

A DIMETHYL SULFOXIDE PREPARATIVE TREATMENT FOR SECTIONING CEREAL GRAINS¹

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ABSTRACT

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Cereal kernel structure can yield useful data for plant breeding and taxonomic research and for subsequent milling and processing of new cultivars or hybrids. The difficulty of sectioning cereals has limited routine examination of kernel structure. A dimethyl sulfoxide (DMSO) pretreatment was developed during evaluation of wheat, barley, rye, and their intergeneric hybrids to permit simple and rapid sectioning. Kernels were supported in cork, treated with 80% (v/v) aqueous DMSO, air dried, and sectioned on a rotary microtome equipped with a razor blade adapter. Sections were

dehydrated, cleared, and mounted for light microscopy. Aleurone cells, endosperm cells, and starch granules also were observed by polarized light and Nomarski interference-contrast microscopy to visualize starch birefringence and other details not possible by bright field illumination. The technique is much less laborious than paraffin embedding, freezing, and glass knife sectioning; maintains the physical integrity of the kernel; and limits artifact development. Its use has been extended to other cereals, legumes, and oilseeds.

Kernel structure has been important in characterizing cereal species (1,2) and in developing milling and other processing techniques (3). Pericarp and endosperm structures have been suggested as predictive indexes of flour yield for breeding wheat cultivars (4,5). In the case of interspecific and intergeneric crosses, hybrid seed may not develop correctly (6), may be shriveled (7-9), or may express various abnormal or inherited traits.³

The dry and brittle nature of cereal grains precludes easy sectioning of the kernels and deters many investigations of grain structure. Conventional methods of paraffin or resin embedding followed by cutting with diamond, glass, or factory-machined knives are laborious, time consuming, and often expensive (10,11). We wanted to overcome present technical difficulties with a simple technique to soften the kernel without altering its structure. Dimethyl sulfoxide (DMSO) penetrates tissue rapidly with little or no damage and is used extensively as a carrier of pesticides, dyes, and nutrients (12,13). DMSO is an antiviral agent (12); markedly inhibits many enzymes, particularly peroxidase, catalase, and α -amylase, in concentrations greater than 20% (14,15); and inhibits respiration, nucleic acid synthesis, and other cellular processes (16-18). On the other hand, DMSO has a protective action on proteins and other cellular constituents (19), can be used as an extraction and storage medium for proteins for electrophoresis (20), and in low concentrations permits some enzyme activities (21). We anticipated these DMSO characteristics would permit development of a rapid sectioning method for routine evaluation of structural changes in wheat, barley, rye, and their intergeneric hybrids. Additionally, we hoped to extend the method to other cereals and dicotyledonous crops, especially those with hard, smooth achene such as buckwheat and safflower.

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MATERIALS AND METHODS

Cork mounts were prepared from No. 1 cork stoppers by removing approximately 5 mm from the narrow end. The remaining cork was punctured to permit pressing and receiving a kernel (germ first). The end of the cork penetrated by the kernel was then secured with a fast-setting epoxy adhesive to a wooden dowel 8 mm in diameter by 4 cm long. The kernel was positioned near the surface of the cork so the epoxy bond would support the kernel and the cork directly. The brush end provided a stronger bond than did the germ structure, although either end may be made equally effective if it is cut off to expose an endosperm section.

After the epoxy had set (30 min), the mounts were cut to expose the endosperm of the kernel and to permit rapid DMSO penetration. The mounts were then inverted and sealed in 6-dr screw-cap vials containing 5 drops of 80% (v/v) aqueous DMSO. Excess DMSO solution softened the epoxy.

The mounts were removed from the solution after four days and air dried four additional days. Each preparation was sectioned over a range of thicknesses from 6 to 20 μm with a rotary microtome equipped with a razor blade adapter. The sections were dehydrated and cleared in methanol, xylene, and clove oil for 15, 10, and 30 min, respectively, and mounted in a synthetic resin. Interfering debris from sectioning the cork mount was removed during dehydration and clearing.

RESULTS AND DISCUSSION

The cork mount is important for two functions. The cork appears to control uniform penetration of the DMSO solution. When not embedded in cork, kernels become soft and distorted, but in cork they maintain their shape during pretreatment and drying. Cork is rigid enough to support a soft kernel, although it is easily sheared. Lateral movement of cork and kernel from DMSO softening action (22), which can cause 3–4- μm variations in section thickness, may be alleviated by using a larger dowel with more mounting base surface for the cork and by changing razor blades more often.

Sections that are 6–10 μm thick are difficult to cut and vary somewhat in thickness. The cell contents can be dislodged easily, which results in unsatisfactory preparations (Fig. 1). Slightly thicker sections—12–15 μm —are preferred, because the cells remain intact (Fig. 2). The additional thickness is not detrimental, since Nomarski interference-contrast permits optical sectioning of cleared kernel sections (Fig. 1,2). The Nomarski technique also eliminates the haloed edges around preparations that are often encountered in phase contrast; it gives a three-dimensional perspective of details, which can be enhanced with various wavelengths of colored light and with different stage orientations.

With high ratios of anhydrous or aqueous DMSO to isolated starches, some granules within any sample are solubilized, while others remain birefringent. Similar variation exists between species (23,24). Under our experimental conditions, however, the starch granules are confined within the cellular matrix, the whole kernel is supported by a relatively rigid cork matrix, and the ratio of DMSO to kernel is small. We observed no swelling or loss of birefringence of starch granules nor any other artifacts from DMSO softening. The technique can be extended readily to large grain cereals such as corn (Fig. 3). Figure 3 illustrates

that all starch granules, regardless of size, remain birefringent in the presence of DMSO. Even broken starch granules remain birefringent.

Maintenance of birefringence is demonstrated also in buckwheat (Fig. 4). In Fig. 5, the pericarp and part of a cotyledon of safflower remain undistorted after

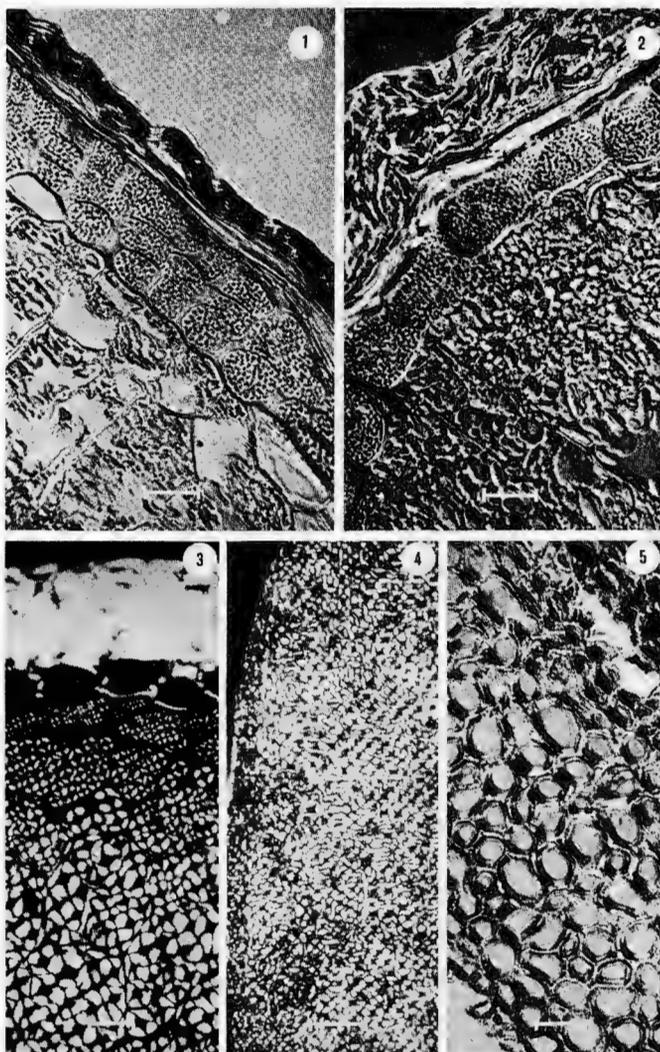


Fig. 1. Cross section of barley. Aleurone and endosperm cell contents dislodged from 6–10 μm thick section, Nomarski interference contrast. **Fig. 2.** Cross section of triticale. Intact cells in 12–15- μm section, Nomarski interference contrast. **Fig. 3.** Cross section of corn. Starch birefringence, polarized light. **Fig. 4.** Cross section of buckwheat. Starch birefringence, polarized light. **Fig. 5.** Cross section of safflower. No pericarp or cotyledon distortion. Nomarski interference contrast. Scale bar on all figures equals 20 μ .

DMSO treatment and sectioning. Soybeans also have been sectioned.

Explaining the effect or effects of DMSO in this technique is difficult when viewed against the wide range of DMSO reactions and effects. Perhaps the easiest explanation is that DMSO may merely substitute for bound and free water (14,25) and remain during the drying step because it is so tightly bound and also nonvolatile. Our technique produces, in effect, a dry but DMSO-"moist" and softened kernel that is easily sectioned.

Acknowledgments

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