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SCANNING ELECTRON MICROSCOPIC PICTURE OF THE CONCENTRATION AND DISTRIBUTION OF PROTEIN STRUCTURES IN THE SEED ENDOSPERM OF LOW AND HIGH PROTEIN VARIETIES OF RYE (*Secale cereale L.*)

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Key words: rye, scanning electron microscopy, endosperm structures, protein structure.

Comparative studies of subaleurone and inner endosperm cells of dry mature caryopses from 8 low-protein and 7 high-protein varieties of rye, grown in similar conditions of isolated collection, were performed by means of scanning electron microscopy technique. Low protein varieties contained starch granules differentiated in the diameter embedded in the less dense protein matrix of the wide subaluerone region. High protein varieties, besides the larger aleurone cells had in some examples wider subaluerone layer with the dense protein matrix, which was very regular and less packed with starch granules. The inner endosperm cells of high-protein caryopses contained much more dense protein matrix all around the starch granules in contrast to low-protein caryopses, which were tightly packed with starch and had only a thin layer of protein matrix around them. Comparative pronase treatment of low- and high-protein rye caryopses confirmed the above observations. No granular protein bodies in any cells, besides the aleurone ones, could be observed.

INTRODUCTION

The results of *Secale cereale* grain protein resources investigations showed a great intra-variability of protein content in rye and its nutritive value [12], as well as the intervarietal variability of protein concentrations in seeds from different sections of spike and within morphological parts of caryopses [15], and also in clones and inbred rye lines [9].

Such a large variability reflects big genetic potential of this genus, flexibility to changes of environment as well as good possibilities for improving the protein content and nutritive value of seeds in cultivated varieties. The progress in breeding of improved varieties is dependent on results of evaluation of crop resources contained in breeding collections.

The DBC method together with reference Kjeldahl method, used in screening purposes to ascertain the approximate nutritive value of protein estimation in

numerous seed samples, has been widely utilized in the determination of the qualitative characteristic of cereals which are used in the breeding process [10, 18, 19], among these rye [13].

However, there was a need to develop a nondestructive method of evaluation of a single grain which provides an opportunity to grow a whole plant from the same grain and then use it for furtheer genetic experiments.

The histochemical method of staining proteins on cross sectioned grain slices fulfiled these conditions. These methods have already been widely used for the distribution of protein and enzymes studies in ceral grains, which allowed to evaluate their losses during the process of milling [26]. A very simple and rapid test of histochemical protein staining in rye and triticale grains, within the half of the grain not containing the embryo and Light Green SF as a dye, was developed [16]. The remaining half can be used to grow the next generation with the possibility of further research upon it. This method proved to be useful for the comparative studies of protein distribution within caryopses among low and high protein cultivars of rye with rater singificant differences being detected.

For more distinctive and detailed description of location of protein in caryopses in several rye varieties, differing in protein content, the Scanning Electron Microscopy method was used.

This method as well as Transmission Electron Microscopy have been widely applied in studies evaluating protein bodies and other forms of protein occuring in storage areas of the plant's reproductive organs [1, 3, 8, 11, 20, 21, 22, 24, 25, 27].

The small production of rye, on a world scale, is the main reason for the comparatively low amount of knowledge on genus *Secale*, and cultivated rye (*S. cereale L.*) though some electron microscopic studies of rye were made for comparison with other cereals [2, 23] and on wild Secale species [14].

MATERIALS AND METHODS

Thirteen rye varieties grown in Skierniewice in the collection of the Botanical Garden of the Polish Academy of Sciences — between them, 6 high-protein (15.9 to 19.3% of total protein on d.w.b. of grains), and 7 low-protein (8.63 to 9.78%) and two rye varieties grown in Powsin (Beaulieu — 10.1%, Kastoria — 17.1% of protein) were used for SEM studies (Table). They were grown on 1. m × 1.5 m plots and were isolated during flowering. Representative sample of particular population of mature caryopses were chosen for studies. The total protein content by micro-Kjeldahl method was determined (N × 5.83).

For histochemical staining of proteins cross sections were made perpendicularly to the long axis in the middle of caryopses, and Light Green SF, according to Łuczak's [16] method, was used.

In order to study the ultrastructure of caryopses, single grains were sectioned in a freezing microtome. To avoid any alterations of protein matrix and liberation of starch granules from it, the sections were unfixed and only dried in air

Table. The total protein content in grains of the studied rye varieties

Low-protein varieties		High-protein varieties	
variety	% protein d.w.b.*	variety	% protein d.w.b.*
Saratovskaja 4	8.63	Castelo Branco	15.9
Dankowskie Nowe	8.70	Guarda	16.5
Chrysanth Hanserrogen	8.80	Jo 090	16.6
Gulzower St. 401	9.09	Van Engelen Hybrid 61:3	16.9
Karlshurder Winterrogen	9.15	Kastoria	17.1
Kustro	9.38	Dominant CAN 2841	17.7
Kortowskie	9.78	Belta	19.3
Beaulieu	10.1		

* Total N × 5.83 on dry weight basis

according to Chabot et al. [5]. The were coated with 50-100 A layer of gold in 5×10^{-5} Thr vacuum. The photographs were taken with a Jeol scanning electron microscope, JSM-2, with high voltage 25 keV. For pronase B treatment the cross section was incubated with a drop of 0.2% solution of the enzyme in pH 5.5 acetate buffer at 40°C for 30-45′, dried on air, afterwords prepared as described above.

RESULTS AND DISCUSSION

The contents of subaleurone and starch endosperm cells were observed on scanning electron micrographs of cross sections from dry, mature caryopses of 8 low protein and 7 high protein varieties of rye (*S. cereale L.*).The protein content of these materials presents Table.

The studied varieties were chosen on the basic of the results of selective staining of proteins and ligh-microscope examination of several rye varieties differing in grain protein content, which showed a large differentiation in the concentration of proteins in the outer and inner layers of caryopses endospem. In both low- and high-protein varieties, the subaleurone layer was much richer in proteins than the inner endosperm cells. The width of this layer may be then decisive for the whole protein content of the grain. However, at the same time, the high-protein rye varieties, contained more proteins in deeper endosperm layers than low-protein ones. Therefore, in high-protein grain the distribution of protein is more even throughout the endosperm. Similar results were received on waxy and nonwaxy sorghums [27] and the connection between the development of the surbaleuron layer, the distribution of the starch granule diameter, and the protein content of barley was also proven [20].

The scanning electron micrographs of low protein rye are shown in Figs. 1

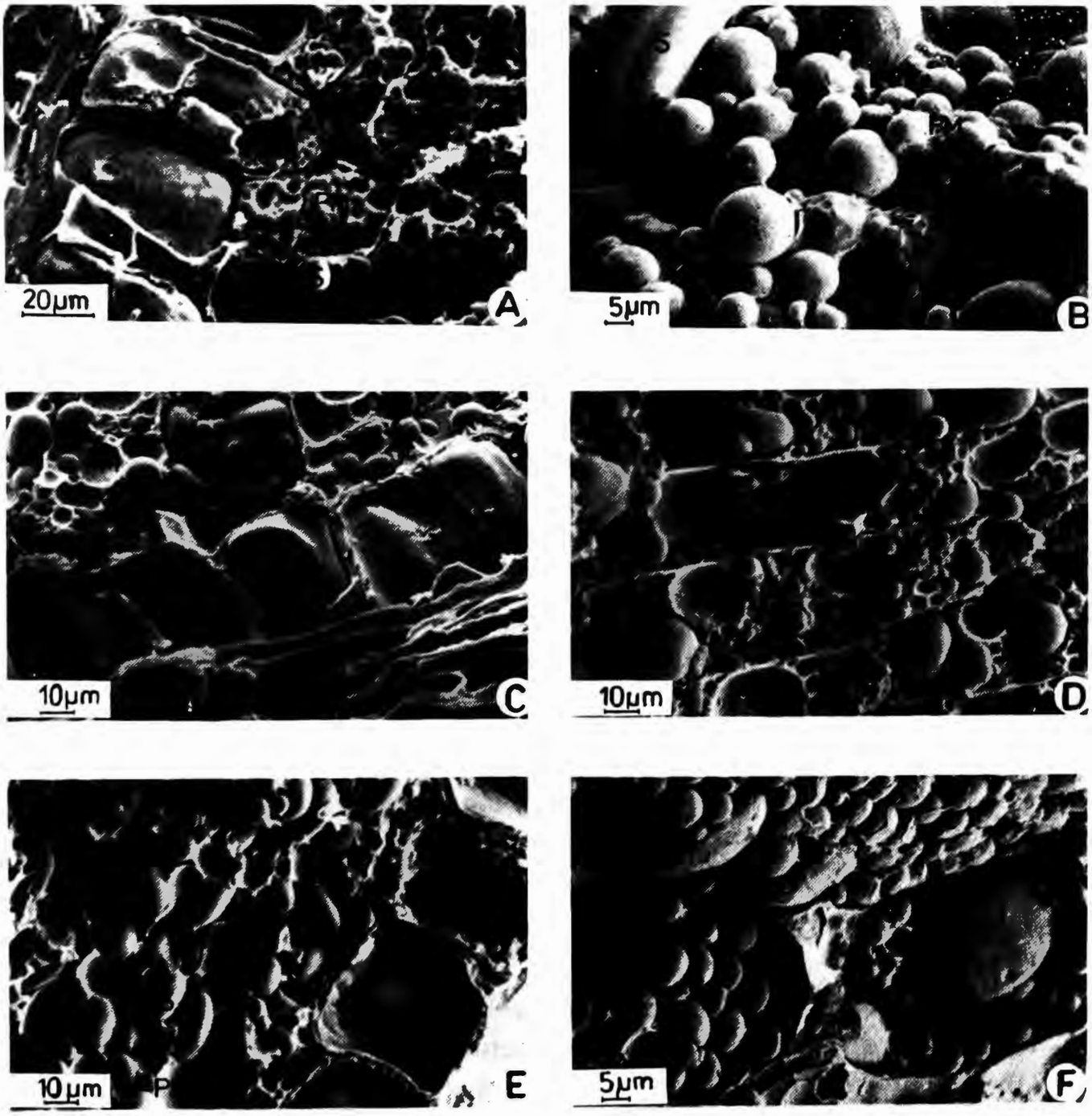

Fig. 1. Scanning electron micrographs of aleurone and subaleurone cells (A, C, E) and inner endosperm cells (B, D, F) of caryopses of low protein rye varieties: A, B — Saratovskaja 4; C, D — Dankowskie Nowe; E, F — Chrysanth Hanserrogen

and 2. Every variety is represented by two sets of pictures. Aleurone, subaleurone and outer layers of starch endosperm cells on the first set (Fig. 1A, C,E; Fig. 2A, C, E, Fig. 3A, C, E), and inner layers of starchy endosperm cells — on the second (Fig. 1B, D, F, Fig. 2B, D, F, Fig. 3B, D, F). The high protein varieties are shown in similar order in Figs. 4 and 5.

Low-protein varieties had in some cases smaller aleurone cells than high-protein ones, e.g. variety Kustro (Fig. 3) and Guarda (Fig. 5), Van Engelen Hybrid 61 : 3 (Fig. 4) and Jo 090 (Fig. 5). The distinct differences in the dimension of subaleurone layer rich in protein and the degree of packing it with starch granules were noted. In some high-protein varieties (Figs. 4 and 6) the starch granules of subaleurone cells seemed to be more regular and were always

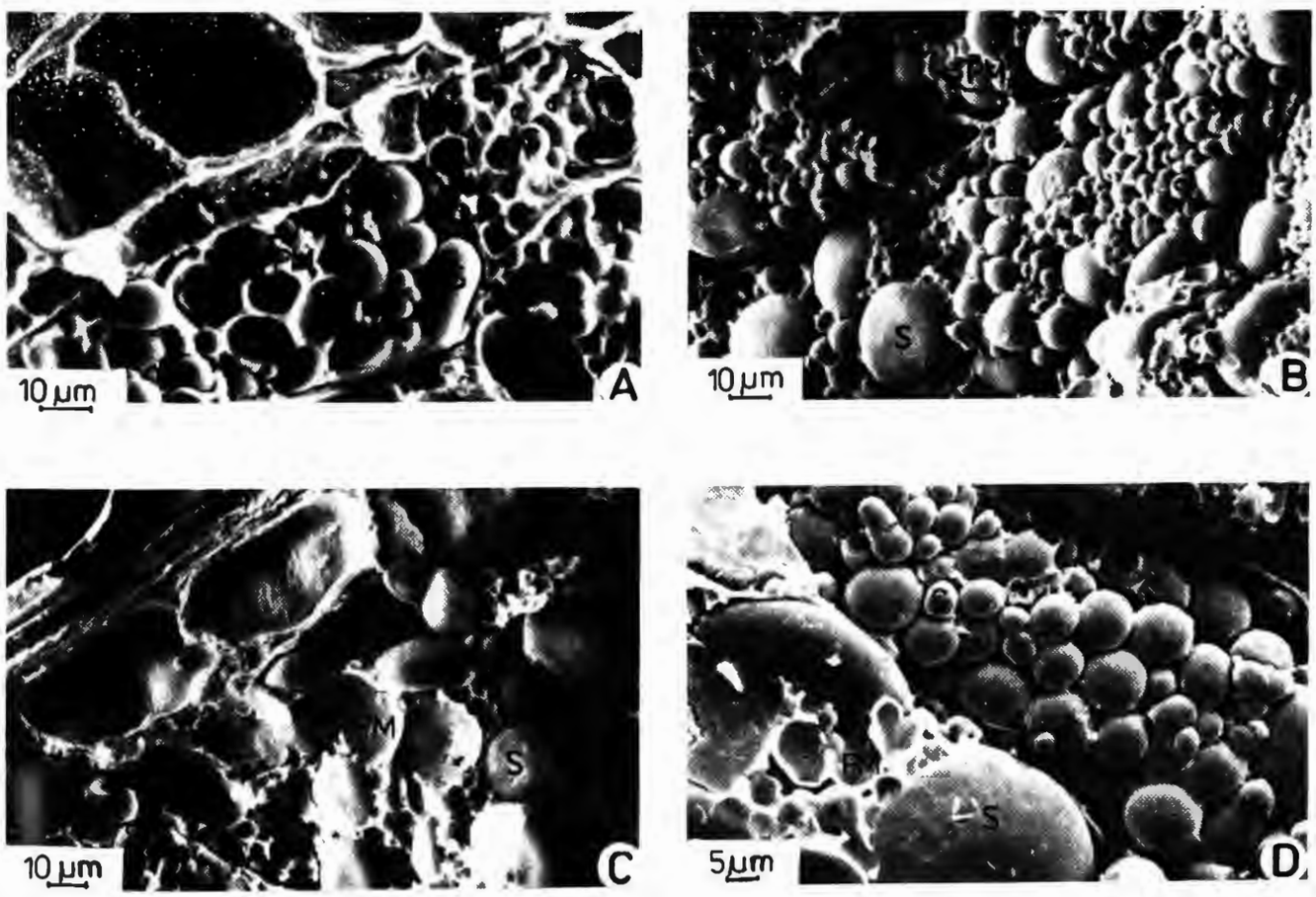

Fig. 2. Scanning electron micrographs of aleurone and subaleurone cells (A, C) and inner endosperm cells (B, D) of caryopses of low protein rye varieties: A, B — Gulzower St. 401; C, D — Karlshurder — Winterrogen

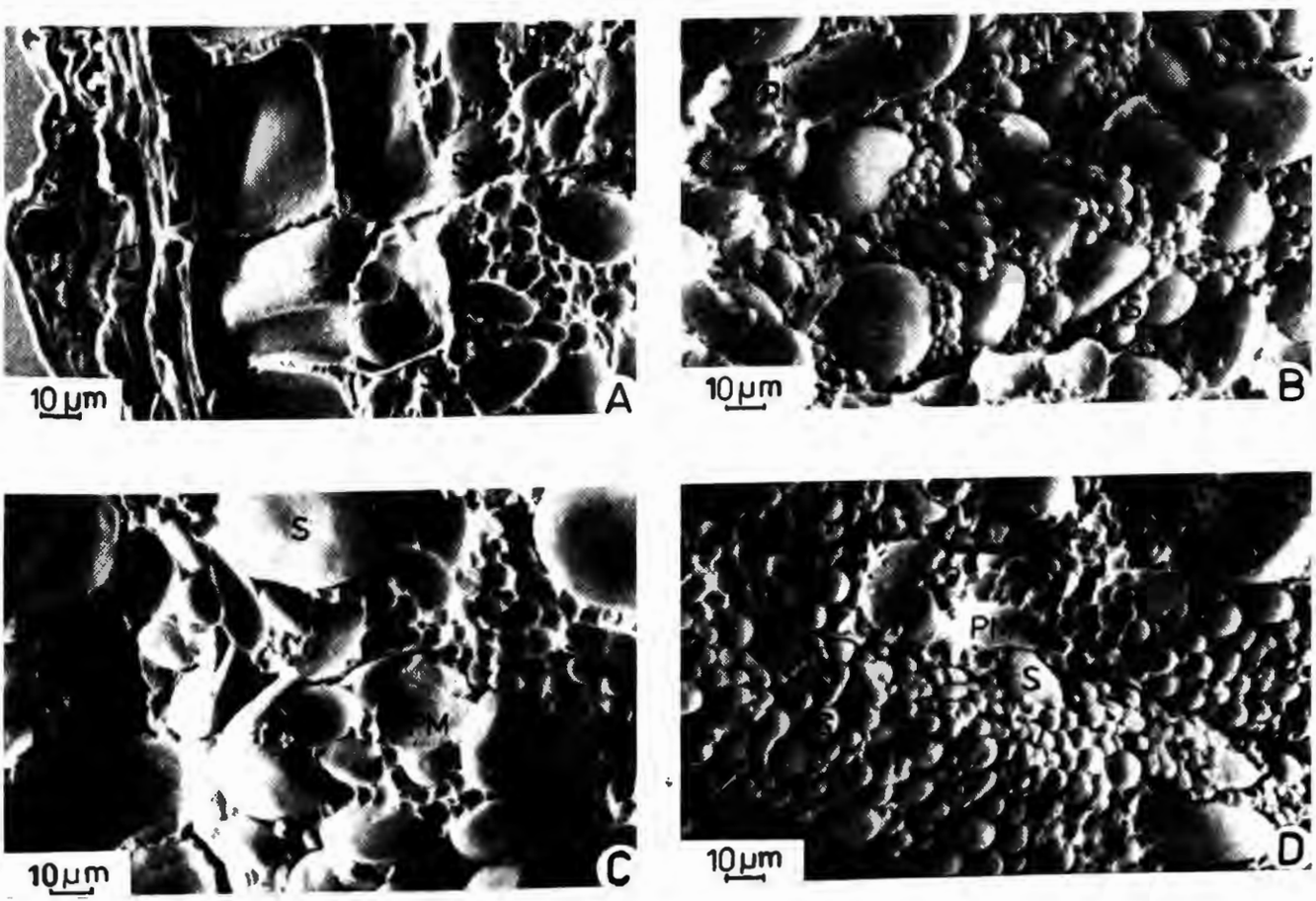

Fig. 3. Scanning electron micrographs of aleurone and subaleurone cells (A, C) and inner endosperm cells (B, D) of caryopses of low protein rye varieties: A, B — Kustro; C, D — Kortowskie

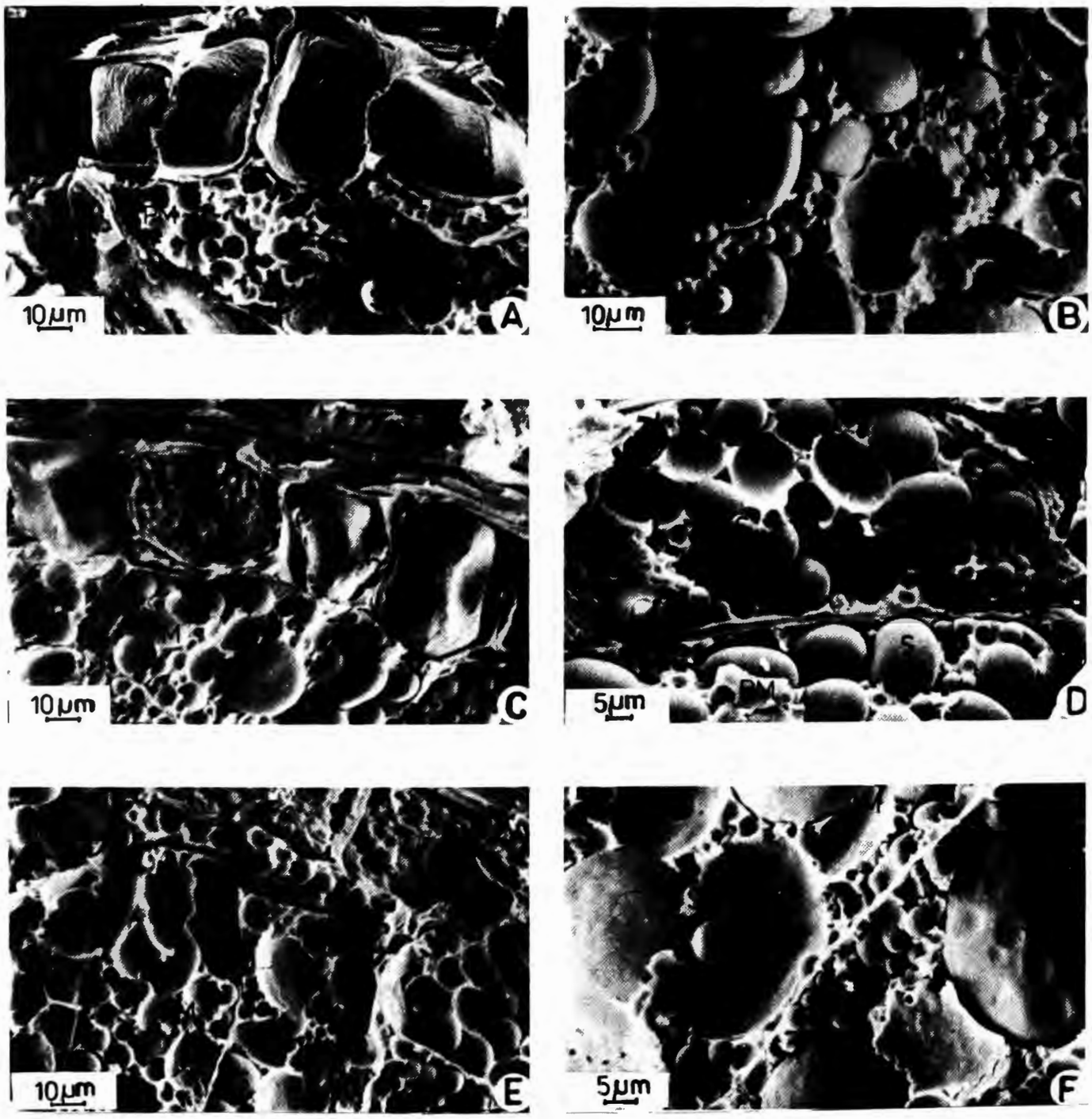

Fig. 4. Scanning electron micrographs of aleurone and subaleurone cells (A, C, E) and inner endosperm cells (B, D, F) of caryopses of high protein rye varieties: A, B — Belta; C, D — Dominant; E, F — Van Engelen Hybrid 61 : 3

surrounded by a thick protein matrix. The protein nature of this matrix was proved by digesting it with pronase (Figs. 6 and 7).

In the subaleurone layer of low-protein ones the starch granules were more differentiated in diameter (cv. Chrysanth Hanserrogen Fig. 1E, Kortowski Fig. 3C).

Subbaleurone cells of low-protein cv. Dankowskie Nowe were very tighly packed with starch granules and not much space remained for protein matrix (Fig. 1C). In high-protein Guarda, there were only a few regular starch granules embedded in dense protein matrix in each subaleurone cell (Fig. 5C).

According to former ligh-microscopy studies, there were noticable differences in the amount of protein matrix surrounding starch granules in deeper layers of

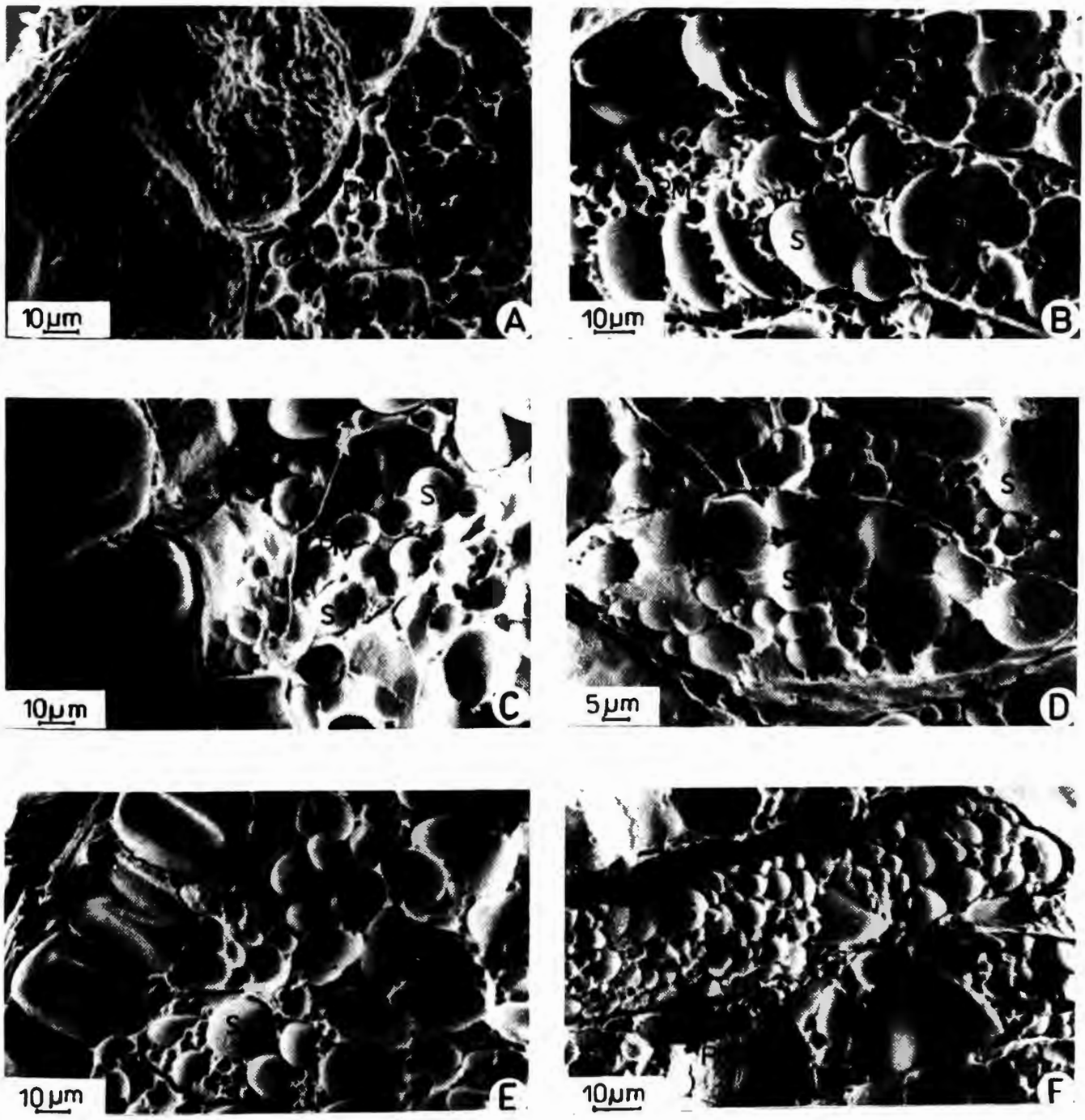

Fig. 5. Scanning electron micrographs of aleurone and subaleurone cells (A, C, E) and inner endosperm cells (B, D, F) of caryopses of high protein rye varieties: A, B — Jo 090; C, D — Guarda; E, F — Castelo Branco

endosperm cells between low and high-protein rye varieties. More dense protein matrix around starch granules is a typical feature of inner endosperm cells of high-protein varieties of rye, no granular protein bodies apart from aleurone ones were identifiable. The results of examining the endosperm cells of the seeds of other *Gramineae* genera indicate their presence [20, 22], with the exception of mature caryopses of wheat [7].

The protein bodies observed by Kocon et al. [11] in mature rye grain endosperm cells were not confirmed either in low or high-protein rye grains. The same size small granules were dyeing brown with Lugol while tested histochemically by ligh microscopy which indicates they are starch granules [16]. This agrees with the results of TEM studies by Parker [21].

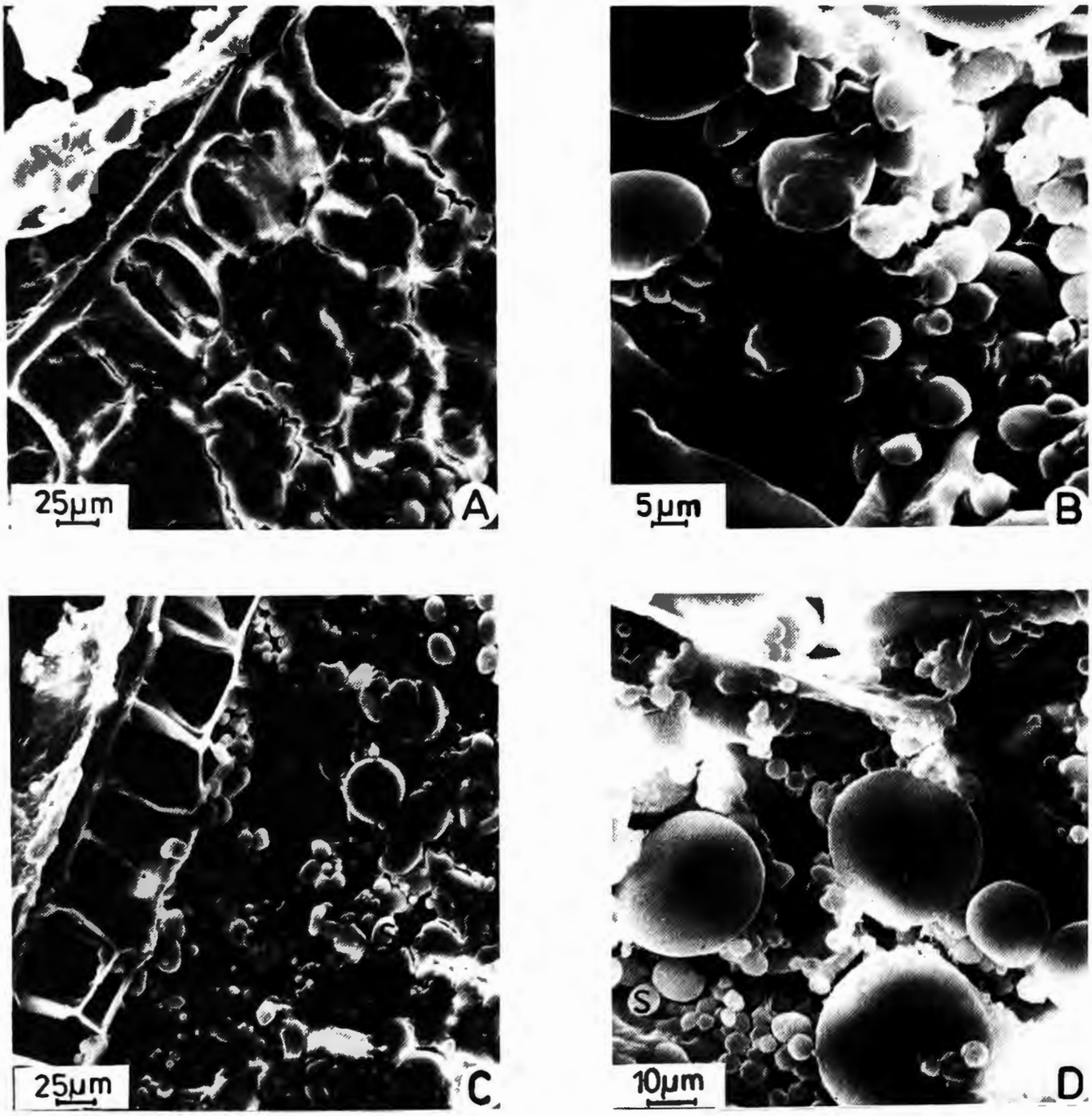

Fig. 6. Scanning electron micrographs of aleurone and subaleurone cells (A, C) and inner endosperm cells (B, D) of high protein rye cultivar Kastoria: A, B — untreated cross sections; C, D — pronase treated cross sections

The pronase treated cross sections of rye caryopses of the low-protein variety Beaulieu, and high-protein Kastoria showed in comparison to the untreated control on SEM micrographs smooth and unlinked starch granules of all different sizes, laying without any protein matrix around within the cells of inner endosperm and subaleurone cells (Figs. 6 and 7). In high-protein Kastoria the smallest starch granules in deep endosperm were about 2 µm in diameter and the largest about 40 µm (Fig. 6D). Whereas, in Beaulieu the smallest had 3 µm in diameter and the largest about 30 µm (Fig. 7B).Starch granules were often not oval but irregular, because of compression between each (Fig. 7D). In untreated control cross sections, all the starch granules were embedded in protein matrix, being more dense in the higher protein Kastoria variety. After digestion, much

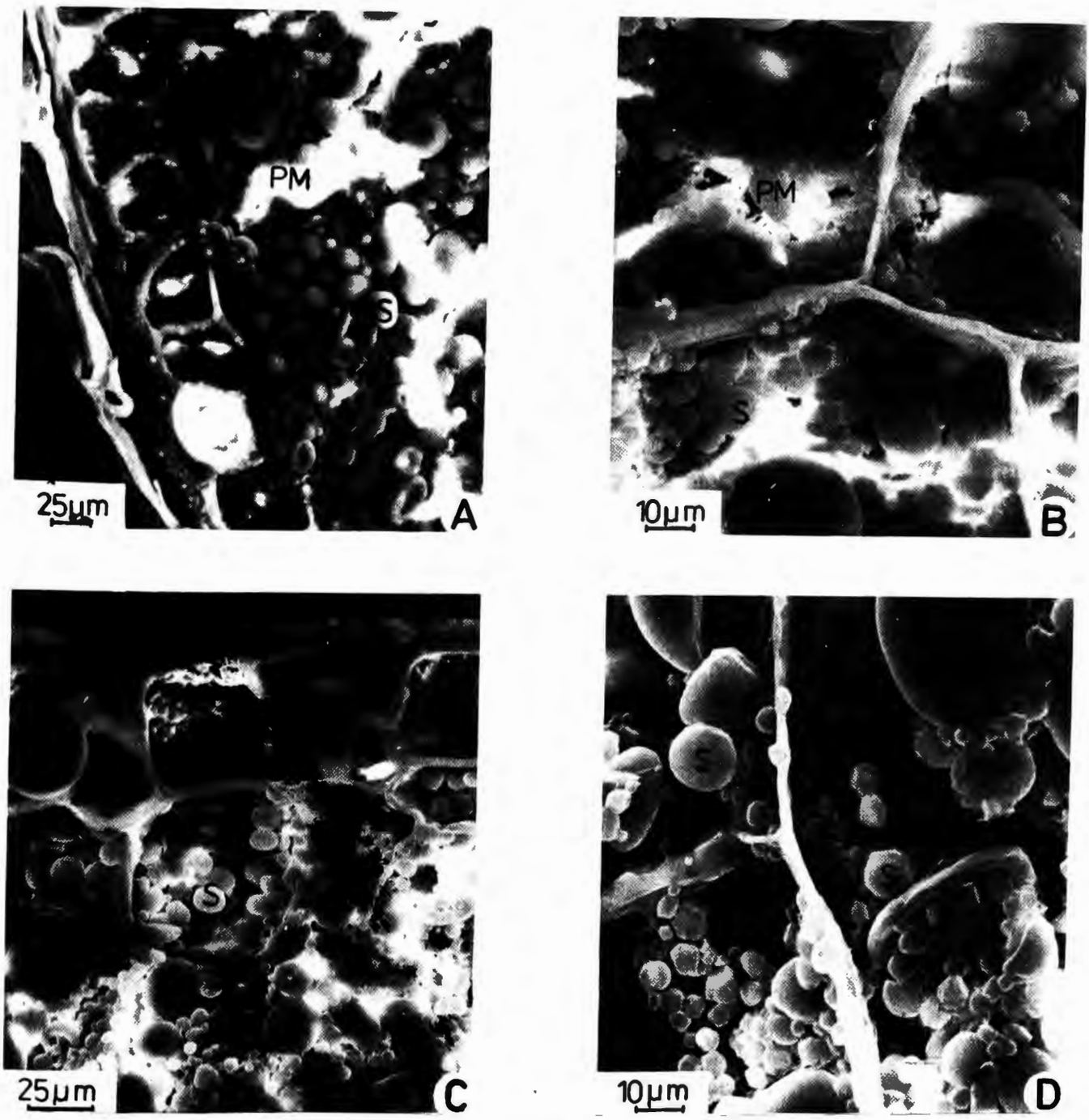

Fig. 7. Scanning electron micrographs of aleurone and subaleurone cells (A, C) and inner endosperm cells (B, D) of low protein rye variety Beaulieu: A, B — untreated cross sections; C, D — pronase treated cross sections

less free space was left between the starch granules of the low protein cultivar (Fig. 7D) than in the high protein one (Fig. 6D).

Smaller concentration of protein matrix covering starch granules in deeper layers of rye grain endosperm, in the ontogenetically oldest storage cells of the grain is explainable due to Evers [6] model of endosperm development. First cells produced by an outer meristematic layer after anthesis as an effect of tangestial division were later on pushed and pressed inside the endosperm. The earlier the synthesis of storage proteins begins — the more protein one can find in the inner parts of grain endosperm. This might be the explanation of higher protein concentration found in high-protein rye varieties in deep endosperm. The proportion of synthesised gliadins to glutelins, both storage proteins but differing

in the lysine content, is decesive for the final nutritive value of grain protein. More glutelins means higher lysine content, but even more important for the amino-acid composition of grain protein is the concentration of albumins and globulins. Albumins due to their high content in grain protein and globulins due to their high lysine content. They can be found mainly in the meristematic layer which after some time ceases to divide and becomes the aleurone layer [4]. If the lenght of a period of filling and development of seeds is extended due to good weather (good supply of water in the soil, high temperatures) and sufficient nutrients supply, the protein synthesis running paralelly with starch synthesis is prolonged. All the subaleurone region is then tightly filled with protein matrix with well developed starch granules embedded in it. This is why bigger, well developed and not shrivelled rye grains were found earlier to contain more protein when examined by means of histochemical staining techniques or total analysis [15].

CONCLUSIONS

Low-protein rye varieties contain less protein matrix in the subaleurone region of the endosperm, tightly packed with different sizes starch granules, in comparison to high protein ryes.

The subaleurone region in high-protein varieties is wider and contains more dense protein matrix than in low-protein rye grains.

High protein varieties contain more protein matrix in cells of the inner layers of endosperm, having the whole protein more evenly distributed within the caryopses than in low-protein varieties.

No granular protein bodies within the protein matrix of mature rye endosperm cells are visible in SEM.

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BADANIA PORÓWNAWCZE ILOŚCI I ROZMIESZCZENIA BIAŁEK W KOMÓRKACH ENDOSPERMY ZIARNIAKÓW NISKO- I WYSOKOBIAŁKOWYCH ODMIAN ŻYTA

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Streszczenie

Przeprowadzono badania porównawcze ilości i rozmieszczenia składników białkowych komórek warstw podaleuronowych i głębszych warstw endospermy w suchych, dojrzałych ziarniakach 8 niskobiałkowych i 7 wysokobiałkowych odmian żyta (tab.), wyhodowanych w 1974/75 r. w Skierniewicach i 1976/77 r. w Powsinie w identycznych warunkach izolowanej kolekcji zachowawczej z wykorzystaniem skaningowego mikroskopu elektronowego (SEM).

W odmianach niskobiałkowych ziarna skrobiowe o zróżnicowanej średnicy były zatopione w grubej warstwie matrycy białkowej w komórkach cienkiej warstwy podaleuronowej (fot. 1, 2, 3). W odmianach wysokobiałkowych, oprócz zdarzających się w wypadku niektórych odmian większych komórek warstwy aleuronowej, warstwa podaleuronowa była lepiej wykształcona z dużą ilością matrycy białkowej i regularnymi i mniej upakowanymi w niej ziarnami skrobi (fot. 4, 5). Również w głębszych warstwach endospermy komórki ziarniaków odmian wysokobiałkowych zawierały więcej matrycy białkowej otaczającej ziarna skrobi w przeciwieństwie do niskobiałkowych, których komórki wypełnione są szczelnie ziarnami skrobi i zawierają niewielkie ilości białek pomiędzy nimi oraz białko cytoplazmatyczne pod ścianami komórek. Porównawcze trawienie pronazą skrawków ziarniaków odmian nisko- i wysokobiałkowych (fot.8) potwierdziło powyższe twierdzenia. Nie zauważono w komórkach innych niż aleuronowe granularnych ciał białkowych w żadnej z badanych odmian żyta.