

# Comparison of Water Absorption Patterns in Two Barley Cultivars, Using Magnetic Resonance Imaging

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## ABSTRACT

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Two barley cultivars, Excel and Prisma (six-row and two-row types, respectively), were obtained from the 1993 harvest at Crookston, MN. Magnetic resonance imaging (MRI) was used to follow water imbibition in single, large seeds of Excel and Prisma barley. A comparison of moisture distribution on longitudinal sections of Prisma and Excel barley during early hours of steeping was then made. MRI analysis revealed that the majority of water initially entered the kernel through the kernel surfaces and into the endosperm tissues. Water was observed under the surface of the hull tissues and appeared to be migrating into the aleurone and endosperm tissues. Then water was detected moving from the embryo

to the scutellum and then into the crushed cell layers. Aleurone and embryo tissue showed more rapid water uptake and distribution than did scutellar tissue. Some transient water distribution was observed in the scutellum, ventral furrow, and vascular tissues. This indicates that the hydration of surface tissues and crushed cell layers may be significant during the early hours of steeping. During the late hours of steeping, the crushed cell and endosperm cavity region appears to become remarkably active in water transport. Water was distributed throughout the endosperm tissue of the Prisma cultivar more efficiently than in that of the Excel cultivar, although Excel absorbed more water initially.

The initial hours of barley steeping are critical to the development of components (enzyme activity and hormonal development and release) that determine the malt quality. Kernel morphology, chemical composition, genetics, and environment affect the physiology of the kernel during these initial hours. Variations in imbibition rates may lead to inefficient hydration and therefore to over- or undermodification of the endosperm. Therefore, to assist in efficient endosperm modification and development of a high-quality malt, breeders and maltsters must understand how kernel hydration is affected by the kernel's physical structure.

It is common practice to separate kernels by size and shape before steeping. This approach optimizes uniformity of hydration and germination. Although the relationship between kernel physical characteristics (e.g., kernel size and pericarp permeability) and hydration has been studied extensively, many of these studies relied on destructive sampling, or the introduction of foreign materials such as dyes, which could reduce the accuracy of the data (Day 1896, Collins 1918, Gruss 1930, Hinton 1955, Axcell et al 1983, Van Eerde 1983, Davies 1991). Researchers have described the hydration pathways for barley using modern techniques such as autoradiography (Axcell et al 1983) and X-ray microanalysis (Davies 1991), but again, these techniques are destructive to the kernel and are indirect measurements of hydration phenomena. Magnetic resonance imaging (MRI) is a nondestructive method that has been used widely as a noninvasive tool to investigate internal phenomena. Examples of its use can be found with various plant tissues (Connelly et al 1987), vegetable drying (Ruan et al 1991), fruit (Wang and Wang 1989), foams (Kauten et al 1991), soybeans (Zeng et al 1996), and maize (Ruan et al 1992).

The principles of MRI, also known as nuclear magnetic resonance imaging, are based on the concepts of nuclear magnetic resonance. The MRI technique used in this experiment was developed by Ruan et al (1992). The technique overcomes obstacles that affect signal decay by using readout gradient reversals for magnetization refocusing. Images are generated by exciting a plane of tissue and applying a long radio frequency pulse and a slice selection gradient perpendicular to the plane of tissue ( $z$  axis), then applying a phase-encoding gradient ( $y$  axis) and a read gradient ( $x$  axis). This pulse

sequence is repeated 256 times, and 2D Fourier transformation is applied to produce an image.

Related imaging research on barley examined the effect of grain size, pericarp quantity, cultivar, and growing season in relation to hydration (McEntyre et al 1995, Yin et al 1996). This research concluded that kernel size and cultivar influenced the hydration rate of the grain. Another study observed the scutellar region of the barley kernel and noted significant increases in moisture in this tissue after 4–6 hr of water imbibition.

The objectives of this study were to: 1) determine how water penetrates and is distributed in barley kernels, 2) evaluate the patterns of water distribution, and 3) compare water content of the endosperm of the cultivars examined using MRI.

## MATERIALS AND METHODS

Samples of barley (*Hordeum vulgare* L.) cvs. Excel (U.S six-row), and Prisma (European two-row) were obtained from the 1993 harvest at the Crookston, MN, Agricultural Experiment Station. Initial moisture was determined for each cultivar using the air-oven method (ASBC 1992).

To determine image acquisition times, a hydration curve was developed. However, to ensure consistent handling and to provide unhindered hydration of the samples, several holders of suitable porous material were designed and constructed using fiberglass screen (18 × 16 openings per square inch, 0.011 in. diameter) (Hanover Wire Cloth Co., Newark, NJ). Screen material was fashioned into 60 × 100-mm envelopes. A twist tie was looped through a lip on the packets to act as a handle, and each packet was weighed.

Fifty seeds from each cultivar were placed in separate packets in duplicate. The packets were weighed, and this measurement was recorded as the weight at 0 hr. The packets were placed in beakers with 300 mL of distilled water (20°C). At hourly intervals (1–48 hr), the seed packets were removed from the steep water, blown dry for 10 sec, and weighed. Water was changed in the beaker every 8 hr to improve oxygenation. The percent weight change (dwb) was plotted against steeping time.

A hydration chamber was fashioned from a plastic pipette tip forming a cone-shaped holder (Fig. 1). Water (≈1.0 mL) was added to this hydration chamber for kernel steeping. The chamber was then sealed with wax. This cone was made to fit inside the radio frequency coil that was tuned to 200 MHz resonance frequency. The unit was then placed in a gradient coil that had a maximum field strength of 20 G/cm. A 4.7-Tesla superconducting magnet with a 330-mm bore was used to generate the external magnetic fields (Spectroscopy Imaging System Co., Fremont, CA.)

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Selected kernels were steeped, and data were acquired after 1.0, 2.0, 8.0, 12.0, 24.0, and 48.0 hr. Hydration times were chosen from the curve developed in the previous hydration kinetics experiment. Measurements were taken during, or at the end of, periods of rapid water uptake.

The acquired images were gray-scale representations of proton density distribution, which in turn represents water distribution. Images were analyzed using NIH image software (V1.55, National Institutes of Health, Bethesda, MD). A density profile was obtained for a selected (longitudinal or transverse) cross section of the kernel. Density profile is an  $x$ - $y$  plot of proton density ( $y$ ) versus spatial position ( $x$ ) and was based on the gray-scale pixel values produced after image processing. A correction factor (actual moisture content [from the hydration chart] divided by average proton density [from the slice image]) was used to determine the actual moisture distribution on the slice. Surface plots based on pixel values were presented for selected gray-scale images. Pseudocolor was applied to the gray-scale images for enhancement. Darker shades represent increased water concentration.

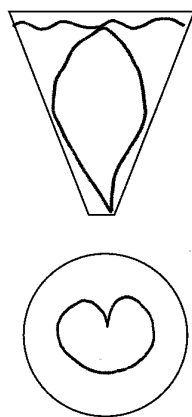
## RESULTS AND DISCUSSION

Hydration curves revealed an initial rapid uptake of water during the initial hours of immersion followed by a gradual rate reduction over time (Fig. 2).

Images for Prisma and Excel were similar, so to simplify the discussion, only one image slice of the longitudinal view and one of the transverse view of Excel barley is shown here for each image capture time. Transverse slice number 4 and longitudinal slice number 3 best illustrate hydration in the kernel because each has the greatest anatomical detail.

Water uptake in kernels is followed by redistribution of the water to internal tissues. However, the time when these two processes occur is not definite and may be overlapping. Water uptake before redistribution is demonstrated by water moving to regions below the hull and penetrating the aleurone and other internal tissues at 1 hr of hydration (Figs. 3a and 4a). The regions just below the outermost layers of the hull have less water than the surface of the grain. It appears that, initially, the outer tissues (pericarp and hull) imbibe water at about the same time that some mechanism (metabolic or vascular) redistributes the surface water to the aleurone. At a similar time, water is imbibed in the embryo region before it reaches scutellum tissue.

At 1 hr, the transverse image shows a triangular configuration of high water concentration made by several circular structures in the embryo region (Fig. 3a). These regions may be the cavity in

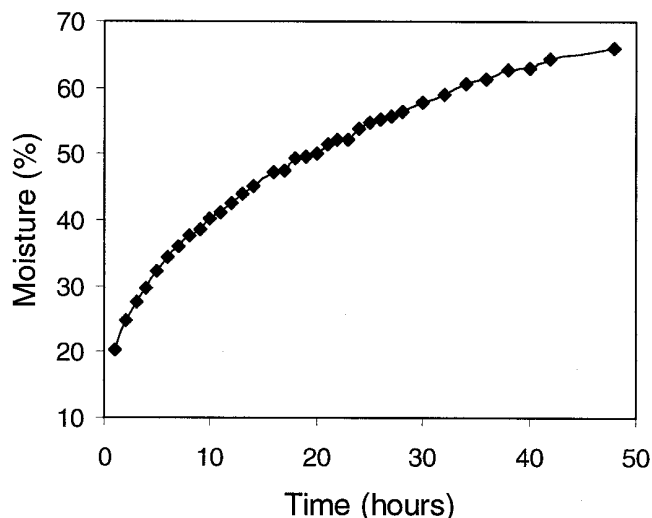


**Fig. 1.** A barley kernel placed in the magnetic resonance imaging (MRI) hydration chamber (sample holder for steeping). Note the orientation of the illustration in relation to slice direction and MRI for longitudinal and transverse sections (Figs. 3 and 4).

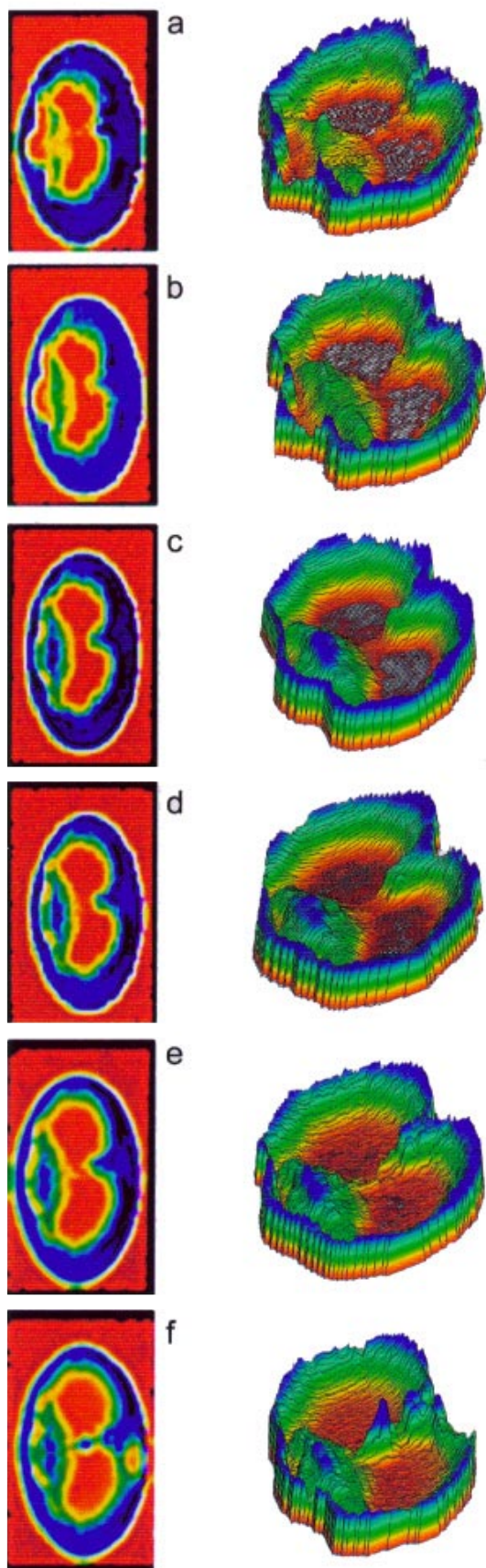
which the coleoptile resides (Briggs 1978). There is an area of very low water concentration on the dorsal side of the kernel behind the embryo that increases in water concentration as hydration time increases but remains relatively dry in relation to surrounding tissues. Initially, little water can be detected in the endosperm and only small amounts in the aleurone. However, as steeping progresses, the aleurone water concentration increases, and the endosperm also appears to gain water in areas adjacent to the aleurone. The crushed cell and endosperm cavity sectors show higher water concentration than surrounding endosperm tissues. These structures divide the starchy endosperm by projection into that tissue and may have a role in water distribution in the surrounding areas (Duffus and Cochran 1993). Water also appears to spread from the scutellum to the surrounding crushed cell layer and then into the endosperm. These observations support the conclusion that water moves from the aleurone and scutellum tissues to the endosperm. But the embryo appears initially to hydrate to a greater degree than these other tissues. The longitudinal image (Fig. 4) shows an increase in the water content of the embryo, scutellum, and aleurone as a function of time. At 1 hr, the ventral furrow region has a higher water content than the surrounding endosperm. This supports the previous finding in the transverse images in which water was seen at higher concentrations during the early hours of steeping in the crushed cell and endosperm cavity areas. The transverse image also shows a continued spread of water from the region of the vascular tissue or cavity in the furrow from 1 to 2 hr (Fig. 3). At 8 and 12 hr, transverse and longitudinal images show that the embryo water concentration has increased considerably, and scutellum and crushed cells show a gradient of high to low water concentration starting from the embryo and moving outward through the depleted layer to the endosperm.

Many significant changes occur in the latter part of steeping. At 48 hr, the endosperm cavity shows a large increase in water concentration and the ventral furrow has enlarged. It had been suggested that this tissue dries and seals off after grain filling (Zee and O'Brien 1970). However, water moves through this region during the early hours and at the end of the steep cycle. In this area there is a large concentration of endosperm cell wall material. The crushed cells in this region undoubtedly help attract moisture to this area because of the hygroscopic nature of 1-3,1-4- $\beta$ -D-glucan, which forms a large part of the endosperm cell wall.

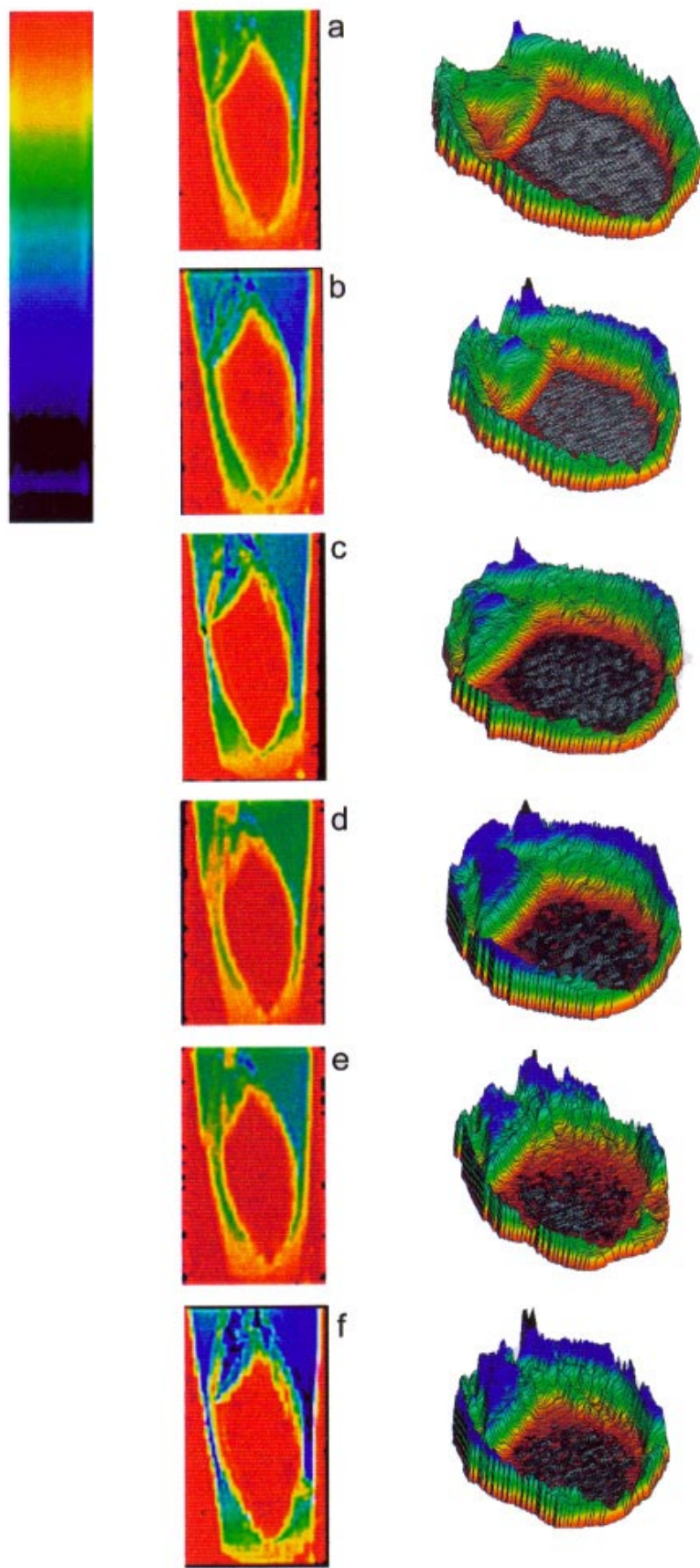
It is apparent that a great deal of information can be gathered concerning the path of grain hydration using MRI. However, the purpose of this study was not only to describe this path but also to contrast the hydration efficiency of the two cultivars. Figure 5 shows the longitudinal transient moisture profiles for Prisma and



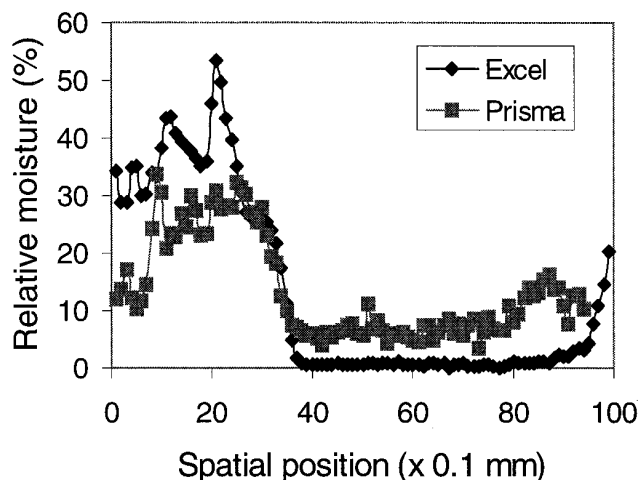
**Fig. 2.** Mean hydration rate of large kernels of Excel and Prisma for the 1993 growing season.



**Fig. 3.** Transverse images of Excel barley slice 4, after 1 (a), 2 (b), 8 (c), 12 (d), 24 (e), and 48 hr (f) of hydration. Range of colors, orange to violet-blue, represents increased hydration.



**Fig. 4.** Longitudinal images of Excel barley slice 3, after 1 (a), 2 (b), 8 (c), 12 (d), 24 (e), and 48 hr (f) of hydration. Range of colors, orange to violet-blue, represents increased hydration.



**Fig. 5.** Relative moisture content at positions inside large kernels of barley cultivars Excel and Prisma, growing season 1993, after 2 hr of hydration.

Excel barley. These are longitudinal views after 2 hr of hydration. At position 0.0–0.5 mm, Excel has greater hydration than Prisma in the micropylar region. Next, at position 0.5–2.0 mm, Excel shows much greater hydration of embryo tissue than Prisma (55 vs. 30%). Position 2.0–4.0 mm, the region of the scutellum, shows similar hydration rates. Finally, at position 4.0–10.0 mm, Prisma endosperm hydration at 8% is greater than that of Excel at ≈0%. Comparison of the moisture profiles for Excel and Prisma barley indicates that Prisma distributes its water to the endosperm more rapidly than Excel. This may be due to the structure of the endosperm cavity or the amount of crushed cell material located in the crease of the cultivars. Prisma may have more crushed cell material or a larger endosperm cavity, which may indicate a greater capacity to store and distribute water into the starchy endosperm. It would be interesting to survey barley for crushed cell content in relation to hydration rate and water distribution in the endosperm.

Functional structures during grain development may also play a role in grain germination. Seed cavities may facilitate water storage, by widening as the kernel matures and hydrates, and by strategically placing water near hygroscopic materials. The early hydration of crushed cells in the crease region suggests that the  $\beta$ -glucan in this region may be hydrolyzed as an energy source; this would free any associated bound water and possibly show as an area of high water concentration during an MRI study.

Variables that may have affected this result include water temperature in the hydration chamber, variability in aleurone and pericarp or hull tissue quantity, and adaptation of a cultivar to its environment. Prisma is a cultivar developed in the Netherlands, while Excel is grown in the upper-midwestern United States. Each of these locations has a specific ecology for which these cultivars were developed. Thus, the morphology and composition of the kernels are likely to be different. MRI procedures did not affect the viability of the grains observed, but inclusion of air rests during the steeping cycle and application of the technique to a range of cultivars will further improve the value of the results.

## CONCLUSIONS

Results from this MRI study indicate that kernel hydration is a two-phase process: initial water uptake by the outer grain layers, followed by internal redistribution. However, distinguishing these

events as occurring independently may not be appropriate because of the overlapping nature of the process.

The path of hydration was determined, and significant hydration rates in the embryo, scutellum, and crushed cell and endosperm cavity regions were observed, while some of the surrounding tissues remained unhydrated. Hydration of the embryo before that of other tissues suggests that the embryo may be the initial source of hydrolytic enzymes during the early stages of germination. Also, the cavities surrounding the embryo tissues contain water, possibly for later distribution to the coleoptile and developing leaves. This finding supports the results of morphological studies indicating that specific, compartmentalized tissues in the grain serve distinct roles in germination. Also, it is apparent that genotype plays a role in water distribution efficiency, which may affect final malt quality.

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