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DAMAGE RESISTANCE AND MICROSTRUCTURE OF BARLEY KERNELS

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A b s t r a c t. Relations between mechanical properties, damage susceptibility and microstructure of five Polish cultivars of barley were examined. Brabender Hardness test, Instron measurements, PSI, PRI, and glassiness were used to characterise mechanical properties. The structure analysis was carried out with scanning electron microscopy and X-ray microscopy. Mechanical properties of grain were related to barley cultivar. Grain of cultivars with glassy endosperm were characterised by higher mechanical resistance than those with floury endosperm. Particle size distribution of the extracted starch granules, as determined by digital image analysis, did not correlate with the mechanical properties of barley grains. Generally speaking, the number of damaged kernels correlated with the fracture resistance and was related both to the direction of compression force and water content of grain. Compression along the axis of kernel thickness always resulted in a high number of damaged creases. When water content was high no damaged to the crease zone and a low number of internal damage was characteristic of kernels compressed along the width axis. It was observed that barley endosperm cracked inside cells, while the spaces between cell walls remained unfractured.

K e y w o r d s: barley, damage susceptibility, mechanical properties, microstructure

INTRODUCTION

In recent years, barley is widely used in human consumption not only in the form of alcoholic beverage but also as a roller-milled product or grits because of its high level of β-glucans which are an important source of dietary fibre [3-5]. Yokoyama *et al.* [17] suggested that cereal food products with reduced glycemic response can be an economic source of soluble fibre, when supplemented with natural ingredients such as β-glucan-enriched barley flours. Yet, some disadvantages are found in the processing and consumption of barley. Mena *et al*. [6] identified a 14.5 kDa barley-endosperm protein that is a major allergen in the baker's asthma disease.

Apart from the traditional physico-chemical techniques (NIR, β-glucan, protein, and starch content or malt extract yield determination) leading to an early assessment of malting quality, new interesting propositions based on the physical properties of grain were presented. Del Moral *et al*. [7] using, among others, image analysis of grain in predicting malting quality, drew attention to the physical properties of grain. Therefore, the use of grain mechanical properties for the accurate prediction of barley processability or in order to ensure satisfactory quality of the final product could be considered. On the other hand, behaviour of barley grain during technological treatments is influenced by their differentiated mechanical resistance [5]. An extensive knowledge about both mechanical properties and factors affecting grain hardness such as microstructure, is necessary for the design of new equipment and processes. Then, the objective of the present work was to determine the relation between grain mechanical properties and/or damage susceptibility and microstructure of some Polish barley cultivars.

MATERIAL AND METHODS

Five Polish barley varieties: Boss, Drop, Edgar, Gil, and Kos, harvested in southern Poland, were examined.

Protein content (Nx 6.25) in whole barley kernels was determined with the standard Kjeldahl's method and starch content was determined by the polarimetric method according to Evers [8]. Determinations were made in triplicate.

Percentage of vitreous kernels was determined for 50 kernels with the Farinotom according to the Polish Standard No PN - 53/R - 74008.

Germination ability was determined according to the Polish Standard No BN- 87/ 9131-13.

Grinding resistance of bulk grain was measured with a Brabender Farinograph (Germany) equipped with a grinding device. Grinding work (L_j) and maximum torque moment (M_{max}) were assumed to be the measures of grain hardness. Determinations were made in triplicate.

Kernel resistance for the compression was measured with an Instron 1011 compression device (Instron Ltd, England) at the crosshead speed of 10 mm min^{-1}. Both ends of the kernel were cut off with a surgical blade. Cylindrical samples were compressed uniaxially. The area of circle, diameter of which was equal to the average diameter of the kernel was assumed as contact surface. Stress, MPa, strain, mm mm^{-1}, and energy, mJ, at rupture-point were taken as characteristic parameters of kernel resistance to fracture. Determinations were made in 30 repetitions.

Particle Size Index was determined according to Williams and Sobering [16] in five repetitions.

Starch granules were isolated according to the method described by Bechtel *et al.*[2]. A drop of suspension of glycerol-rehydrated starch granules was mixed with iodine on a microscope slide, covered with cover glass, and examined with a Biolar microscope. Granule size distribution was measured using the DIA method [10].

Model damaging of kernels with 11± 0.2% and 14.5 ± 0.2% water content was carried out using an Instron 1011 compression device, (Instron Ltd, England) at the crosshead speed of 10 mm min^{-1}. Undamaged kernels were compressed perpendicularly to the kernel crease along the width and thickness axes (Fig. 1). Compression was stopped below the kernel yield-points (different for each cultivar). Internal damage of raw and model-damaged kernels were determined by the X-ray method using an Elektronika 25 Apparatus (St. Petersburg, Russia) [14]. Determinations were made for 72 kernels.

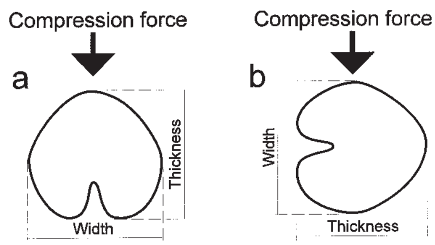

Fig. 1. Position of barley kernels during compression.

Specimens of cross-sectioned barley kernels (1.5 mm thick) coated with carbon and gold were examined in a JS 5200 scanning electron microscope (SEM) at 15 kV. Microphotographs weretaken with a Nicon camera. SEM micropictures of damage were taken for the carefully sectioned kernels in which damage was previously localised using the X-ray analysis. Five glassy and five mealy kernels from each cultivar were observed.

Statistical analysis of the results was carried out with the Statistica ver. 5 (StatSoft, USA) programme [13].

RESULTS

Protein content in barley grain of the examined cultivars ranging from 8.93 for cultivar Gil to 11.51 % of dry matter (d.m.) for the cv. Edgar (Table 1) was rather low when compared to Canadian, USA and Japanese barleys, i.e., from 12.8 to 17.4 % d.m. [3], from 8.7 to 17.8 % d.m. [4], and 12.5 to 14.9 % d.m. [5], respectively. Glassiness values, ranging from 13.0 (cv. Gil) to 69.0% (cv. Edgar), allowed for characterising cv. Drop, Gil, and Kos as mealy and Edgar and Boss as glassy grains (Table 1). Starch, the largest single component in barley, representing up to 60% of kernel dry matter, affected many of their physical properties (Table 1). Isolated starches generally had similar bimodal granule size distribution ranging from 2 to 30 μm, except a trace amount of granules larger than 30 μm in Boss and Drop starches. Large granules (≥ 8 μm) accounted for 13.1 to 29.0% of the total starch granules (Fig. 2). Granule size distributions of the examined starches were similar to those of the non-waxy barley starches reported by Vasanthan and Bhatty [15], who examined small (2-10 μm) and large (12-26 μm) granules in starch from waxy, regular and high-amylose barley types.

Extremely high percentage of internally damaged kernels (from 78.7 to 100%) and high percentage of defected germs (1.5 to 6.0% damaged and 2-16.2% dark germs) decreased germination ability of the examined barleys (from 59 to 73%) (Table 1). Savin *et al.* [12], also using the X-ray method for determining the amount of seed damage, confirmed little effect of cracking of the endosperm on seed germinability, in contrast to the marked effect of damage to the germ. They calculated that not more than 10.8% of seeds had more than 4

T a b l e 1. Characteristics of barley grain

Cultivar	Protein content (% d.m.)	Starch content (% d.m.)	Glassiness (%)	Internal damages (%)	Germ (%)		Germination ability (%)
					dark	damaged	
Boss	10.51 ± 0.09	65.70 ± 1.57	40.00 ± 7.92	91.2	6.7	1.5	64 ± 3.21
Drop	10.59 ± 0.11	62.92 ± 0.91	13.00 ± 7.22	98.0	9.5	6.5	65 ± 4.15
Edgar	11.51 ± 0.06	64.66 ± 2.03	69.00 ± 9.01	78.7	3.5	1.5	77 ± 2.88
Gil	8.93 ± 0.07	64.97 ± 1.20	13.00 ± 3.84	98.0	16.2	1.5	59 ± 4.09
Kos	10.00 ± 0.09	58.42 ± 0.44	17.00 ± 4.60	95.0	2.0	6.0	73 ± 5.59

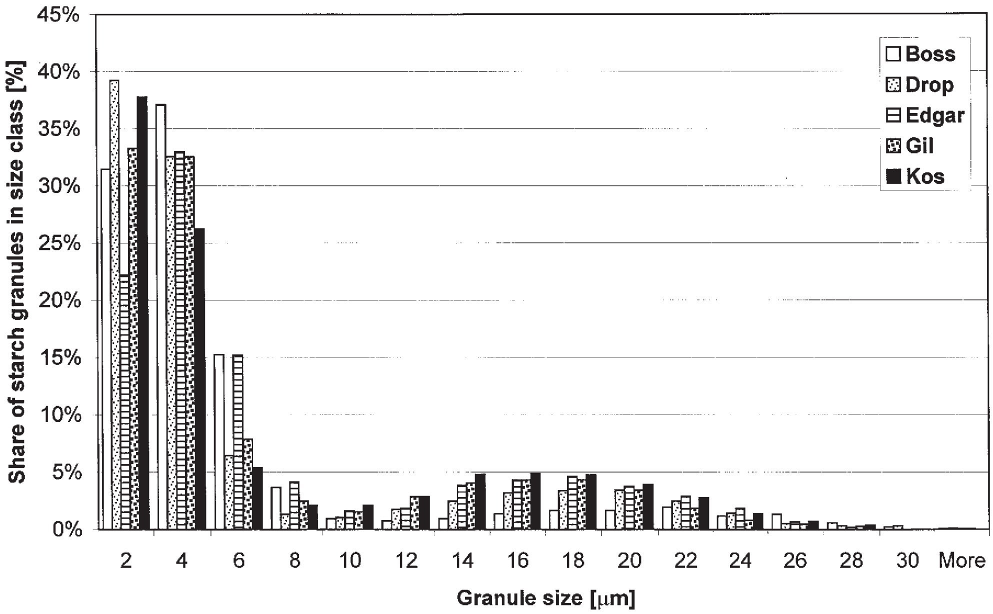

Fig. 2. Size distribution of starch granules in barley grains.

cracks in the endosperm, 7.4% of seeds had germ damaged and 7.6% of seeds were unfilled which reduced grain yield by >5% [12].

The nature and extent of damage is related to the rheological and mechanical characteristics of grain. So, Brabender hardness and Particle Size Index test were used to examine barley grain. Since mechanical damage of agricultural product usually results from compressive load, the compression test was used to characterise a single barley kernel, the viscoelastic behaviour of which can be adequately described by a three-term Maxwell model [1]. Although, correlations between the results of the Brabender hardness, PSI, and compression tests are rather weak, the determined indices of mechanical resistance for the examined cultivars showed the same tendency (Table 2). The values for the PSI test (from 40.0 to 47.1) allowed for the classification of all the barley grain as very hard (according to Williamson [16] wheat is very hard at PSI < 50). Boss and Edgar cv. grain with glassy endosperm were characterised by higher compression resistance, grinding energy, and torque moment, and lower PSI than Drop, Gil, and Kos cv. grain with mealy endosperm (Table 2). Different size of kernels, which influenced especially L_j (Brabender test), and small number of the examined cultivars was probably the main reason for weak correlation.

Granule size distribution of wheat starch was assumed to be a good factor for the discrimination between hard, soft, and durum wheat [18]. Similarity in the relations between starch size distribution and grain hardness in barley was looked for. Size distribution of starch granules, as determined by the digital image analysis, did not, however, correlate with the mechanical properties of the barley grain examined. Vasanthan and Bhatty [15] also concluded that, since small granules comprised a minor proportion of total starch mass (because of a low percentage of the total number of granules), differences in the physico-chemical properties of barley starches were greater among the genotypes than between small and large granules.

It is known that mechanical properties of grain, related to species and varieties of cereals, are closely related to kernel-endosperm microstructure. Wide variation in the structure of barley endosperm was found in respect to compaction of small starch granules and protein matrix [11]. Microscope observations (SEM) of the microstructure showed, however, only small differences in the endosperm of the examined barley cultivars. Barley kernels with glassy endosperm (Photo 1a) were characterised by a little thicker cell walls and stronger compaction than those in mealy endosperm and by starch granules embedded in the protein network (Photo 2a). Palmer [9] obtained different microphotographs of barley endosperm when investigating potential importance of endosperm-structure for determining quality of malting barley. He found mealy endosperms to be more readily degradaded by enzymes during malting than glassy endosperms. This suggests that compact areas localised in glassy endosperms may still release β-D-glucan which retards wort separation and restricts beer filtration.

Grain susceptibility to damage related to their water content was studied in a model experiment. Higher total number of internal damage for the compression tests along the axis of thickness than width was observed. The number of damage at both directions of compression were strongly related to the water content (Table 3). It is worth noticing that damage appeared mainly in the kernel medium zone, indepeden of its water content and direction of compression. Close correlations ($0.7321<R<0.9649$) were found between the number of damage for different force directions and water content levels. This suggests that the number of internal damage was correlated to fracture resistance of kernels. X-ray photos (1b and 2b) confirmed different kernel susceptibility of mealy (low mechanical resistance) and glassy (high mechanical resistance) barley cultivars to damage. Photos 1c,d and 2c,d present details of a typical crack in a mealy and glassy endosperm. It was observed that the crack of barley endosperm appeared inside the cells, while

T a b l e 2. Mechanical properties of barley grain

Cultivar	PSI (%)	Brabender test		Compression test		
		Lj (kJ kg^{-1})	M_{max} (N m)	fracture stress (MPa)	fracture strain (mm mm^{-1})	fracture energy (mJ)
Boss	42.0 ± 2.20	32.21 ± 0.580	4.77 ± 0.193	37.2 ± 6.92	0.214 ± 0.042	160.5 ± 19.86
Drop	47.1 ± 1.31	25.71 ± 0.202	4.25 ± 0.328	26.3 ± 5.63	0.213 ± 0.045	107.7 ± 21.46
Edgar	40.0 ± 3.19	28.58 ± 0.699	4.64 ± 0.193	37.9 ± 6.12	0.199 ± 0.034	152.0 ± 26.70
Gil	46.7 ± 1.11	27.09 ± 1.018	3.23 ± 0.184	21.1 ± 2.57	0.179 ± 0.056	59.0 ± 12.25
Kos	44.1 ± 4.25	26.12 ± 0.152	4.01 ± 0.193	26.7 ± 5.18	0.203 ± 0.043	93.0 ± 19.77

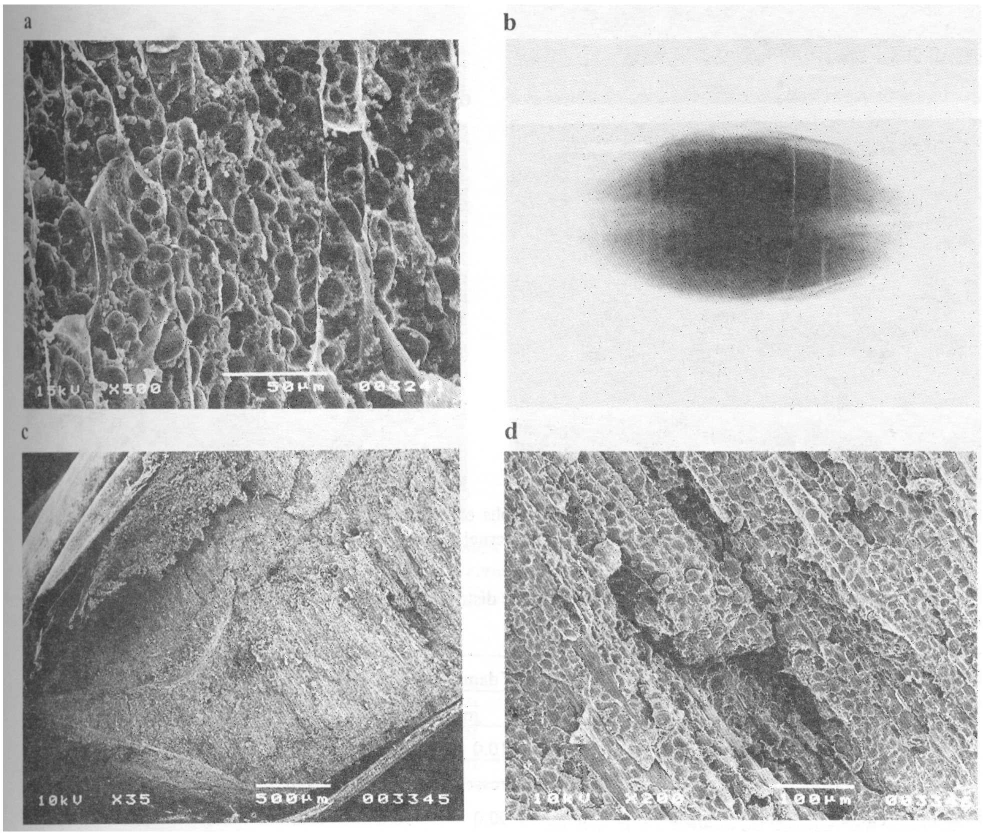

Photo 1. Micrographs of a glassy endosperm. SEM micrographs of a glassy kernel endosperm (a), X-ray picture of kernel (b), SEM micrographs of damage details: longitudinal section of a kernel (c), crack across endosperm (d).

the spaces between cell walls were not fractured. Damage of crease was mostly related to the direction of compression. While the compression along the thickness axis resulted in a high percentage of crease damage (from 12 to 48) irrespective of water content, the compression along the width axis did not damaged creases even in kernels with 14.5% water content. Damage in the kernel germ zone resulted in an extremely disadvantageous decrease of the sowing potential and easy enzyme and microbe action during storage. Photos 3a-d shows details of the typical forms of germ damage found in the examined barley grain.

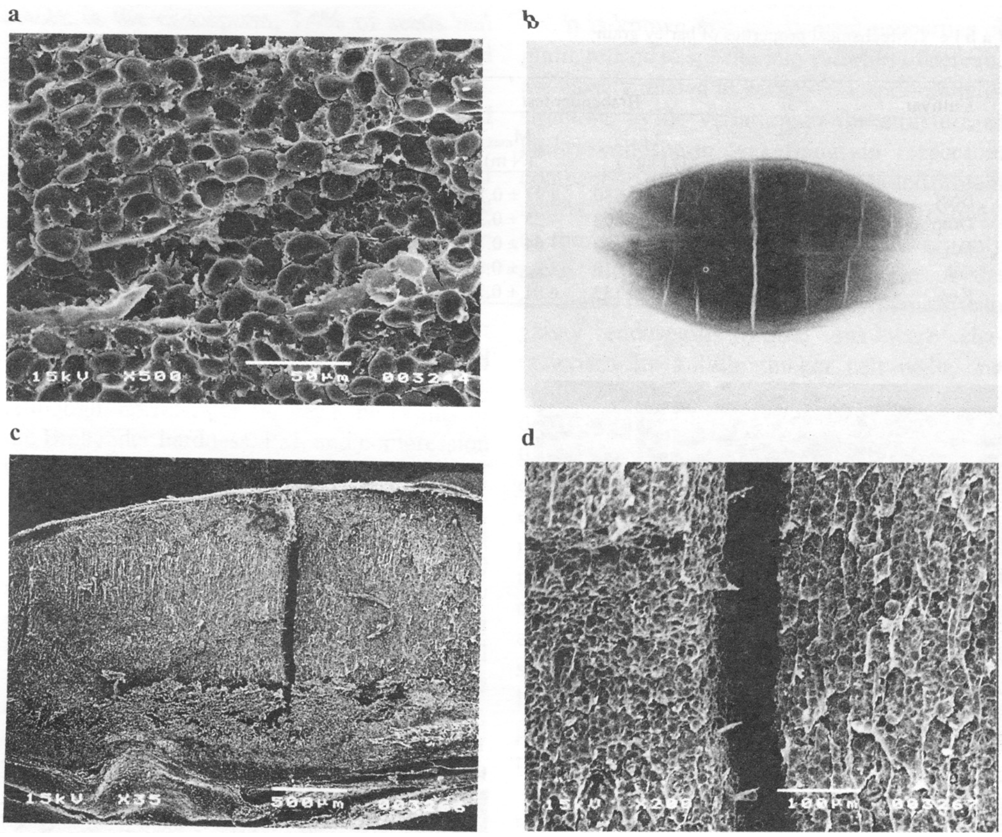

Photo 2. Micrographs of a meal kernel. SEM micrographs of a meal kernel (a), X-ray picture of a kernel (b), SEM micrographs of damage details: longitudinal section of a kernel (c), crack across endosperm (d).

T a b l e 3. Effect of water content on the internal damage distribution in the kernel compressed along the thickness and width axes

Cultivar	Number of internal damages (%)		Percentage of damages in the kernel zone						Percentage of damged creases	
			upper		medium		germ			
	10.0	14.5	10.0	14.5	10.0	14.5	10.0	14.5	10.0	14.5
	kernel compressed along thickness axis									
Boss	2.40^{a}	2.04^{a}	0	0	90.0	90.2	10.0	9.8	16.0^{a}	16.0^{a}
Drop	3.44^{b}	2.92^{d}	7.0	5.5	88.4	87.7	4.6	6.8	25.0^{a}	28.0^{b}
Edgar	2.65^{a}	2.20^{b}	0	0	90.2	100.0	9.8	0	34.7^{b}	48.0c
Gil	4.12^{c}	2.96^{d}	2.9	0	89.4	98.7	7.7	1.3	12.0^{a}	13.0^{a}
Kos	3.52^{b}	2.64^{c}	2.3	0	90.9	100.0	6.8	0	20.0^{a}	28.0^{b}
	kernel compressed along the width axis									
Boss	2.33^{a}	1.56^{a}	7.1	0	83.2	92.0	9.7	8.0	5.5^{a}	0
Drop	3.05^{c}	2.27^{b}	3.6	0	89.1	87.8	7.3	12.2	11.1^{b}	0
Edgar	2.11^{a}	1.72^{a}	0	3.2	84.2	93.6	15.8	3.2	0	0
Gil	2.76^{b}	2.33^{b}	2.1	2.4	95.8	83.3	2.1	14.3	27.8^{c}	0
Kos	3.11^{c}	2.22^{b}	3.6	0	92.8	97.5	3.6	2.5	11.1^{b}	0

Values are means of 30 determinations.The same superscripts in the column denote the Duncan's homogenous subsets.

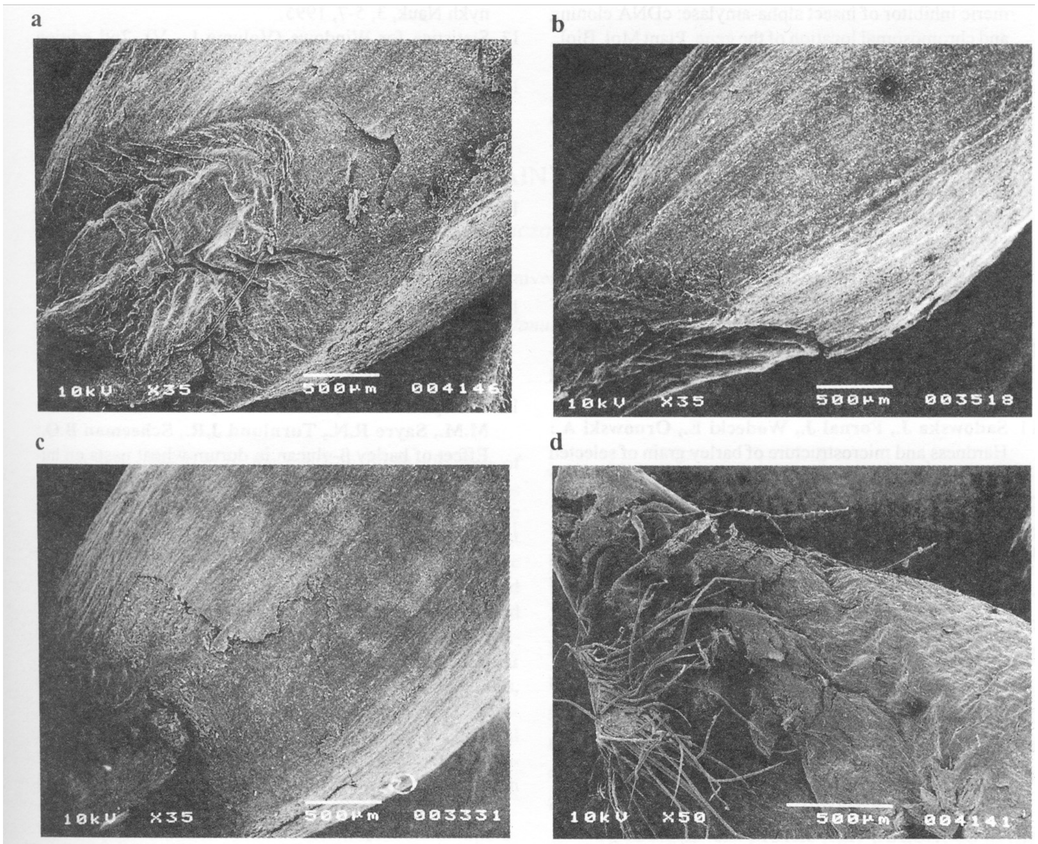

Photo 3. Micrographs of a meal kernel. SEM micrographs of a meal kernel (a), X-ray picture of a kernel (b), SEM micrographs of damage details: longitudinal section of a kernel (c), crack across endosperm (d).

CONCLUSIONS

1. Statistical analysis of the results for all the tests confirmed the significant influence of the cultivar on the mechanical resistance of barley grain.

2. Number and distribution of internal damages were mostly related to both moisture, that affected mechanical resistance, and direction of the compressing-force action.

3. A significant number of internal cracks across the cells and undamaged intercellular spaces prove that the bonds between walls of the adjacent cells were stronger than those between the cell structural compounds.

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