DISTRIBUTION OF MINERALS IN BARLEY AT THE CELLULAR LEVEL BY X-RAY ANALYSIS[1]

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ABSTRACT

Distribution of minerals in the barley caryopsis was determined with an X-ray analyzer attached to a scanning electron microscope by three techniques: spot analyses; X-ray image profiles; and line-scan profiles. Silicon was concentrated on the surface and the outer part of the lemma. Calcium and P were higher in the inner, than in the outer, layer of the lemma. The two main mineral components in the pericarp were K and Ca. Mineral composition of cell walls in the aleurone and starchy endosperm was similar. Presence of S in the cell walls is indicated. Aleurone grains were richest, among studied barley tissues, in K, P, Mg, and Ca. Concentration of K in the subaleurone layer was higher than in the center of the starchy endosperm.

Electron microprobe analysis measures X-rays emitted from inner shell ionizations of elements upon impact of an electron beam. This phenomenon can be used to determine in situ chemical composition and distribution of elements in microareas of biological specimens (1,2,3). The usual way to determine the composition and distribution is by recording X-rays emitted from the specimen. Energies of emitted X-rays can be identified with a solid crystal detector and a multi-channel analyzer. The detector can be placed close to the specimen to collect the X-rays; it is virtually 100% efficient for X-rays up to 20 keV. This detector is well suited to low probe currents of the scanning electron microscope (SEM)[4] and can be attached to it without limiting the other uses of the instrument.

Three types of analyses can be performed (4,5,6). In spot analyses, the area subjected to elemental analysis is selected by use of the SEM, an electron probe of less than 1-μm diameter is placed in position on the part of the sample that is to be analyzed and the X-rays emitted by the electron-bombarded area are recorded on a chart. Individual elements are identified according to the energy of emitted X-rays; the relative amounts of each element are related to the areas under the peaks on the record chart.

In a second method, an X-ray image of the distribution of a specific element in a large area is recorded by a camera. Finally, relative distribution of an element can be determined by a line scan in which the electron beam is moved above a stationary specimen. The X-ray signal is recorded on a chart. Concentration profiles for specific elements can be related to the cellular structures recorded photographically by the SEM.

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[4]SEM is an abbreviation for both scanning electron microscope and scanning electron microscopy.

Information from spot analyses can be used for quantitative assays. Information provided by the X-ray line scan and X-ray image methods is semiquantitative (2). According to Lehrer and Berkley (7), without appropriate standards, only relative measurements can be made in biological materials with electron microprobes. The authors (7) described a rather elaborate procedure for the preparation and application of gelatin standards in the quantitative elemental analyses of tissue sections by the electron microprobe. Applicability of this procedure to analyses of cereal grains is yet to be determined.

Probably the greatest benefit of electron-probe X-ray analysis is that it enables one to locate the mineral components of biological tissues and, thus, to interpret results of mineral assays which were independently obtained by chemical and/or instrumental methods. We have previously determined (8,9), by atomic absorption spectroscopy, mineral components in barley sections and in milled barley products. The present electron-probe microanalyses were to determine the precise sites and relative distribution of these components.

MATERIALS AND METHODS

Barleys

Three barleys were grown in 1971 at the Montana Agricultural Experiment Station, Ft. Ellis. These included a hulled six-row cultivar, 'Larker,' and two naked barleys, 'Hiproly' (CI 3947) and its sister line CI-4362. The protein contents of the barleys were, respectively, 13.7, 18.3 and 17.8% (N × 6.25, dry matter basis).

Structure and Composition

Transverse sections, about 1 mm thick, were cut from about the midpoint of barley kernels, mounted on circular (diameter, 9 mm) graphite specimen holders with an adhesive, and coated with a 200–300 Å carbon layer. The sections were examined on a JEOL JSM-50A[5] scanning electron microscope.

Secondary electron images were used to examine the specimens and to select areas for X-ray analyses. Mineral components in selected spots, areas, or lines were determined with an NS-880 X-ray analyzer (Tracor Co., Middleton, Wis.) by analyzing X-rays emitted from inner shell ionizations of mineral elements. X-Rays resulting from excitation of K shell electrons (K_α X-rays) were recorded. The scanning electron microscope was operated at an accelerating voltage of 20 kV, no tilt, 5×10^{-11} to 2×10^{-9} A specimen current, and 13 mm working distance. The take-off angle was 52.5°. The diameter of the electron beam was about 1000 Å when the current was 10^{-9} A and about 100 Å when the current was 10^{-12} A.

RESULTS

Results of detailed SEM studies of the barley kernel were reported elsewhere (10,11,12). Only a brief discussion of appropriate SEM pictures is presented here

[5]Mention of a trademark name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

to describe the tissues that were studied and to illustrate those tissues in barley cultivars that were not included in previous publications.

An SEM micrograph of a cross section of the hull (lemma), pericarp, seed coat, and aleurone of Larker barley is shown in Fig. 1. The lemma and pericarp are both multilayered. The aleurone tissue of barley consists of up to four layers of cells; in other cereals, generally, only one layer is present (12). The aleurone cells in Fig. 1 contain no aleurone grains. Presumably, they were removed during the preparation of the section. The starchy endosperms of Larker and CI-4362 barleys are shown in Figs. 2 and 3. Large and small starch granules can be seen in the endosperm of both varieties, but CI-4362 appears to have more of the small starch granules than Larker. A cell from the center of the starchy endosperm of CI-4362 is shown in Fig. 4. The cell is apparently filled almost entirely by small starch granules embedded in a protein matrix. This type of cell was not observed

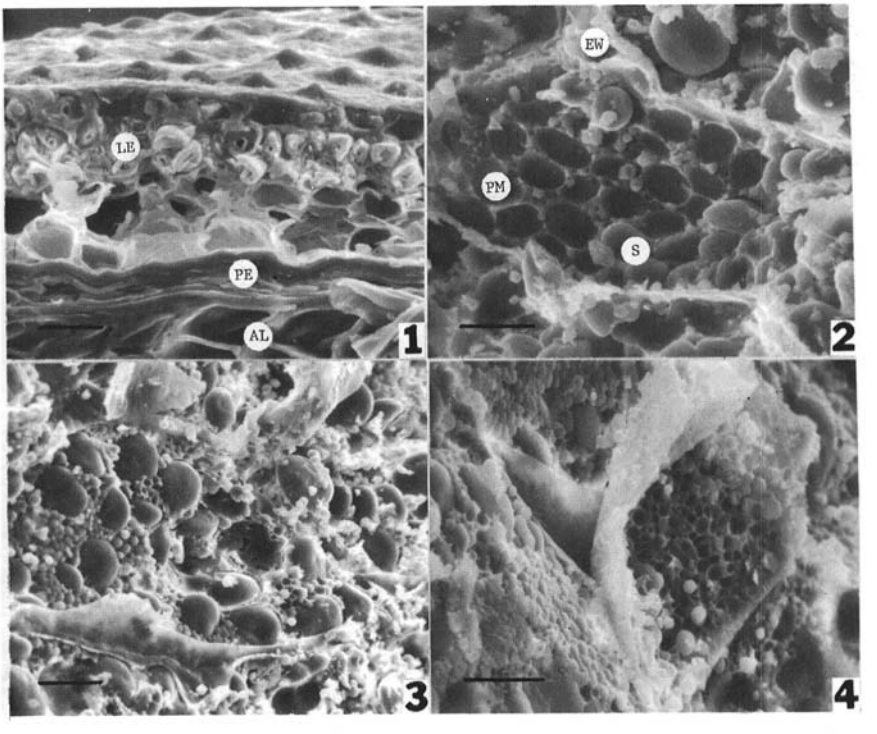

Fig. 1. Scanning electron microscope (SEM) micrograph of a cross section through the lemma (LE), pericarp (PE), and aleurone layer (AL) of Larker barley. Scale bar = 20 μm.

Fig. 2. SEM micrograph of a cross section through the center of the starchy endosperm of Larker barley. S = starch granule; EW = cell wall; and PM = protein matrix. Scale bar = 20 μm.

Fig. 3. SEM micrograph of a cross section through the center of the starchy endosperm of CI-4362 barley. Note presence of large and small starch granules. Scale bar = 20 μm.

Fig. 4. SEM micrograph of a cross section through the center of the starchy endosperm of CI-4362 barley. Note predominance of small starch granules in the cell enclosed by the cell wall. Scale bar = 20 μm.

in Larker used in this study or in the endosperm of any of the other hulled barleys that were studied previously (10,11,12).

Analyses of Microareas (Spot Analyses)

Relative concentrations of mineral components in barley tissues are summarized in Table I. The results of spot analyses are based on counts (per 500-sec) determined in areas about 1 μm in diameter. Each value in Table I is an average of several spot analyses of selected areas in Figs. 1-4. Concentrations of elements for which no values are recorded were below the detectability limit of the analyzer. Note that the K_β radiation of K contributed in most cases to the "Ca peak." Consequently, the actual Ca content may have been much lower than indicated in the table.

The results summarized in Table I show that whereas some elements are distributed throughout the barley kernel, others are concentrated in some tissues only. Silicon is the only prominent mineral element in the lemma. X-Ray counts of Si in the outer part of the lemma are about 20 times as high as the counts in the inner part. The minor mineral components in the hull are K, P, and Ca. Whereas K was more concentrated in the outer lemma, P and Ca were mainly in the inner lemma. The only mineral elements present in measurable amounts in the pericarp are K and, probably, Ca. The cell walls in the aleurone layer and in the starchy endosperm are similar in mineral composition. Both types of cell walls are low (compared to aleurone components) in K and P and relatively high in S and Cl.

The aleurone tissue is rich in K, P, Mg, and Ca; the aleurone grains contain more of these minerals than the protein matrix. K and P were the only mineral elements present in measurable amounts in the starchy endosperm. As the levels of these elements in the starchy endosperm are very low, it is questionable whether the relative differences in K and P, between the protein matrix and the starch granules, are significant. Similarly, there were no significant differences in concentrations of K and P in small and large starch granules.

TABLE I
Relative Concentrations[a] of Mineral Components in Barley Tissues

	Concentration, Counts/500 sec in								
				CI 4362					
				Aleurone			Endosperm		
	Lemma of Larker Barley		Peri-	Cell			Cell	Protein	Starch
Element	Outer[b]	Inner[b]	carp	Wall	Matrix[c]	Grain[c]	Wall	Matrix	Granule
Si	1445	77	...[d]	...	...	...	...	...	...
K	325	202	398	180	1900	3390	135	83	178
Ca	71	132	113	118	440	670	121	...	...
Mg	...	...	...	98	690	950	125	...	...
P	...	89	...	303	3800	5110	253	101	159
S	...	...	...	227	...	...	275	...	...
Cl	...	...	...	142	...	...	176	...	...

[a]X-Ray spectra were obtained under the following conditions: 20 kV, 5×10^{-11} A, no tilt.
[b]200 sec × 2.5.
[c]50 sec × 10.0.
[d]Dashes indicate that concentration of elements were below detectability limit of the analyzer.

Distribution Images

Distribution images of P, K, Ca, Mg, and S in the pericarp and aleurone of Hiproly and CI 4362 are shown in Figs. 5 and 6. P and K are concentrated in the interior of the aleurone cell; the grains contain substantially more of these elements than the matrix. Magnesium, present in smaller concentrations, shows a similar pattern. Calcium is slightly more concentrated in the grains and the

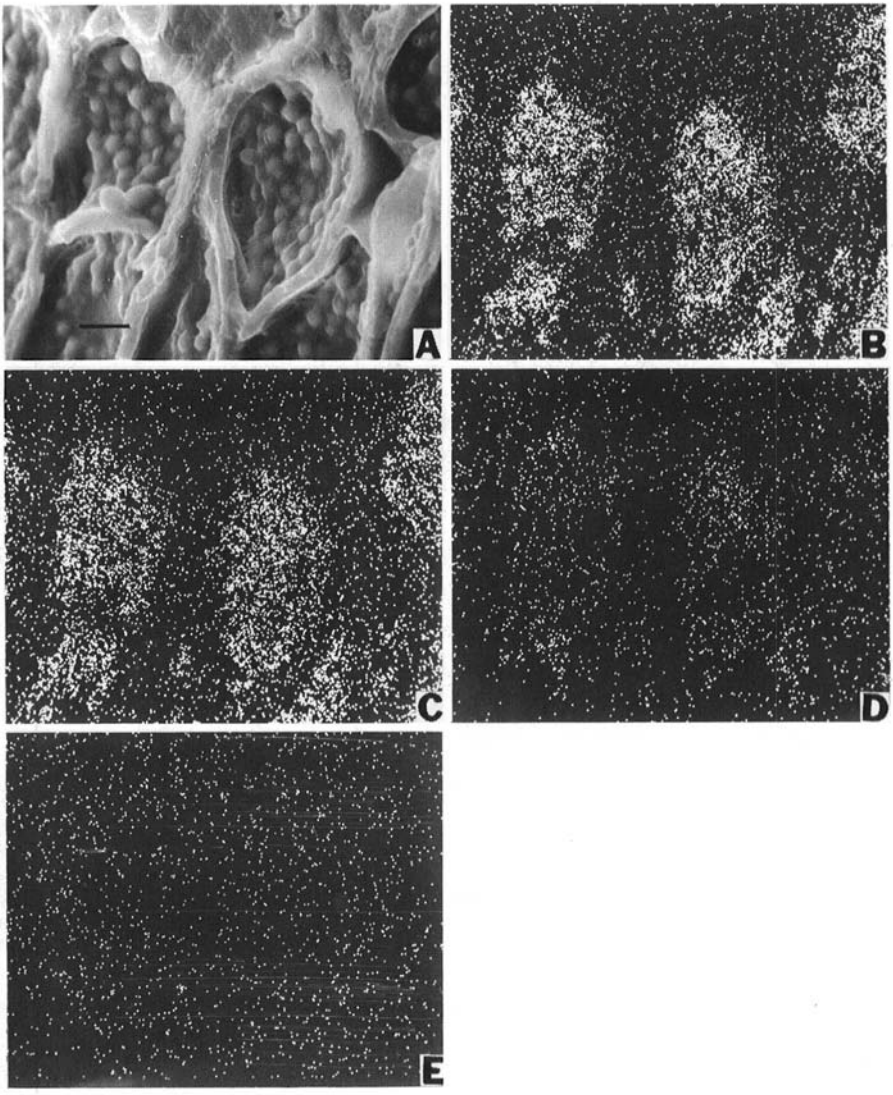

Fig. 5. SEM micrograph and X-ray distribution images of P, K, Mg, and S of the aleurone layer in CI-4362 barley are shown in A, B, C, D, and E, respectively. Analyses were performed at 20 kV with specimen current of 1×10^{-10} A. The areas were scanned for 500 sec. Scale bar = 5 μm.

protein matrix of the aleurone layer than in the cell wall of the aleurone layer or in the pericarp (Fig. 6E). However, the difference in distribution for Ca is not as large as the differences for P, K, and Mg. Note that the X-ray analyzer used in this study records the K_β radiation of K and the latter may affect the results of

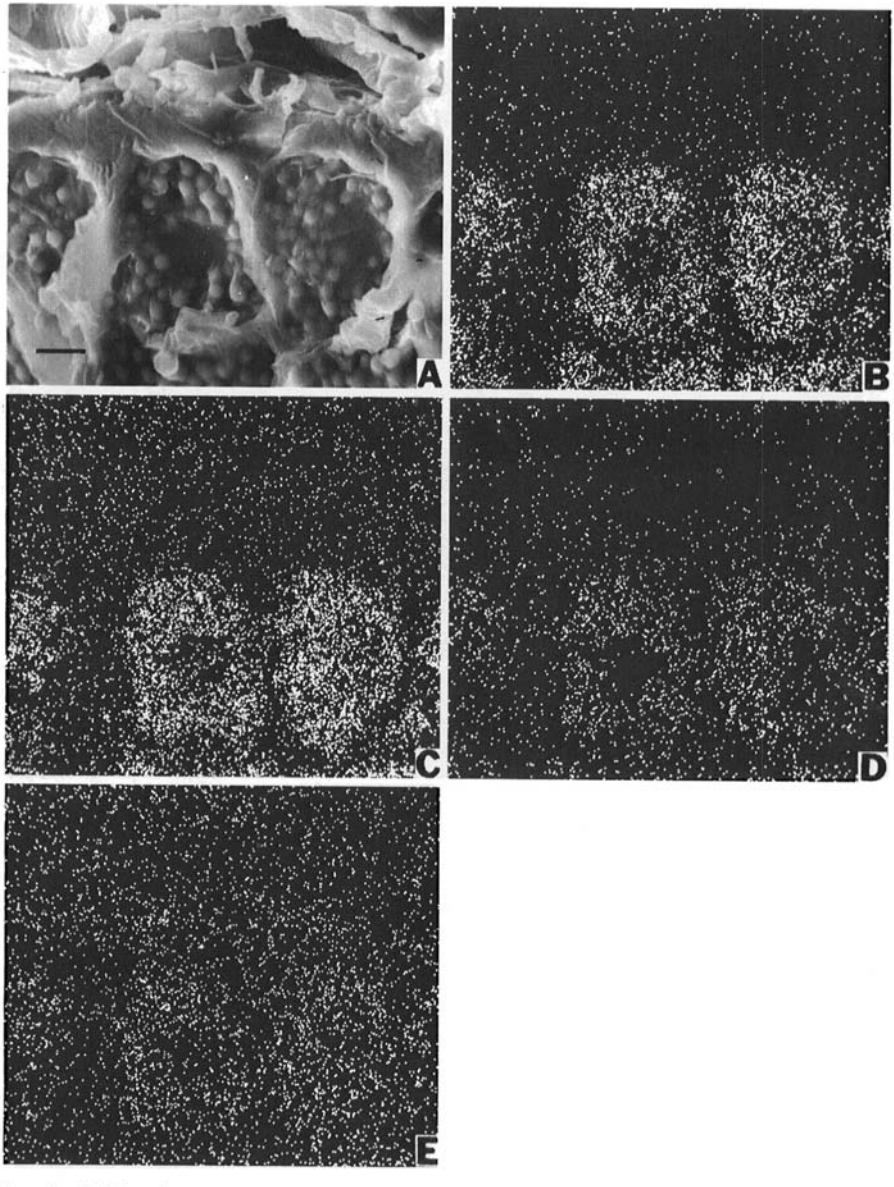

Fig. 6. SEM micrograph and X-ray distribution images of P, K, Mg, and Ca of the pericarp and aleurone layers of Hiproly barley are shown in A, B, C, D, and E, respectively. Analyses were performed at 20 kV with a specimen current of 1×10^{-10} A (0.8×10^{-10} A for P). The areas were scanned for 500 sec (1000 sec for Ca). Scale bar = 5 μm.

determining the K_α radiation of Ca. No distribution pattern could be established for S in the studied specimens (Fig. 5E). This is due, in part, to the relatively low S content of the tissues that were studied and to the limitations of the analyzer in the distribution image mode of analysis (13).

Line Scans

A line scan for Si through the lemma, pericarp, and aleurone of Larker barley is shown in Fig. 7. The concentration profile confirms the data summarized in Table I which show the high concentration of Si in the outer lemma and low concentration (or absence) in the interior lemma, pericarp, and aleurone. Hayward and Parry (5) have recently shown that all detectable Si in transverse sections of a barley grain was in the lemma and palea and was confined to the abaxial epidermis and subepidermal sclerenchyma or hypodermis. None could be detected in the adaxial epidermis of the palea or lemma, or in the pericarp, or in the aleurone and starchy endosperm of the caryopsis.

Apparently K is distributed rather uniformly in the lemma and in the pericarp; P is low (or almost absent) in the outside layer and relatively high in the inside layer of the lemma (not shown here). Several chart recordings for K and P indicated variations across the aleurone and a higher concentration of K (but not of P) in the subaleurone layer than in the starchy endosperm. Line scans showed a rather spurious distribution for P in the subaleurone layer and in the starchy endosperm. No attempt was made to relate the relatively low concentrations of P in the endosperm to structural grain components. Those findings will require confirmation by additional studies and by use of independent methods.

Line scans through the aleurone layer (not shown here) for minor elements indicated a uniformly low concentration profile for Fe and a low but consistent level of S in the cell wall. The latter is in agreement with previous results (12) and the data in Table I.

Interpretation of line scans must be made with caution. In the line scan method, the depth of penetration of the beam changes continuously because of differences in densities of the heterogenous material (5). The uneven surface results in topographical variations in the X-ray take-off angle and decreases precision of X-ray intensity measurements.

DISCUSSION AND CONCLUSIONS

We have reported recently (8,9) the atomic absorption spectroscopy determinations of 11 elements (including P, K, Mg, Ca, and Fe) in the ash of whole barley, in caryopsis components separated by hand, in products of tangential abrasion, in roller-milled flours, and in air-classified flours. Germ and bran contained the highest, and central endosperm the lowest, concentrations of mineral components. Decrease in concentration of mineral elements from the bran to the central endosperm varied widely; *i.e.*, some elements were more uniformly distributed throughout the kernel than others. Tailings and shorts of roller-milled barley, which were rich in germ and bran particles, contained higher concentrations of mineral elements than hulls or flour. Hand-dissected central endosperm contained up to 100 times as much of some mineral components as starch. During air-classification, shifts in protein concentration were accompanied by shifts in concentrations of mineral components.

The results summarized in Table I confirm the high concentrations of mineral components in the aleurone layer and the low concentrations in the starchy endosperm. The high ash content of bran can be attributed to the presence of particles originating with the aleurone, rather than to hull and pericarp particles.

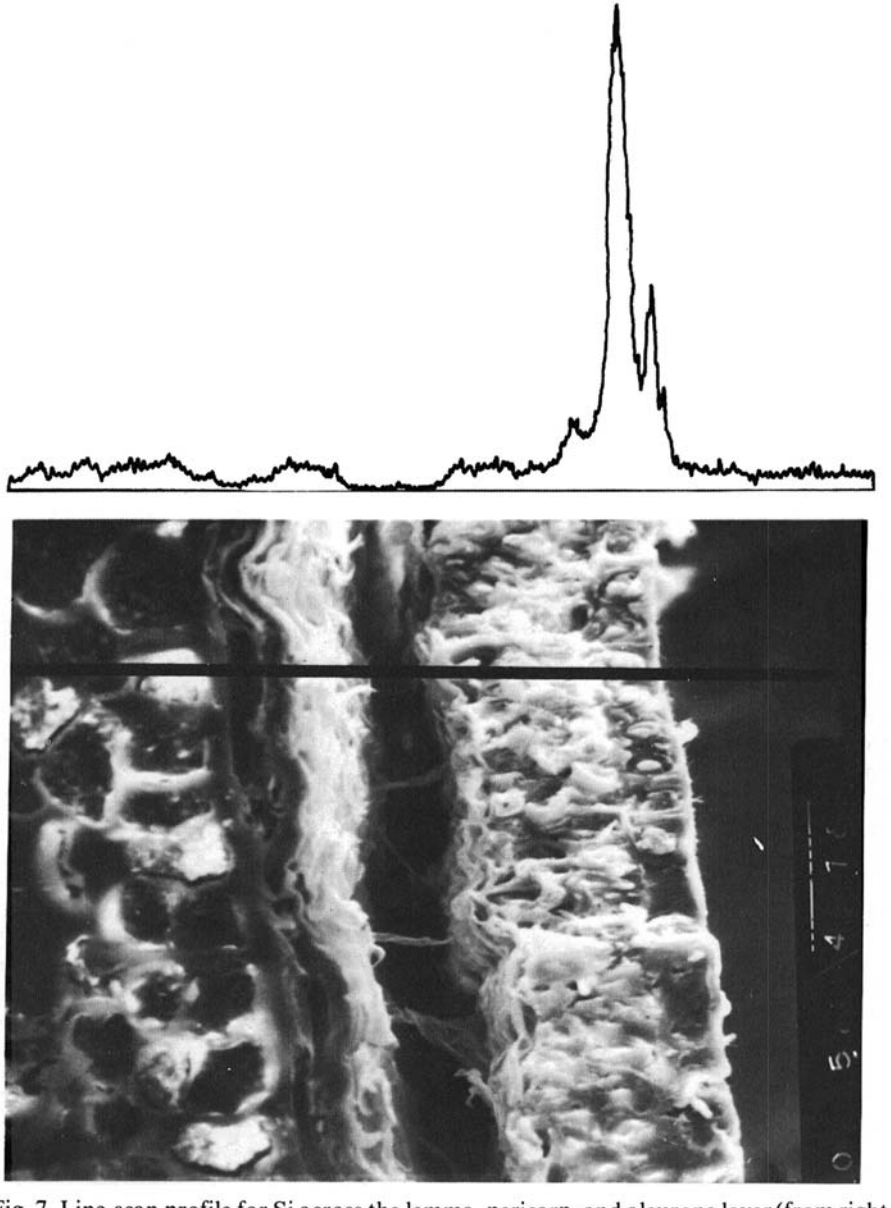

Fig. 7. Line-scan profile for Si across the lemma, pericarp, and aleurone layer (from right to left) of Larker barley; scanned for 250 sec at 20 kV, 2×10^{-9} A. Bottom: SEM microphotograph (600×), top: Si = line-scan profile (10^3 counts per sec).

Microprobe analyses showed only small differences in P and K contents of the starch granules and the protein matrix. It would seem, therefore, that shifts in mineral components and proteins during air-classification are not directly related.

Analyses by atomic absorption spectroscopy (8) indicated that Ca was rather uniformly distributed throughout the barley kernel. Calcium, as Ca pectate, is the major cation of the middle lamella of the cell wall (14,15). Yet, microprobe analyses show that Ca was more concentrated in the aleurone grains and in the protein matrix of the aleurone layer than in the aleurone cell wall (or endosperm cell wall and pericarp).

The presence of S in the aleurone and starchy endosperm cell walls is of interest. According to Lamport (16), peptides isolated from cell walls of plants contain no sulfur-amino acids. However, the review of Mühlethaler (17) indicates that cystine and cysteine are present in cell-wall proteins. The role of S in metabolic (enzymatic) activities of the cell walls is yet to be determined. One can assume, however, that disulfide bridges, which are important cross-links between protein chains, could contribute significantly to the strength of cell walls. Interchanges between disulfides and sulfhydryl groups could govern flexibility of the walls.

The nutritional aspects of trace elements in plants are well documented (18,19). The data have shown that the mineral content of a plant has some controlling effect on growth and fruitfulness. Agronomically, trace metals are important as their presence (or application) may govern crop yield. In order to determine the optimum level of application, amounts and availability of the elements in the soil and in the plant tissue are determined routinely. The value of the empirical approach to plant nutrition has been significantly enhanced by numerous studies of the biochemical aspects of mineral nutrition. Such studies have been useful in developing sensitive indicators of the nutritional status.

It has become increasingly clear that to better understand the role which mineral elements play in plants, it is important to pinpoint the site of these elements in vital metabolic processes. Despite the limitations and problems encountered during microprobe analyses of biological specimens, the use of the techniques is rapidly expanding and promises to make major contributions to elucidating the function of mineral components in production and utilization of foods of plant origin.

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