JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Barley Viability during Storage: Use of Magnetic Resonance as a Potential Tool To Study Viability Loss

MARCO L. H. GRUWEL,*,† XIANG S. YIN,‡ MICHAEL J. EDNEY,§ STEVE W. SCHROEDER,§ ALEX W. MACGREGOR,§ AND SUZANNE ABRAMS†

National Research Council, Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9 Canada; Prairie Malt Ltd./Cargill Malt Americas, Box 1150, Biggar, SK, S0K 0M0 Canada; and Canadian Grain Commission, Grain Research Laboratory, 1404-303 Main Street, Winnipeg, MB, R3C 3G8 Canada

Malting-quality barley samples of the varieties Harrington, Manley, and TR118, each from two locations in Saskatchewan, were collected directly from the producers and sent to China for storage. At regular intervals samples were shipped back to Canada for analysis consisting of germination studies, α-amylase tests, magnetic resonance imaging (MRI), and magnetic relaxation (NMR) studies. Samples showing a decrease in germinative energy and elevated levels of α-amylase also showed a rapid uptake of water in the area between the embryo and the endosperm as observed by MRI. Using NMR relaxation experiments, viable and nonviable barley samples could be distinguished after 2 h of imbibition.

KEYWORDS: **^{1}H NMR; imbibition; relaxation time; MR imaging; germination; α-amylase**

INTRODUCTION

Malting barley must exhibit high levels of germination (≥95%) and be able to maintain its viability during transportation and during storage periods of up to one year (*1*). Loss of barley viability during storage is of concern to producers, marketers, and end users of malting barley. It results in the down-grading of malting barley to feed barley with an associated negative economic impact on producers.

In recent years, some shipments of malting barley to countries such as China have lost viability. These shipments were not always stored under ideal conditions. However, it was not clear if the loss of viability was caused by the storage conditions or by damage to the barley before shipment or a combination of the two.

The reasons for the loss of barley viability during storage are not clear, but it is often associated with low levels of sprouting or pregermination (*2*). This can occur if the grain is exposed to excess moisture just before harvest or at some time between harvest and grain utilization. During 1998, the growing and harvesting conditions in western Canada were drier and warmer than normal. There was no significant problem with preharvest sprouting in selected samples of malting barley.

In general, maximum seed viability and vigor are reached after the final drying stage of seed maturation (*3*). From this point in time gradual deterioration occurs. Many factors such as genetics, preharvest effects, mechanical damage during harvest and handling, storage conditions (moisture, temperature), and microbal infections influence the rate at which seeds lose viability. However, at present no clear picture of why seeds lose viability has emerged. This is partially due to different aging conditions, different species, and different analytical techniques used by different researchers.

However, with the actual cause frequently unknown, the loss of seed viability is often attributed to the reduction of plasmalemma integrity (*3*, *4*). Using accelerated aging techniques on cucumber seeds, Koostra and Harrington (*5*) showed that membrane deterioration, observed as a decrease in total phospholipid content, could be linked with a reduction in seed viability. The increasing loss of cell membrane functionality leads to an inability to maintain turgor and to osmotically respond to environmental changes, resulting in seed death (*6*). During water uptake plasmalemma degradation becomes more pronounced. Parallel to plasmalemma dysfunction, changes in cell compartmentalization indicate disruption to internal membranes (*7*). Changes in membrane integrity can be quantitatively characterized by electron paramagnetic resonance (EPR) of water soluble spin probes, which have to be added to the seeds (*8*).

Our main research objective was to identify specific parameters associated with a loss of barley viability during storage and handling in order to develop a test to predict loss of seed viability using simple nuclear magnetic resonance (NMR) techniques.

MATERIALS AND METHODS

Barley Sample Handling. *Collection.* Malting-quality samples of Harrington barley (*Hordeum vulgare* cv. Harrington), Manley (*Hordeum vulgare* cv. Manley), and TR118 (*Hordeum vulgare* cv. TR118), each

* Author to whom correspondence should be addressed [e-mail marco.gruwel@nrc.ca; fax (306) 975-4839].
† National Research Council.
‡ Prairie Malt Ltd./Cargill Malt Americas.
§ Canadian Grain Commission.

10.1021/jf0108617 CCC: $22.00 Published 2002 by the American Chemical Society
Published on Web 01/17/2002

Table 1. Results of Germinative Energy Studies of Barley Samples

cultivar (origin)	germinative energy (%)					
	storage time (months)					
	control	5	6	7	8	control[a]
Harrington (St. Brieux)	97	100	100 100[b]	99	100	99
Harrington (Carrot River)	99	99	84 91[b]	72	63	96
Manley (Tisdale)	99	95	77 90[b]	59	51	98
Manley (St. Brieux)	100	99	100 100[b]	99	98	100
TR118 (Arborfield)	96	72	44 47[b]	23	20	87
TR118 (Naicom)	99	99	100 100[b]	100	99	100

[a] This control was performed in December 1999, whereas the first control was performed in December 1998. [b] Percent viable embryos as determined by tetrazolium chloride staining (*10*).

Table 2. α-Amylase Content of Control Samples[a]

cultivar (origin)	α-amylase (IDC units·g^{-1} dm)
Harrington (St. Brieux)	37
Harrington (Carrot River)	130
Manley (Tisdale)	365
Manley (St. Brieux)	75
TR118 (Arborfield)	643
TR118 (Naicom)	65

[a] Malt check contained ~145000 IDC units·g^{-1}.

Table 3. Storage Conditions of Barley Samples Shipped to China, Measured in the Storage Unit

date	temp range (°C)	relative humidity range (%)
April 12–15, 1999	10–20	50–90
May 3–31, 1999	20–20	60–80
June 1–30, 1999	~25	70–90
July 1–18, 1999	~25	70–90

from two locations in Saskatchewan, were collected directly from producers (1998 crop) by Prairie Malt Ltd./Cargill Malt Americas and sent to Nanjing, China, in January 1999 following standard procedures for bulk shipments. In China, samples were stored in burlap bags. Subsamples of these bulk samples were kept in Canada and stored at 5 °C in plastic containers in December 1998. These are referred to as the control samples.

Sample shipments from China were received at intervals from May to October 1999. Samples were shipped from China in sealed plastic containers with screw caps and contained ~1 kg of material. The containers were completely filled to prevent kernel damage due to physical stress. Upon arrival in Canada, the samples were immediately stored at 5 °C.

Kernels for NMR and Magnetic Resonance Imaging (MRI) Measurements. For the purpose of relaxation time measurements (NMR), small batches of 15 kernels were imbibed at room temperature in distilled water using a 50 mL beaker while shielded from air and light. At selected intervals a single kernel was removed and blotted with filter paper to remove any excess water attached to the husk. The blotted kernels were centered in a capped Zirconia MAS spinner (2 cm length and 0.7 cm o.d.) and inserted into the NMR probe. For every NMR relaxation time experiment a new kernel was used, and NMR relaxation time measurements were performed up to four to five times per imbibition time point used in the NMR experiments.

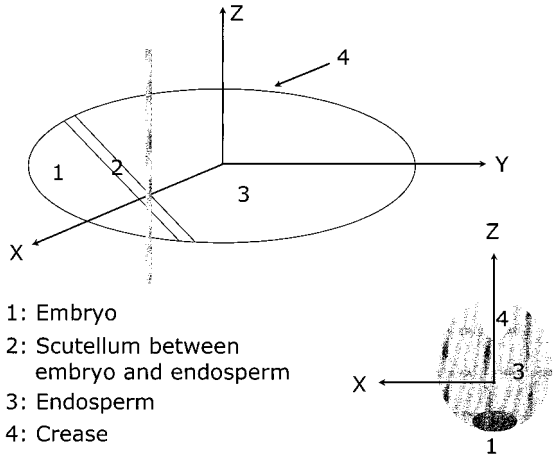

Figure 1. Very simple model of a barley kernel indicating the positions of the embryo and crease. An axis system has been inserted to use as a guide for the interpretation of the MR images.

Microimaging experiments were performed on samples taken from a germination dish. As only small samples of barley were available, Petri dishes (Fisher Scientific Ltd., Nepean, ON, Canada) with a diameter of 54 mm were used for 35 kernels in 1.5 mL of water on Whatman No. 1 filter paper (Whatman Ltd., Maidstone, U.K.). This method was adapted according to the germinative energy protocol (vide infra). Germination was performed in a dark cabinet at 20 °C, and, as needed for MRI measurements, kernels were removed at regular intervals. After microimaging, kernels were returned to a different Petri dish to follow germination progress with time. This allowed us to correlate MRI observations with the presence or absence of germination after visual inspection.

NMR Spectroscopy. All magnetic relaxation experiments were performed on a Bruker (Rheinstetten, Germany) Avance DRX 360 wide-bore machine, operating at 360.13 MHz for ^{1}H. Barley kernels were studied with a Bruker broad-band magic angle spinning (MAS) probe. With the exception of dry barley kernels, the samples were studied without spinning at 20 °C. Relaxation studies on dry kernels used a sample spinning frequency of 2.5 kHz. The ^{1}H $\pi/2$ pulse length was 4.5 μs. Using a spectral width of 15.2 kHz and a recycle time of 4 s, ^{1}H NMR spectra were acquired in 64 scans and stored in data blocks containing 4096 time domain points. Spin−lattice relaxation rates were measured with an inversion recovery sequence of $\pi-\tau-\pi/2-$acq., with 14−16 different τ values. After Fourier transformation, the signal amplitudes were fitted to a single exponential using a three-parameter expression of the form

$$M(t) = C[1 - B\exp(-R_1 t)] \quad (1)$$

where C and B are adjustable parameters, $M(t)$ is the signal amplitude at time t, and R_1 is the spin−lattice relaxation rate ($1/T_1$).

MRI/Microimaging. MRI experiments were also performed on the Avance DRX 360, using a Bruker Micro-2.5 probehead and gradient system. All imaging experiments were performed at room temperature (20 °C) on single seeds mounted on a plexiglass foot and inserted in a 10 mm NMR tube. The relative humidity in the tube was maintained at 100%, using a piece of wet cotton wool inserted in the cap of the NMR tube.

For the acquisition of microimages a Hahn spin−echo pulse sequence was used. Each echo experiment took 17 min and used a field of view (FOV) of 1.0 cm and a slice thickness of 0.6 mm. Each image consisted of a 256 × 256 data matrix, resulting in a pixel resolution of 39 μm. The Hahn spin−echo pulse sequence consisted of 2 ms SINC3 pulses for selective excitation and refocusing with an echo time of 6.35 ms and a recycle delay of 1s.

α-Amylase Assay. α-Amylase assays were performed according to the method of Briggs (*9*) as modified by MacGregor et al. (*10*). The activity of the enzyme is expressed in iodine dextrin color (IDC units).

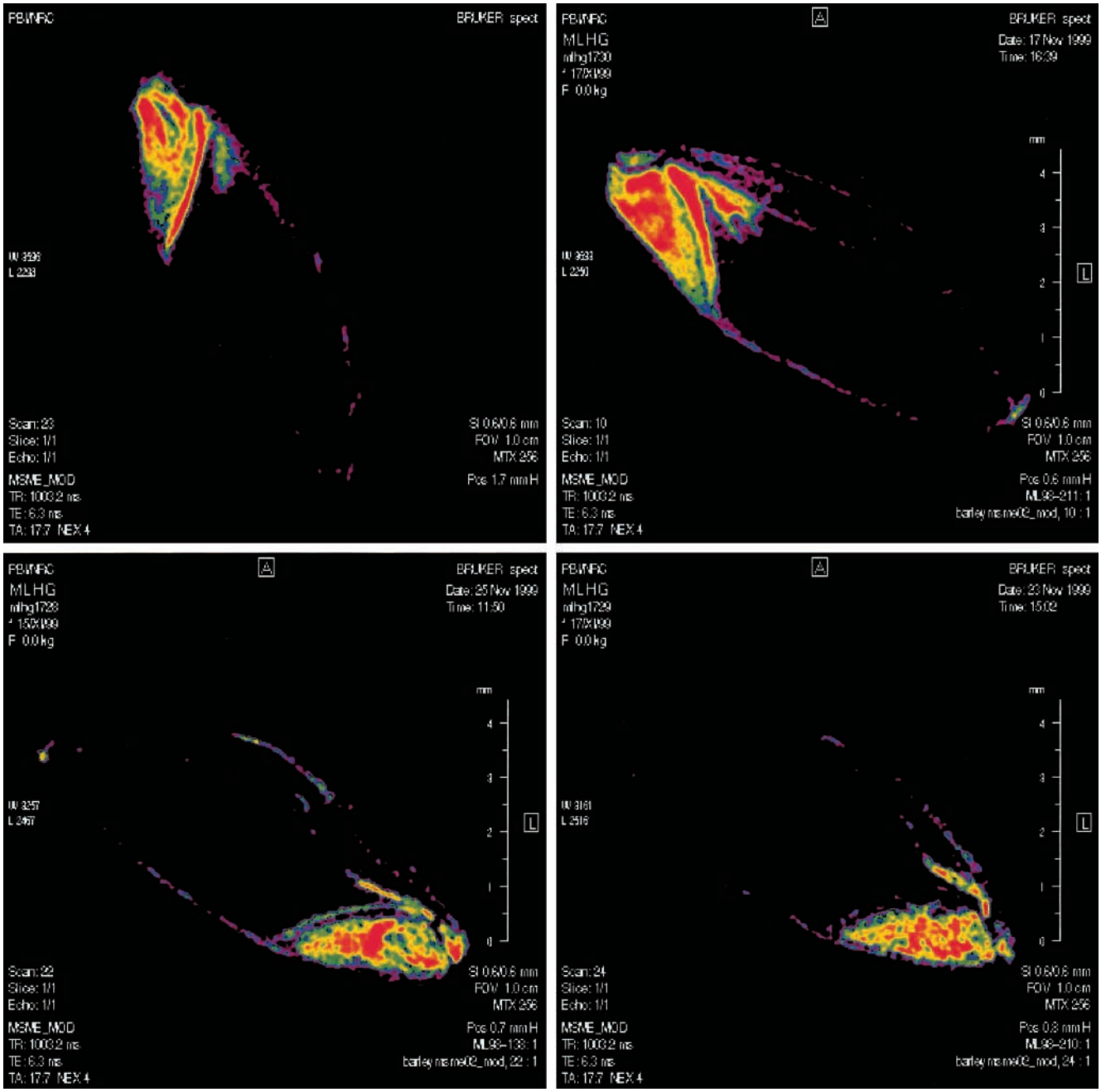

Figure 2A. (A) MR images of nonviable TR118 (Arborfield) (top, left and right, after 6 and 7 months of storage, respectively) and TR118 (Naicom) (bottom left, after 6 months of storage) and Manley (St. Brieux) (bottom right, after 7 months of storage) as viable control. The TR118 images clearly show an increased water resonance intensity in the embryo/scutellum areas compared with the TR118 Naicom (bottom left) and Manley St. Brieux (bottom right). Areas corresponding to a high water intensity are displayed in red, whereas the absence of water is marked by black, using a scale going from high to low as going from red, yellow, green, blue, purple, to black. Note that the images shown are typical for the ZY-plane orientation as explained in **Figure 1**.

One IDC unit corresponds to the amount of enzyme required to lower the absorbance of a standard amylopectin β-limit dextrin digest from 0.6 to 0.4 in 100 min (*9*, *10*). α-Amylase activity levels reported were the average of replicated analyses.

Germinative Energy. Barley kernels (100) were placed on two pieces of Whatman No. 1 filter paper in a 9 cm Petri dish. Deionized water (4 mL) was added, and the samples were kept in the dark at 20 °C and 90% relative humidity. Germinated kernels were counted and removed at 24, 48, and 72 h, but only the 72 h values are reported here (*11*).

Replicates of 50 seeds from the subsamples returned from China after 6 months of storage were stained with a 10 g·kg^{-1} solution of tetrazolium chloride, according to the ISTA method (*12*). Kernels with nonstaining embryos were classified as nonviable.

RESULTS AND DISCUSSION

Germination energy levels of the barley samples are shown in **Table 1**. The results clearly show that of the six samples sent to China, three samples Harrington (Carrot River), Manley (Tisdale), and TR118 (Arborfield), had lost a substantial amount of germinative energy after storage of 5 months or longer. These samples continued to lose viability while the other samples maintained high levels of germinative energy, even after 8 months of storage in China. Except for TR118 (Arborfield), the control samples showed high levels of viability after storage at 5 °C for 1 year (results not shown). Tetrazolium chloride staining of the 6-month-storage samples showed that the samples

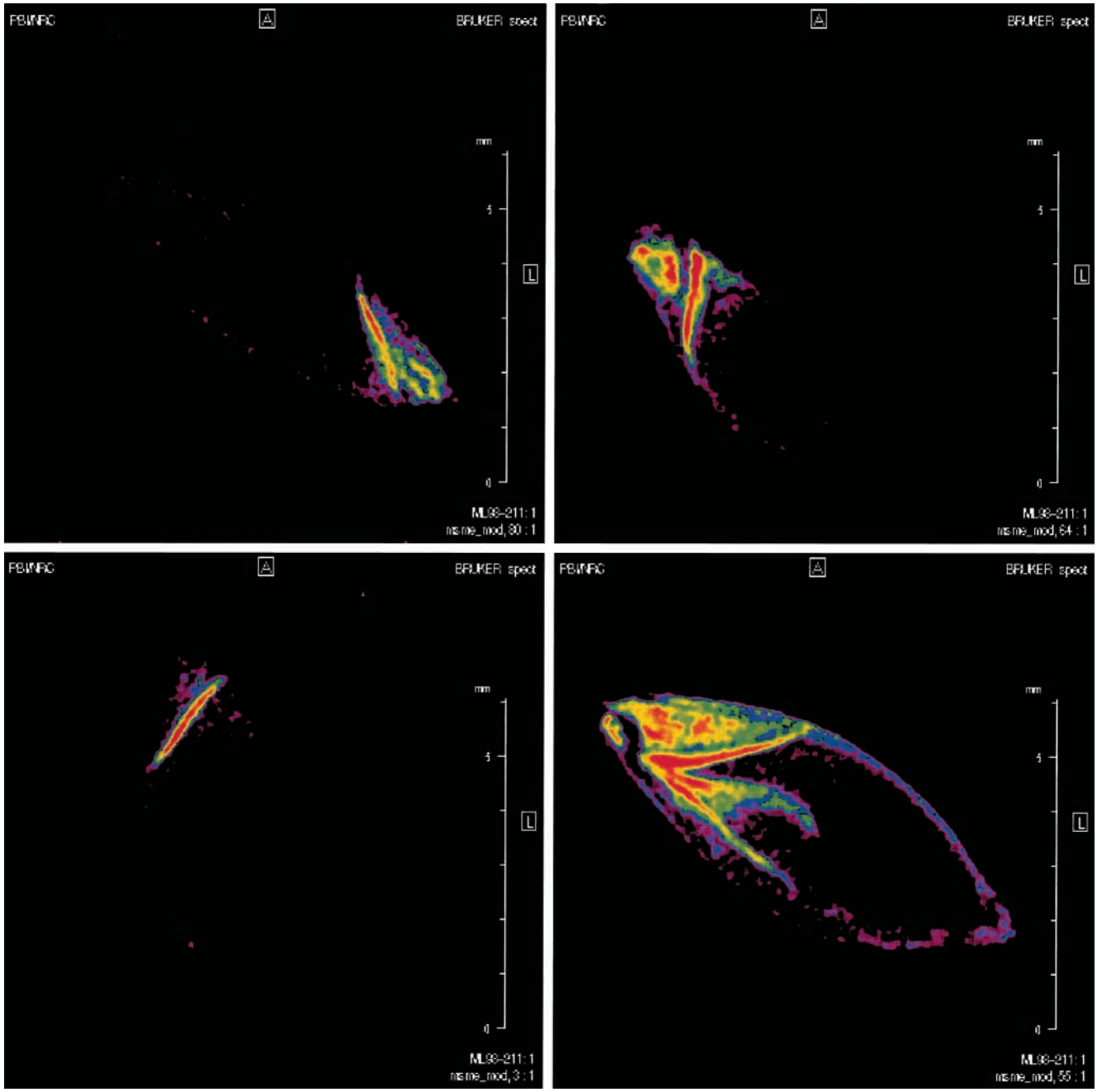

Figure 2B. (B) MR images of the ZY orientation of a nonviable TR118 (Arborfield) sample after 7 months of storage. The two top images show two different kernels, obtained 6 h after the initiation of the germination protocol. The two bottom images, also of different kernels, were obtained 25 h into the germination protocol.

that were losing viability also contained significant numbers of kernels with dead embryos; that is, kernels in these particular samples were dying because of the poor storage conditions. Aleurone cells of these samples appeared to be still viable, however (results not shown).

α-Amylase levels in the control samples are shown in **Table 2**. Three samples contained elevated levels of α-amylase, Harrington (Carrot River), Manley (Tisdale), and TR118 (Arborfield), suggesting that these samples may have been subjected to low levels of pregermination. Unpublished data (MacGregor and Schroeder) showed that 90% of the α-amylase present was malt α-amylase-2. The contribution from microflora α-amylase was small. It should be noted that α-amylase levels in commercial malt are ~145000 IDC units·g^{-1}. The highest α-amylase level recorded in **Table 2** represents <0.5% of this value, indicating that the degree of pregermination in these samples is exceedingly low. It appears to be sufficient, however, to damage the samples so that they lose viability when stored under unfavorable conditions of heat and high humidity (**Table 3**). Undamaged samples, those exhibiting low levels of α-amylase, maintain their viability under the same conditions.

Barley samples with reduced viability (see **Table 1**) still contained viable kernels, making the selection of individual kernels for microimaging difficult. To maintain efficient data acquisition of nonviable kernels, batches with a known viability of ~50% or less were selected for imaging. According to this criterion the TR118 (Arborfield) samples stored for 6−8 months and the Manley (Tisdale) sample stored for 8 months were selected. Using the standard Petri dish germination protocol, water uptake in barley kernels was followed as a function of

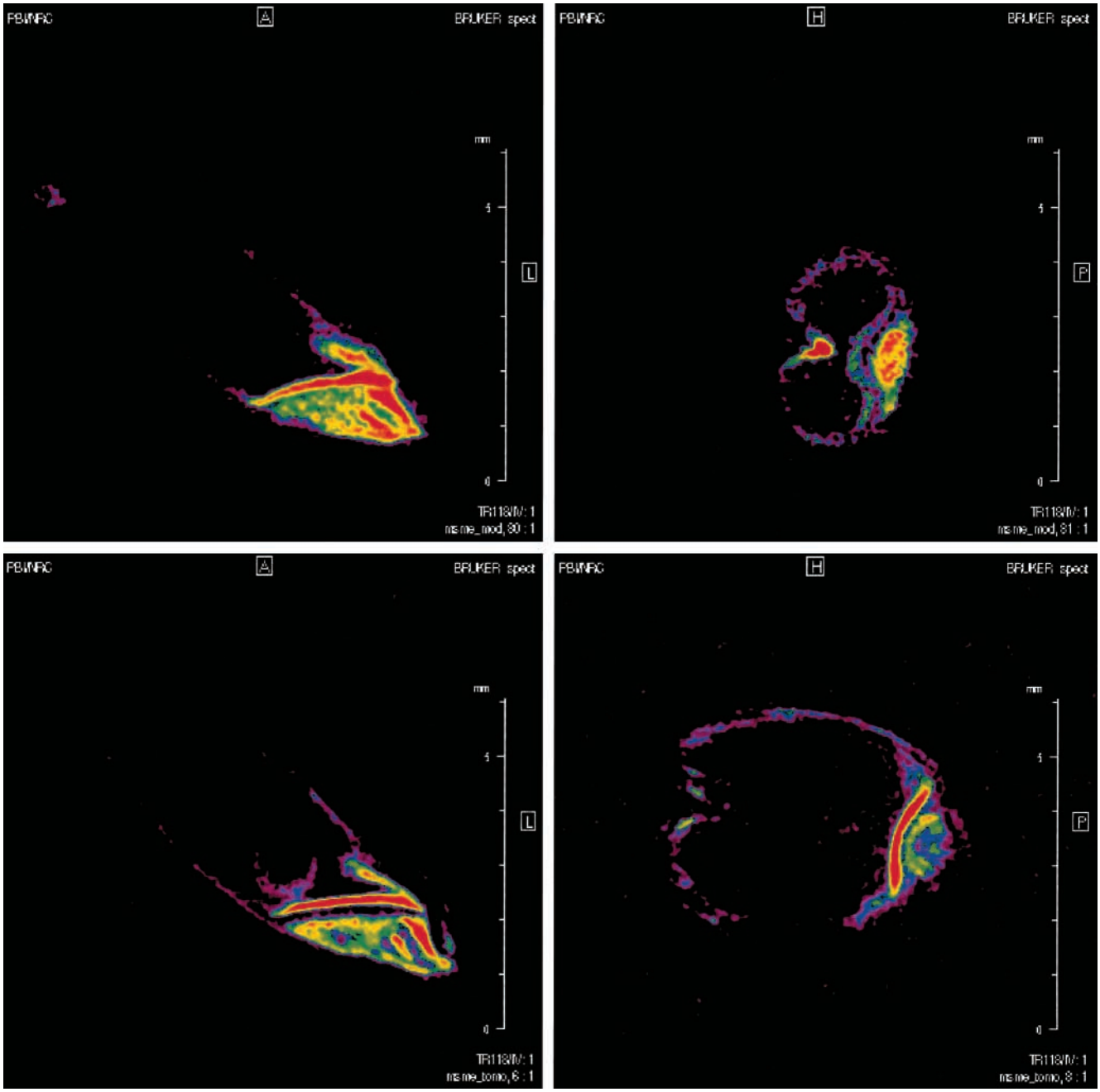

Figure 3A. (A) MR images of a nonviable TR118 (Arborfield) sample after 8 months of storage. The two top images show two orientations of the same kernel (ZY orientation on the left and XZ orientation on the right), obtained 7 h after the initiation of the germination protocol. The two bottom images, also of the same kernel (ZY orientation on the left and XY orientation on the right), were obtained 10 h into the germination protocol.

time for selected samples. As it is not always clear whether a kernel germinates, especially during the early stages of germination, all kernels used for MRI were returned to a separate Petri dish to follow germination after imaging. Using this control, MRI observations could directly be correlated with the germination history of the seed at a later stage.

Figure 1 shows a very simple model of a barley kernel in a reference frame. This frame will be used to explain the orientation of some of the images. The shaded rectangle in the top diagram indicates the XZ orientation used for some of the images. At the bottom of **Figure 1** this orientation is shown as seen looking parallel to the *Y*-axis. **Figure 2A** shows four typical images of different kernels with, at the top, TR118 (Arborfield) images and, at bottom left, TR118 (Naicom) and, at bottom right, Manley (St. Brieux) images, each used as a control. The left-hand side corresponds to samples stored for 6 months, whereas the right-hand side represents samples stored for 7 months. The images show sections parallel to the long axis of the kernel, and the embryo can be seen in all images. Hydration of the endosperm takes more time, hence, the absence of signal intensity from this part of the seed (*13*). Images are displayed using a false color scheme. Images of hydrated seed show water signal intensity to vary from red (high moisture) to yellow, green, blue, purple, and black (very low or lack of moisture). The TR118 Arborfield images show an increased water resonance intensity compared with the control samples at the bottom of the figure, especially in the area between the embryo and the endosperm. That the area between the embryo and endosperm could be damaged, either physically or chemically, is shown by more images obtained from TR118 (Arborfield)

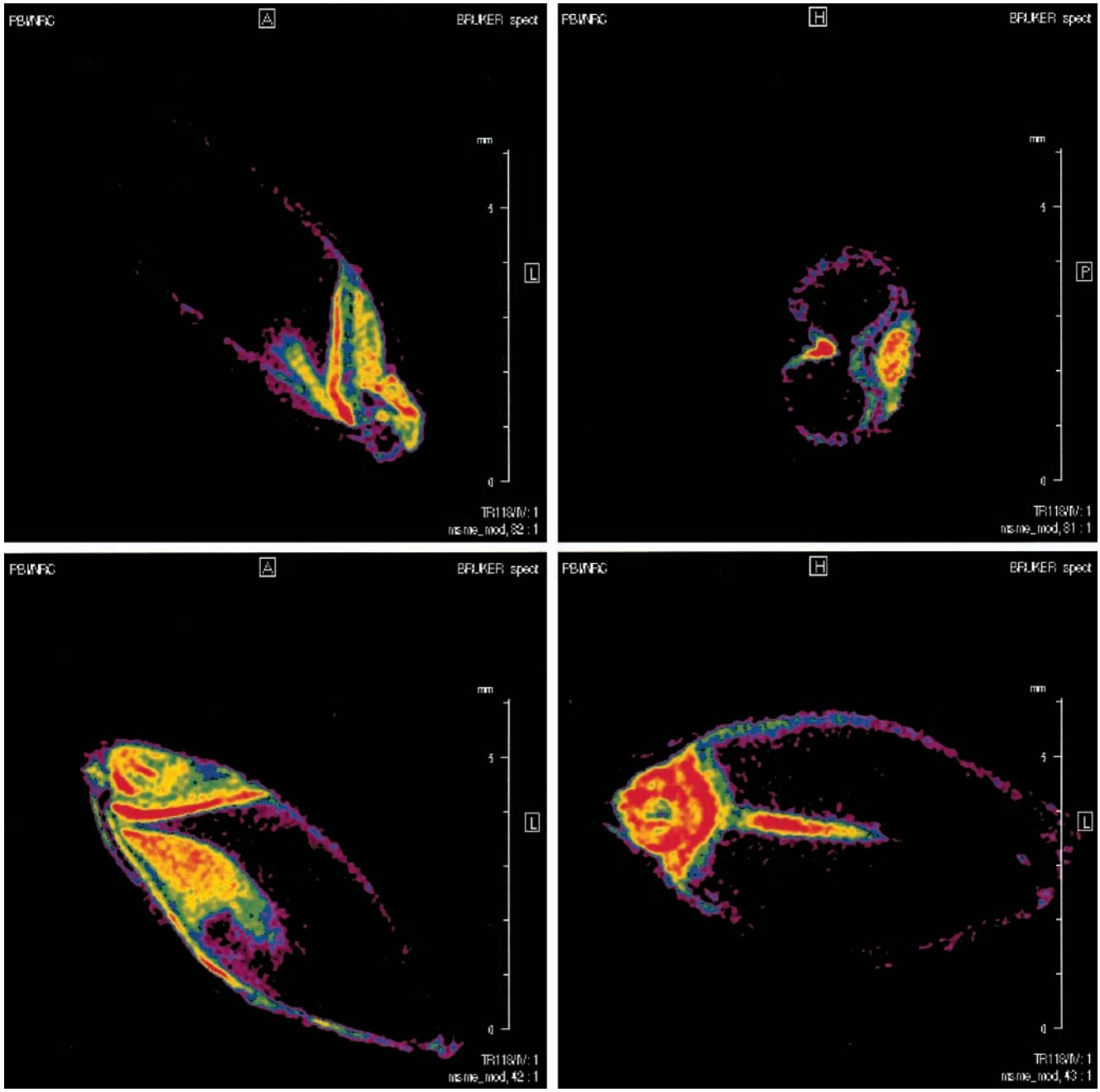

Figure 3B. (B) MR images of a nonviable TR118 (Arborfield) sample after 8 months of storage. The orientations are the same as in **Figure 3A**. The two top images show two orientations of the same kernel, obtained 17 h after the initiation of the germination protocol. The two bottom images, also of the same kernel, were obtained 33 h into the germination protocol.

kernels, shown in **Figure 2B**. This area is visible as a red band in the TR118 images, separating the embryo from the endosperm. This is not observed in viable control samples.

Corresponding MR images for a TR118 (Arborfield) sample after 8 months of storage are shown in **Figure 3A**,**B**. Each figure shows two perpendicular images of the same seed. The TR118 MR images shown are representative of nongerminating kernels, as germination, checked for after imaging, did not occur. These images show that for the TR118 (Arborfield) sample, hydration of the seed embryo occurs rapidly in comparison with viable kernels, as shown in images of the control sample in **Figure 2A** (bottom) and **Figure 4**. In **Figure 4**, images of a control sample, Manley (St. Brieux) after 7 months of storage, are shown. The bottom images clearly show the growth of the roots from the embryo. Note that the image on the right-hand side displays another orientation of the kernel, perpendicular to the image on the left. **Figure 3A,B** strongly suggests a degradation of the area between the embryo and endosperm in the TR118 (Arborfield) samples. In a few of the kernels studied, nontypical hydration patterns were observed, which also indicate damage to this area. **Figure 5** shows a TR118 (Arborfield) kernel imaged 35 h into the germination protocol. Hydration of the endosperm, from multiple points along the scutellum, indicates severe damage to the cell layer between the endosperm and embryo. A similar event occurs in **Figure 3A** at the bottom left.

Low seed viability (51%) was also observed for the Manley (Tisdale) sample after 8 months of storage (**Table 1**). **Figure 6** shows typical MR images of kernel water distributions of this sample. As observed for the TR118 (Arborfield) samples, these images show that hydration of the seed embryo occurs rapidly in comparison with images of viable control samples, especially in the scutellum area between the embryo and endosperm.

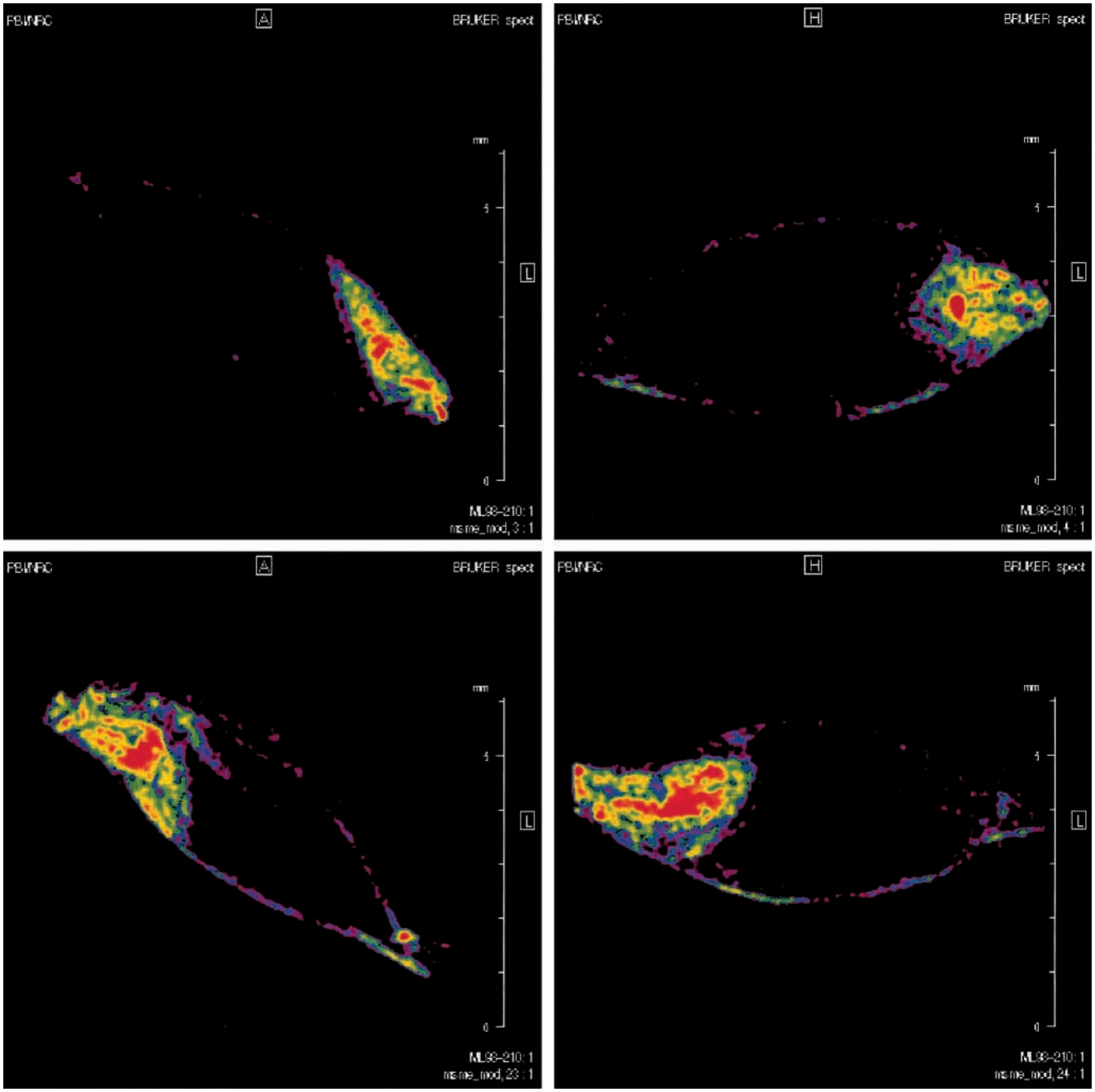

Figure 4. MR images of viable Manley (St. Brieux) samples after 7 months of storage. The two top images show two orientations of the same kernel, obtained 13 h after the initiation of the germination protocol. The two bottom images, also of the same kernel, were obtained 21 h into the germination protocol. All images represent ZY orientations, except the top right image, which shows an XY orienation.

MRI experiments indicate that samples with a known, reduced viability seem to be preferentially hydrated in the scutellum area prior to full hydration of the embryo. This was never observed for any of the control samples. Our observation of a rapid hydration of the scutellum in nonviable kernels can in principle be explained by three basic scenarios:

- membrane damage in some parts of the embryo due to radicals (peroxides etc.);
- a germination event prior to our experiments (during this attempt small molecules were produced after which hydration and germination were halted, followed by drying of the seeds);
- quick pick-up of water by embryos during imbibition of barley kernels (*14*) (therefore, under moist conditions, conducive to pregermination, barley embryos would become hydrated and swell; when the kernels are subsequently dried, the embryos shrink and become relatively loose within the embryo cavity of the kernel; during rehydration, water could rush into the cavity as well as being absorbed by the embryo).

The first scenario can be excluded as it does not explain the large α-amylase content of the seeds. This leaves us with the second and third scenarios as likely explanations.

The third scenario requires a physical separation of the embryo from the rest of the kernel. If this occurs, a void between the endosperm and embryo would be created, which, in principle, could be filled with water upon hydration. To exclude this possibility, 8-month-storage samples of TR118 from both Arborfield and Naicom were subjected to hyperpolarized ^{129}Xe NMR studies (*15*). These experiments did not show any significant penetration of xenon gas into the kernels of the TR118 Arborfield sample. Additional experiments were per-

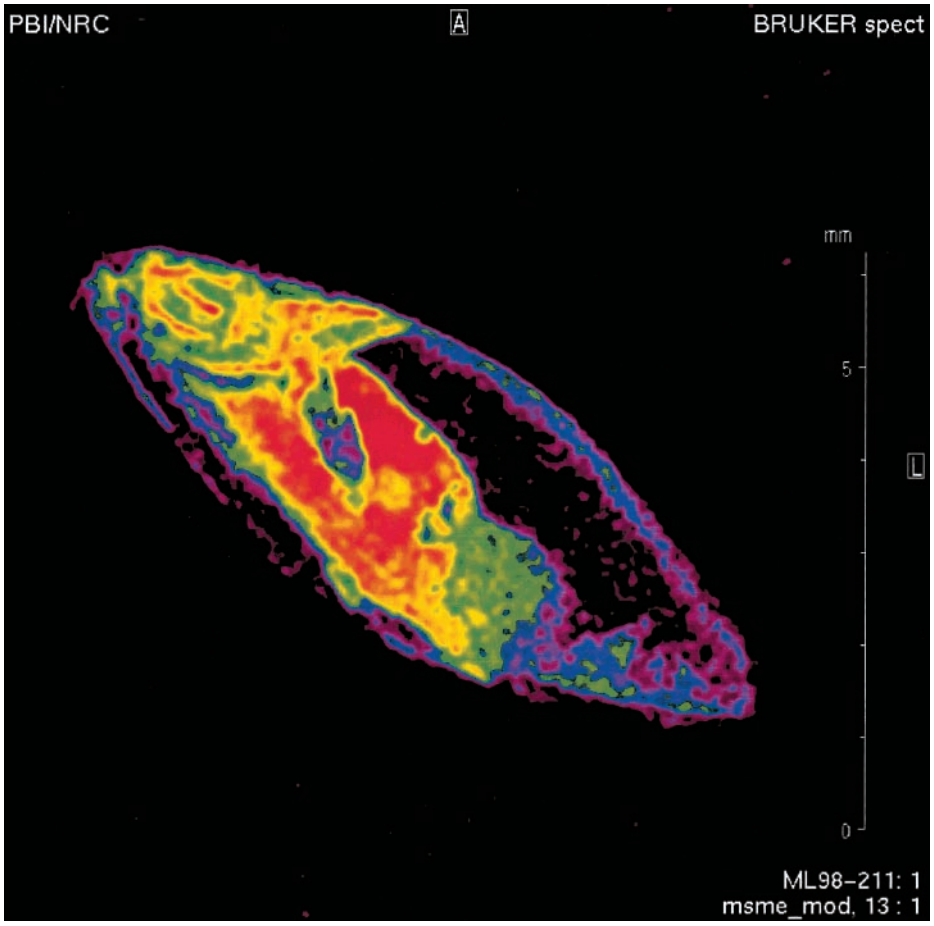

Figure 5. ZY orientation MR image of a TR118 (Arborfield) kernel after 7 months of storage. This image was obtained 35 h into the germination protocol and clearly shows the unconventional hydration pattern at the scutellum.

formed with [^{19}F]hexafluoroethane and kernels punctured with hypodermic needles prior to exposure to gas. No penetration of gas into the kernels could be observed, indicating that the voids are not likely to occur in these samples.

Assuming a previous hydration or germination attempt of the TR118 (Arborfield) and Manley (Tisdale) samples, carbohydrates, proteins, and lipids in the scutellum could have partially degraded to simple sugars, amino acids, and fatty acids, which were left behind when this first germination attempt was halted when the seeds dried again. Upon a second germination attempt these sugars and amino acids created an osmotic potential, rapidly attracting water compared to other compartments of the embryo.

If this model is correct, profound hydration of the scutellum should result in a different overall water relaxation rate, as measured by NMR, compared to values obtained from sound samples. Water mobility in kernels can be measured with NMR spectroscopy (*16*) and can be used to characterize the physicochemical state of water in biological materials using the ^{1}H water spin−lattice relaxation time (T_1) or rate ($R_1 = 1/T_1$). T_1 is very sensitive to water mobility and thus allows the indirect study of the water environment in kernels. During imbibition, different parts of the kernels become hydrated at different times. This reflects itself in a continuous change in T_1 with time. **Figure 7** shows the relaxation rates obtained for viable and nonviable TR118 samples.

Imbibition of barley is characterized by a rapid increase in ^{1}H R_1 during the first 6−7 h of imbibition, followed by a small but steady decrease after reaching a maximum (*16*). The initial rapid increase in R_1 can be explained in terms of water uptake in porous materials. This process can be described by a simple diffusive pathway and predicts that the number of absorbed water molecules is proportional to the square root of the imbibition time (eq 2). In other words, the number of water

$$N_{ABS}(t) = \text{constant}(\sqrt{Dt}) \tag{2}$$

molecules diffused into the barley kernels (N_{ABS}) during the imbibition time t is proportional to the water distribution at time t. The width of the distribution is described by the water translational diffusion constant D.

During imbibition two populations of water exist, a so-called bound water fraction and a fraction representing more mobile water molecules. From experiments in similar materials it is known that water molecules in these two states exchange rapidly compared to the T_1 of each of the sites. Dry barley still contains ~10% (by weight) water. This water occupies sites for bound water, indicated by the index b. Sites for mobile water are indexed with m. Accordingly, the observed T_1^{obs} can be described by

$$\frac{1}{T_1^{obs}} = p_m \frac{1}{T_1^m} + (1 - p_m)\frac{1}{T_1^b} \tag{3}$$

With the help of eq 1 this can be written as

$$\frac{1}{T_1^{obs}} = \frac{1}{T_1^b} + \frac{\sqrt{t}}{\text{constant} + \sqrt{t}} \cdot \left(\frac{1}{T_1^m} - \frac{1}{T_1^b}\right) \tag{4}$$

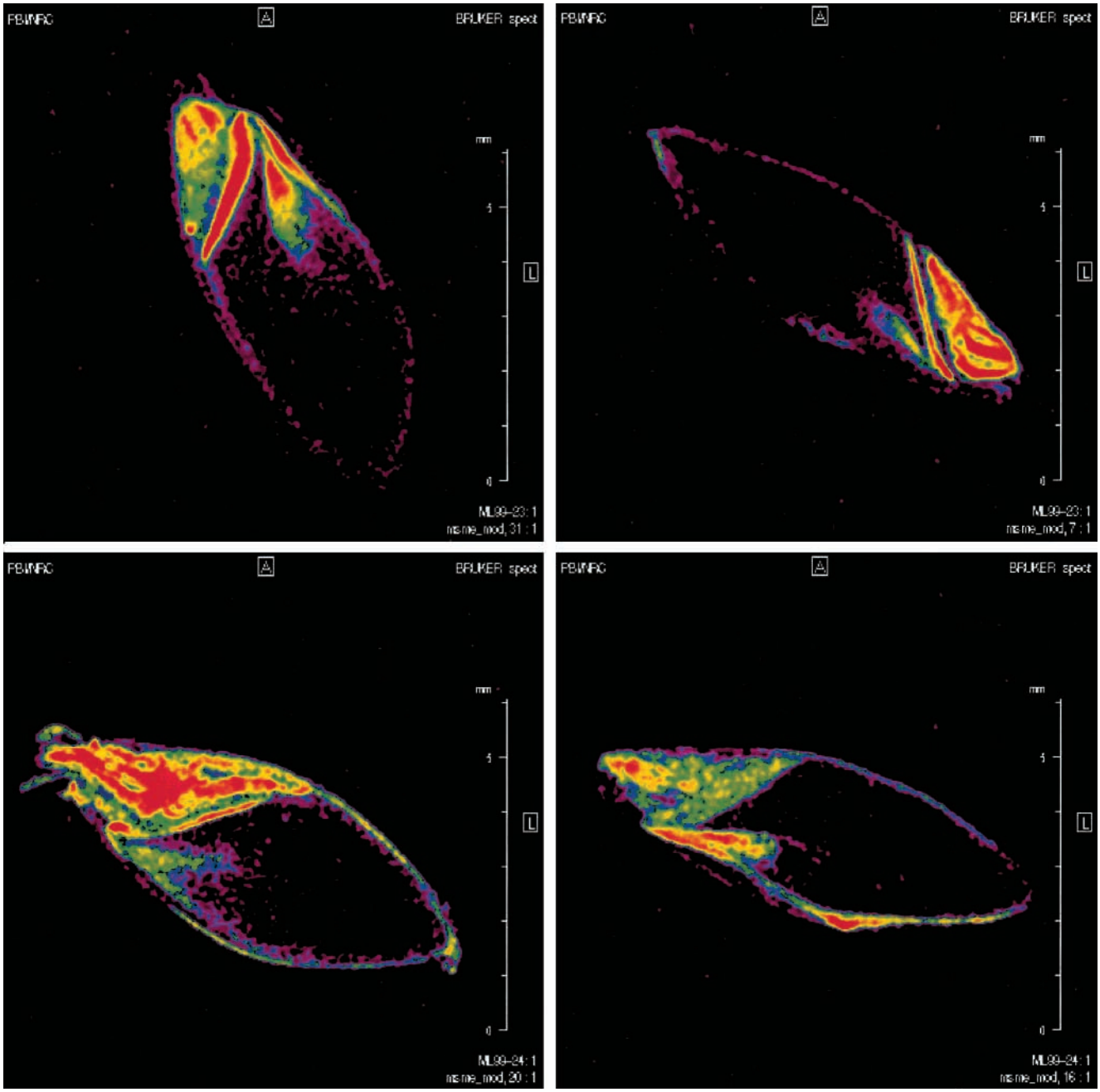

Figure 6. MR images of nonviable Manley (Tisdale) after 8 months of storage, obtained, from left to right, 40 and 21 h into the germination protocol. The two bottom images represent viable Manley (St. Brieux) after 8 months of storage and were obtained after 38 and 19 h, going from left to right, respectively. All images show ZY orientations.

Figure 7 shows a fit of $1/T_1^{obs}$ versus the first 6 h of imbibition of viable TR118 (Naicom) barley after 8 months of storage. Each data point represents an average measurement of R_1 performed on at least four different kernels. The kernels were not checked for germinative energy after the NMR measurement; however, it was known that the TR118 (Arborfield) sample had a very low germinative energy (20%, see **Table 1**). **Figure 7** clearly shows that the water uptake in the TR118 (Arborfield) sample is different from that of the TR118 (Naicom) control sample after the first 2−3 h. During the first 2−3 h of imbibition both TR118 samples display similar relaxation rates. After ∼2−3 h of imbibition, the TR118 (Arborfield) sample shows a rapid increase in relaxation rate. The relaxation rates measured after 3 h of imbibition for TR118 (Arborfield) are significantly different from those measured for the control and represent a second hydration phase. This observation could be explained by introducing an additional, new, porous area within the seed which has come into contact with water. This area could likely be the layer between the embryo and the endosperm. These relaxation results could form the basis of a simple test for barley viability.

These results suggest that the loss of viability during storage was caused by low levels of sprouting either before or after harvesting. Using NMR relaxation measurements, this type of seed degradation found for the TR118 (Arborfield) samples can easily be identified within 3−4 h after imbibition.

ABBREVIATIONS USED

NMR, nuclear magnetic resonance; MRI, magnetic resonance imaging; EPR, electron paramagnetic resonance; ISTA, International Seed Testing Association.

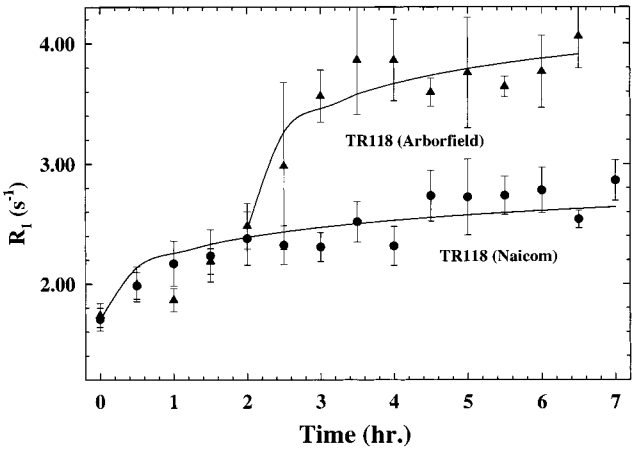

Figure 7. ^{1}H relaxation rates of TR118 (Arborfield) (△) and TR118 (Naicom) (○) barley, both after 8 months of storage, with a germinative energies of 20 and 99%, respectively. After ∼3 h of imbibition, the observed relaxation rate for TR118 (Arborfield) has become significantly different from that of the TR118 (Naicom) control. Data from the Naicom sample was fitted to eq 4, whereas the curve for the Arborfield sample was drawn as a guide to the eye.

ACKNOWLEDGMENT

We thank the Canadian Wheat Board for their help with sample handling and Drs. I. L. Moudrakovski and J. Ripmeester of the NRC Steacie Institute for Molecular Sciences for performing polarized gas NMR experiments.

LITERATURE CITED

(1) Brooks, P. A. The significance of pre-harvest sprouting of barley in malting and brewing. *Cereal Res. Commun.* **1980**, *8*, 29−38.
(2) Carn, J. D. α-amylase indicates problems with malting of stored barley. *Food Technol. Aust.* **1982**, *34*, 82.
(3) Bewley, J. D.; Black, M. *Seeds, Physiology of Development and Germination*, 2nd ed.; Plenum Press: New York, 1994.
(4) Roberts, E. H. Cytological, genetical and metabolic changes associated with loss of viability. In *Viability of Seeds*; Roberts, E. H., Ed.; Chapman and Hall: London, U.K., 1972; pp 253−306.
(5) Koostra, P. T.; Harrington, J. F. Biochemical effects of age on membranal lipids of *Cucumis sativus* L. seed. *Proc. Int. Seed Test. Assoc.* **1969**, *34*, 329−340.
(6) Priestley, D. A. *Seed Aging: Implications for Seed Storage and Persistence in Soil*; Comstock Publication Association: Ithaca, NY, 1986.
(7) Smith, M. T.; Berjak, P. Deteriorative changes associated with the loss of viability of stored desiccation-tolerant and desiccation sensitive seeds. In *Seed Development and Germination*; Kigel, J., Galili, G., Eds.; Dekker: New York, 1995; pp 701−746.
(8) Buitink, J.; Claessens, M. M. A. E.; Hemminga, M. A.; Hoekstra, F. A. Influence of Water Content and Temperature on Molecular Mobility and Intracellular Glasses in Seeds and Pollen. *Plant Physiol.* **1998**, *118*, 531−541.
(9) Briggs, D. E. A modification of the Sandstedt, Kneen and Blish assay of α-amylase. *J. Inst. Brew.* **1961**, *67*, 427−431.
(10) MacGregor, A. W.; LaBerge, D. E.; Meredith, W. O. S. Changes in barley kernels during growth and maturation. *Cereal Chem.* **1971**, *48*, 255−269.
(11) American Society of Brewing Chemists. *Methods of Analysis*, 8th rev. ed.; American Society of Brewing Chemists: Chicago, IL, 1992.
(12) International Seed Testing Association (ISTA). International rules for seed testing. *Seed Sci. Technol.* **1993**, *21* (Suppl.).
(13) Gruwel, M. L. H.; Chatson, B.; Yin, X.; Abrams, S. A nuclear magnetic relaxation study of water uptake in barley kernels. In *Molecular Biology and Physiology of Water and Solute Transport*; Hohman, S., Nielsen, S., Eds.; Kluwer Academic/Plenum Publishers: New York, 2000; pp 289−295.
(14) Reynolds, T.; MacWilliam, I. C. Water uptake and enzymatic activity during steeping of barley. *J. Inst. Brew.* **1966**, *72*, 166−170.
(15) Moudrakovski, I. L.; Lang, S.; Ratcliffe, C. I.; Simard, B.; Santyr, G.; Ripmeester, J. Chemical shift imaging with continuously flowing hyperpolarized xenon for the characterization of materials. *J. Magn. Reson.* **2000**, *144*, 372−377.
(16) Gruwel, M. L. H.; Chatson, B.; Yin, X. S.; Abrams, S. A magnetic resonance study of water uptake in whole barley kernels. *Int. J. Food Sci. Techn.* **2001**, *36*, 161−168.

Received for review July 6, 2001. Revised manuscript received November 7, 2001. Accepted November 7, 2001. Part of this research was funded by a grant from the Agricultural Development Fund (ADF Project 97000335) of the government of Saskatchewan, Canada.

JF0108617