

Pre-harvest stress cracks in maize (*Zea mays* L.) kernels as characterized by visual, X-ray and low temperature scanning electron microscopical analysis: effect on kernel quality

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Abstract

Internal cracks caused by high temperature or excessive moisture during maize (*Zea mays* L.) kernel development were characterized, and their effects on kernel quality were assessed. Pre-harvest stress cracks are often located near the middle of the kernel along the embryo axis, but they were also detected in other positions, irrespective of the shape of the kernel. X-ray analysis enabled visualisation of stress cracks that are invisible to the human eye and, therefore, gave a better estimate of the percentage of cracks. However, low temperature scanning electron microscopy of the surface of milled kernels revealed small cracks not noticed by visual or X-ray inspection. All kernels tested in this way had a crack of some sort in the endosperm tissue. Cracks were also frequent in the scutellum, but rare in the embryo axis. Endosperm cracks followed the boundary of the starch granules, but did not extend into the pericarp tissue. In contrast to external cracks caused by mechanical impact, pre-harvest internal stress cracks generally are not detrimental to germination and vigour. However, if the crack is located inside or perpendicular to the embryo axis, it may affect the quality of the kernel, probably by impeding nutrient translocation to the embryo.

Keywords: cracks, germination, kernel, scanning electron microscopy, vigour, X-ray analysis, *Zea mays* L.

Introduction

Many attributes of the seed influence its quality, some of which are physical in nature. The relationship between physical attributes and quality is interesting because of the potential for quality improvement during seed production and processing. Various defects in maize kernels can be induced during production, harvest, drying, storage, and handling (Escasinas and Hill, 1988; Peterson et al., 1995; Burris et al., 1997). Defects such as pericarp cracks, caused by mechanical stress are easily detected. However, internal cracks caused by thermal, moisture, or mechanical stresses may not be readily identifiable.

Seed producers from the northern region of the Minas Gerais State in Brazil have observed that stress cracks in maize kernels ensue when the temperature in the field fluctuates considerably during the final stages of kernel development. This also occurs when the drying rate of the harvested kernels is very fast (Gunasekaran and Paulsen, 1985; Burris et al., 1997). The type of damage induced by pre-harvest stress has not been well characterized, but Gunasekaran et al. (1985), using scanning electron microscopy (SEM), noted that some stress cracks were internal fissures in the maize kernel endosperm which may not extend into the lower surface of the pericarp. These stress cracks originate at the centre of the floury endosperm and propagate toward the kernel periphery along the boundary of starch granules. Such fissures do not necessarily develop during drying, but can do so during subsequent storage (Sharma et al., 1979; Gunasekaran et al., 1985).

The usual method to detect cracks is visual inspection of maize kernels by holding the embryo side towards a light source (Chowdhury and Buchele,

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1976; Chowdhury, 1977; Gonçalves, 1981; Carvalho *et al.*, 1994). However, if the mechanical damage or stress cracks are internal or invisible to the human eye, there is a definite need for a more accurate and automatic system of evaluation, such as X-ray and image analysis. Girardin *et al.* (1993), for example, concluded that X-ray radiography was the best non-destructive method to estimate the characteristics of maize kernels that differed in weight, width or position on the ear. Cicero *et al.* (1996), using a combination of X-ray techniques and digital image processing, have demonstrated that X-ray analysis can be used to relate internal and external mechanical damage of maize kernels with abnormal seedling development. However, damage assessment is not always sufficient to predict the quality of the resulting plant (Chowdhury and Buchele, 1976; Zaleski *et al.*, 1992). Moreover, mechanical damage, independent of the extent, can increase the rate of kernel deterioration in storage (Escasinas and Hill, 1988; Sato and Cicero, 1992). These studies on the relationship between kernel physical attributes and vigour or plant performance apparently have produced variable results, which means that no clear relationship between damage to kernels and subsequent performance of the plant has been established. This is understandable because reduction of kernel quality may depend on many factors apart from physical damage, such as kernel hardness (Peterson *et al.*, 1995) and variability between different genotypes (Adegbuyi and Burris, 1988; Vyn and Moes, 1988; Herter and Burris, 1989; Plett, 1994).

The current project was aimed at characterizing stress cracks that developed in kernels before harvest and their effects on kernel quality.

Materials and methods

Plant material

The hybrid maize kernels, AG 122, used in this project, were produced in a seed production field in 1996 at Paracatu, Minas Gerais State, Brazil. The kernels developed under conditions of excessive moisture (heavy rains) and high day-temperature (33–42°C), and were hand-harvested at 24% moisture content (fw-basis) and hand-shelled. The kernels were divided into two sublots, one with and one without apparent visual cracks in the endosperm.

Internal cracks detected by X-rays

The X-ray inspection was made with the same samples as used for visual stress crack determination, using 12 replications of 25 kernels per treatment (with or without cracks). The X-ray machine (FAXITRON,

model 93805N, Hewlett Packard, Oregon, USA) was operated at 15 keV, 2 mA. The kernels were placed on a small cassette-type holder with a transparent bottom, directly on top of the film at a distance of 35 cm from the X-ray source, for 2.5 min. The professional copy film N 4125 was developed in Agfa Neutol general developer and examined using a microfiche reader.

Kernel quality evaluation

After visual selection of the kernels according to the presence of pre-harvest cracks, further X-ray inspection was performed. Both evaluation methods are non-destructive. Three categories were assembled:

- 1 Visually perfect kernels, also without cracks according to X-ray analysis.
- 2 Kernels with visual cracks, also with cracks according to X-ray analysis.
- 3 Kernels without visual cracks, but with internal cracks as determined by X-ray analysis.

These kernels were submitted to a cold test without soil according to the AOSA (1983). The kernels with and without visual cracks were evaluated by the standard germination test according to the MARA Rules of Seed Testing, Brasil (1992), an established tetrazolium test to estimate vigour and viability level (MARA, 1992), determination of the dry matter, and length of seedlings (Dias and Barros, 1995). These tests were conducted on four replicates of 50 kernels. The substrate moisture for the germination test was 3 times the weight of the filter paper that was used in the germination test, i.e., 600 ml H₂O for 200 g of filter paper.

Kernel crack characterization by scanning electron microscopy

Maize kernels with and without visual internal cracks were maintained at 25°C for 12 h in a dry environment or in distilled water. After these treatments, the kernels were vertically glued in a brass specimen holder with TBS (Tissue Freezing Medium, Electron Microscopy Sciences, Washington DC, USA). The electrical conductivity of the TBS was improved by mixing this glue with charcoal powder. To avoid freezing stress that may result in cracks, the samples were frozen slowly. The specimen holders with the maize kernels were placed in a Styrofoam (Tempex) box and placed in a low temperature freezer (−70°C) where the temperature dropped at approximately 5°C min^{−1}. After 16 h, the box was taken out of the freezer, and the samples were further cooled very slowly to −196°C by filling the box stepwise with small amounts of liquid nitrogen over a period of 2 h.

The internal structure was exposed by using a low temperature milling apparatus (Polycut, Reichert Jung, Wien, Austria). During milling at 10 μm per round with a diamond knife, the samples were kept at liquid nitrogen temperature. After milling, the samples were placed in a cryo-preparation stage (CT 1500 HF, Oxford Instruments, Oxon, UK), and the temperature was raised to -90°C at 10^{-6} torr to sublimate contaminating water vapour and to freeze-dry the milled surface quickly. After sputter-coating with 5 nm platinum, the samples were examined at -193°C with a field emission low temperature scanning electron microscope (LTSEM) (JSM 6300F, JEOL, Japan). A total of 20 kernels were inspected.

Experimental analysis

All kernels were arranged in a completely random design. Statistical tests were applied as described by Snedecor and Cochran (1989). For comparisons between means, analysis of variance and t-test were used.

Results and discussion

Visual characterization of preharvest stress cracks

The internal cracks of kernels, which are visible to the eye are shown in Fig. 1. Most pre-harvest stress cracks were located near the middle of the kernel, along the embryonic axis (Fig. 1A), but they were also detected in other positions, irrespective of the shape of the kernel (Fig. 1B). Sometimes, cracks perpendicular to the embryonic axis and at various angles were found. Although the kernels were hand-harvested and hand-threshed, almost 85% of the kernels had an internal crack of some sort, as judged by visual inspection. Fig. 1C shows kernels after treatment for 2 min. with a 2% amaranth dye solution. External cracks as a result of mechanical impact were stained by dye, whereas the internal preharvest stress cracks remained unstained. We interpret this to mean that the internal endosperm cracks did not reach the periderm surface. These results are in accordance with those of Peterson *et al.* (1995), who observed that the fast green test can discriminate between internal cracks and external cracks that are caused by mechanical harvesting and processing.

Internal cracks characterization by X-ray analysis

The percentages of internal stress cracks in maize kernels calculated on the basis of X-ray analysis and visual evaluation are shown in Table 1. In kernel lots that were classified as perfect kernels by visual analysis, 38% had cracks when inspected by X-ray analysis, mainly of the internal type, perpendicular to

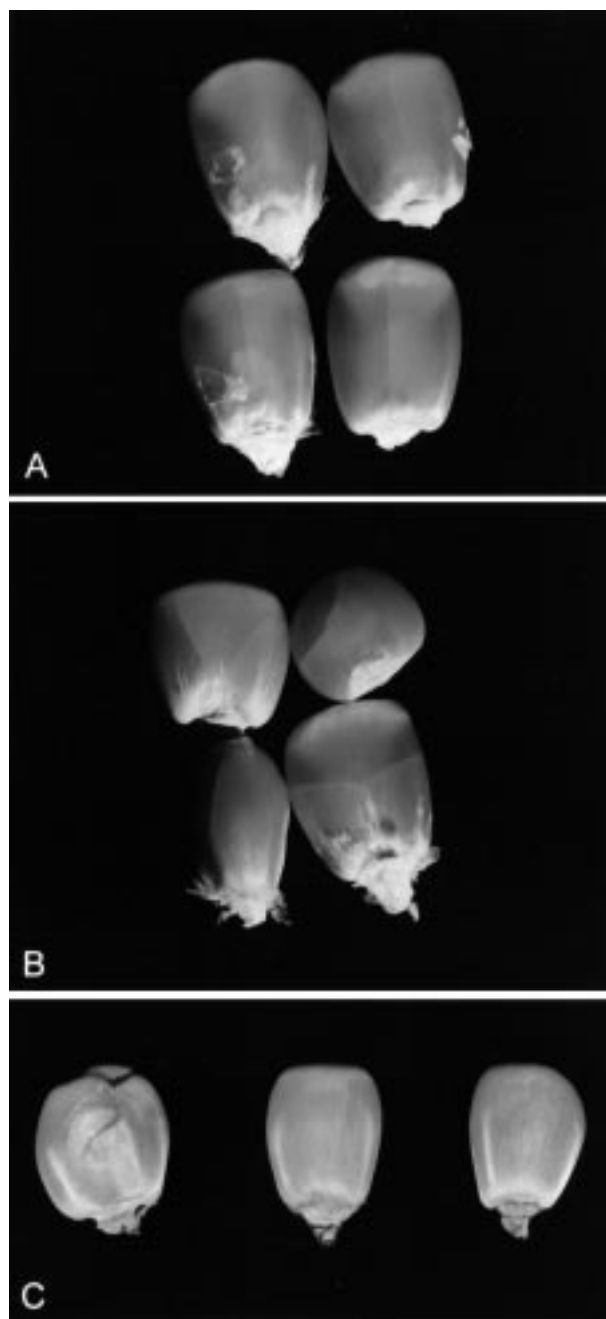


Figure 1. Internal preharvest stress cracks in maize kernels. (A) cracks parallel to the embryo axis; (B) cracks at several angles; (C) kernels treated with amaranth dye, left: external crack; middle: internal crack; right: perfect kernel.

the embryo axis (33.7%). Apparently, X-ray analysis permits visualisation of cracks that are invisible to the human eye and, therefore, provides a more precise estimate of the percentage of cracks. Nevertheless, some cracks visible to the human eye were not

Table 1. Percentage of X-ray-detected internal stress cracks in two maize kernel sublots (600 kernels each) that were selected on the basis of visual intactness. One subplot consisted of visually perfect kernels and the other of visually cracked kernels.

Sublots	Cracked kernels (%)		
	Visual	X-ray	X-ray (perpendicular cracks)
Visually perfect kernels	0	38.0	33.7
Visually cracked kernels	100	94.3	32.2

detected by the X-ray method, because 5.7% fewer cracks were observed with X-ray analysis in the subplot containing cracked kernels. This underestimation can be partially explained by the orientation of the crack within the kernel. If the crack is parallel to the X-ray beam, it will appear as a dot on the film, which is sometimes not recognized as a crack. If the crack is perpendicular to the X-ray beam, it will appear as a line and will be counted. In addition, the different shapes, weights and thicknesses of the kernels may reduce the chance that cracks are detected. A similar explanation for the underestimation of the percentage of cracks has been given by Reid *et al.* (1991), using computer vision sensing of stress cracks in maize kernels. To overcome the problem of overlooking cracks that are parallel to the X-ray beam, two X-ray images from different angles were made to improve precision (Girardin *et al.*, 1993), which was particularly effective in the case of round kernels that are situated at the base of the ears. Likewise, for better diagnosis of the effects of mechanical damage on germination of maize kernels, Cicero *et al.* (1996) advised X-ray radiographs to be taken from both the front and the back side of kernels. It is interesting to note that in the visually cracked kernels, approximately the same percentage of internal cracks perpendicular to the embryo axis was observed as in the visually perfect kernels. The visually unnoticed cracks apparently go together with the visually detected cracks.

Fig. 2A-D shows the different types of kernel cracks detectable by X-ray analysis: A, a perfect kernel; B, with cracks along the embryonic axis; C, with cracks perpendicular to the embryonic axis; and D, with both types of cracks. The most common type of damage found in maize kernels concerns the crack parallel to the axis (B), while the crack only detectable by X-rays occurs perpendicularly to the long embryonic axis (Fig. 2C).

Kernel crack characterization by LTSEM

A LTSEM study was conducted to characterize in detail cracks in the preselected sublots. LTSEM analysis may reveal small cracks that are unnoticed by either visual or X-ray inspection. Also, information can be obtained as to how far cracks protrude from the endosperm into the different kernel tissues, such as

pericarp, aleurone layer, scutellum and embryonic axis. To prevent development of new cracks during cooling of the samples for milling and LTSEM inspection, a slow cooling rate was applied.

In all of the eight visually perfect kernels inspected, some type of crack was observed, most often thin cracks around the scutellum inside the endosperm tissue. Also observed at high frequency were thin cracks in the scutellum, whereas very thin cracks in the embryo and the aleurone layer were observed in only one kernel. These observations have to be considered with caution, because for every individual kernel only one transverse image was obtained. Examples of the different damage types found are shown in Fig. 3. A visually perfect hydrated kernel with a crack around the scutellum inside the endosperm can be observed in Fig. 3A, with a detail of the intact embryonic axis of this kernel shown in Fig. 3B. A small crack in the scutellum of a dry visually perfect kernel is shown in Fig. 3C, with Fig. 3D giving details. A crack through the embryonic axis of a hydrated, visually perfect kernel is presented in Fig. 3E, with a detail in Fig. 3F.

As expected, large cracks in the endosperm tissue were found in the kernels selected for visual cracks [dry kernels in Figs 4A and B (detail)]. In all the twelve cracked kernels inspected, thin cracks were observed around the scutellum inside the endosperm tissue. Also thin cracks in the scutellum were common in the cracked kernels (85% of cases). Figures 4C and D show an example of a crack in the endosperm and scutellum of a dry kernel, but the embryo axis was intact. Embryos and aleurone layers were generally intact. Cracks in the embryo were only observed from two kernels; a crack in the aleurone layer was observed in only one kernel (not shown) out of 12 kernels.

On hydration, the cracks in the endosperm of the cracked kernels had a thinner appearance than in the dry state (Figs 4E and F for detail). Most likely, the swelling of the starch granules in the imbibed kernels caused the cracks to close. Examples of starch granules in the dry endosperm and after rehydration, are shown in Figs 5A and B, respectively. The starch granules were more angular in the dry kernels than in the imbibed kernels, in which the starch granules were rounder and more turgid.

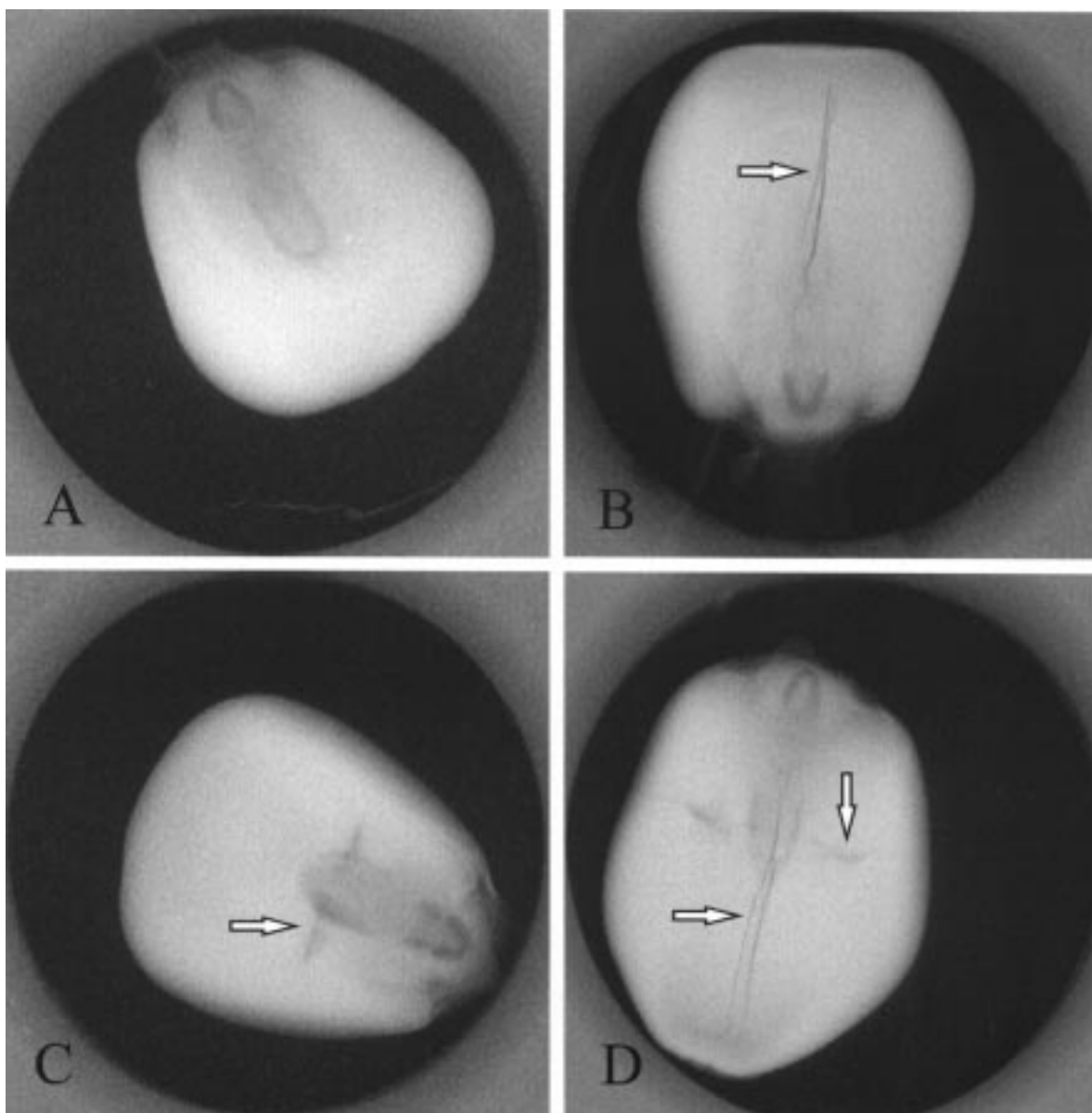


Figure 2. Internal preharvest stress cracks in maize kernels as detected by X-ray analysis. (A) perfect kernel; (B) cracks along the embryo axis; (C) cracks perpendicular to the embryo axis; (D) cracks as in (B) and (C).

The cracks followed the boundary of the starch granules (Fig. 5C). In no case were the preharvest stress cracks found to extend into the pericarp tissue (Fig. 5D).

Kernel quality evaluation

The kernels of the two sublots were analysed for their germination capacity, viability and vigour (Fig. 6A). The germination percentages were high (approximately 90%) and not significantly different for the

kernels selected visually as perfect or as having internal stress cracks. Considering the general occurrence of cracks around the scutellum inside the endosperm (100%) and in the scutellum (65-85%) in both the visually perfect and the cracked kernels (Figs 3 and 4), we conclude that such cracks have no major impact on germination. This is understandable for the endosperm cracks, because the endosperm is dead tissue in mature maize kernels (cf. Golovina *et al.*, 1997). The intact pericarp and aleurone layer (Fig. 5D) will likely keep leakage from the endosperm to a

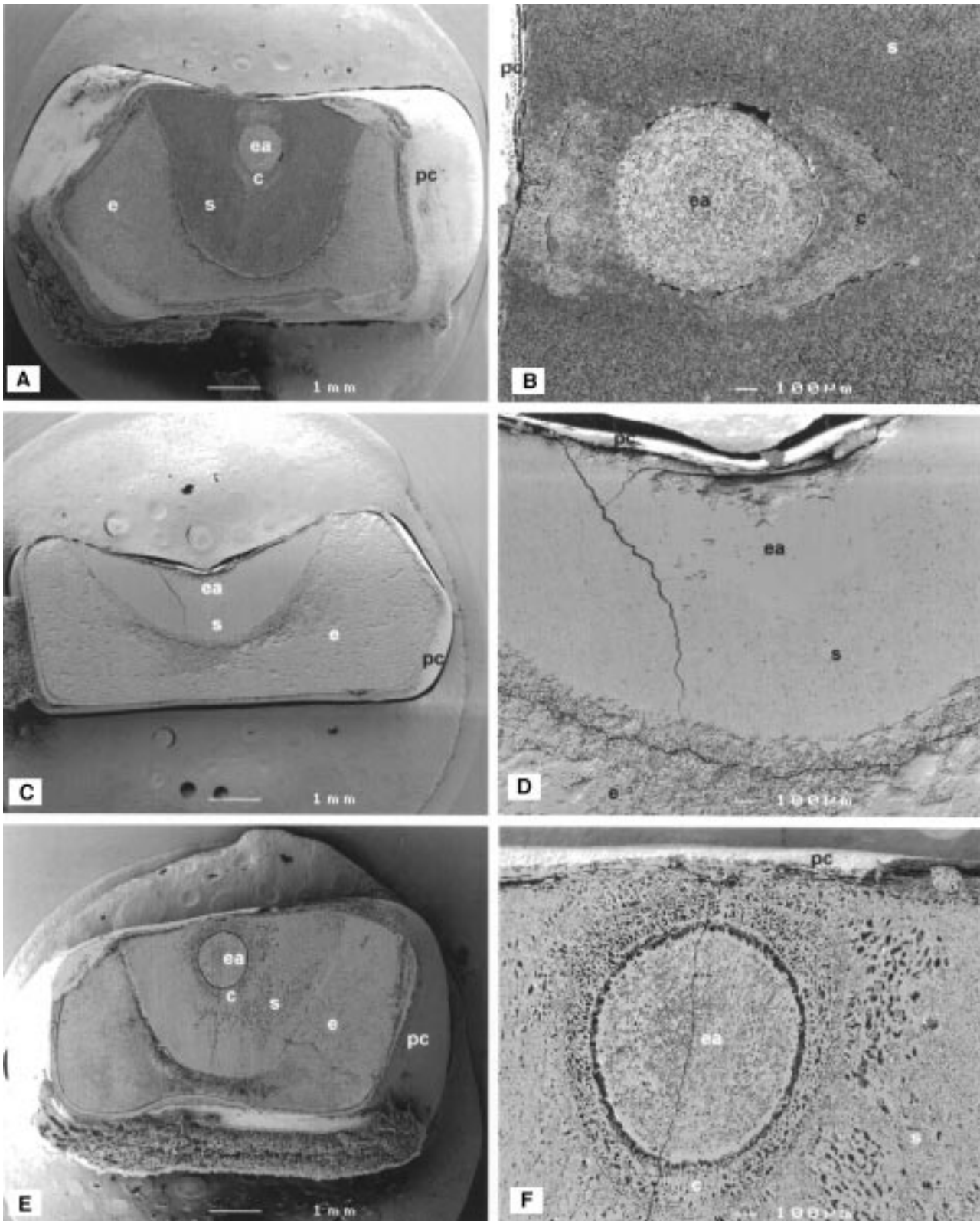


Figure 3. SEM images of the milled surface of visually perfect maize kernels. (A), (B), (E), and (F): seeds hydrated for 12 h; (C), (D): dry kernel. Abbreviations: c = coleoptile, e = endosperm, ea = embryo axis, s = scutellum, pc = pericarp.

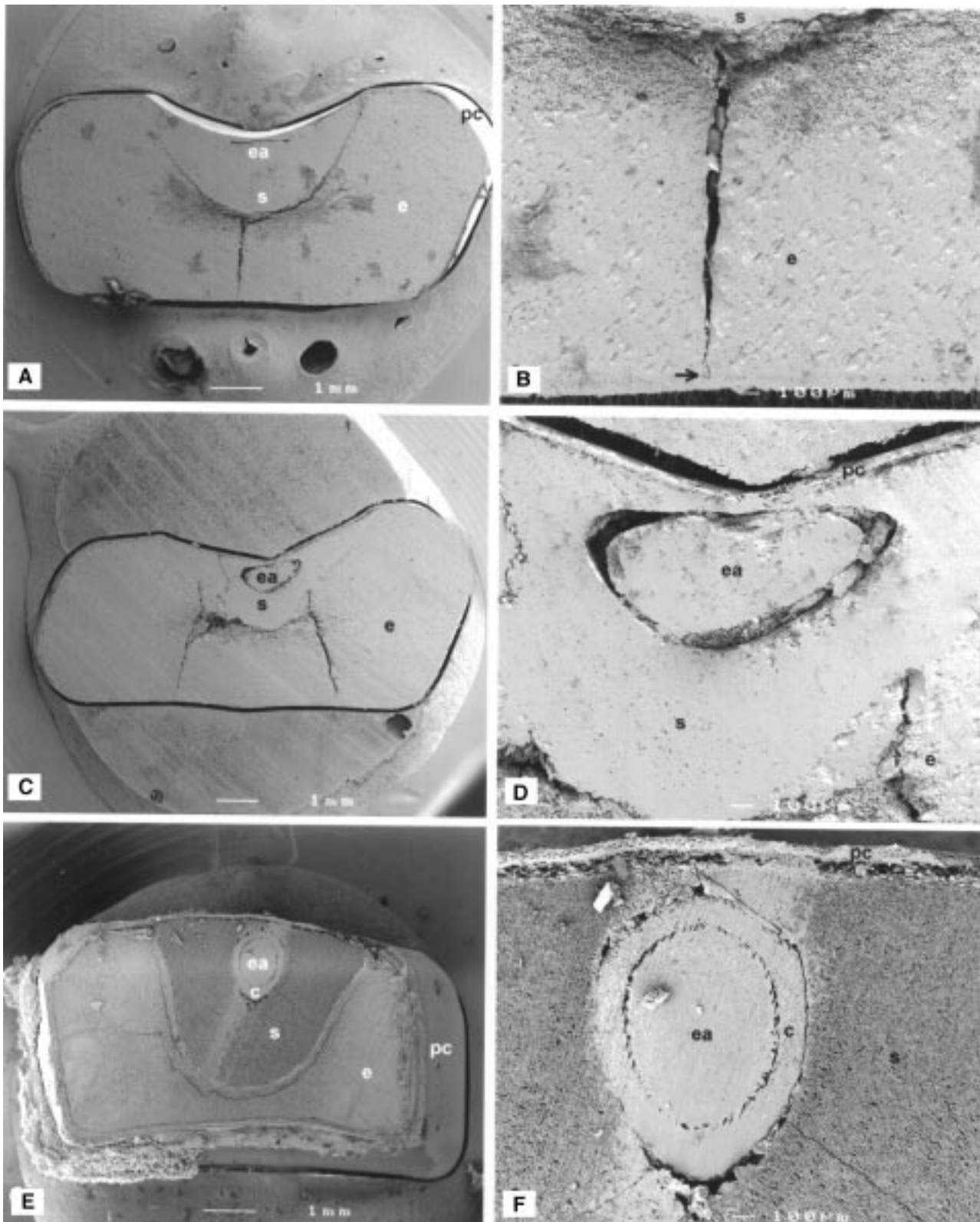


Figure 4. SEM images of the milled surface of visually cracked maize kernels. (A), (B), (C), and (D), dry kernels; (E), (F), kernel hydrated for 12 h. Abbreviations as in Fig. 3.

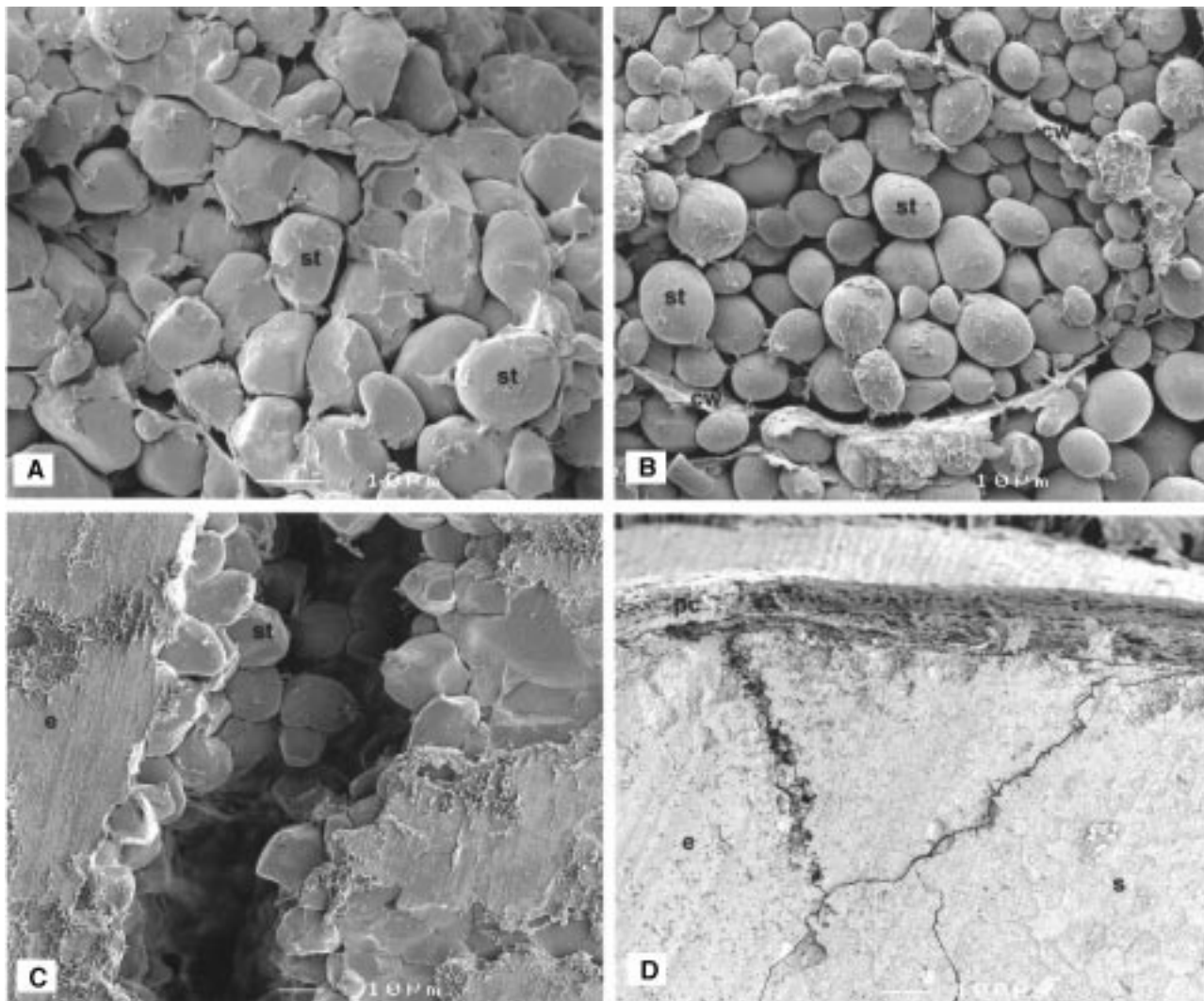


Figure 5. SEM images of (A) starch grains in a dry kernel; (B) starch grains in a kernel hydrated for 12 h; (C) detail of an endosperm crack in a dry kernel; (D): Crack in the endosperm and scutellum of a dry kernel, ending at the aleurone layer. Abbreviations: cw = cell wall, e = endosperm, s = scutellum, st = starch granule, pc = pericarp.

minimum. The scutellum cracks apparently have little effect on the germinative capacity of the kernels. The few cracks in the embryo axis as revealed by LTSEM, which are invisible to the human eye, may be responsible for the lack of germination in approximately 10% of the kernels.

The score of the tetrazolium test was slightly, but significantly, higher in the case of the cracked kernels but the vigour test did not reveal significant differences between the cracked and perfect kernels (Fig. 6A).

When kernels selected as perfect or cracked based on both visual or X-ray analysis (Fig. 6B) were subjected to the cold test, the cracked kernels performed significantly better than the perfect

kernels. This may result from the possibly better water access to the endosperm tissue in the cracked kernels. When visually perfect kernels that were selected by X-ray analysis as having cracks, mostly perpendicular to the axis (see Table 1), were subjected to this cold test, an even lower germination percentage was obtained than with the perfect kernels not having X-ray detectable cracks. This adds to the suggestion that cracks through the axis are responsible for the germination reduction.

The influence of visible internal cracks was also assessed at the level of seedling performance (Table 2). Seven days after incubation, the dry weight and length did not differ between seedlings that were grown from the visually perfect or cracked seeds. The

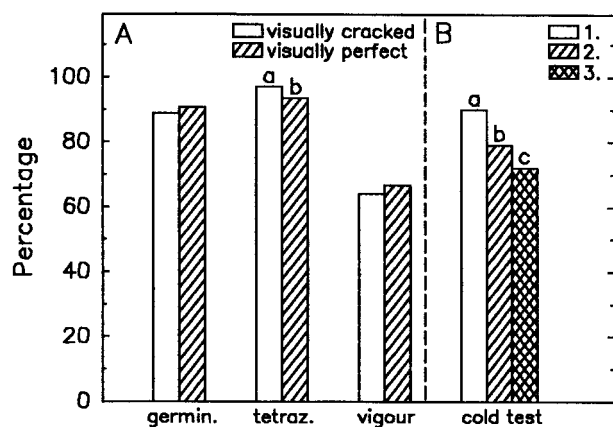


Figure 6. Quality evaluation of maize kernels. (A) Percentage of germination, and viability and vigour as evaluated from tetrazolium tests, of visually perfect and cracked maize kernels. (B) Percentage of germination in the cold test for three categories of maize kernels: 1 – both visually and X-ray cracked kernels; 2 – both visually and X-ray perfect kernels; and 3 – Kernels without visual cracks, but with internal cracks as determined by X-ray analysis. Different letters indicate significant differences at $P=0.05$.

methods and the number of kernels used in these vigour tests are, in our opinion, sufficient to estimate the kernel quality.

In other studies, in which the quality was determined for kernels that were mechanically harvested and processed, varying results were obtained (Herter and Burris, 1989; Sato and Cicero, 1992; Peterson *et al.*, 1995). In contrast to mechanical stress, temperature stress during dehydration (causing only internal cracks) does not affect maize kernel vigour (Naplava and Weingartman, 1994). We show that internal preharvest cracks are not detrimental to germination and vigour, but external cracks can affect these parameters (Escasinas and Hill, 1988). Therefore, it is important to determine whether the cracks are internal or external, and caused by stress or mechanical impact.

General considerations

Visual inspection can give a reasonable estimate of the extent of internal cracks in maize kernels caused by stress in the field. X-ray analysis, that reveals cracks

invisible to the eye, permits better assessment of the effect of internal cracks on kernel quality than visual inspection does. LTSEM analysis reveals small cracks that remain unnoticed by any other method. The aforementioned internal stress cracks generally have no impact on kernel germination and seedling performance. However, if the crack is located in the embryo or occurs perpendicular to the embryo axis, it may affect the nutrient translocation to the embryonic axis and have negative consequences for the quality of the kernel lots. With the present demonstration of internal stress cracks as a result of high temperature and moisture conditions during kernel maturation, we show that such growth conditions can exert an effect on the kernel physical characteristics. Although a number of other hybrids in the same field did not show this type of damage (data not shown) as observed with the hybrid used, the phenomenon needs further evaluation with other maize varieties under agronomically variable conditions.

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Table 2. Seedling performance of visually perfect and cracked maize kernels. Seedling dry matter and length are shown (analyzed 7 days after imbibition).

Sublot	Seedling DW \pm SE (g)	Seedling length \pm SE (cm)
Visually perfect kernels	6.6 \pm 0.2	20.8 \pm 0.5
Visually cracked kernels	7.0 \pm 0.3	21.8 \pm 0.5

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