Distribution of (1→3),(1→4)-β-D-Glucan in Kernels of Oats and Barley Using Microspectrofluorometry[1]

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ABSTRACT

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The distribution of (1→3),(1→4)-β-D-glucan (β-glucan) in grains was studied using scanning microspectrofluorometry of cell-wall-bound Calcofluor in selected cultivars of oats and barley. Microspectrofluorometric imaging showed a high concentration of β-glucan in the depleted layer adjacent to the embryo in all oat cultivars examined. In the low β-glucan oat OA516-2, a high concentration of β-glucan was also seen in the subaleurone region in cross sections of the proximal, central, and distal areas of the kernel. In the high β-glucan cultivar Marion, the relative fluorescence intensity of the bound Calcofluor was high throughout the central endosperm. Morphological differences were observed in the central endosperm of the two cultivars, with Marion having somewhat smaller cells and slightly thicker walls, which would result in a greater concentration of β-glucan per unit volume than that in OA516-2. Very little β-glucan was observed in the embryo in either oat cultivar. In a comparison of maps of central cross sections of five cultivars of differing β-glucan content (range 3.7–6.4%), there was a trend for the high subaleurone concentration of β-glucan to become less distinct as the total β-glucan content of the cultivars increased. The distribution of β-glucan in barley was more uniform, with no high subaleurone concentration of β-glucan in any of the five cultivars examined (β-glucan range: 2.8–11%). The highest concentration of β-glucan was in the central endosperm.

(1→3),(1→4)-β-D-Glucan (β-glucan) is the major cell-wall polysaccharide in the endosperm of oats and barley (Fincher and Stone 1986). Reports of specific benefits from inclusion of β-glucan (in the form of oat bran or other oat products) in the diet (Wood et al 1989) have generated an increased public demand for these products and prompted an interest in the manufacture of products with increased levels of β-glucan. Attempts to increase the β-glucan concentration in raw materials, ingredients, and resultant food products have followed different strategies: searches for existing varieties with high β-glucan contents, increasing the β-glucan content of existing varieties through different types of manipulation, and production of high β-glucan fractions through milling.

The search for a high β-glucan oat among cultivated varieties has an apparent limit of 6–7% β-glucan (Welch and Lloyd 1989, Peterson 1991, Miller et al 1993a). Although this value is fairly high on a groat basis, there is a dilution factor when the oats are incorporated into finished products such as ready-to-eat cereals. Greater flexibility would be possible with materials of even higher β-glucan content. Similar limits in β-glucan content have also been reported in commercial barley varieties (Gill et al 1982, Henry 1986, Lehtonen and Aikasalo 1987).

Although environmental factors have influenced β-glucan content in oats and barley, the dominant source of variation is genetic in both these grains (Bourne and Wheeler 1984, Åman and Graham 1987, Peterson 1991, Miller et al 1993a). Low β-glucan (<3.5%) mutants of barley have been produced by chemical treatment of grain (Aastrup 1983). Some waxy, hull-less cultivars with very high levels (>10%) have been developed also. However, the specialized waxy, hull-less varieties have lower fertility and yield than do commercial varieties (Fox 1982, Nam and Lee 1990). Similar information on oats is sparse, although very low β-glucan levels have been reported in some primitive species of *Avena* (Miller et al 1993b).

To date, the most successful way to increase β-glucan contents in raw materials for food products is to use high β-glucan grain fractions. Wood et al (1991) reported an average β-glucan enrichment factor of 1.5 in oat bran (obtained in about 50% yield), as compared to that in whole groats of several oat varieties. Knuckles et al (1992) used dry milling and sieving to obtain fractions from defatted oats and barley that were enriched (16–27%) in β-glucan.

Microscopy indicates differences in distribution of β-glucan in kernels from different oat cultivars (Fulcher 1986). Such differences, not well-characterized to date, might be related to differences in overall β-glucan content and could also have a profound effect on the processing performance of various cultivars. This article describes differences in β-glucan distribution in some cultivars of oats and barley detected by using scanning microspectrofluorometry. Our ultimate aim is to identify appropriate sources of high β-glucan fractions for inclusion in various food products.

MATERIALS AND METHODS

Oat cultivars of known β-glucan content (Donald, 3.7%; OA516-2, 4.0%; Tibor, 4.6%; Woodstock, 5.1%; Marion, 6.4%) were obtained from the Eastern Cooperative Oat Test (Agriculture Canada) and dehulled manually. The β-glucan content of samples was determined enzymatically by a modification (Miller 1992) of the method of McCleary and Glennie-Holmes (1985). Barley cultivars (M-737, 2.8% β-glucan; Chalky Glenn, 3.2%; Minerva, 6.0 %; Arizona, 11.0%.) were the generous gift of S. Aastrup, Carlsberg Research Laboratory, Copenhagen, Denmark, who also provided β-glucan analyses for these cultivars. Agriculture Canada provided the cultivar Leger (5.7% β-glucan).

Distribution of β-Glucan Within the Caryopsis

Oat and barley kernels were embedded in blocks of polyester resin (Motomaster Liquid Auto Body Resin, Canadian Tire Corporation; prepared according to the manufacturer's directions) using the method of Symons and Fulcher (1988). Each block contained 25 kernels from a single cultivar. The blocks were cut to the desired level using glass paper. The surfaces of cut blocks, containing exposed transverse sections of the embedded kernels, were treated for 2 min with 0.01% Calcofluor White in potassium phosphate (50 m*M*, pH 8) buffered ethanol (50%) and then rinsed with 50% ethanol. The blocks were counterstained for 30 sec with Fast Green FCF (0.1% in 50 m*M* acetate buffer, pH 4.0), rinsed with distilled water, and blotted dry.

Stained blocks were placed on the scanning stage of a scanning microspectrophotometer (Zeiss UMSP80, Carl Zeiss, Oberkochen, FRG) equipped with an epi-illuminating condenser and HBO 100-W mercury illuminator. Both the scanning stage and the photomultiplier of the UMSP80 are software-controlled. The fluorescence filter combination had a dichromatic beam splitter and an exciter-barrier filter set with maximum transmission at 365 and 420 nm. A 10× Neofluar objective (Zeiss) was used for scanning. The microspectrophotometer was cali-

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brated at the beginning of each session using a solution of 0.002% 4-methylumbelliferone (Sigma), freshly diluted from a 0.01% stock solution that was prepared weekly. Using Zeiss software (Automatic Photometric Analysis of Microscopic Objects by Scanning, APAMOS), a 2,500 × 3,000 pixel field was marked over each kernel cross section in each block and scanned in a matrix fashion to generate a plot of fluorescence intensities across the field. For presentation purposes, rotation, tilt, and perspective factors were applied to the plots so that kernel characteristics were more readily visible.

Preparation and Examination of Methacrylate-Embedded Grains

For microscopic examination of thin sections, grain pieces were fixed and embedded in glycol methacrylate as described by Fulcher and Wong (1980). Sections 3 μm thick were cut, mounted on slides, and examined using a Zeiss Universal research photomicroscope equipped with a IIIRS epi-illuminating condenser and fluorescence filters as described above. The staining procedure was the same as described above. Photomicrographs were obtained using 35-mm Kodak Ektachrome 400 film.

RESULTS AND DISCUSSION

Distribution of β-Glucan in Oats

The distribution of β-glucan in oats was mapped in cross sections of three different parts of the kernel: the proximal end (containing the embryo), the central portion, and the distal, or brush, end. Calcofluor White, which binds specifically to β-glucans in oats and barley (Wood and Fulcher 1978, 1983) was used as a fluorescent probe for β-glucan. The relative fluorescence intensity (RFI) of Calcofluor bound to β-glucans was measured and plotted as intensity profiles to map β-glucan distribution. Each profile is oriented such that the ventral furrow, or crease, is positioned at the left of the section, thus the dorsal side of the caryopsis is to the right. In solution, the RFI of bound Calcofluor is approximately proportional to the amount of β-glucan present within a narrow concentration range (Jorgensen 1988). The present method is based on the assumption that peak height (RFI) is proportional to the β-glucan content at any given point on the intensity profile (in discussion, high intensity is taken to mean high concentration).

Intensity profiles representing proximal, central, and distal scans of a single kernel of OA516-2 (4.0% β-glucan) are shown in Fig. 1. The proximal region contains the embryo (Fig. 1a) and had the highest concentration of β-glucan in the starchy endosperm immediately adjacent to the embryo. Fluorescence

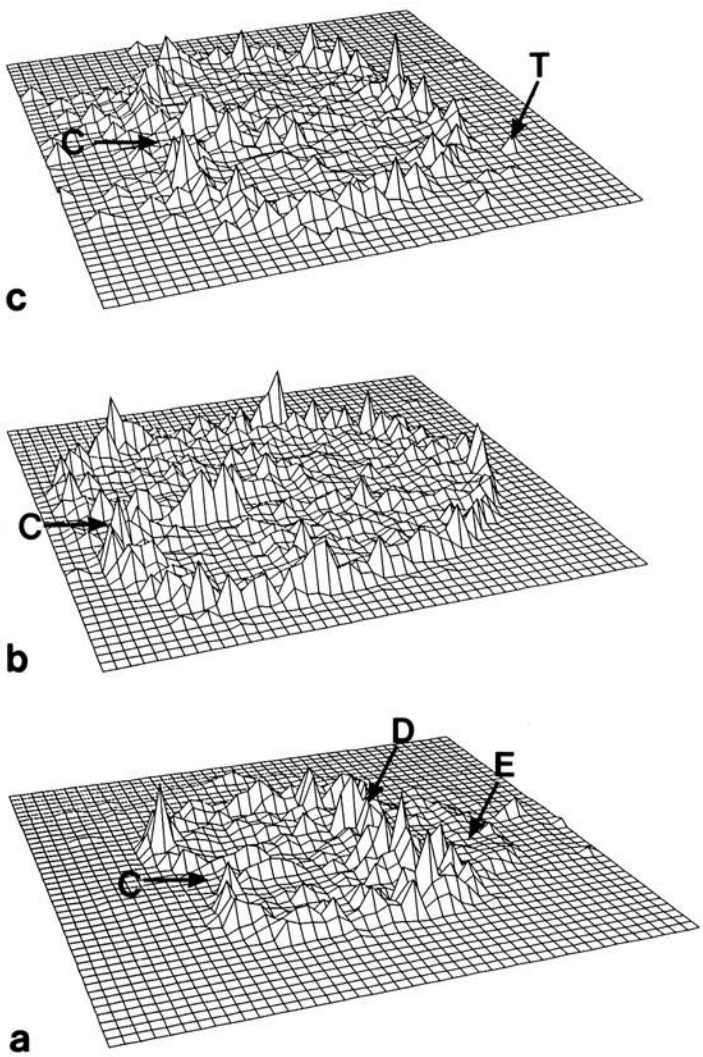

Fig. 1. Distribution of β-glucan in a kernel of OA516-2 oats as detected by microspectrofluorometric scanning of the relative fluorescence intensity of bound Calcofluor at the proximal end (**a**), central region (**b**), and distal end (**c**). E = embryo, D = depleted layer, C = crease, T = trichomes.

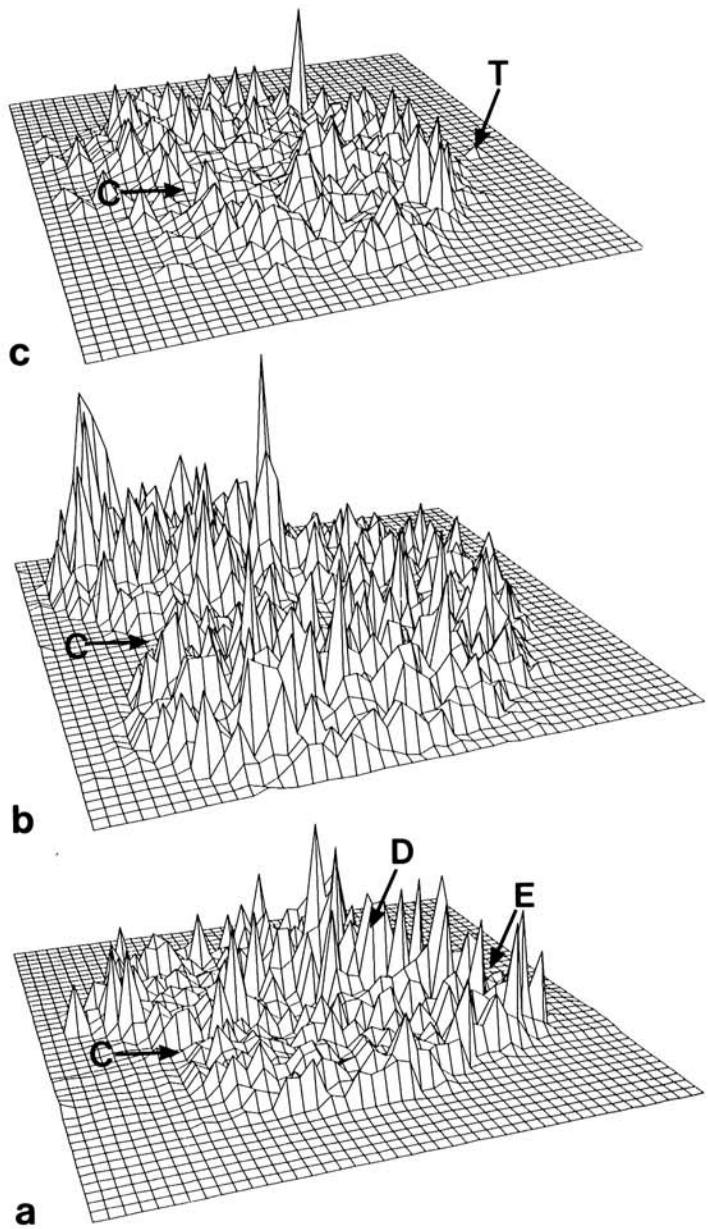

Fig. 2. Distribution of β-glucan in a kernel of Marion oats as detected by microspectrofluorometric scanning of the relative fluorescence intensity of bound Calcofluor at the proximal end (**a**), central region (**b**), and distal end (**c**). E = embryo, D = depleted layer, C = crease, T = trichomes.

microscopy of thin sections of OA516-2 showed very thick walls and little or no cell content in this area. This area is known as the depleted layer. It consists of walls left behind as the growing embryo pressed against the endosperm during development of the caryopsis (Brown and Morris 1890, Fulcher 1986). Thickened walls in the depleted layer were a consistent feature of all the cultivars examined, although the degree of this deposition varied. The walls in the embryo itself were very thin and showed little staining by Calcofluor (Fig. 1a). A high concentration of β-glucan was also found in the peripheral regions of the kernel in the layer of cells just inside the aleurone layer (subaleurone layer). Examination of thin sections showed that, as in the depleted layer, the walls in the subaleurone layer were very thick; wall thickness decreased in the interior of the endosperm. Microscopic demonstration of thinner walls in the interior of the endosperm is reflected in the lower RFI observed in those areas (Fig. 1). In the central region of the kernel (Fig. 1b), the β-glucan content was highest in the subaleurone layer just inside the periphery of the kernel. The β-glucan content was lower in the interior of the section. The distribution of β-glucan in the distal portion of the kernel (Fig. 1c) was similar to that of the central region.

Small points of fluorescence were visible outside the kernel at the distal ends of the kernels (Figs. 1c, 2c). These were caused by autofluorescence of the trichomes (hairs) attached to the kernel. Hydroxycinnamic acids in the aleurone layer show autofluorescence similar to the excitation-emission wavelengths of wall-bound Calcofluor (Fulcher et al 1972, Fulcher 1986), but the autofluorescence observed was of much lower intensity than that of wall-bound Calcofluor. Autofluorescence in the aleurone layer was quenched to some extent by Fast Green, which was used as a counterstain in this work and did not interfere with the fluorescence detection and measurement of β-glucan using Calcofluor.

The pattern was different in the central and distal regions of the cultivar Marion (Fig. 2), which contained 6.35% β-glucan. In these areas of the kernel (Fig. 2b,c), the RFI was high throughout, although there was some indication of a slightly higher subaleurone concentration of β-glucan in the distal region. When thin sections of the central region of both Marion and OA516-2 were compared microscopically (Fig. 3a,b), the differences in β-glucan content in the interior of the starchy endosperm reflected differences in cell size as well as wall thickness. In OA516-2, the cells in the endosperm were, in general, larger (Fig. 3a) than those in the endosperm of Marion (Fig. 3b). The cell walls in the endosperm of Marion (Fig. 3b) were thicker than those of OA516-2.

Intensity profiles were generated from central scans of five oat cultivars with different levels of β-glucan content to further compare varietal differences (Fig. 4). The central region of the kernel was chosen for comparison because it constitutes the largest proportion of the caryopsis (roughly 50%). Therefore, we anticipated it would provide the most accurate reflection of the whole kernel. A pattern of higher β-glucan concentration around the periphery of the kernel and lower amounts in the interior was a consistent feature of all three cultivars with lower β-glucan (Donald [Fig. 4a], OA516-2 [Fig. 4b], and Tibor [Fig. 4c]). The pattern became less pronounced as the β-glucan content increased (Woodstock [Fig. 4d]); it was not discernible spectrofluorometrically in the cultivar with the highest β-glucan content (Marion [Fig. 4e]). In thin sections, however, thickened walls were observed in the subaleurone layer of kernels of Marion. The lack of a distinctly elevated subaleurone concentration of β-glucan, as determined microspectrofluorometrically, may be a result of a higher concentration of β-glucan in the central endosperm.

Thickened walls in the subaleurone layer of oats are clearly important to the production of oat bran. Wood et al (1991) reported a range of values for the enrichment factor of β-glucan content between whole groats and brans from a selection of oats. Significant changes in ranking were observed in the β-glucan contents of kernels and resultant brans. The differences in distribution observed in the present study could provide some explanation for the observations of Wood et al (1991). In addition, it has become obvious that scanning microspectrofluorometry is a useful method for selection of appropriate oat cultivars for production of high β-glucan brans. The method allows complete cross sections of kernels to be analyzed at one time, with very

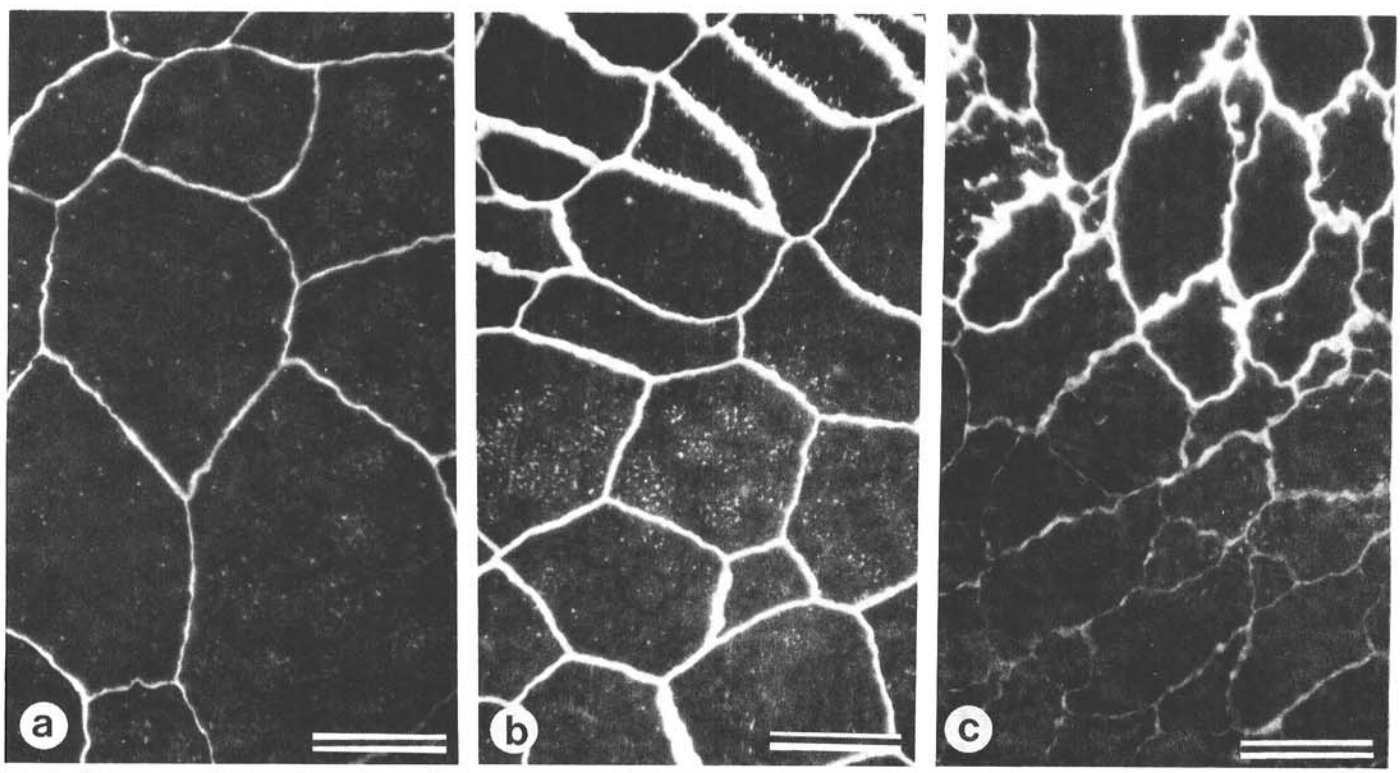

Fig. 3. Fluorescence micrographs of thin sections of oat and barley kernels stained with Calcofluor and counterstained with Fast Green. **a,** Large cells with thin walls in the central endosperm of OA516-2 oats. **b,** Slightly smaller cells and thicker walls in the central endosperm of Marion oats. **c,** Thick and thin walls in the endosperm of M-737 barley. Bars represent 100 μm.

little preparation time. A block containing a large number of kernels can be prepared in less than a day. Using thin sections requires considerable preparation time (up to two weeks), and, as a rule, it is not possible to examine a complete cross section of the kernel in a single thin section.

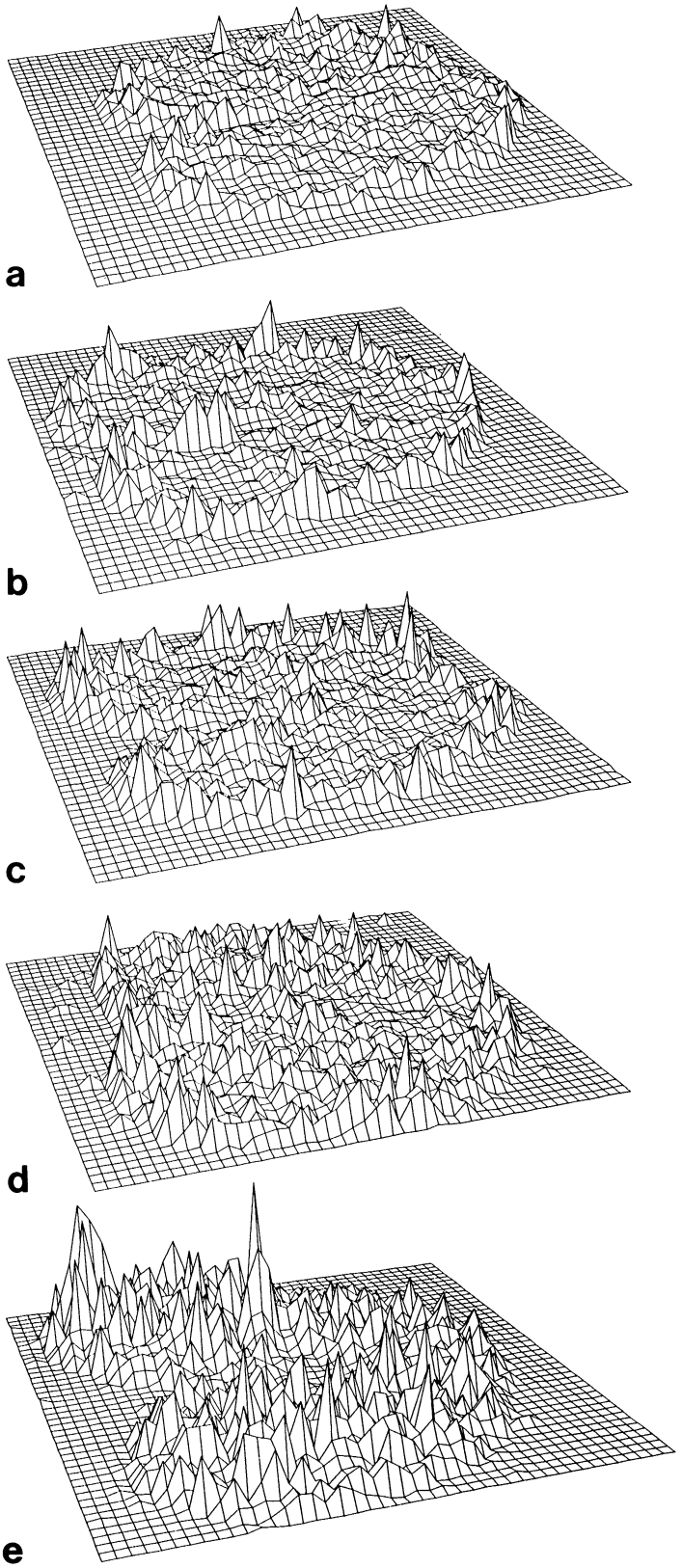

Fig. 4. Distribution of β-glucan (%, dwb) in the central region of five oat cultivars as detected by microspectrofluorometric scanning of the relative fluorescence intensity of bound Calcofluor. **a,** Donald, 3.7%; **b,** OA516-2, 4.0%; **c,** Tibor, 4.6%; **d,** Woodstock, 5.1%; **e,** Marion, 6.4%.

Distribution of β-Glucan in Barley

Barley cultivars with a broad range of β-glucan contents were selected to compare with oats. Results suggest that, regardless of β-glucan level, the distribution of β-glucan in barley (Fig. 5) is similar to the distribution found in the central region of Marion oats. In the limited set of cultivars examined, there appeared

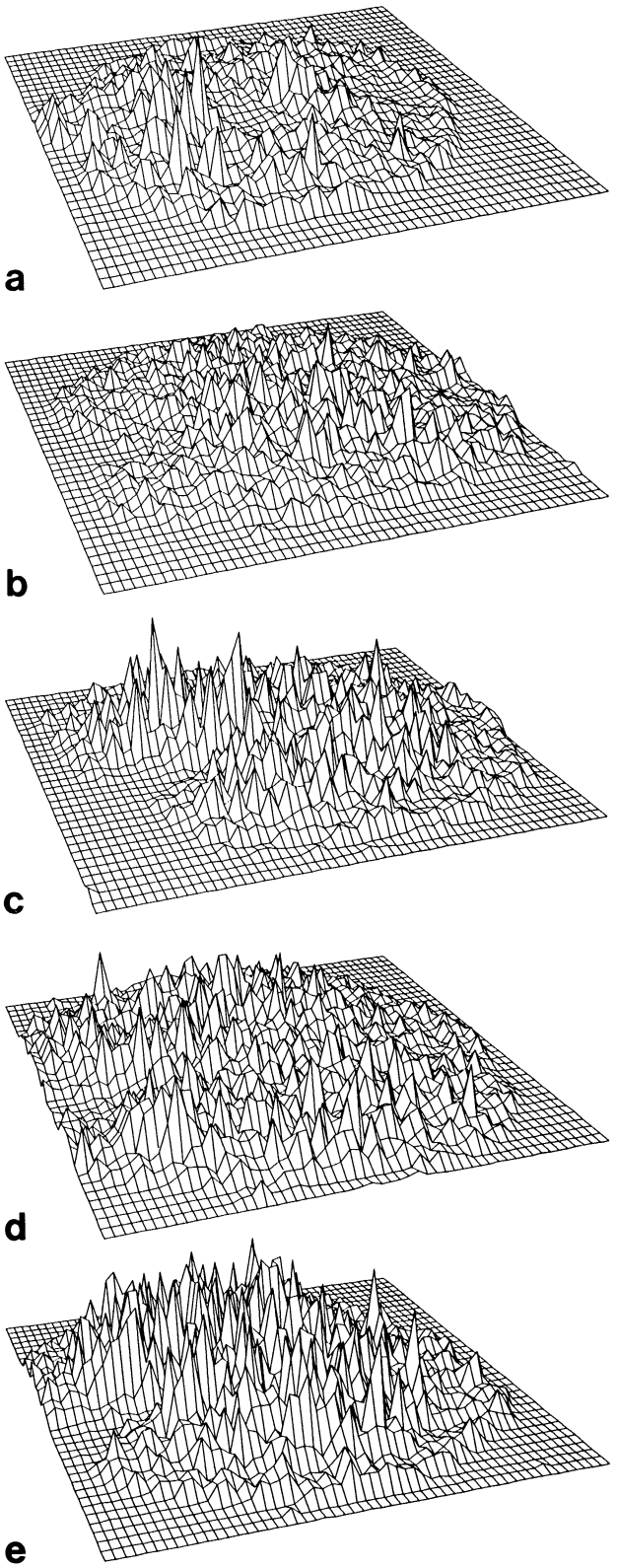

Fig. 5. Distribution of β-glucan (%, dwb) in the central region of five barley cultivars as detected by microspectrofluorometric scanning of the relative fluorescence intensity of bound Calcofluor. **a,** M-737, 2.8% ; **b,** Chalky Glenn, 3.2%; **c,** Leger, 5.7%; **d,** Minerva, 6.0%; **e,** Arizona, 11.0%.

to be no particular concentration of β-glucan in the subaleurone region (with the possible exception of M-737, which is discussed below), and the highest levels were observed in the center of the kernel.

The range of β-glucan content in the five barley cultivars was wider than the range in the oat cultivars. The barley samples contained two mutants with low β-glucan contents (M737, 2.8%; Chalky Glenn, 3.2%) and one waxy, hull-less cultivar with an extremely high β-glucan content (Arizona, 11.0%). The low β-glucan cultivar M-737 (Fig. 5a) was produced by mutation with sodium azide from the higher β-glucan cultivar Minerva (Fig. 5d). M-737 contained walls approximately half as thick as those in the parent variety Minerva (Aastrup 1983). In the present study, examination of thin sections showed two different populations of cells in M-737 (Fig. 3c). A "normal" population with walls similar to those in Minerva was found in the exterior regions of the kernel. A "mutant" population with very thin walls was present in varying proportions in the interior of the kernel. The differences in wall thickness observed correspond to different levels of β-glucan in different parts of the kernel shown by the intensity profile (Fig. 5a). Similar, but lesser, variations in wall thickness were observed in Chalky Glenn, which is also a low β-glucan mutant. The high β-glucan cultivar Arizona had very thick walls and very high RFI levels throughout most of the endosperm (Fig. 5e). The data presented in Figure 5 are not comprehensive as they represent only five cultivars of barley. Three of these are more unusual than most commercially available varieties, being mutants with extremes of β-glucan content. Nevertheless, the data does confirm the reported (Aastrup 1983) relationship between wall thickness and β-glucan content in barley.

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