CO1970QUA01

CELLULAR STRUCTURE OF RAPESEED

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INTRODUCTION

Rapeseed has recently become very important as a source of both oil and protein (1,2). In comparison with cereal grains such as wheat[3] however, relatively little is known about the cellular structure of these seeds. Chemical analyses show that typical rapeseeds contain about 40 percent oil, 25 percent protein, 20 percent polysaccharides and up to 3 percent glucosinolates. It is not exactly known where in the seeds these various substances are located, nor in what cellular organelles they occur. To answer such questions we began some cytological studies of rapeseeds two years ago at the Institute of Physiological Botany at the University of Uppsala in Sweden. Our work has been carried out in collaboration with Professor Jerker Porath and his co-workers at the nearby Institute of Biochemistry where the protein concentrates and toxic constituents of rapeseeds are being characterized. We have also studied several rapeseed products obtained from the Karlshamns oil factories and samples from Dr. Tape in Ottawa here in Canada. Some of our material has been obtained from the Swedish Seed Association at Svalov in southern Sweden, where an extensive breeding project is in progress.

The purpose of our microscopic work has been to study the anatomical and ultrastructural changes which occur during maturation of rapeseed and to compare the structure of seeds which differ in their content of protein, oil and toxic compounds. Let me first discuss the gross morphology and anatomy of rapeseeds and then show the results of some of our ultrastructural studies.

GROSS MORPHOLOGY

For our studies we have used both, oilseed rape, Brassica napus (Svenska Gylle) and turnip rape, Brassica campestris (Rapido II) obtained from the Swedish Seed Association at Svalov.

Traditional paraffin-embedding and sectioning techniques are not always suitable for studies of the anatomy of seeds which are hard and frequently difficult to fix and stain. To overcome these difficulties, I have embedded rapeseeds in a plastic resin

called Epon, and cut sections with glass knives in an ultra-microtome. The hard outer parts of the seed coat were peeled off and the inner part of the seed were fixed in solutions containing glutaraldehyde and potassium permanganate. After dehydration in a series of ethanol solutions of increasing concentration, the cell material was impregnated with Epon and polymerized at + 60°C. The sections were cut at a thickness of about 1 micron and stained with lactophenol.

Intact seeds of oilseed rape and turnip rape look very similar when observed in a low power dissecting microscope. In contrast to cereal grains Brassica seeds have very little so-called endosperm tissue, and almost the entire seed consists of the embryo itself. It contains two cotyledons and a central meristematic tissue from which the root, stem and bud will develop, if the seed is allowed to germinate. A slide showed (Note: not reproduced here) a cross-section through a rape seed of Brassica napus. Outermost is the remaining part of the seed coat consisting of a palisade layer of sclerified cells. Underneath these is a narrow layer with protein-rich cells. The cotyledons are double (conduplicate) and surround the central embryonic root bud and stem. The dark blue spots in the cells are nuclei which contain desoxyribonucleic acid, DNA. It is difficult to study the distribution of oil and protein in the individual cells with this technique, but it gives a better general view of the seed than electron microscopy.

The various types of cells undergo marked cytological changes during different stages of maturation. These changes are clearest, when sections are observed in an interference microscope. Another slide showed the starch granules which predominate in the thin-walled cells of immature seed. The mature seed contains distinct yellow oil droplets and dark blue regions of protein-rich aleurone grains.

The seed coat, which has been peeled off can be seen clearly in the stereo scanning electron microscope. This instrument gives high contrast combined with good depth of focus, when the cell material is covered with a very thin film of gold particles. Figure I shows part of a seed coat where the fibrous net-work is very clear. At higher magnification this pattern shows up even more beautifully, as shown on Figure II. This technique could probably be very useful in distinguishing between different rapeseed varieties. Another showed some cells of the outer part of the cotyledon. Here the cells are almost collapsed, since all the oil has been extracted from the seed.

ELECTRON MICROSCOPY

With the aid of a transmission electron microscope it is possible to obtain a more detailed understanding of the cytology of rapeseed than can be obtained in the light microscope. Parts of the seeds were impregnated with the plastic Epon, in the same way as for light microscopy. But each section which shall be studied in the electron microscope must be cut at a thickness of only about 30 nm. This means that it would require about 100,000 sections to cut up a whole rape seed, across its diameter. To analyze the thin sections we have used three different microscopes. A Cambridge stereoscanning electron microscope, an AEI 801 transmission electron microscope and an Akoshi TRS-50 electron microscope.

To illustrate the difference in magnification which can be obtained with the light and electron microscopes I will again show a cross-section of almost a whole seed. We shall then look at some ultrathin sections; first at a cell from this hypocotyl region, then at some lipid-rich cells from the cotyledons, and also at some of the outermost cells of the seed.

Figure III shows that the cells of the central region of the mature seed contain large protein grains. These are called aleurones(4) - which in Greek means flour. The oil is distributed in the form of small droplets in the rest of the cytoplasm. On some sections one can see the irregular cell nucleus, and we can also note that the cell walls are thin. This picture shows a seed of oilseed rape, and Figure IV shows that the corresponding cells of turnip rape are very similar (aleurone, fat, cell wall).

The cells in the cotyledons also contain both aleurone grains and lipid droplets. But some of the cells are extremely rich in oil (Figure V). The outermost cell layer is bordered by a heavier cell wall, but they seem to contain as much protein and oil as other cells.

Before we leave this series of pictures, it is worth noting that the oil droplets appear to be bounded by thin protein layers. In this picture the pattern of droplets is irregular, but this is because the outer cells are more dry and deformed. Between many of the cells there are intercellular channels. These are considered to be important for translocation of substances within the seed.

MATURATION

All these pictures were of mature seeds where the protein and oil are storage products.(5) During maturation of the seed many cytological changes take place before these substances have been synthethized. About ten days after pollination the seed cells contain large nuclei and a net-work of synthesizing membranes (Figure VI). Many starch granules are also present, but no aleurones or oil droplets are evident.

The lipid droplets become common twenty days after pollination and the aleurone grains often have a net-like structure. As long as the seeds are green they contain chlorophyll in chloroplasts (Figure VII). These organelles of course are of fundamental importance for photosynthesis in the leaves. But we do not know if they have a special function in the seeds. The chloroplasts seem to disappear completely when the seed is fully mature. But as you well know there are peculiar toxic compounds which simultaneously accumulate in most rapeseeds.

TOXIC COMPOUNDS

As far as I know, nothing is known about the exact cytological distribution of the toxic thioglucosides. Antinutritional phytic acid derivatives have also been identified in various seeds, and these are supposed to be associated with aleurone grains.

Rapeseed contains as much as 3 percent thioglucosides, but there is considerable variation between different strains. We are particularly interested in finding out the ultrastructural distribution of these compounds both, during the ripening process and in the mature seed.

In the aleurone grains one can see large numbers of inclusions, of a type which often are called globoids, round bodies, by cytologists. Another slide showed a thin section of embryonic cells of Brassica napus, oilseed rape. The dark areas are the aleurone grains, but these organelles contain lots of these peculiar globoids. Many of them are lost during the sectioning of the embedded seeds, so that only white holes remain. But I think one can see that there are several very dark particles left. This cell also contains a distinct nucleus.

Brassica campestris, the turnip rape, has similar inclusions (Figure VIII). It appears that these seeds often contain very large globoids. Another slide showed two globoids at a high

magnification. I think that this slide shows part of a rapeseed cell at a magnification of about one million times on this screen. It is very peculiar that the globoids seem to be surrounded by a double-membrane structure. I would very much like to get your opinion about the chemistry and function of these globoids, since I think they must be important in connection with the preparation of protein concentrates.

At Uppsala we have started to study the thioglucosides by tracer technique through incorporation of S^{35} labeled sulfate. We hope that this work will give us both, biochemical information and an opportunity to study the cytological distribution of the toxic thioglucosides.

GENETIC LINES

At the Swedish Seed Association at Svalov genetic lines containing different amounts of thioglucosides, have been studied during the last two years. I have recently started studies on the ultrastructure of some of these strains. It is too early to draw definite conclusions from this work yet, but we have found globoids also in less toxic seeds of the Bronowski strain.

PROTEIN PRODUCTS

Finally, I would just like to show a couple of electron micrographs, which fall slightly outside the theme of my lecture. Since we are particularly interested in the protein products from rapeseed, we have looked at both, fat-free meal and non-toxic protein flour in the scanning electron microscope. Samples of these products were very kindly provided by Dr. Tape here in Canada, who has prepared them together with Dr. Eapen and Dr. Sims.(6)

The meal is grey in colour, and contains many cell wall fragments with much protein adhering to them. At high magnification the protein particles appear as a fluffy popcorn - like structure (Figure IX).

The meal contains about 30-40 percent protein, but the white detoxified protein flour has a protein content of about 60-70 percent. This flour (Figure X) contains no cell walls and has a more flaky appearance. This flour shall soon be used for human consumption, whereas the hull-rich meal is used only as an animal feed. I believe that scanning electron microscopy

will also be useful in studies of rapeseed products prepared by different techniques.

I hope that what I have said about the cellular structure of rapeseeds has convinced you that this is an interesting, but far from complete story. My work has so far only dealt with a few rapeseed strains and much more remains to be done on the maturation process in the seeds. With new advanced electron microscopic techniques, which are now available it should also be possible to analyze the distribution of the antinutritional compounds. Such work requires the collaboration of both, biologists and chemists. I think that the results could be most useful for the oilseed technologists.

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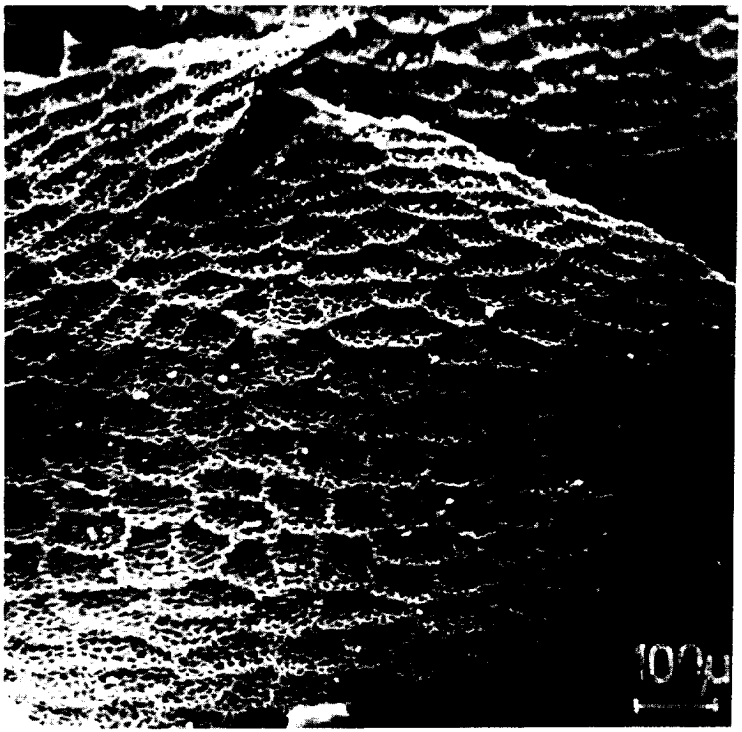

Fig. 1

Scanning electron micrograph of the seed coat of Brassica napus.

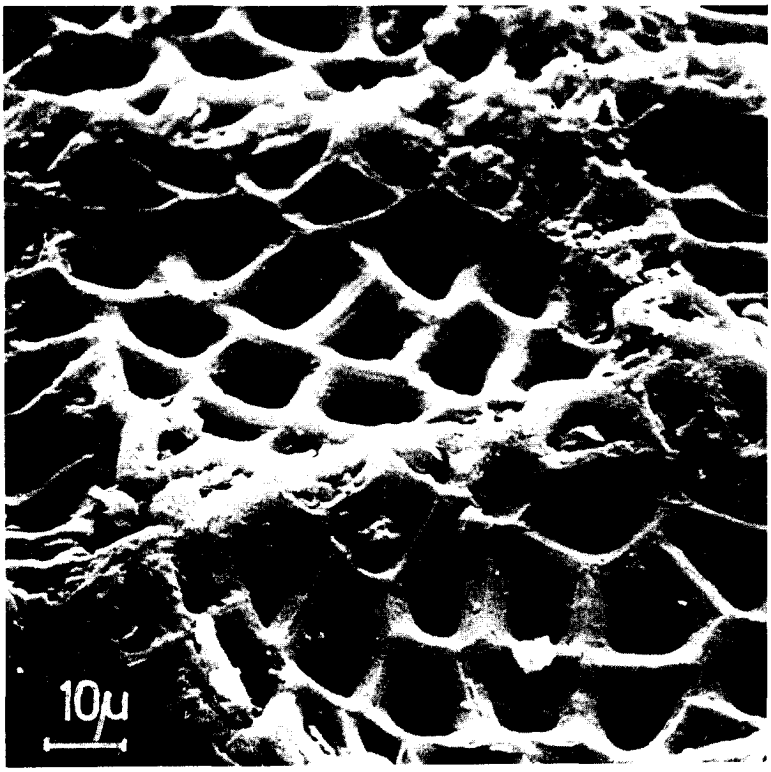

Fig. 2 Scanning electron micrograph of the coat structure with several holes (Brassica napus).

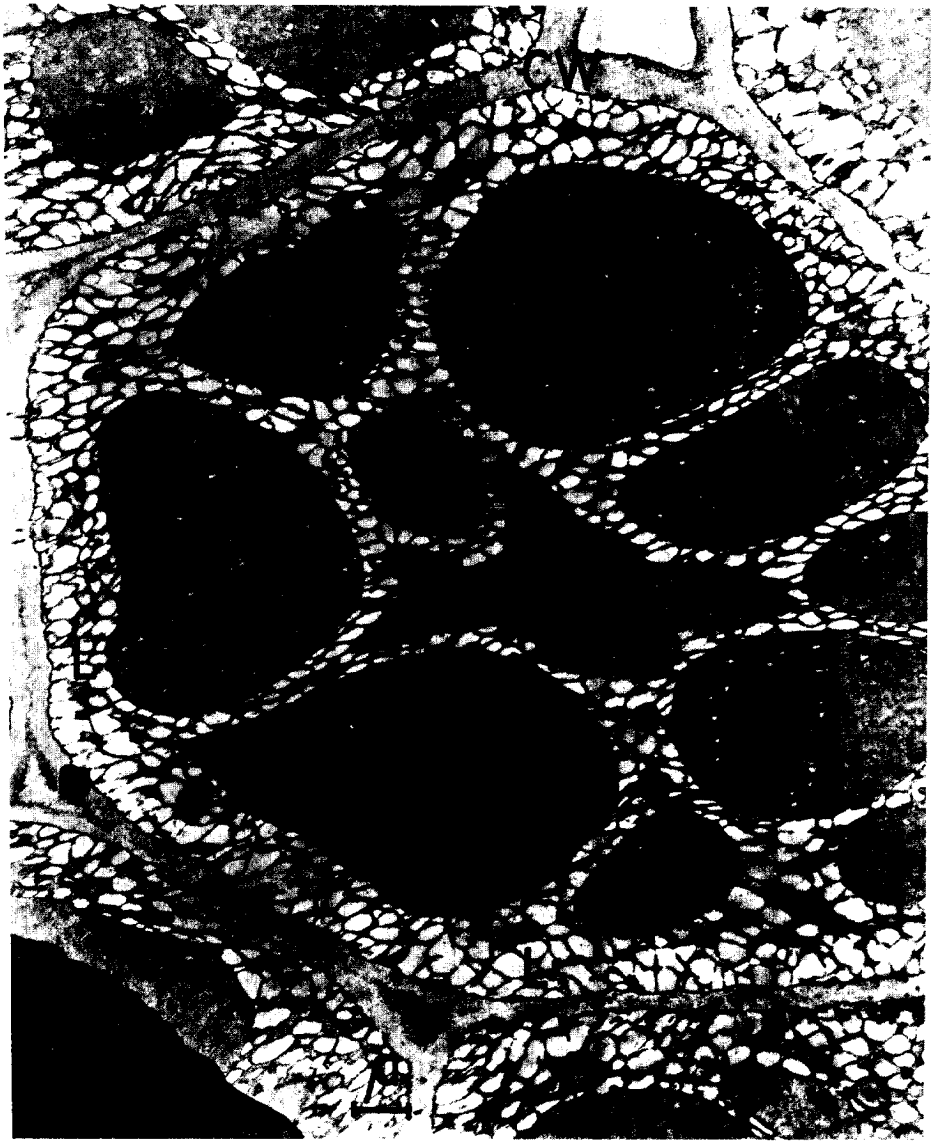

Fig. 3

Electron micrograph of oilseed rape (Brassica napus) with cell wall (CW), nucleus (N), lipids (L) and protein rich aleurones (A).

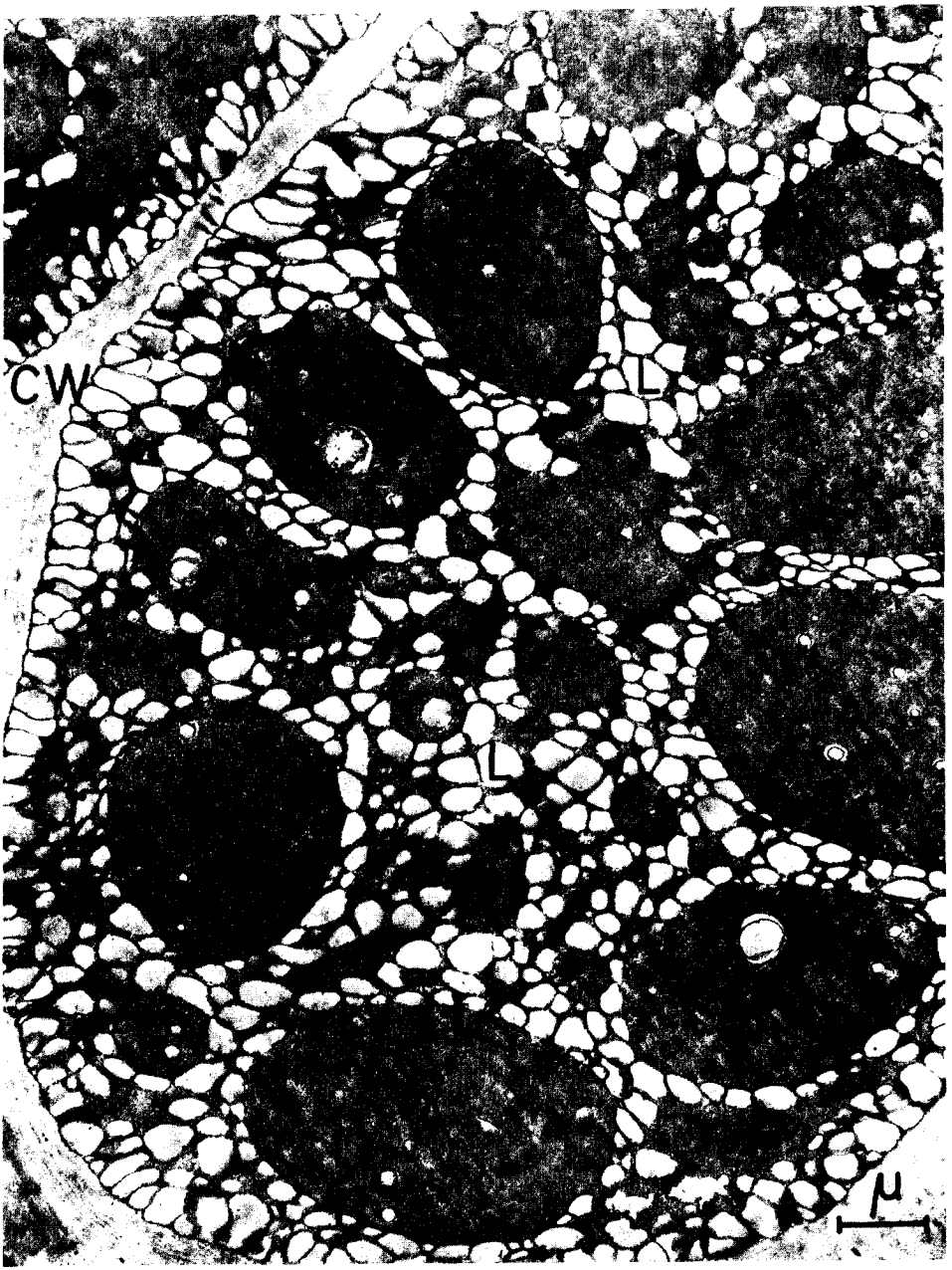

Fig. 4 Electron micrograph of turnip rape (Brassica campestris).

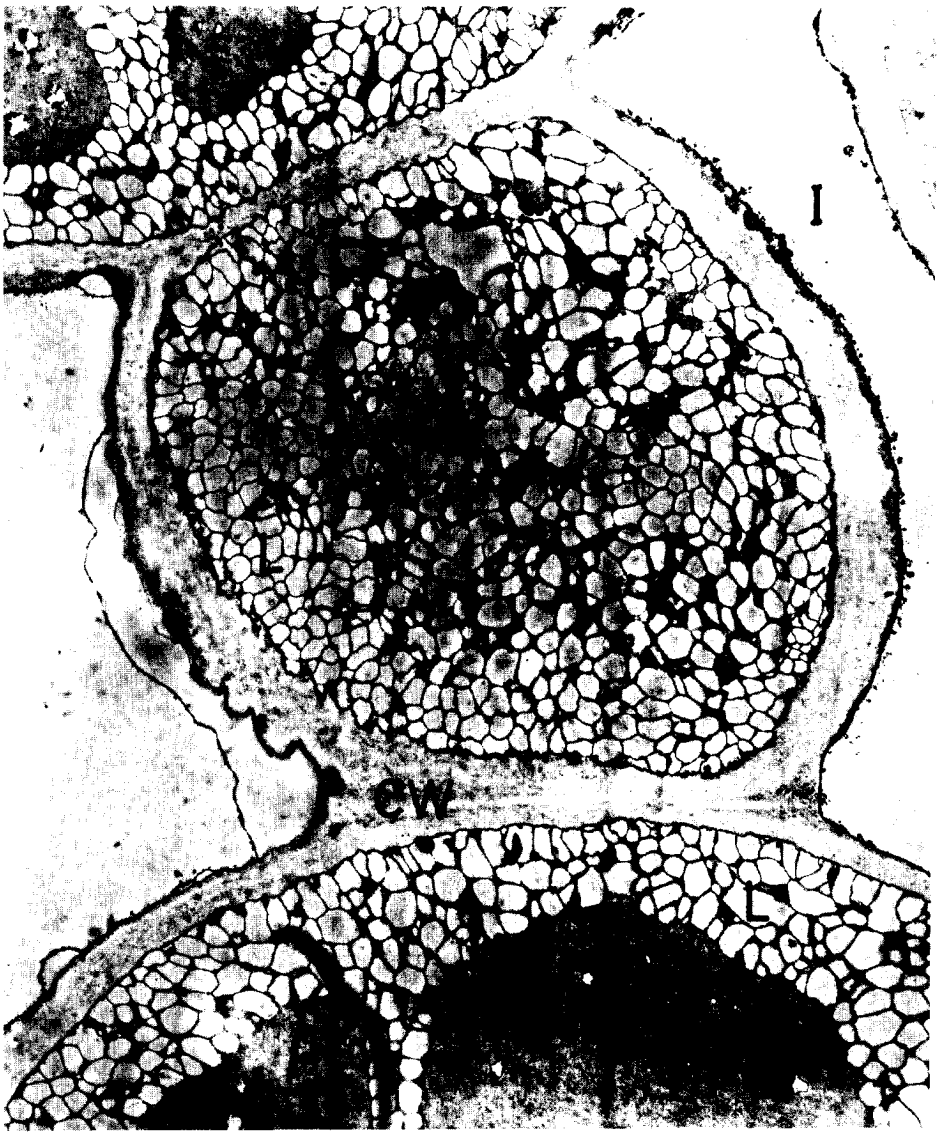

Fig. 5 Electron micrograph of Brassica napus, with an extremely lipid rich cell, and large intercellular chanels (I).

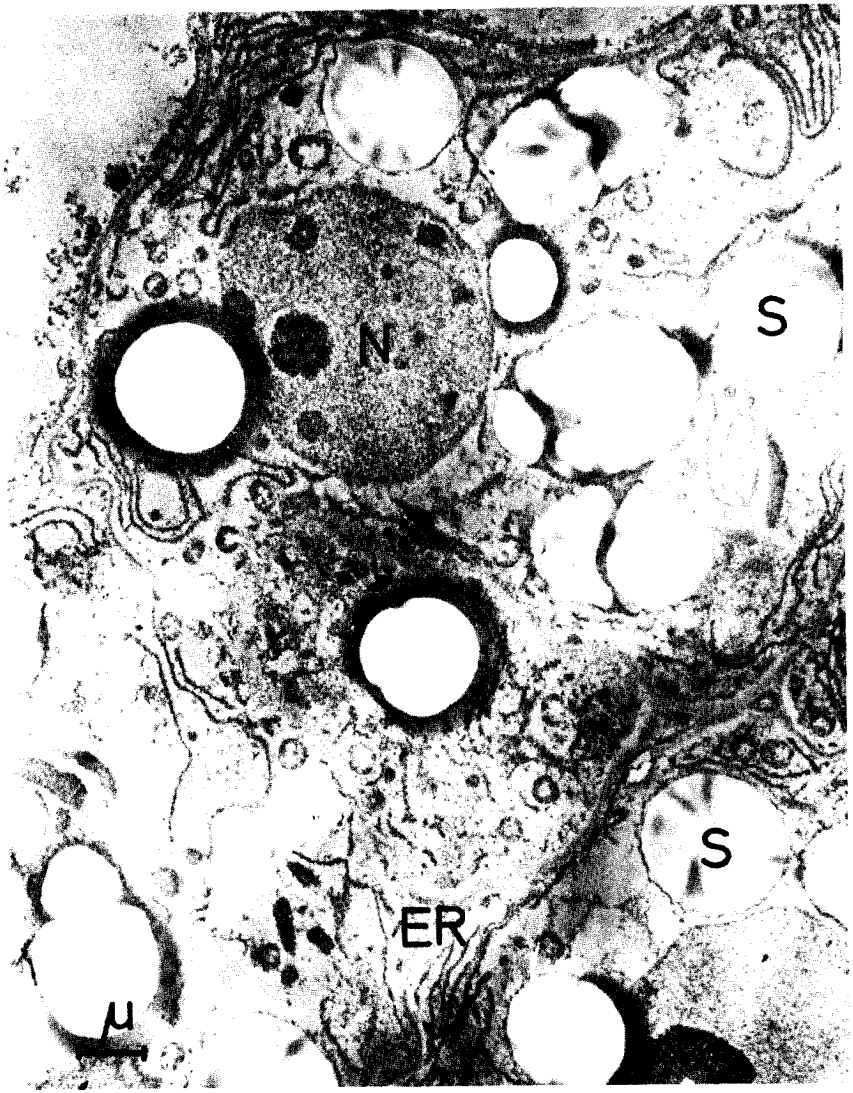

Fig. 6 Electron micrograph of Brassica campestris, ten days after pollination. Endoplasmic reticulum (ER) and starch (S) occupy the cell.

Fig. 7 Chloroplasts in green seed of Brassica napus.
Magnification 20000 X

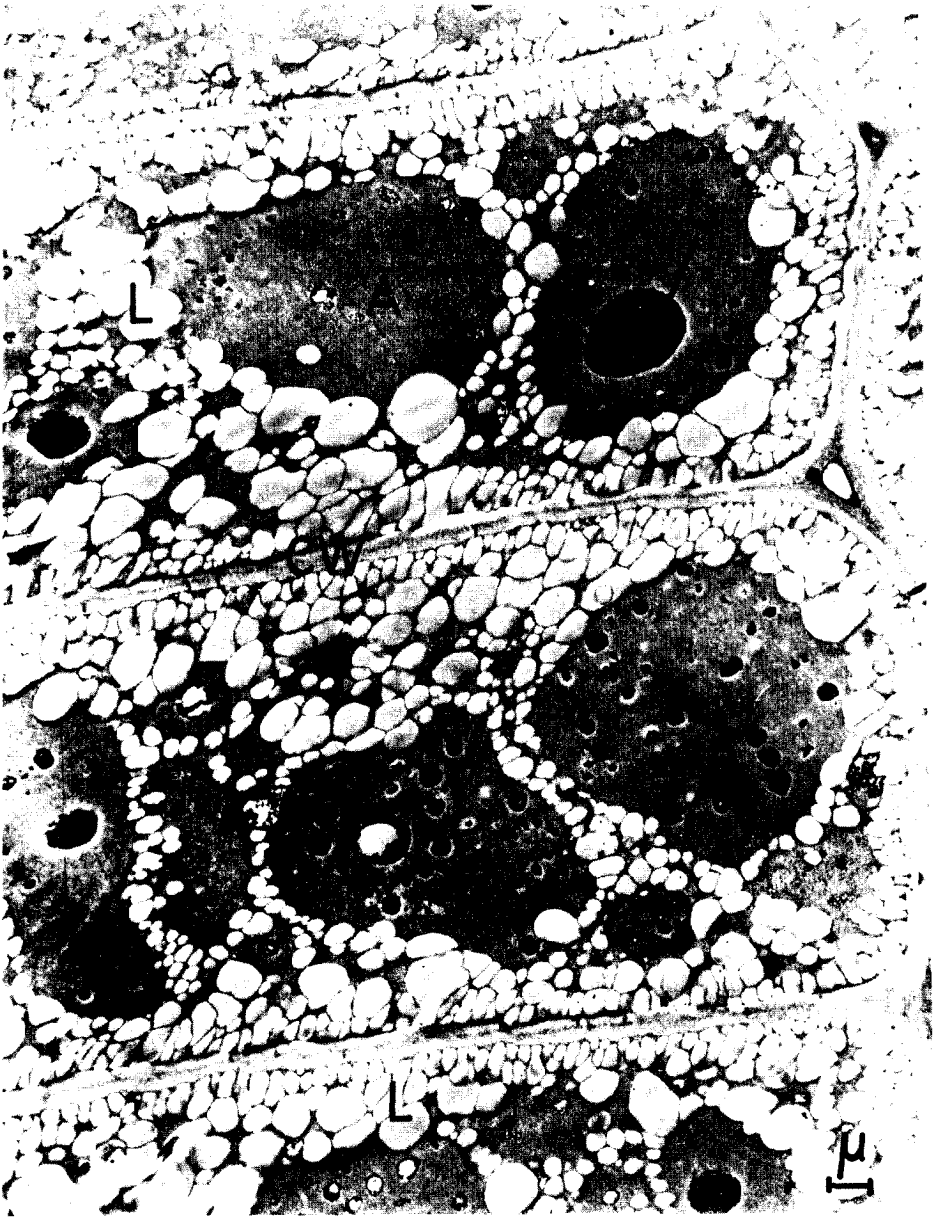

Fig. 8 Electron micrograph of embryonic cells of Brassica campestris, with dark globoids (G) in the protein grains (A).

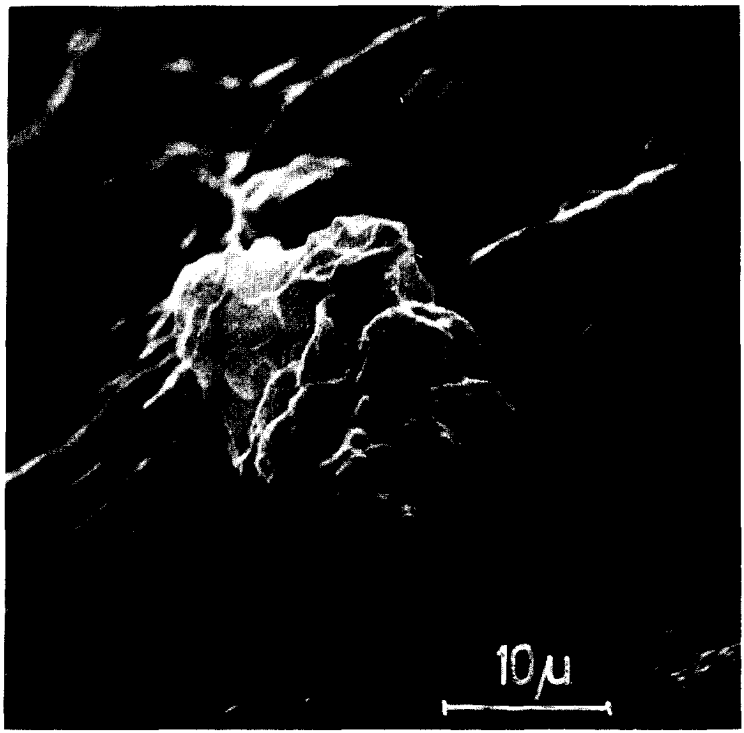

Fig. 9 Scanning electron micrograph of rapeseed meal with 37 per cent protein.

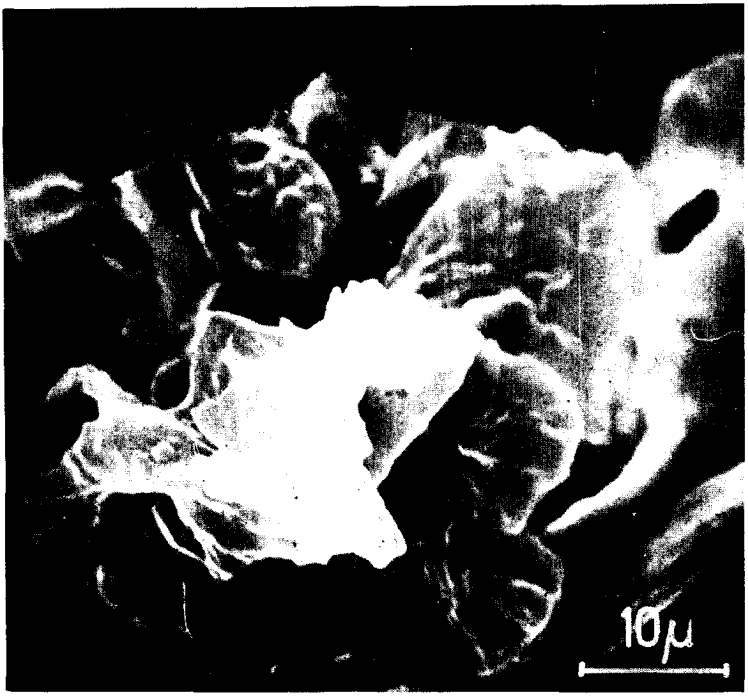

Fig. 10 Scanning electron micrograph of rapeseed flour with 65 per cent protein.