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MUSÉUM NATIONAL D'HISTOIRE NATURELLE

Publication trimestrielle. Vol. XX — N° 1. 1978.

POLLEN et SPORES

E.P.H.E.
LABORATOIRE de PALYNOLOGIE

EXTRAIT

PARIS
61, Rue de Buffon
—
1978

PUBLIÉ AVEC LE CONCOURS DU CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

POLLEN MORPHOLOGY OF SELECTED TRITICEAE AND TWO INTERGENERIC HYBRIDS (*)

BY

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SOMMAIRE. — Utilisation de la microscopie à balayage — qui grossit jusqu'à 25 000 fois — pour étudier le pollen de 23 échantillons de *Hordeum vulgare* L., *H. bulbosum* L. (2 × and 4 ×), *Secale cereale* L., *Triticum monococcum* L., *T. speltoides, T. timopheevi, T. turgidum,* and *T. aestivum* plus deux hybrides intergénériques (*H. vulgare* × *S. cereale* and *H. vulgare* × *T. turgidum*). Toutes les espèces de *Triticum* ainsi que les cultivars y compris *T. monococcum* ont une exine aréolée, alors que *H. vulgare* et *S. cereale* ont une exine scabre. *H. bulbosum* a une exine aréolée comme les espèces de *Triticum*. Le pore et la taille de l'annulus chez *H. bulbosum* et chez toutes les autres espèces de *Triticum* augmentent parallèlement à la ploïdie. *H. bulbosum* est peut-être un chaînon évolutif entre l'orge et le blé. L'étude du pollen a été mise en corrélation avec les analyses cytogénétiques et les analyses de stomates. Ces études ont montré l'utilisation possible de l'analyse détaillée de la sculpture de l'exine en tant que marqueur génétique pour l'étude de la transmission et de l'expression des caractères.

Introduction.

Comparative cereal pollen morphology studies have been reported by phase contrast microscopy (ERDTMAN and PRAGLOWSKI, 1959 ; FAEGRI and IVERSEN, 1964), transmission electron microscopy (ROWLEY, 1960), and by scanning electron microscopy (SEM) [ANDERSON and BERTELSEN, 1972 ; GRANT, 1972]. ANDERSON and BERTELSEN reported that pollen grains of *Hordeum vulgare* L., *Secale cereale* L., *Triticum monococcum* L., *Agropyron repens,* and *Elymus arenarius* L. had scabrate exine sculpturing whereas pollen from all other *Triticum* species (*T. aestivum, T. compactum, T. dicoccum,* and *T. spelta*) and *Avena sativa* L. had areolate sculpturing. In addition,

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GRANT (1972) recognized island and diffuse types within scabrate sculpturing.

In the present SEM study, Triticeae pollen sculpturing is examined in greater detail and for additional species and accessions within *Hordeum, Secale,* and *Triticum* than previously reported. Exine sculpture analysis was studied to establish genetic markers for inheritance and expression. Two F_1 intergeneric hybrids (*H. vulgare* × *S. cereale* and *H. vulgare* × *T. turgidum*) were also evaluated by detail SEM analysis.

Materials and Methods.

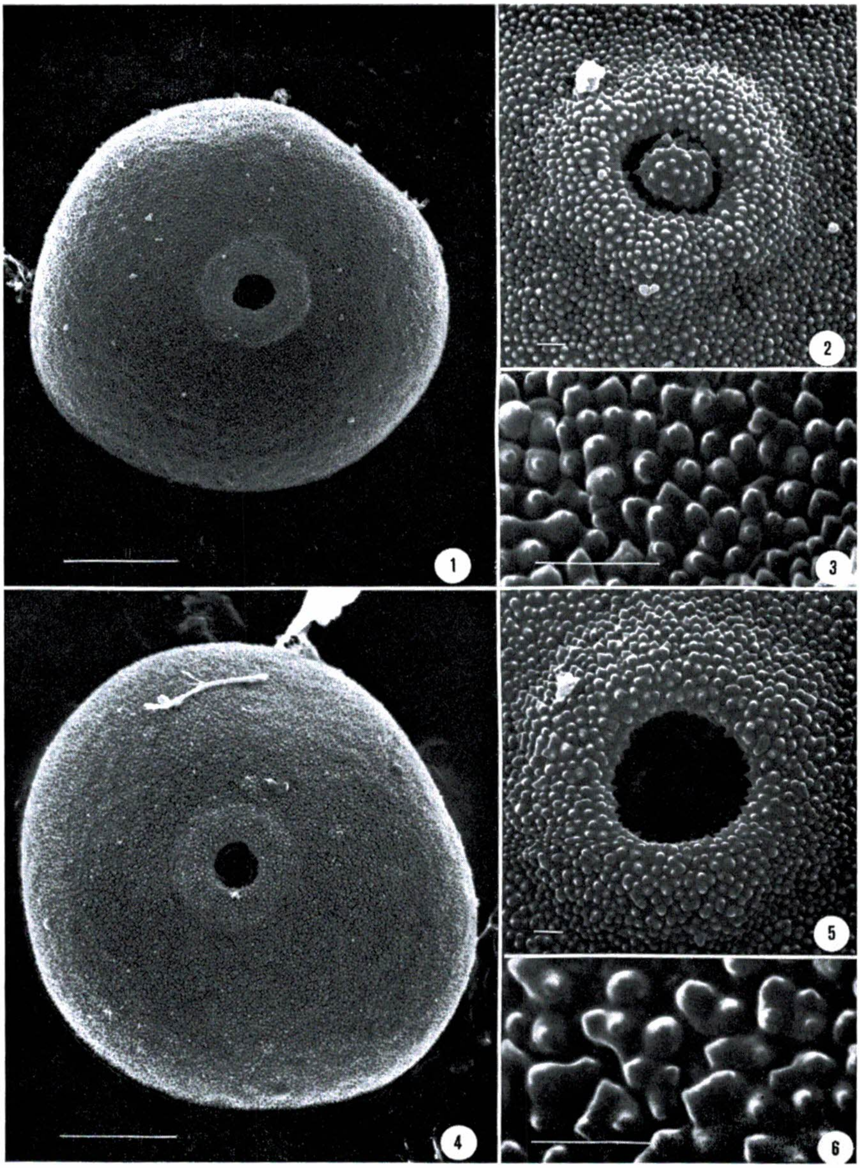
Pollen was collected from several species and accessions of *Hordeum, Secale,* and *Triticum* and two of their intergeneric hybrids (Table I). Samples were acetolyzed by the method of FAEGRI and IVERSEN (1964). After acetolysis, a water suspension of pollen was placed on a specimen stub and freeze-dried. The stubs were vacuum coated with approximately 100 Å of gold-palladium while rotating at a 45° angle to the vapor source. Samples were viewed with an ETEC Autoscan electron microscope operating at 10 or 20 KV depending on the magnification used. Twenty pollen grains were randomly picked from each sample and viewed at × 1800, × 6000, and × 25,000 magnifications. Representative pictures were taken of the whole pollen (× 1800), of the pore region (× 6000), and of a predetermined area approximately midway between the annulus and the apparent « edge » of the pollen granule (× 25,000).

Permanent glycerine-jelly slides have been deposited in the pollen reference collection of the Kansas State University Herbarium.

Results and Discussion.

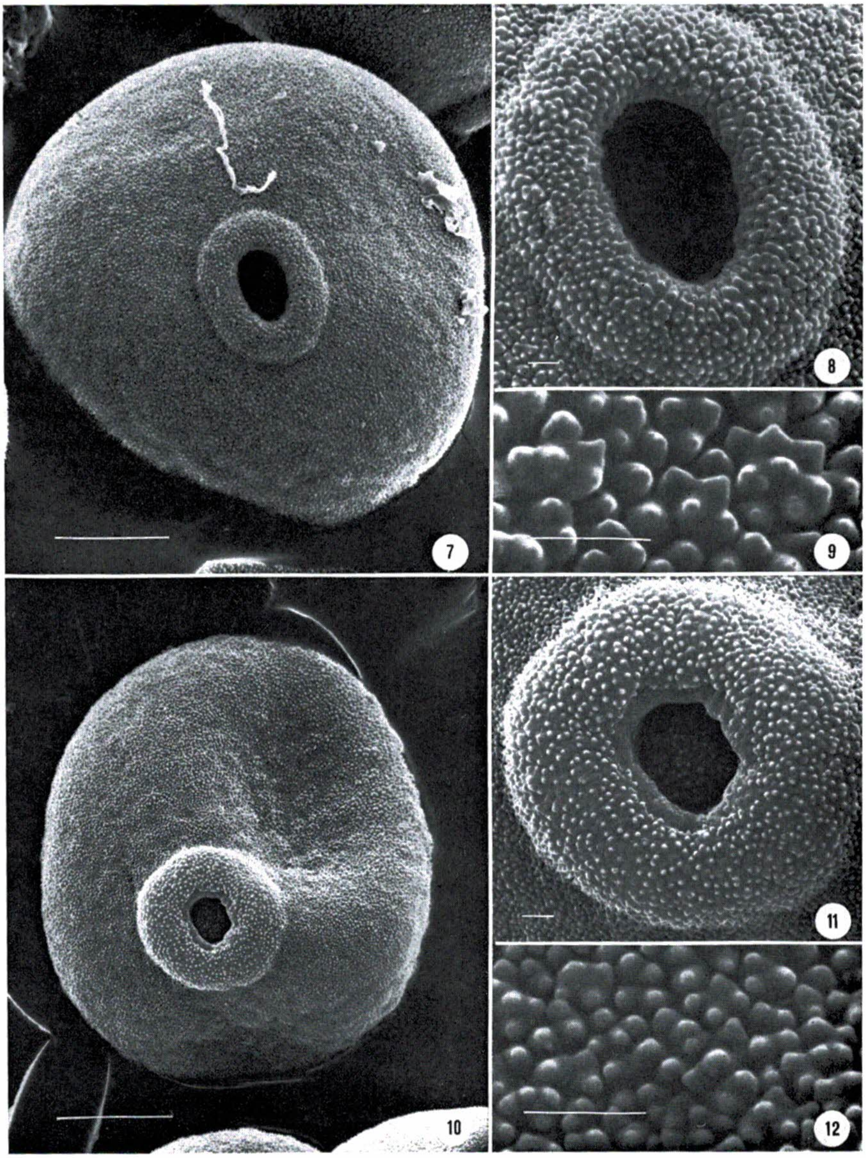
Triticum : All species and cultivars of *Triticum* have spherical pollen with areolate exine sculpturing with branched spinules isolated on islands (Figs. 1-15). As the ploidy level increases, the size of the spinules, pollen, pore and annulus also increases (Figs. 1-9). Influence of ploidy on pollen size has been reported in *Oryza sativa* (SREE RANGASAMY and RAMAN, 1973). Annuli of all *Triticum* species (Figs. 2, 5, 8, 11 and 14) are more prominent than *S. cereale* or *H. vulgare* (Figs. 17 and 20).

ANDERSON and BERTELSEN (1972) reported that unacetolyzed pollen of *T. monococcum* possessed scabrate exine sculpturing resembling barley and rye. Tapetal debris obscured the small areolate spinules that are easy to observe at the tetraploid and hexaploid levels but

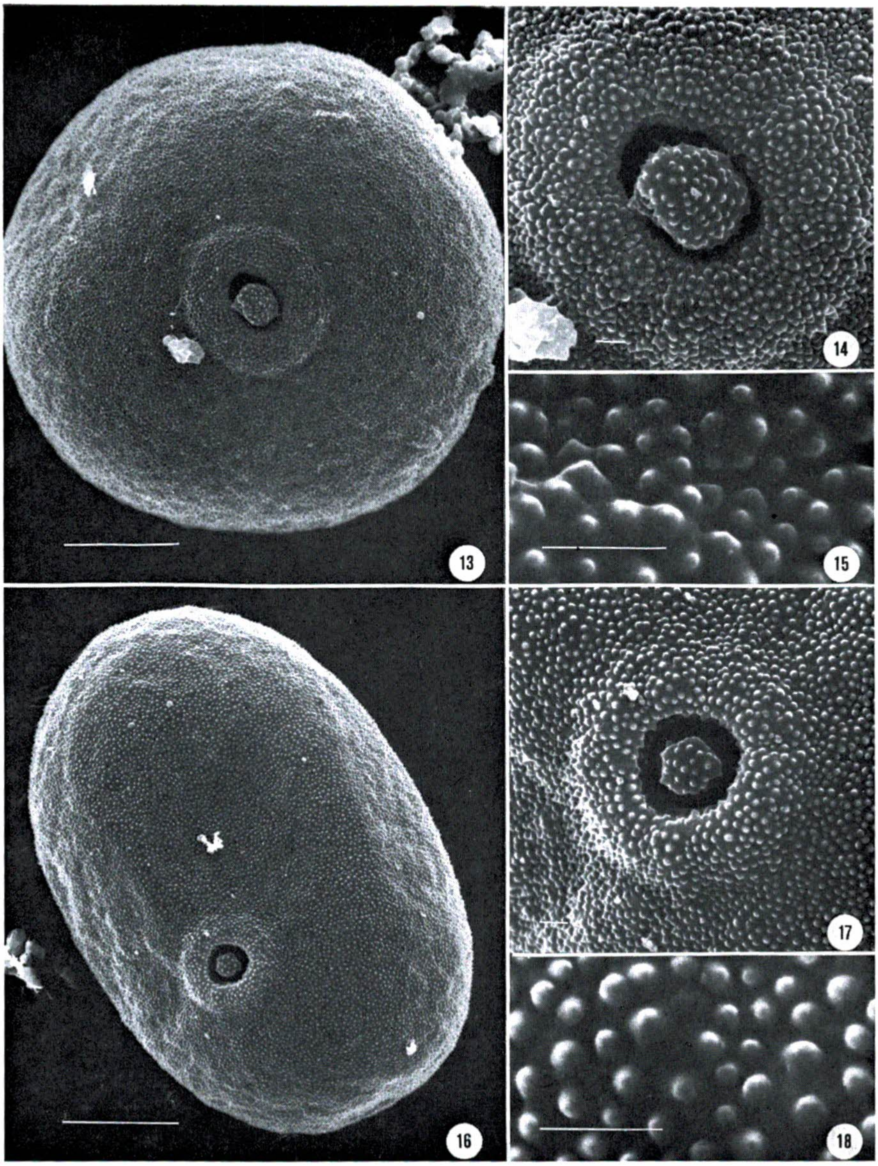


FIGS. 1 - 3. — Pollen of *T. monococcum* showing prominent annulus and areolate exine sculpturing. Note branching of spinules (× 1800, × 6000 and × 25,000). Scales represent 10 μ, 1 μ and 1 μ respectively, in all figures.

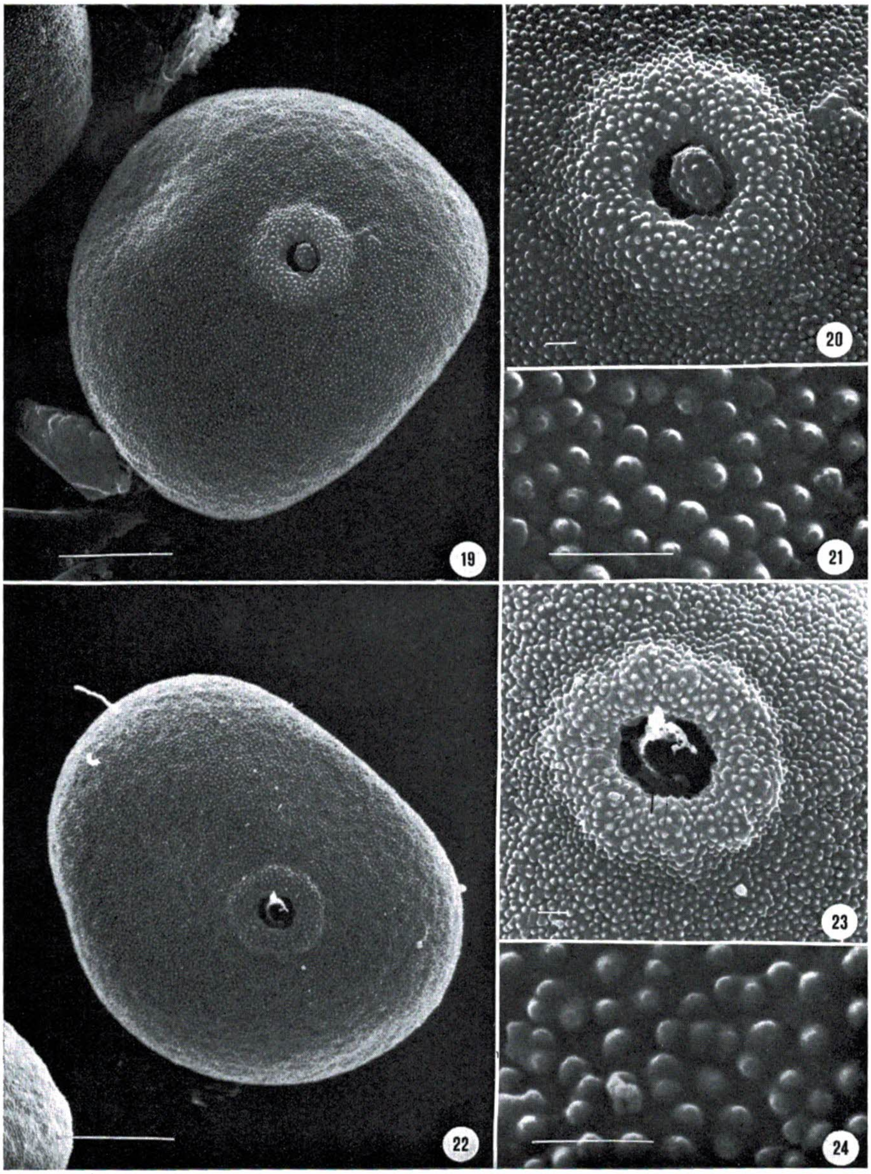
FIGS. 4 - 6. — Pollen of *T. turgidum*.



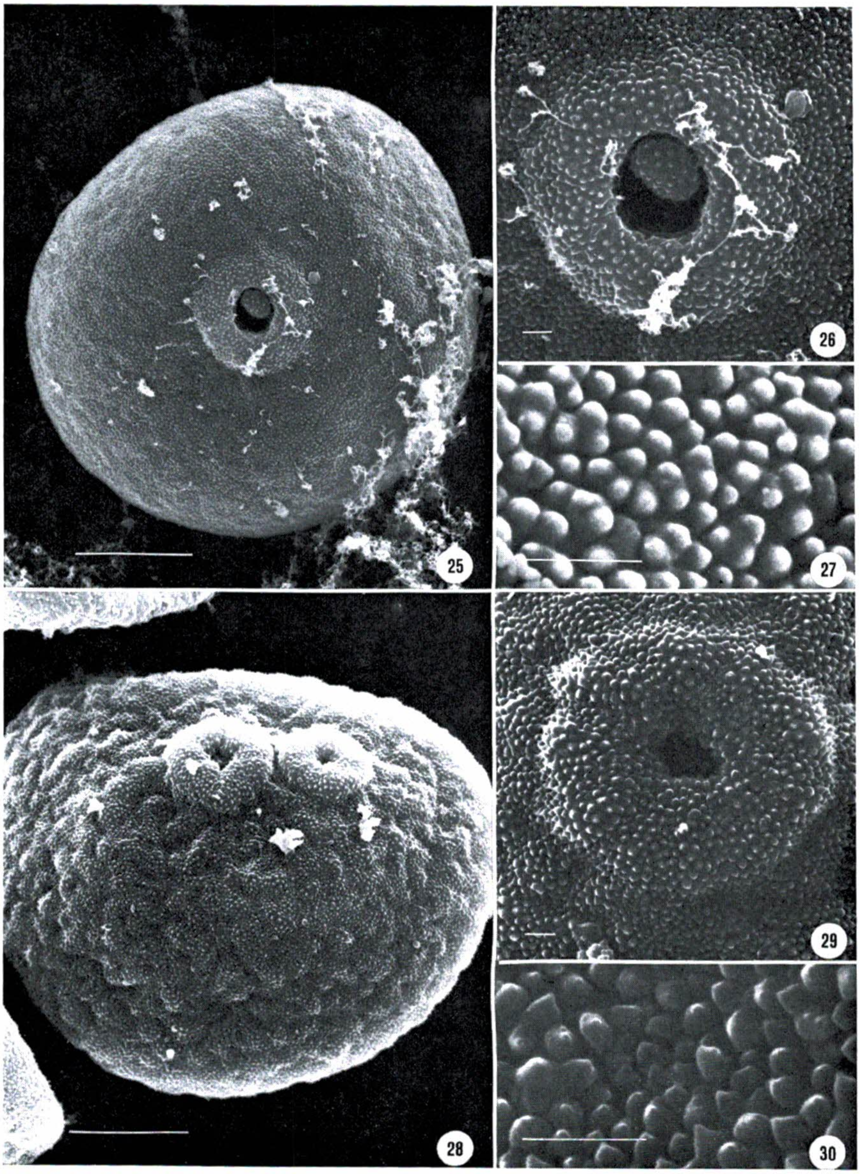
FIGS. 7 - 9. — Pollen of *T. aestivum*.
FIGS. 10 - 12. — Pollen of *T. speltoides*.



Figs. 13 - 15. — Pollen of *T. timopheevi*.
Figs. 16 - 18. — *S. cereale* pollen showing small annulus, scabrate exine sculpturing and ellipsoid granules.



FIGS. 19 - 21. — *H. vulgare* pollen.
FIGS. 22 - 24. — Pollen of *H. bulbosum* (2 ×) showing areolate branched spinules.

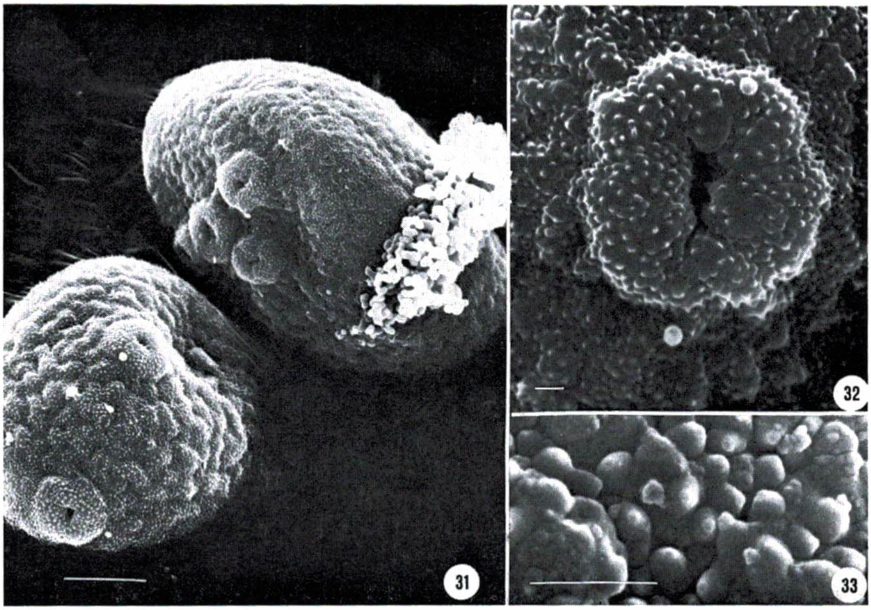


FIGS. 25 - 27. — Pollen of *H. bulbosum* (4 ×) with prominently branched areolate spinules.

FIGS. 28 - 30. — Biporate pollen from the *H. vulgare* × *T. turgidum* F_1 hybrid. Note mixture of areolate and scabrate spinules.

require higher magnifications for the diploid accessions. At × 25,000 we observed areolate sculpturing in all accessions of *T. monococcum* (Figs. 1-3).

Hordeum and *Secale* : All cultivars of *S. cereale* and *H. vulgare* had scabrate exine sculpturing (Figs. 18 and 21) which agrees with the results obtained by ANDERSON and BERTELSEN (1972). Within species, significant cultivar differences were not observed.



FIGS. 31 - 33. — Bi- and Tetraporate pollen from the *H. vulgare* × *S. cereale* F_1 hybrid. Note the undulating exine sculpturing and pinched pores.

Although *H. vulgare* and *S. cereale* show scabrate sculpturing, they can be identified in a mixture on the basis of shape of pollen grains. Both cultivars of rye showed larger and slightly ellipsoid pollen grains (Fig. 16) compared to the almost spherical grains of *H. vulgare* (Fig. 19). *H. bulbosum* (2 x and 4 x) pollen was surprisingly wheat-like with areolate exine sculpturing (Figs. 22-24 and 25-27). It did not show any similarity to *H. vulgare* pollen. As in wheat, ploidy level also influenced pollen pore and annulus size.

F_1 Hybrid pollen : Pollen from the two polyhaploids (i) barley × rye and (ii) barley × *durum* wheat showed some very interesting features. Both polyhaploids are completely sterile and show paternal dominance (THOMAS, *et al.*, 1977). Mitosis in the barley × rye poly-

haploid gave the cross-consistent count of 14. The barley × *durum* wheat polyhaploid consistently showed a somatic count of 21, but somatic and gametic instability has been observed (MUJEEB *et al*, 1977). Micronuclei also have been encountered at mitotic interphase in both polyhaploids. Figures 31, 32 and 33 show pollen from *H. vulgare* × *S. cereale polyhaploid*. A number of two, three and four pored pollen grains were observed and on closer examination at × 25,000, it was evident that the sculpturing was intermediate between the two parents (Fig. 33). GRANT (1972) found differences in exine morphology between *Zea* and *Tripsacum* and the hybrids bet-

TABLE I.

List of Triticeae genera, species and cultivars studied.

Genera	Species	Cultivars
Hordeum	*H. vulgare* L. ($2x = 14$)	Apizaco Porvenir Dickson Hi-Proly CM-67 Bomi Manker
	H. bulbosum L. ($2x = 14$) *H. bulbosum* L. ($4x = 28$)	
Secale	*S. cereale* L. ($2x = 14$)	Prolific Snoopy
Triticum	*T. monococcum* L. ($2n = 2x = 14$)	CI 2433 PI 221413 PI 170916 PI 168806
	T. speltoides (TAUSCH) GRAN. ($2n = 2x = 14$) *T. timopheevi* ZHUK. Var. *timopheevi* ($2n = 4x = 28$)	
	T. turgidum L. Var. *durum* DESF. ($2n = 4x = 28$)	Cocorit-S Cocorit-71
	T. aestivum L. em THELL. Var. *aestivum* ($2n = 6x = 42$)	Tobari-66 Torim-73 Jupateco-73 Bonza-55 WS 1809

F_1 Intergeneric Hybrids (*) :

1. *H. vulgare* ($2x = 14$) CV. BOMI × *S. cereale* ($2x = 14$).
2. *H. vulgare* ($2x = 14$) CV. MANKER × *T. turgidum* ($2n = 4x = 28$) CV. Cocorit-71.

(*) These F_1 hybrids are polyhaploids, resemble the paternal parents for plant and spike morphology, and produce sterile pollen. A clone of *H. vulgare* cv. Bomi × *S. cereale* was provided by Dr. A. KRUSE, Royal Veterinary and Agricultural University, Taastrup, Denmark.

ween the two had intermediate exine sculpturing. Sterile pollen from male sterile mutants of barley are known to be inaperturate or show double poral sites (AHOKAS, 1975). In our studies we did not encounter inaperturate pollen in either polyhaploid. All pollen were either uniporate and shrunken or multiporate and large. The pores were smaller and flattened compared to either parents. In the polyhaploid from *H. vulgare* × *T. turgidum*, pollen exine sculpturing was predominantly areolate showing the characteristic branched spinules (Figs. 28, 29 and 30). Although the pollen size resembled the paternal parent, a number of singular spinules like that of barley were found to be intermingled with the areolate branched spinules

TABLE II.

Mean frequencies (mm^2) and Size (μ) of stomates in *H. vulgare* (2 x = 14), *H. bulbosum* (2 x = 14), and *T. aestivum* (2 n = 6 x = 42).

Surface	H. vulgare		H. bulbosum		T. aestivum	
	Frequency	Size	Frequency	Size	Frequency	Size
Adaxial ...	103.0	40.0	87.6	44.6	66.0	61.0
Abaxial ...	103.0	41.0	76.2	45.5	52.0	61.0

(Fig. 30). A number of twin-pored large pollen grains were observed but to a lesser degree than in the *H. vulgare* × *S. cereale* polyhaploid. These pollen had extremely small pores (Figs. 28 and 29) compared to normal *T. turgidum* (Figs. 4 and 5). Such aberrant pollen was reported by ERDTMAN (1952) in *Triticale* where he observed 0-7 pores. DOVER (1972) was able to induce multiaperturate pollen using colchicine which disrupts the meiotic spindles whose axes are involved in the positioning of the pores. In normal meiosis, the four spindle axes determine the site of the pores and at the end of the second meiotic division tetrad cross-cell walls form to give four uniporate pollen grains. DOVER observed tetraporate pollen in *T. aestivum* containing alien addition chromosomes of *Aegilops mutica*. These addition lines showed meiotic irregularities and formation of micronuclei. DOVER (1972) also stated that the tetraporate pollen formed due to defective development of cross-cell walls. In the two polyhaploids we studied. the 2, 3, and 4-pored pollen were much larger than uniporate pollen. The tetraporate pollen in the *H. vulgare* × *S. cereale* polyhaploid (Fig. 31) originated due to the absence of chromosome pairing (MUJEEB, *et al,* 1977) which results in meiotic disturbances leading to abnormal cross-cell wall formation analogous to DOVER's observations (1972). Similar abnormal meiotic disjunction and aberrant cytokinesis may explain the origin of bi- and tri-porate pollen.

We also studied the size and frequency of stoma per unit area in *H. bulbosum*. It is known that *H. vulgare* has similar frequencies of stoma per unit area on both the abaxial and adaxial surfaces (MISKIN *et al*, 1970, RAJENDRA *et al*, 1977 b). In wheat, at all ploidy levels, stomatal frequencies are significantly higher on the adaxial surfaces compared to the abaxial surfaces. The abaxial surfaces of wheat leaves have characteristic wrinkling of the epidermal cell-walls which is absent in *H. vulgare* (RAJENDRA, *et al*, 1977 a). In *H. bulbosum* there was a significantly higher number of stoma per unit area on the adaxial surface compared to the abaxial surface, similar to wheat (Table II). On the basis of these similar pollen and stomata observations we believe that *H. bulbosum* could be an evolutionary link between *H. vulgare* and *Triticum*. This contention has additional support from the crossability studies of KASHA and KAO (1970), BARCLAY (1975) and ISLAM *et al*, (1975) which show, in essence, that it is easier to hybridise *H. vulgare* with *H. bulbosum* (KASHA and KAO, 1970) and *T. aestivum* cv. Chinese Spring with *H. bulbosum* (BARCLAY, 1975) than *H. vulgare* with *T. aestivum*.

This SEM study, correlated with both cytogenetic and stomatal data, demonstrates the potential usefulness of micromorphological pollen characteristics as genetic markers in the Triticeae. Detailed pollen morphology offers a new tool for inheritance and gene expression studies in interspecific and intergeneric hybridization.

Abstract.

Scanning electron microscopy, up to × 25,000, was used to examine pollen from 23 accessions of *Hordeum vulgare* L., *H. bulbosum* L. (2x and 4x), *Secale cereale* L., *Triticum monococcum* L., *T. speltoides, T. timopheevi, T. turgidum*, and *T. aestivum* plus two intergeneric hybrids (*H. vulgare* × *S. cereale* and *H. vulgare* × *T. turgidum*). All *Triticum* species and cultivars including *T. monococcum* had areolate exine sculpturing whereas *H. vulgare* and *S. cereale* had scabrate exine. *H. bulbosum* had areolate exine, like the *Triticum* species. Pollen pore and annulus size in *H. bulbosum* and in all the *Triticum* species was increased as ploidy increased. *H. bulbosum* was suggested as an evolutionary link between barley and wheat. Pollen observations were correlated to stomate analyses and to cytogenetic analyses. The studies demonstrated the potential use of detailed pollen exine sculpturing analysis for genetic markers in inheritance and expression studies.

REFERENCES.

AHOKAS, H. (1975). — Male sterile mutants of barley. I. Inaperturate pollen of the msg6cf mutant. *Ann. Bot. Fenn.*, 12, 17-21.

ANDERSON, S. Th. and R. BERTELSEN, (1972). — Scanning electron microscope studies of pollen of cereals and other grasses. *Grana*, **12**, 79-86.

BARCLAY, I. R. (1975). — High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. *Nature*, **256**, 410-411.

DOVER, G. A. (1972). — The organization and polarity of pollen mother cells of *Triticum aestivum*. *J. Cell Sci.*, **11**, 699-711.

ERDTMAN, G. (1952). — Pollen morphology and plant taxonomy-Angiosperms. Almquist and Wiksell, Stockholm.

ERDTMAN, G. and J. R. PRAGLOWSKI (1959). — Six notes on pollen morphology and pollen morphological techniques. *Bot. Notiser*, **112**, 175-184.

FAEGRI, K. and J. IVERSEN (1964). — Textbook of pollen analysis. Hafner, NY.

GRANT, C. A. (1972). — A scanning electron microscopy survey of some Maydeae pollen. *Grana*, **12**, 177-184.

ISLAM, I. K. M. R., K. W. SHEPHERD and D. H. B. SPARROW (1975). — Addition of individual barley chromosomes to wheat. In Proc. Third Int. Barley Genet. Symp., pp. 260-270.

KASHA, K. J. and K. N. KAO (1970). — High frequency haploid production in barley (*Hordeum vulgare* L.) *Nature*, **225**, 874-876.

MISKIN, K. E. and D. C. RASMUSSON (1970). — Frequency and distribution of stomata in barley. *Crop. Sci.*, **10**, 575-578.

MUJEEB, K. A., J. B. THOMAS, R. RODRIGUEZ, R. F. WATERS and L. S. BATES (1978). — Chromosome instability in F_1 hybrids of *Hordeum vulgare* L. with *Triticum turgidum* L. and *T. aestivum* L. *J. Hered.*, **69** (in press).

RAJENDRA, B. R., K. A. MUJEEB and L. S. BATES (1977a). — A modified technique to study leaf epidermis in Triticeae. *Stain Tech.*, **52**, 9-12.

RAJENDRA, B. R., K. A. MUJEEB and L. S. BATES (1977b). — Relationships between 2 X *Hordeum* sp., 2 X *Secale* sp. and 2 X, 4 X, 6 X *Triticum* sp. for stomatal frequency, size and distribution. Environmental and Experimental Botany (In Press).

ROWLEY, J. R (1960). — The exine structures of cereal and wild type grass pollen. *Grana Palynol.*, **2**, 9-15.

SREERANGASWAMY, S. R. and V. S. RAMAN (1973). — Pollen production in diploids and autotetraploids of Rice (*Oryza sativa*). *Pollen et Spores*, **15**, 189-193.

THOMAS, J. B., K. A. MUJEEB, R. RODRIGUEZ and L. S. BATES (1977). — Barley X Wheat hybrids. *Cereal Res. Comm.*, **5**, 181-188.

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