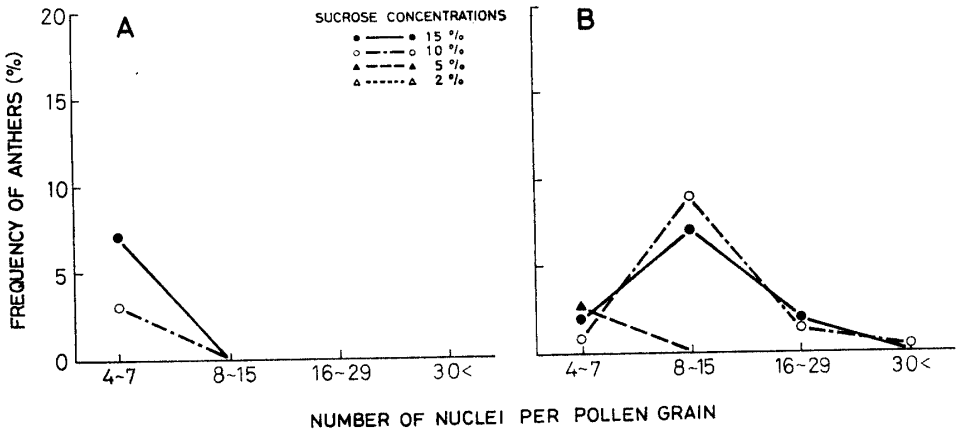


Fig. 8. Frequency of anthers with dividing pollen grains as affected by varied sucrose concentrations in *Solanum verrucosum*. For the letters A and B, see Fig. 1.

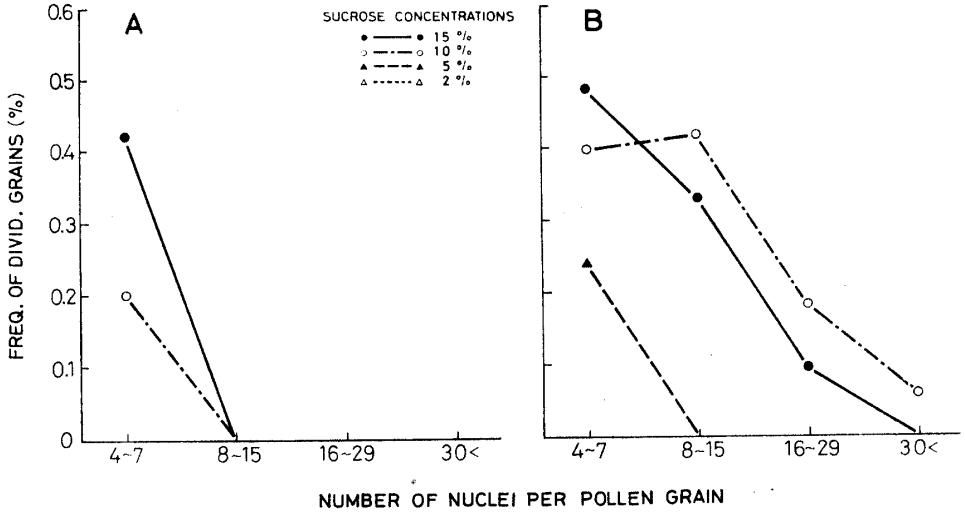


Fig. 9. Frequency of dividing pollen grains per anther as affected by varied sucrose concentrations in *Solanum verrucosum*. For the letters A and B, see Fig. 1.

intact in most cases, though with occasional occurrence of grains undergoing starch deposition or plasmolysis.

Solanum species : Among the five species examined, *S. verrucosum* responded fairly and *S. phureja* negligibly, but the others showed no response (Table 2). With *S. verrucosum*, as Figure 7 shows, dividing pollen grains were scored, on the 10th day of culture, in about 3% of anthers cultured at the 10% sucrose level and in slightly more than 7% at the 15% level. All of them had nuclei ranging 4 to 12 nuclei (Fig. 13-j). After the next 10 days, such grains became occasionally found even in anthers cultured at the 5% sucrose level, and at the 10% level 9% of the anthers had dividing grains, a few of them having 30 or more nuclei. A similar response was also observed when sucrose was used at 15% level. The frequency of dividing grains per anther was 0.2 and 0.4% of the grains counted in anthers cultured on the media added with sucrose at 10 and 15% concentrations, respectively, on the 10th day of culture (Fig. 8). When counted on the 20th day, these frequencies were raised to a more or less extent at any level of sucrose. In *Solanum* species, therefore, higher sucrose concentrations than usual appeared to be effective for initiating pollen grains into embryogenic development.

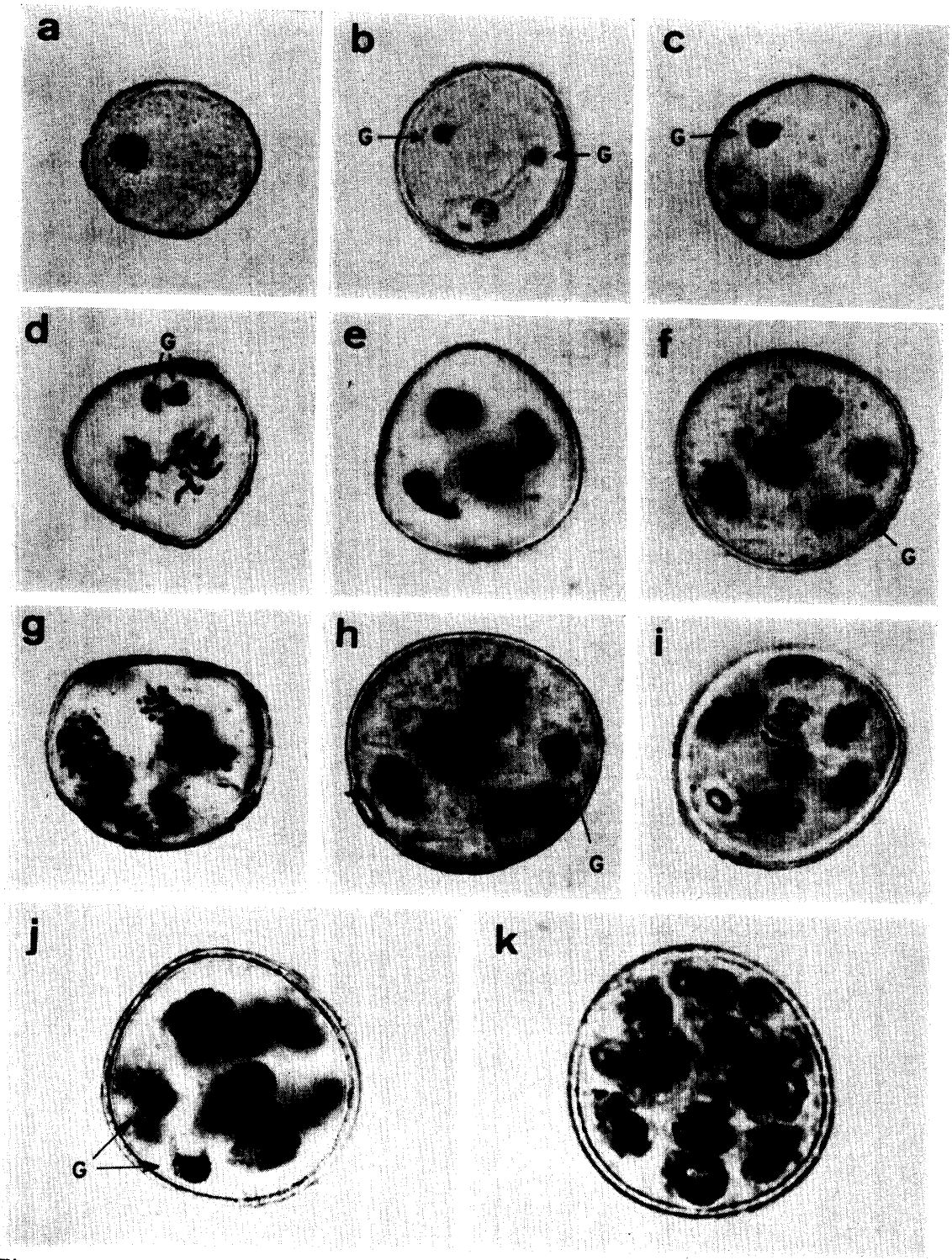


Fig. 10. Early developmental sequence in pollen grains of *Hordeum distichum*. The letters G and V indicate the generative and vegetative nuclei, respectively. (*ca.* x 2500)
a ; Microspore remaining in a uninucleate state (after 20 days of culture).
b : Trinucleate grain with 2G+V (after 10 days). c : Trinucleate grain with G+2V (after 10 days). d : Grain with 2G+V, the latter being at anaphase (after 10 days). e : Grain with 4 equal-sized nuclei (after 10 days). f: Grain with 2G+4V (after 10 days). g : Dividing grain, there being seen nuclei at anaphase and interphase (after 10 days). h : Grain with G+5V (after 10 days). i: Grain with 8 nuclei, 4 of them entering prophase (after 20 days). j : Grain with 12 nuclei, 2 of which are generative (after 20 days). k : Multinucleate grain consisting of as many, or more nuclei than 30 (after 20 days).

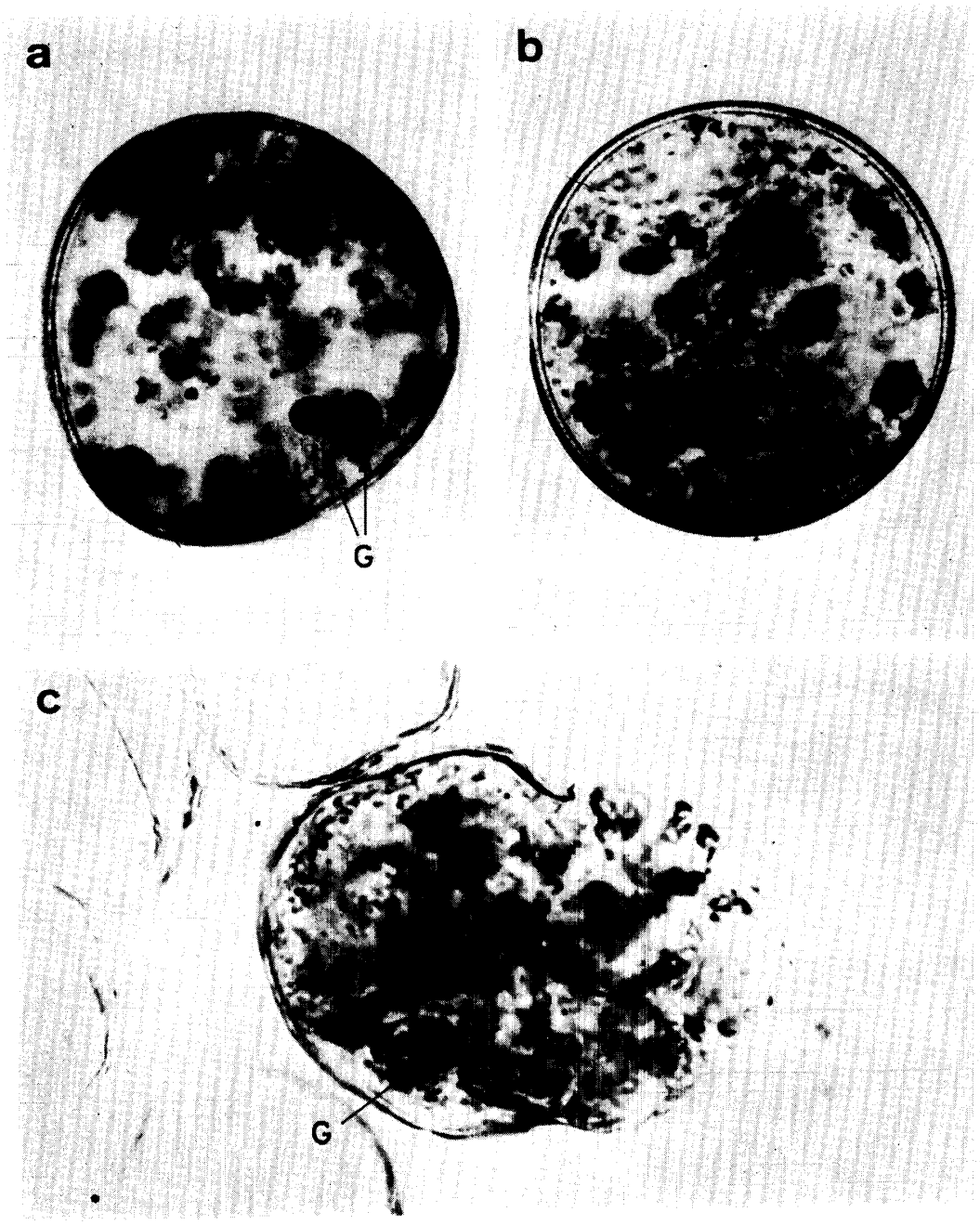


Fig. 11. Non-embryogenic grains of *Hordeum distichum*, which enlarged with starch deposition. (*ca.* x 2500)
a and b : Giant grains both filled with starch (after 20 days of culture). c : Giant grain getting out after bursting the exine (after 20 days).

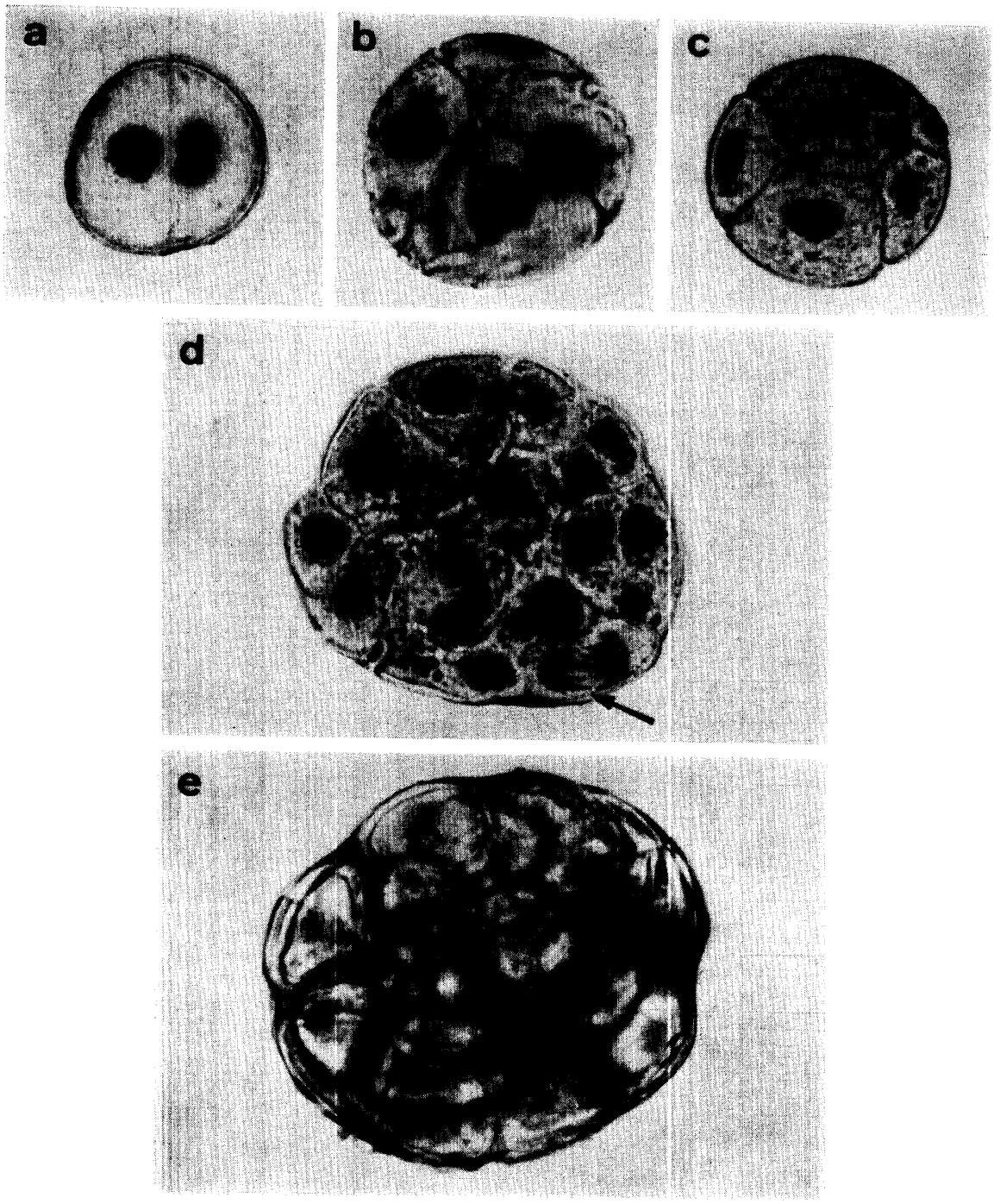


Fig. 12. Early embryogenic sequence in pollen grains of *Hordeum distichum*, with the successive formation of cell walls. (*ca.* x 2500)
a : Bicellular grain with 2 equal-sized nuclei comparted with cell walls (after 10 days of culture). b : Four-celled grain, its nuclei being nearly equal-sized and comparted with cell walls (after 10 days). c : Eight-celled grain with nearly equal-sized nuclei (after 20 days). d : Multicellular grain consisting of cells as many as 30, its nuclei all being nearly equal in size (after 20 days). e : Pro-embryoidal grain at globular stage (after 20 days).

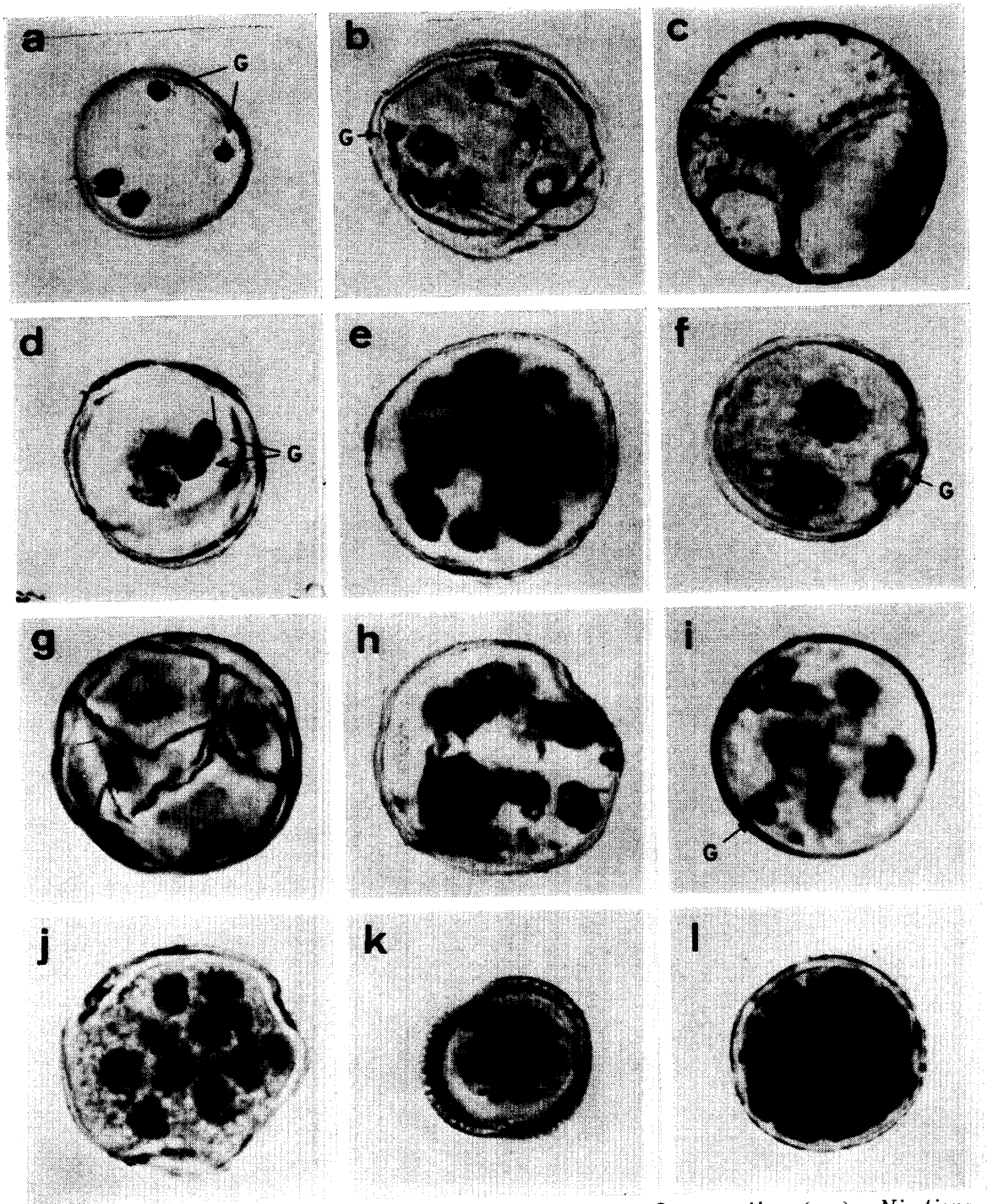


Fig. 13. Early developmental features of pollen grains in *Oryza sativa* (a–c), *Nicotiana tabacum* (d–e), *Lycopersicon esculentum* (f–i), *Solanum verrucosum* (j), *Brassica oleracea* (k) and *Glycine max* (1). (*ca.* x 2500)
a : Grain with 2G+2V (after 10 days of culture). b : Multinucleate grain with G+8V (after 10 days). c : Multicellular grain which was comparted irregularly with cell walls, being indicative of dedifferentiation (after 20 days). d : Grain with 2G+2V (after 10 days). e : Multinucleate grain composed of free nuclei of 20 or so (after 20 days). f : Tricellular grain with the relatively large compartment of 4 or more cells. Such a grain is possibly embryogenic (after 20 days). h and i : Multinucleate grains with 8 nuclei in a free state, the latter's nuclei being irregular-sized and vaguely outlined (after 10 days). j : Multinucleate grain with 12 free nuclei, v and its derivatives being not descernible (after 10 days). k and 1 : Degenerating grains, both containing unknown particles stained densely with FEULGEN reagent (after 20 and 10 days, respectively).

Brassica oleracea : Anthers with dividing pollen grains occurred slightly more frequently when cultured on media containing sucrose at increased levels than at decreased levels, but their frequency was very low on the whole (Table 2). The number of nuclei involved in a pollen grain was four in most instances, with a maximum of 16. With this species, however, it was not ascertained whether sucrose has an immediate effect or not on induction of pollen development, owing to rare occurrence of dividing grains. Besides, there were frequently encountered degenerating grains that were filled with numerous unknown particles densely stained with FUELGEN reagent (Fig. 13-k).

Glycine max : Anthers with dividing pollen grains were of no occurrence in any case, with every grain remaining quiescent. In this instance there were most frequently found degenerating grains that were of the same entity as in *Brassica* (Fig. 13-l).

Early Embryogenic Processes of the Pollen

Cytological behavior of pollen grains in anther cultures has been traced in detail during the early processes of their embryogenic development in the species responsive to anther culture, particularly in *Hordeum*. First, the features observed with *Hordeum* will be described below.

In certain instances, the pollen nucleus divided into two distinctive daughter nuclei, the generative and vegetative ones, after the first pollen mitosis, and either of them soon underwent the next mitoses resulting in the formation either of one generative nucleus plus two vegetative ones (Fig. 10-b) or of the reverse (Fig. 10-c). Thereafter, these nuclei, regardless of the generative and vegetative ones, underwent further repeated divisions without the formation of cell walls, divided into as many as 20 to 30 nuclei in a free state and eventually became to form a multinucleate grain (Fig. 10-j, k). These free nuclei were remarkably variable in their size. Subsequent processes were bifurcated ; in one pathway multinucleate grains remained quiescent, and in the other they enlarged by depositing starch grains (Fig. 11-a, b) and then got out of the exine after its bursting (Fig. 11-c). These grains sooner or later resulted in degeneration. Such features were fairly frequently encountered.

In other instances, there were observed the following features. One of them, as pointed out by some workers[9,12], was that the generative nucleus or its few derivatives degenerate remaining attached to the intine and only the vegetative nucleus undergoes repeated divisions to become multinucleate. On rare occasions, the generative derivatives did contribute to the multinucleate formation with a few degenerating vegetative derivatives. The multinucleate grains were formed in either way, and most of them appeared to degenerate without forming cell walls in a brief period later but some to proceed on their own embryogenic pathway, their divided nuclei being comparted by the cell walls formed at an earlier or later stage.

Another feature was comparable to the type B described by SUNDERLAND[11] for *Nicotiana tabacum*. In this case the daughter nuclei formed by the first pollen mitosis divided repeatedly without distinction of the generative and vegetative nuclei and with the successive formation of cell walls from the beginning of division. The multicellular grains formed in this way, with few exceptions, were characterized by their possessing nearly equal-sized nuclei separated by the cell walls. This feature seems to be in a reasonable similarity to that in embryogenesis *in vivo*, and so such grains may be regarded as embryogenic, being capable of forming pollen plantlets. In Figure 12 (-d, e) are shown proembryoids at globular stage, which had been formed in such a way.

The various features described above were found in anthers cultured at any level of sucrose concentrations. Therefore, the varied concentrations of sucrose appeared to have little effect on developmental pathways of the pollen, even if effective for triggering pollen grains to divide.

The other species observed all, with a

few exceptions, followed a similar pattern, at least in initial behevior of their pollen grains. The following description is for the exceptional features. In *Oryza*, all of responsive grains had dividing nuclei in a free state up to six to eight-nucleate stages (Fig. 13-a, b), and then some of them became irregularly comparted with cell walls and resulted in multicellular grains consisting of cells of various sizes, being indicative of dedifferentiation into calli (Fig. 13-c). In *Lycopersicon*, there were seen two features ; one proceeding on embryogenic pathway with regular formation of cell walls (Fig. 13-f, g) and the other undergoing free nuclear divisions (Fig. 13-h, i). Further development, however, ceased at six to eight-nucleate stages in almost all cases. The pollen behavior observed in *Nicotiana* was basically the same as described already by earlier workers[2,9,11] (Fig. 13-e). The type B grains as designated by SUNDERLAND[11], however, were not encountered at all, even though their occurrence might have been extremely rare. In *Solanum* (sp. *verrucosum*), responsive grains became multinucleate with up to as many as 10 nuclei by divisions either of the generative or vegetative nuclei (Fig. 13-j). This process was not accompanied by the formation of cell walls. Of the resultant grains, most remained quiescent, and only a few retained further development with cell wall formation leading to proembryoids. In *Brassica*, most of the responsive grains remained at four-nucleate stage and a few became of 16-nucleate grains. The cell walls comparting these grains were not recognizable at all. As described already, there were most frequently found grains filled with innumerable particles which were stained densely with FEULGEN reagent (Fig. 13-k).

Discussion

Embryogenic Rates of the Pollen to Varied Sucrose Concentrations

With regard to the inductive effects of sucrose on pollen embryogenesis *in vitro*, attempts have been so far made by earlier workers[1,2,8,10] with several plant genera, and the results have been presented informing that sucrose at increased levels of concentration gave no or little effect in *Nicotiana* but was effective to a more or less extent in *Hordeum*, *Lolium* and *Lycopersicon*, although its effect being on cell proliferation from the pollen. From these results, it can be seen that pollen responses to sucrose vary widely with different species.

Among the species used in the present study, there has been also found a remarkable variation in response of the pollen to sucrose. On the basis of the sucrose concentrations at which the anthers with dividing pollen grains occurred with a relatively high frequency, the present species may be grouped into four types as follows : 1) species making greater response at lower sucrose levels (*Nicotiana*), 2) ones making it at higher sucrose levels (*Brassica*, *Hordeum*, *Oryza* and *Solanum*), 3) ones responding no matter what sucrose levels may be used (*Lycopersicon*), and 4) ones with no response at any level of sucrose (*Glycine*, *Petunia* and *Zea*). Although responsive alike, these species differed considerably in the frequencies not only of anthers with dividing pollen grains but of dividing grains per anther. The same was true of the number of nuclei per pollen grain. It is not explainable from the present study why different species differ in such responsiveness. This may be due probably to species differing from one another in their requirements to exogenous factors as well as in their possessing endogenous factors inherent in them. In *Nicotiana*, in fact, it has been found not only in the present work but also by NITSCH[8] that pollen plantets arised even from anthers cultured on the medium containing 2% sucrose only. This fact is an example of how the above factors play a major role in embryogenic response of the pollen to anther culture. To substantiate such a view, however, analytical investigations are needed of each of the factors involved.

Embryogenic Processes of the Pollen and Sucrose Effect to Them

Embryogenic sequence of pollen grains *in vitro* has been in detail traced by NITSCH[8,9] and SUNDERLAND[11,12] with *Nicotiana tabacum*. With other genera, however, information is still fragmentary, though it has been derived from *Datura* and *Hordeum* by some workers[5,9]. In the present study observations along this same line have been carried out with all of the responsive species, especially in detail with *Hordeum*.

The embryogenic processes observed here may be classified into the following patterns according to their pathways.

Unresponsive
- i) Microspores remaining quiescent.
- ii) Bi- or trinucleate grains not undergoing further development.

Responsive
- a) Non-embryogenic
  Multinucleate grains are formed by repeated free-nuclear divisions and eventually result in degeneration.
  - i) Multinucleate grains remaining quiescent.
  - ii) Multinucleate grains enlarging with starch deposition and getting out of the exine after its bursting.
- b) Embryogenic
  Dividing grains become sooner or later to form multicellular structure by the formation of cell walls and then lead to embryoid formation.
  - i) Multicellular grains having their origin in the generative nucleus.
  - ii) Multicellular grains having their origin in the vegetative nucleus.
  - iii) Multicellular grains arising from the pollen nucleus, without distinction of generative and vegetative, by its divisions and being accompanied by the successive formation of cell walls.

Of these embryogenic patterns, ii) and iii) correspond to the types A and B designated by SUNDERLAND[11], respectively. He has suspected of the type B grains for their embryogenic capability on account of they arising in the least productive anthers. However, these grains show a reasonable similarity to initial embryos *in vivo* in that the nuclei involved in both are almost equal in size and comparted regularly with the cell walls formed successively. In addition, in *Nicotiana*, there exists a certain similarity between the frequency of the grains in question per anther and that of plantlets arising from an anther, though a correlation between both has yet not been evidenced experimentally. Such being the case, it is the present authors' view that the pathway presented in iii) of the above embryogenic patterns may be the most responsible for the formation of pollen plantlets.

Of the emryogenic patterns mentioned above, all have occurred in anthers cultured at any level of sucrose concentrations. Sucrose seems to be, therefore, litte effective on determining which pathway pollen development takes. In this case, a cessation of further development of dividing pollen grains is rather noticeable. Such a cessation has been most frequently observed after divided grains became to have as many as 20 nuclei, especially with its increased frequency when anthers were cultured at higher sucrose levels. The same is true even in more highly responsive *Hordeum* and *Nicotiana*. The definite reasons for this are not clear at present. A probable explanation, however, seems to come from the view that although sucrose at increased levels acts effectively on initiation of pollen division as a trigger, it has little or no effect on further development of divided grains which reached a multinucleate state. If so, as suggested by NITSCH[8] for *Nicotiana* and by SHARP *et al.*[10] for *Lycopersicon*, it is necessary for successful induction of pollen plantlets that anther cultures are transplanted from high sucrose medium to low sucose medium as long as two weeks after inoculation.

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各種糖濃度に対する培養葯内花粉粒の分裂反応

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要　約

葯培養による花粉起源胚状体の形成は，培養時における物理的，化学的ならびに生物的諸要因の相互作用にその成否がかかっているものと考えられる。本研究では，これら諸要因のうち，まず化学的な外生要因の一つとしての糖の効果を検討するために，数種の植物につき，2，5，10および15%の糖濃度をもつ培地で葯培養を行ない，培養10日目と20日目に花粉粒の分裂行動を調べた。

各糖濃度に対する花粉粒の反応には，種による著しい差異がみられた。すなわち，分裂花粉粒が高頻度に認められたときの糖濃度に基づいて供試植物を類別すると，1）低濃度（2～5%）でよく反応するもの（*Nicotiana tabacum*），逆に2）高濃度（10～15%）でよく反応するもの（*Brassica oleracea*, *Hordeum distichum*, *Oryza sativa*, *Solanum verrucosum*），3）いずれの濃度でも反応するもの（*Lycopersicon esculentum*）および 4）いずれの濃度でも全く反応を起さないもの（*Glycine max*, *Petunia hybrida*, *Zea mays*）の4型となる。同じく反応を示しても，その程度は植物によって大きく異なる。それぞれ最も有効な糖濃度における培養20日目の調査によると，*Hordeum* や *Nicotiana* では，それぞれ置床葯の50.9%と73.7%が分裂粒を示したのに対し，*Brassica*, *Lycopersicon*, *Oryza* および *Solanum* ではそれぞれ8.0, 16.7, 3.9および11.4%の葯がそれを示したにすぎない。これと似た傾向は葯当り分裂花粉粒の頻度や花粉粒当り分裂核数についてもみられた。高濃度の糖によく反応する植物でも，培養日数の経過に伴う分裂花粉粒の頻度の増大はさして認められなかった。これは高濃度の糖が花粉粒の分裂誘起には有効であっても，その後の多核化もしくは多細胞化にはほとんど効果を現わさないためと考えられる。

一方，培養葯内花粉粒の分裂行動も著しく多様で，これを整理すると凡そつぎのように類型化できる。まず培養に対して花粉粒が全く反応を示さない場合とそれを多かれ少なかれ示す場合に大別される。後者には，さらに，分裂花粉粒が数10核で発育を停止したり，あるいは澱粉を蓄積して肥大する場合と，生殖核か栄養核のいずれかが分裂を重ねて多核化し，数核から10数核のとき膜を形成して多細胞化するか，あるいは最初から膜の形成を伴って生殖核と栄養核の別なく分裂をくりかえして多細胞粒になる場合とがある。花粉植物誘導の最も大きい可能性をひめているのは，分裂様式や出現頻度からみて，最後にあげた場合のようである。これらの分裂様式はいずれの糖濃度区においても認められたので，それに対する糖濃度の影響はないものと考えられる。