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RESEARCH PAPER

Water movement into dormant and non-dormant wheat (*Triticum aestivum* L.) grains

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Abstract

The movement of water into harvest-ripe grains of dormant and non-dormant genotypes of wheat (*Triticum aestivum* L.) was investigated using Magnetic Resonance Micro-Imaging (MRMI). Images of virtual sections, both longitudinal and transverse, throughout the grain were collected at intervals after the start of imbibition and used to reconstruct a picture of water location within the different grain tissues and changes over time. The observations were supplemented by the weighing measurements of water content and imbibition of grains in water containing I₂/KI which stains starch and lipid, thereby acting as a marker for water. In closely related genotypes, with either a dormant or a non-dormant phenotype, neither the rate of increase in water content nor the pattern of water distribution within the grain was significantly different until 18 h, when germination became apparent in the non-dormant genotype. Water entered the embryo and scutellum during the very early stages of imbibition through the micropyle and by 2 h water was clearly evident in the micropyle channel. After 12 h of imbibition, embryo structures such as the coleoptile and radicle were clearly distinguished. Although water accumulated between the inner (seed coat) and outer (pericarp) layers of the coat surrounding the grain, there was no evidence for movement of water directly across the coat and into the underlying starchy endosperm.

Key words: Dormancy, germination, imbibition, MRI, *Triticum aestivum*, water, wheat.

Introduction

The movement of water into ripe and dry grains is a critical step in germination, preharvest sprouting, expression of dormancy, and processes such as conditioning for optimum milling performance. Imbibing and maintaining grains under warm, moist conditions is the only means of determining the dormancy or germinability of wheat grains. Variation in the rate or the pathway of water movement may affect measurement of the dormancy phenotype and might also be involved in the dormancy mechanism. Recent developments in Magnetic Resonance Micro-Imaging (MRMI) (Kockenberger, 2001; Kockenberger *et al.*, 2004) now allow non-destructive real-time visualization of grain water distribution with high spatial resolution and allow the determination of relative rates and pathways of water movement into living grain tissues. MRMI is the visualiza-

tion of small objects through the same principles of MRI and was first used in wheat to visualize water distribution in developing grains (Jenner *et al.*, 1988) and more recently in even smaller seed species, such as those of tobacco (Manz *et al.*, 2005).

Preharvest sprouting, or the germination of grain in the spike, can occur prior to harvest maturity if the crop is exposed to wet, humid conditions. The majority of wheat-growing climates throughout the world experience rain during harvest, which can result in sprouted grain and a loss of quality premiums. Although there are a number of management techniques and varietal differences that can reduce the risk of sprouting, these are largely ineffective if adverse environmental conditions persist. Grain dormancy is the most reliable protection against preharvest sprouting

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in a broad range of environments and will significantly protect against preharvest sprouting.

Dormancy in some plant species is controlled, at least in part, by differential permeability to water of the seed coat between dormant and non-dormant genotypes (Bewley and Black, 1982*a*). The possibility that differences in grain water uptake or seed coat water permeability could be associated with dormancy in wheat, as in other species (Finch-Savage and Leubner-Metzger, 2006), has not been discounted. Wheat caryopses, commonly called grains, like the caryopses of other cereals and grasses, are indehiscent fruit having a single seed surrounded by a seed coat and, in turn, enclosed within a thin, adherent pericarp (fruit coat) (Bradbury *et al.*, 1956; Black *et al.*, 2006). In this investigation, the term coat is used to refer to the tissues, pericarp (inner and outer) and the true seed coat (testa and nucellus), that surround the germ (embryo, scutellum, and epiblast) and endosperm (aleurone and starchy endosperm) of the wheat grain. In wheat, therefore, the pericarp and/or the seed coat could affect water permeability and water entry into the germ.

In white-grained wheat, dormancy is relatively rare, but genetic studies have indicated that there appears to be more than one factor which contributes to dormancy (Mares, 1999). According to the model proposed by Mares (1999), there is at least one gene expressed in the embryo that controls a transient sensitivity to abscisic acid (ABA) which, on its own, gives rise to an intermediate dormant phenotype. Another unknown factor controlled by a gene that is expressed in the coat of dormant genotypes, can interact with the sensitive embryo to produce a stronger complete dormant phenotype (Mares, 1999). The coat factor could involve germination inhibitors, a reduced permeability of the seed coat to water or oxygen, or other unknown mechanisms.

The aim of this investigation was to use MRMI to provide both a non-destructive and detailed picture of water uptake and distribution in a wheat grain, as well as a clearer indication of water uptake in dormant and non-dormant genotypes. MRMI was initially employed over a period of 0–18 h after the start of imbibition, i.e. prior to the rupture of the seed coat and the first physical evidence of germination in the non-dormant genotype, to address the question of whether dormancy involved differences in grain water uptake or seed coat permeability and to determine the pattern of water movement into imbibing grains. Subsequently the experiments were extended to later stages of germination in order to examine the movement of water into the starchy endosperm. In addition, MRMI data was supplemented with the weights of grain tissues at intervals following imbibition and staining of the starchy endosperm following imbibition with I₂/KI solution.

Materials and methods

Plant material

Grain of the following bread wheat (*Triticum aestivum* L.) genotypes, representing the extremes in the range of genetic

variation for grain dormancy in white-grained wheats, was used: Hartog, an Australian, genetically non-dormant (sprouting susceptible) cultivar and SUN325B, a genetically dormant (sprouting resistant) breeding line with pedigree Hartog/Vasco//Aus1408/3/Hartog. Large samples of grain were harvested from field plots of each genotype at harvest-ripeness by gentle hand-threshing and stored at –20 °C until required (Mares, 1983). Storage at –20 °C preserves dormancy in wheat grains for at least 9 months (Mares, 1983) and all grains were used within this time period. A smaller sample of SUN325B from plants that had been grown in a cool greenhouse to promote a higher level of dormancy (no germination of harvest-ripe seed after 7 d imbibition at 20 °C) was separated into two portions; one being stored at –20 °C until required whilst the other was after-ripened at room temperature for 8 weeks until all dormancy had disappeared. These samples were used for gravimetric measurements of water uptake into the embryo and scutellum to confirm observations from the large samples.

Grain water uptake

The dormancy phenotype of the grains was confirmed with germination tests (Mares *et al.*, 2005). These tests were conducted with 10 replicates of 50 grains incubated in Petri dishes at 22 °C for 7 d, before commencing the experiments. For the weighing measurements of grain water uptake, four replicates of 23 grains were weighed initially to determine the initial grain dry weight (DW). Grains were subsequently imbibed with reverse osmosis (RO) water at 20 °C and samples of four grains were taken every 10 min for the first hour of imbibition, then every hour following for 18 h and weighed to provide a whole grain fresh weight (FW). At each sampling time, grains were also dissected and changes in the weight of the germ and de-embryonated grains (endosperm plus coat) were calculated as: $(FW - DW) / DW \times 100$. The increase in moisture content was thus expressed as a percentage of the initial dry weight of each grain tissue. The data were analysed by Genstat (Version 8.2.0158, Lawes Agricultural Trust) and fitted with a rectangular hyperbola [$y = ax / (b + x)$].

Imbibition of grains of Hartog and SUN325B was continued for 24, 36, 48, 72, and 96 h in four replicates to extend the initial 18 h and to determine the phases of grain water uptake in dormant and non-dormant wheat genotypes (Manz *et al.*, 2005; Terskikh *et al.*, 2005). Germ tissue from each grain was excised with a scalpel, weighed, and the data fitted to an exponential asymptotic curve with the software program WinCurveFit (Version 1.1.8, Kevin Raner Software, 2002).

Imaging of grain water uptake using MRMI

Grains were soaked in RO water and analysed at hourly intervals from 2 h up to 18 h imbibition, to determine the general pattern of water uptake. For comparison of water uptake between dormant and non-dormant lines, grains

were soaked for a total of 18 h, with images taken at six representative time points during early imbibition (0–18 h). These representative time points were selected within blocks of 3 h, for example, a point between 1–3 h, 4–6 h, 7–9 h, 10–12 h, 13–15 h, and 16–18 h. For the imbibition experiments designed to compare the effect of applying water to different parts of the grain, grains were wrapped in Teflon tape with only the embryo/proximal or brush/distal end section exposed. Grains were then suspended from the lid of the Petri dish with the same tape, to ensure that only the exposed end was in contact with the moist filter paper.

Grains were also germinated in a Petri dish for 72 h, with the protruding roots and shoots removed to avoid interference from the high water content of these tissues, before being placed in the MRI machine as described below.

After soaking for a specific time, grains were removed from the Petri dishes and the seed blotted dry on tissue. Teflon tape was used to completely cover the grains to prevent any water loss. The grain was subsequently wrapped in tissue and suspended in a 60 mm glass tube, which was then placed into the bore of the MRI machine. Images were collected in the transverse (horizontal) and longitudinal (vertical) orientation to provide a complete three-dimensional perspective of the grains.

All magnetic resonance experiments were performed at the Centre for Magnetic Resonance, University of Queensland, using a Bruker AMX300 (Germany) console interfaced to a Bruker 7T superconducting magnet with a 15 cm bore. Proton density images were acquired using three-dimensional spin echo pulse sequence (SE3D). The 90° pulse time employed was 13 μs and the echo time was 4.5 ms. Repetition time of 1.5 s was sufficient for full relaxation of the magnetization. For the grains imaged prior to any physical sign of germination (0–18 h imbibition), longitudinal images consisted of four slices 1.25 mm thick, with 15×6.0 mm field of view for each slice. The resolution in each slice (pixel size) was 117×62 μm. Transverse images consisted of eight horizontal slices 6.0×6.0 mm, with slice thickness 1.0 mm. For these images resolution (pixel size) was 46×62 μm. For the grains imaged after germination, the longitudinal images consisted of four slices 3 mm thick with a 12×10 mm field of view for each slice. The resolution in each slice (pixel size) was 98×75 μm. Transverse images consisted of eight horizontal slices 12×12 mm, with a slice thickness of 0.125 mm. For these images, resolution (pixel size) was 93×93 μm. Acquisition times for all the images were 20 min for longitudinal and 34 min for transversal images. The images obtained accurately represent mobile water content throughout the sample.

The MRMI images were compared with previously published micrographs of grain sections and tissues. The main structures, illustrated in the diagrams of transverse and longitudinal sections of wheat grains (Fig. 1A, B), were identified in the MRMI images, and the grain diagrams have been presented here to assist in the interpretation of these images. In all images, the colours red through bright yellow to white represent increasing water contents in the particular cellular tissues.

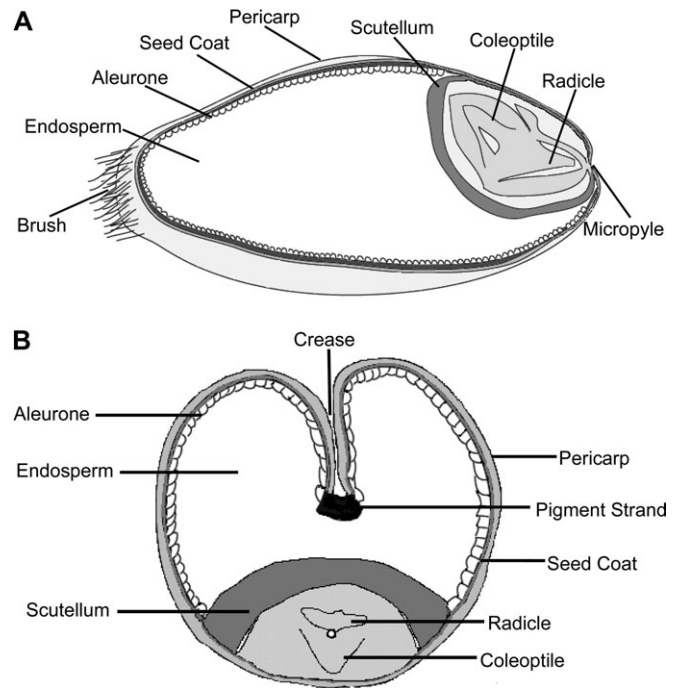


Fig. 1. Diagram of a wheat grain showing major structures in (A) longitudinal and (B) transverse sections, identifiable in the MRMI images (Figs 4–6).

Visualization of grain water uptake with I_2/KI solution

Grains were imbibed in a solution containing 1 g I_2 and 2 g KI in 100 ml water for 25 h, and samples were taken at intervals, blotted dry, and cut longitudinally through the crease with a scalpel. The exposed section of the grains was observed with an Olympus microscope and images captured with Olysia BioReport imaging software version 3.2 (Olympus, Australia). Other dyes were applied in solution to the grains, including 0.1% Methylene blue, 0.1% Congo red, and 1% tetrazolium chloride (Sigma, USA), for 25 h and sampled as described above.

Germination of grains where imbibition was restricted to the proximal or distal end

Fifty grains in five replicates of Hartog and SUN325B were imbibed proximally or distally for 7 d, by placing grains vertically into 30 ml 1% agarose gel in a Petri dish. Germinated grains were removed daily, with germination defined as the protrusion of the radicle through the pericarp.

Results

Germination rates for genotypes used in the study

The germination rates, defined as protrusion of the radicle through the pericarp, of the larger samples of the two genotypes were compared to determine their dormancy phenotype. Hartog (white-grained, non-dormant) germinated

rapidly, while the genotype SUN325B (white-grained, dormant) germinated at a much slower rate (Fig. 2). Whilst some grains (<2%) of SUN325B had germinated by the first day, the rate of germination was much slower than Hartog and more than 70% of grains had not germinated by day 7. By comparison, no grains in the small, harvest-ripe sample of SUN325B from the cool greenhouse germinated by day 7 whilst the germination curve for the after-ripened sample was similar to Hartog (data not shown).

Measurements of grain water uptake by weighing

Whole grains in four replicates were incubated in RO water and weighed at 10 min intervals for the first hour of imbibition and then hourly for the following 17 h. The quantity of water taken up by the grain was estimated from the increase in weight as a percentage of the initial weight. Water uptake into the grain was initially rapid but slowed by about 5 h, based on the rectangular hyperbola curve, with a good fit ($P < 0.001$) of $R^2 = 0.98$ for Hartog (Fig. 3A) and 0.99 for SUN325B (Fig. 3B). Both the genotypes took up water at a comparable rate and the final grain water content, was also similar between the genotypes.

At each time point, whole grains in four replicates were dissected into germ and non-germ portions and also weighed to determine water uptake. The data are presented as an increase in the weight of the de-embryonated grain as a percentage of the initial grain weight and was also fitted significantly ($P < 0.001$) for Hartog ($R^2 = 0.89$) and SUN325B ($R^2 = 0.92$) with a rectangular hyperbola (Fig. 3B, C). Water entered the germ very rapidly during early imbibition in both Hartog and SUN325B, but reached a plateau by approximately 4 h in both genotypes. The most substantial increase in embryo hydration occurred in the first 10 min of imbibition and there was no significant

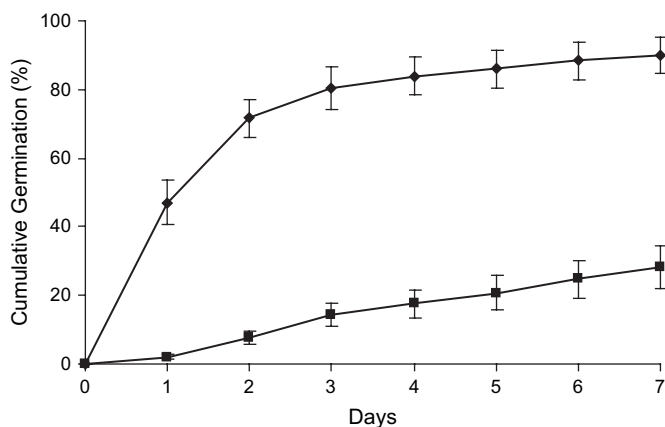


Fig. 2. Cumulative germination (%) over 7 d of Hartog (white-grained, non-dormant) compared with SUN325B (white-grained, dormant). The arrow and dashed line indicate the point of earliest pericarp rupture by the radicle in the non-dormant genotype. All time points are an average of 10 replicates of 50 grains incubated on moist filter paper at 22 °C. Standard error represented by bars.

difference between the rate and amount of water taken up by the embryo of Hartog compared to SUN325B. The time taken to 50% hydration was 39 min for Hartog and 15 min for SUN325B. In a further experiment, the water content of germ tissues from the harvest-ripe (highly dormant) and after-ripened (non-dormant) samples of SUN325B grown in the cool environment were compared at 30 min, 1 h and 2 h after the start of imbibition. Mean water contents were 48% and 48.5% after 30 min, 59% and 58% after 1 h, and 65% and 65.5% after 2 h for harvest-ripe and after-ripened SUN325B, respectively, and similar to the previous experiment. Standard errors varied from 2 to 4%.

By contrast, the rate of entry of water into the non-germ portion of the grain, primarily endosperm, was substantially slower than the germ (Fig. 3E, F). The endosperm weight ($P < 0.001$) increased in a similar way to the whole grain throughout the period of the experiment in both genotypes, with water uptake initially rapid but slowing after about 5 h of imbibition. The rectangular hyperbola curve significantly fit the data, with R^2 values of 0.98 for Hartog (Fig. 3E) and 0.99 for SUN325B (Fig. 3F). The final weight after 18 h of imbibition of the non-germ portion of the grain, as a percentage of the initial fresh weight, was similar for both Hartog and SUN325B (Fig. 3E, F).

When measurement of water uptake by the germ tissues was continued beyond 18 h, three distinct phases (Phase I, II, III) were apparent (Fig. 4). Phase I corresponded to initial rapid imbibition to the first point of inflection in the water uptake curve. After this early stage of imbibition, water uptake slowed for a brief period in the case of Hartog or levelled out for approximately 40 h in SUN325B (Phase II). Phase III marked the initiation of germination, and, following the rupture of the pericarp, the water content of the germ or seedling increased rapidly again and towards a plateau at 100% (water taken up as a percentage of initial fresh weight, w/w). Phases I and III were similar in both dormant and non-dormant grains, however, Phase II was extended in dormant grains because of the delay in germination (Fig. 4).

MR micro-imaging of imbibing wheat grains

After 2 h imbibition (Fig. 6A), the grain was still relatively dry and consequently the image was very noisy and lacked detail. Nevertheless, some localized hydration was already evident in the embryo (Fig. 6A, images 1 and 3), the scutellum (Fig. 6A, image L, and Fig. 5, image 1), the coat (Fig. 6A, images 1 and 2) and, in particular, the channel of the micropyle (Fig. 6A, images 3 and 4) compared to other grain structures. With increasing time of imbibition, the amount of water as well as the detail in the images of these embryo structures increased, but there was little evidence of movement to other parts of the grain. At 12 h (Fig. 6B), there was a greater level of hydration than at the earlier time points and, in particular, the coat appeared to have taken up more water and is clearly distinguishable. Embryo structures such as the radicle (Fig. 6B, image 4 as seen in

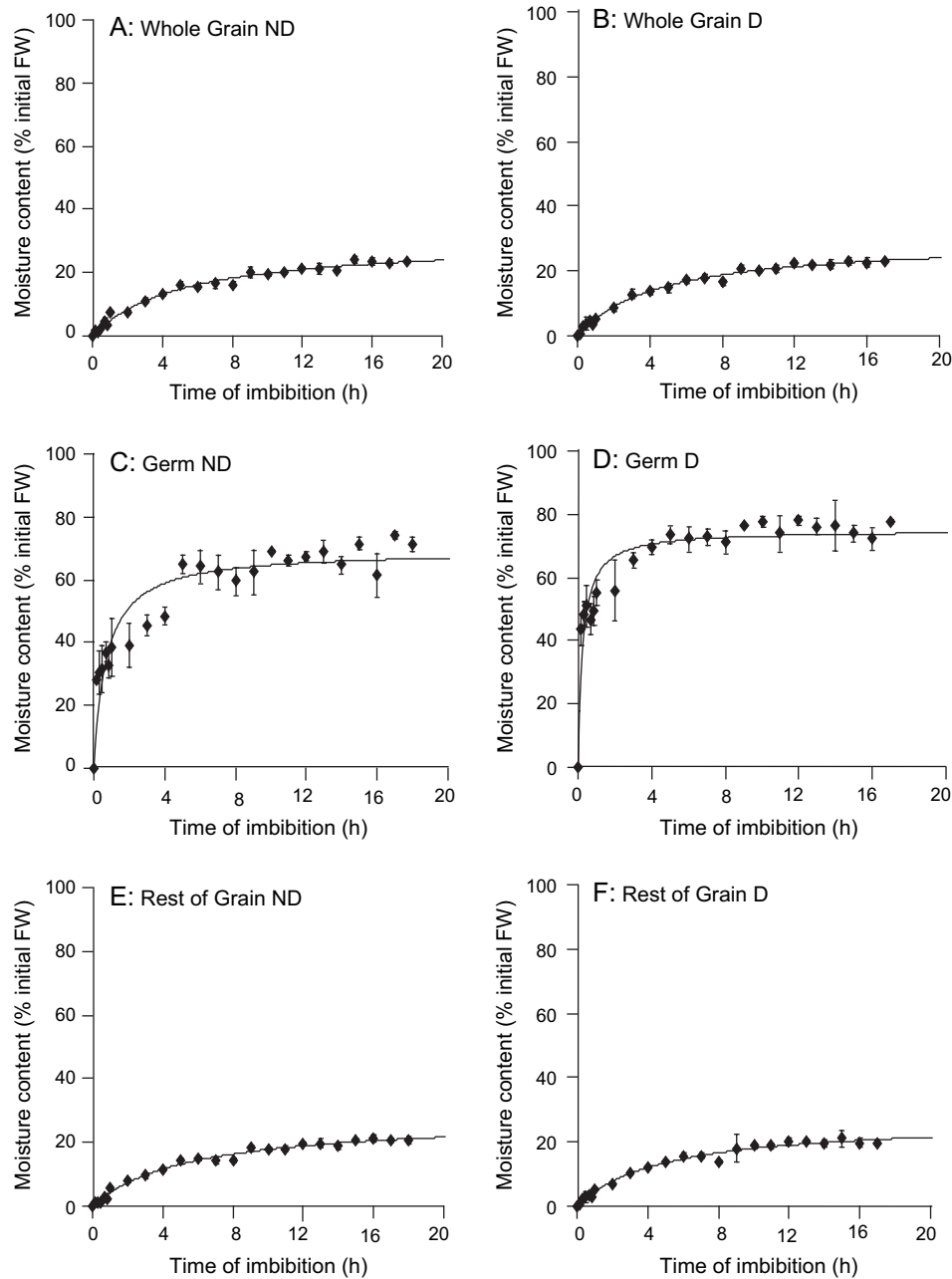


Fig. 3. Time-course of grain water uptake measured from 10–50 min and hourly from 1–18 h, and presented as an increase in moisture (% of initial fresh weight, w/w). (A) Hartog and (B) SUN325B whole grain; (C) Hartog and (D) SUN325B are germ (embryo/scutellar) tissue; and the remainder of the grain of (E) Hartog and (F) SUN325B. Graphs are all fitted significantly with rectangular hyperbola curves [$y=ax/(b+x)$]. Grains were incubated on moist filter paper at 22 °C. All time points are independent measurements and are an average of four replicates, with standard errors indicated by bars.

Fig. 5, image 5) were also more clearly defined and the micropyle stood out as a distinct ring around a saturated (white) pixel (Fig. 6B, image 3 as seen in Fig. 5, image 2). In particular, the coleoptile was visible in Fig. 6B, image 4 (as seen in Fig. 5, image 3). These fine embryo structures initially became evident at 7 h imbibition (data not shown). The signal intensity associated with the embryo continued to increase throughout imbibition, with the water distribution possibly becoming less localized and more widely distributed through the embryo region (Fig. 6B). This

observation is consistent with weighing measurements of embryo water uptake (Fig. 3). The coat was also significantly more hydrated after 12 h imbibition, with water appearing to be concentrated in the outer layers of the seed coat and in the crease rather than at the dorsal surface (Fig. 6B). Water appeared to be concentrated between the pericarp and the true seed coat, image 2, Fig. 6B (as seen in Fig. 5, image 4). There was no evidence of significant movement of water directly across the testa layer and into the underlying endosperm.

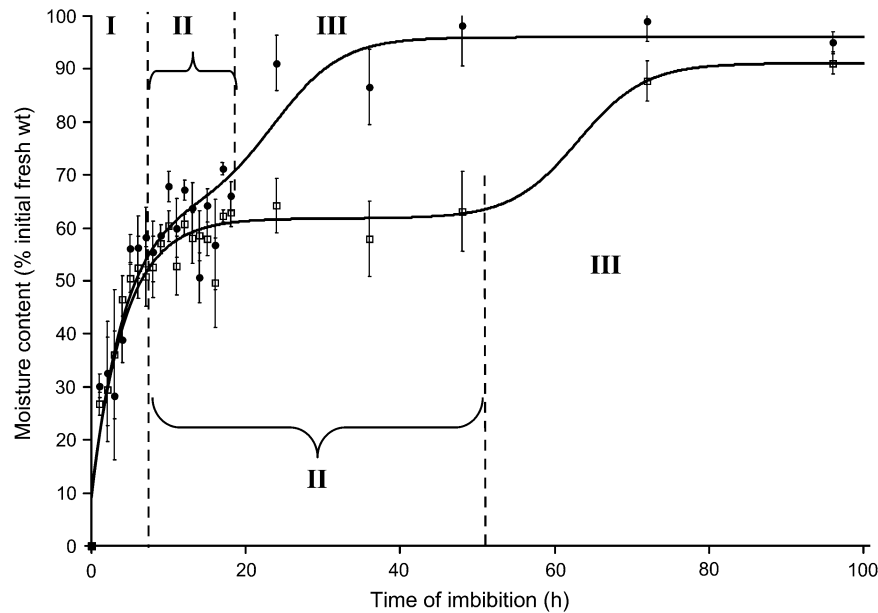


Fig. 4. Pattern of water uptake by the germ fraction of grains showing three distinct phases, early imbibition (phase I), a plateau stage (phase II) where a relatively constant moisture content was maintained, and finally germination (phase III). Hartog (closed symbols) and SUN325 (open symbols), with phase II extended in SUN325. Grains were incubated on moist filter paper at 22 °C. Measurements were conducted in four replicates, with standard errors indicated by bars.

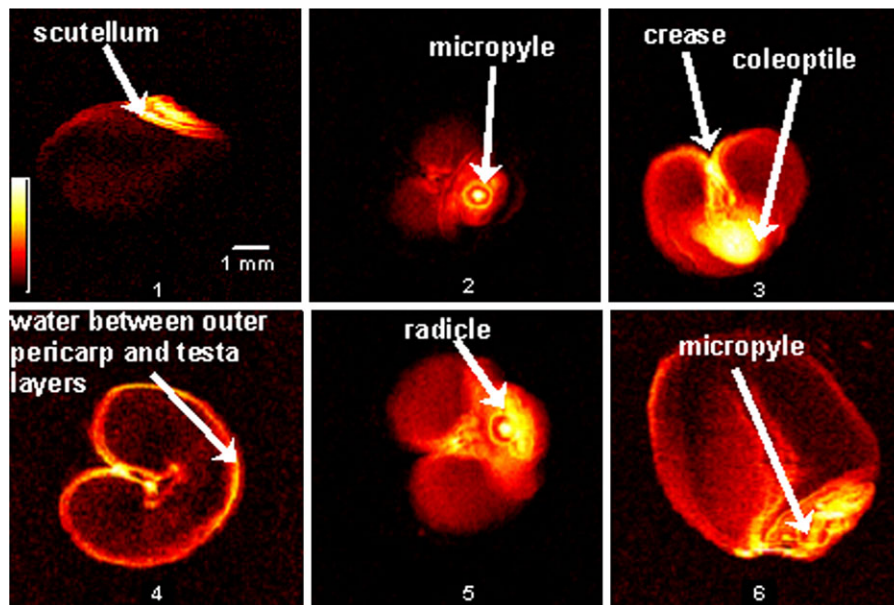


Fig. 5. MRMI images of transverse (images 2–5) and longitudinal (images 1 and 6) virtual slices of a wheat grain, showing the major grain structures involved in water uptake, including water located between the pericarp and true seed coat layers (image 4). Grains of Hartog (images 3 and 6) and SUN325 (images 1, 2, 4, and 5) were imbibed for 11 h (images 1–5) and 14 h (image 6). Water content was graded by colour, with red being low, yellow intermediate, and white the highest.

MR micro-imaging of imbibing wheat grains of differing dormancy

For this part of the investigation, images were taken at representative time points (in 3 hourly blocks) from 1 h to 18 h, although images for only two time points are presented here for each genotype. At the first observation point, water had entered the embryo and scutellum (Fig. 7A,

image L), the coat was only barely hydrated and the grains were still relatively dry (Fig. 7A, B). There was no discernible difference in either the amount of water taken up or the distribution between Hartog and SUN325B (Fig. 7A, B).

At 18 h (Fig. 7C, D), both genotypes appeared to have a higher level of hydration in the coat and the embryo than in the early stages of imbibition. Scutellar tissue was highly hydrated in Hartog (Fig. 7C) and the micropyle was clearly

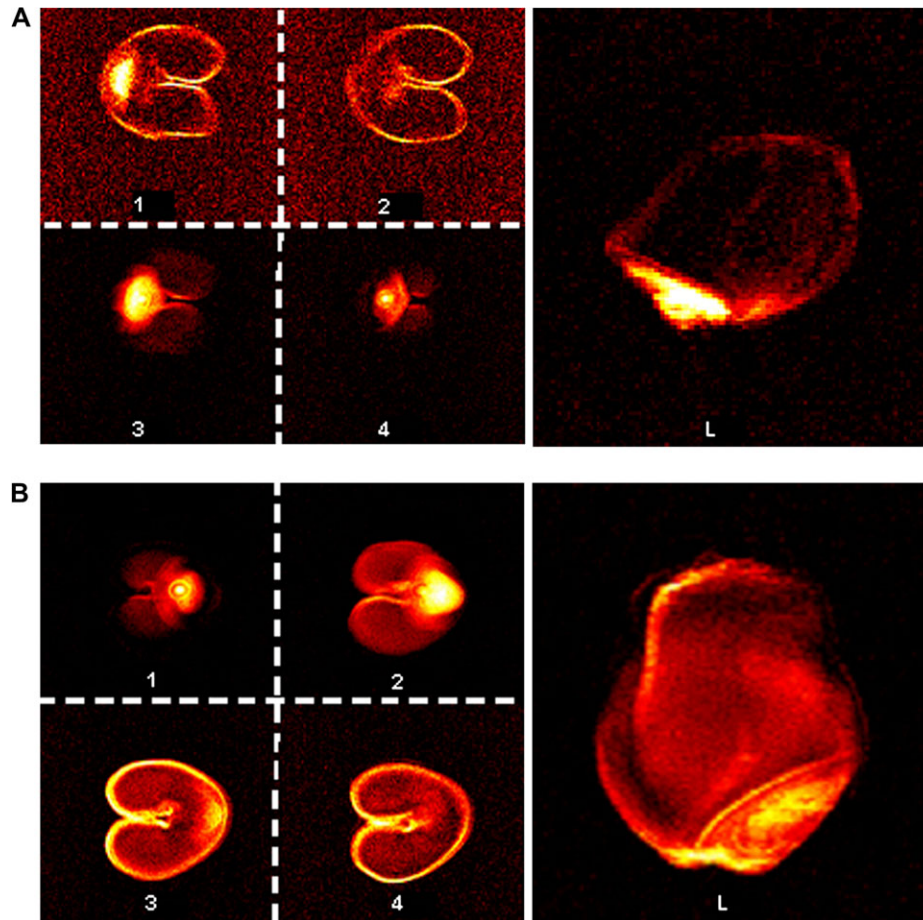


Fig. 6. Comparison of MRMI images (transverse and longitudinal slices) of grains of Hartog imbibed for (A) 2 h and (B) 12 h. Images 1–4 are transverse slices and image L is the longitudinal slice.

visible as a tube leading to the embryo in the longitudinal slices of both genotypes (Fig. 7C, D, images 4, as seen in Fig. 5, image 6). Discrete embryo tissue structures were also apparent in both genotypes (Fig. 7C, D) and from 7 h imbibition (data not shown), for example, the radicle in image 4 (Fig. 7D). Both Hartog (Fig. 7C) and SUN325B (Fig. 7D) had a moderate level of hydration in the coat but with substantially more water concentrated in the crease than other areas of the coat (Fig. 7C, D). Again, there was no evidence of water permeating directly across the coat into the aleurone starchy endosperm. There was no obvious difference in water content or distribution between Hartog and SUN325B at any stage prior to the onset of germination in the non-dormant genotype, Hartog.

MR micro-imaging of grains, where access to water was restricted to either the proximal (embryo) or distal (brush) end, and germinated grains

When only the embryo end of the grain was exposed to water, the extent and pattern of hydration of the embryo appeared similar to embryos in grains imbibed normally on moist filter paper (Fig. 8A). The outer layers of the coat were also hydrated, indicating that water appears to diffuse through the pericarp layer (Fig. 8A), although

to a lesser extent than for grains imbibed on moist filter paper where a much greater proportion of the grain surface was in contact with the water. In addition, water was also concentrated in the crease region (Fig. 8A).

Surprisingly, the restriction of water for imbibition to the distal (brush) region also did not significantly change the water distribution in the grain (Fig. 8B). Again the embryo clearly contained the highest water content and the micropyle structure was starkly apparent in images 1 to 4 (Fig. 8B). There was again no evidence of water permeating directly across the coat into the underlying endosperm (Fig. 8A), or entering the embryo by any other mechanism other than the micropyle, in either of these two experiments (Fig. 8A, B). Only data for Hartog is presented, however, there was no significant difference in the corresponding images obtained for the dormant cultivar, SUN325B.

Germinated grains that had been imbibed for 72 h showed high concentrations of water in the remnants of the embryo left after excising the roots and shoot of the growing seedling (Fig. 8C). The scutellum appeared to have enlarged after germination, compared with the non-germinating grains in Fig. 7A. Importantly, there was now a suggestion of more water in the endosperm, with this tissue showing more hydration than the early imbibition periods in the absence

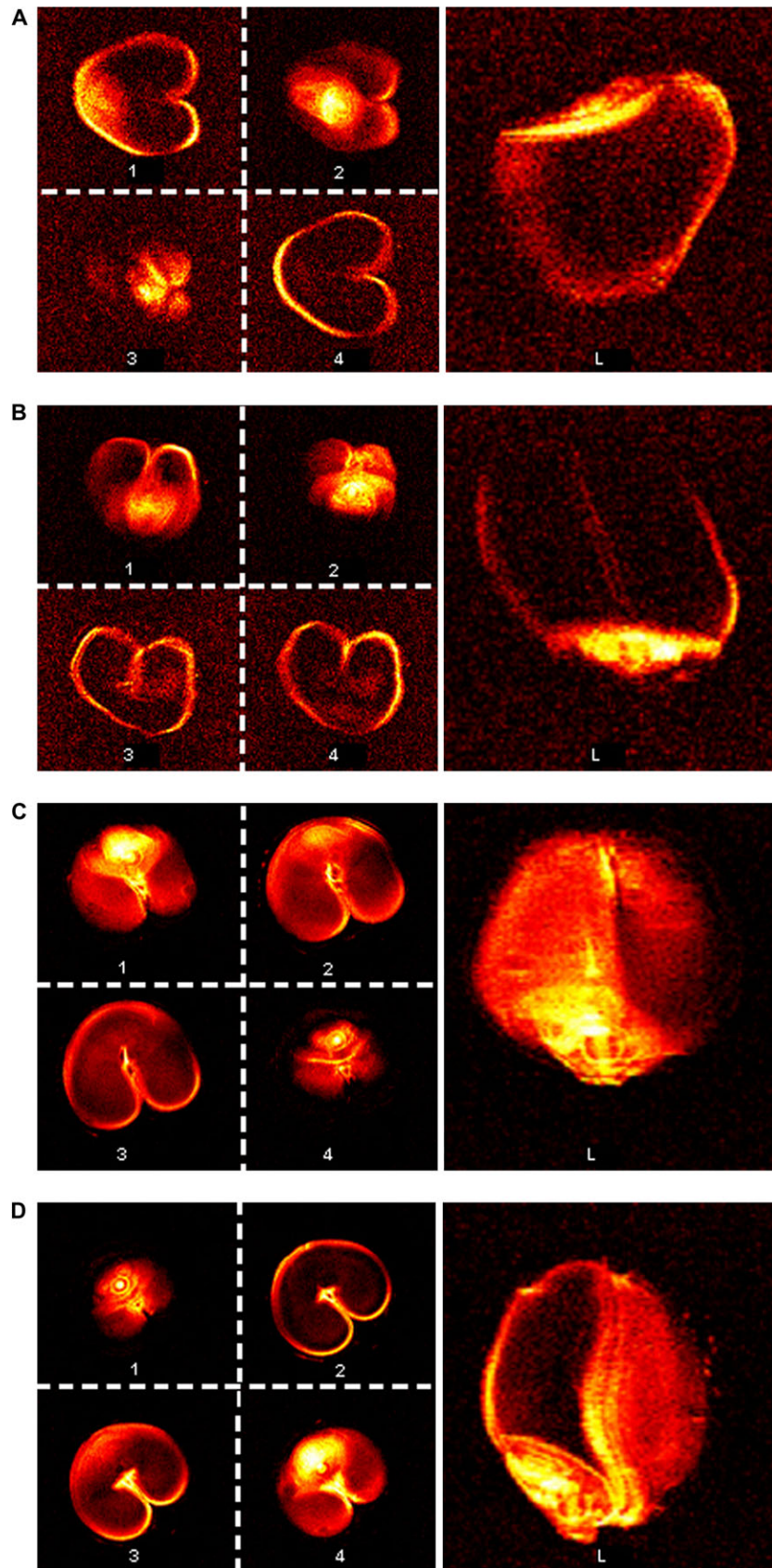


Fig. 7. Comparison of MRMI images (transverse and longitudinal slices). Images of grains of non-dormant Hartog (A, C) and dormant SUN325 (B, D) were taken at representative time points between 1–3 h and 15–18 h of imbibition. Images 1–4 are transverse slices and image L is the longitudinal slice.

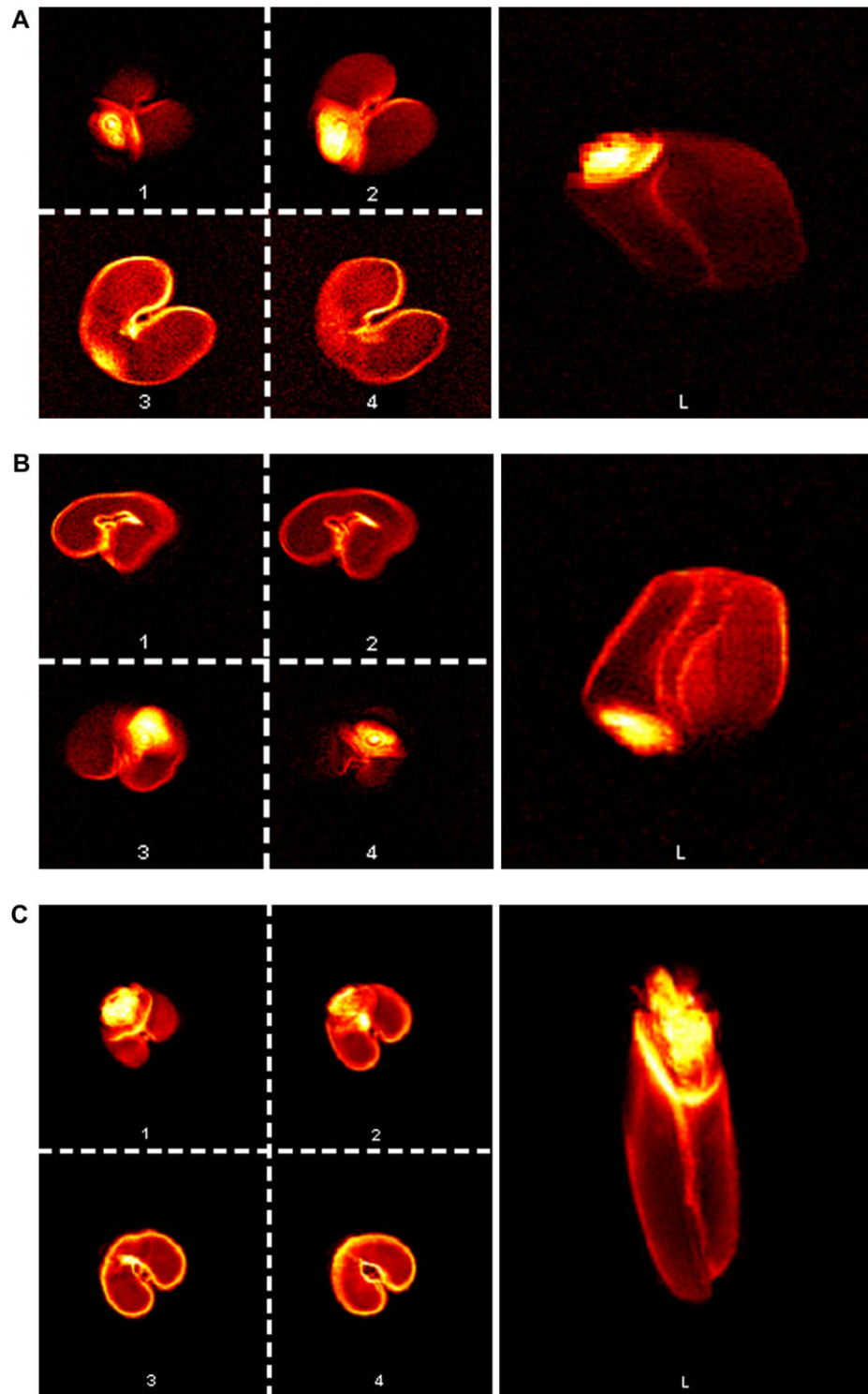


Fig. 8. MRMI images (transverse and longitudinal slices) of Hartog grains, in contact with water at only the proximal (embryo) (A) or the distal (brush) end of the grain (B) for 17 h, and grain that had germinated for 72 h (C). Images 1–4 are transverse slices and image L is the longitudinal slice.

of germination (dorsal regions of images 2 and 4 in Fig 8C). Despite this, the weak intensity of the MRMI signal from the endosperm was indicative of a much lower amount of free water compared with the embryo tissue (Fig. 8C). The scutellum of the germinated grain is also highly hydrated and significantly larger than was observed in imbibing grains (Fig. 8C, image L).

Visualization of water uptake by grains using I_2/KI as a marker

I_2/KI was found to be the most effective marker for visualizing water uptake into the endosperm, due to the small solute size and the ability to bind with and stain starch tissue. Imbibition of grains in I_2/KI solution allowed

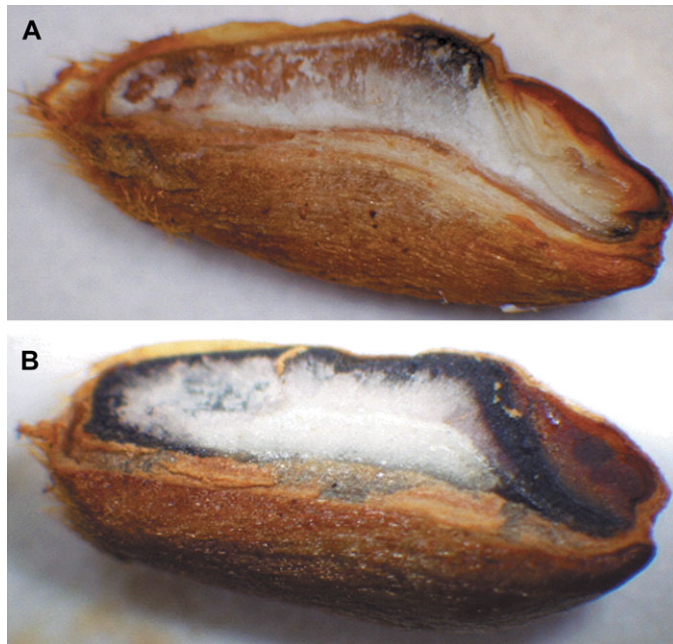


Fig. 9. Staining of embryo and endosperm tissue following imbibition of Hartog grains with a solution of I_2/KI for 7 h (A) and 18 h (B).

water uptake to be visualized by the staining of cellular membranes and starch tissue. After 7 h, the embryo was stained a dark brown whilst the endosperm adjacent to the dorsal edge of the scutellum was stained a brown/purple colour (Fig. 9A). There was also some staining in the endosperm adjacent to the ventral edge of the scutellum, although to a lesser extent. After 18 h, the I_2/KI solution had stained the entire embryo dark brown as well as the entire outermost layer of the starchy endosperm within the grain (Fig. 9B). Methylene blue and Congo red dyes failed properly to infiltrate the grain beyond the outer embryo tissue after 25 h, even though it was clear that the grain was significantly hydrated (data not shown). The tetrazolium chloride only showed faint staining of grain tissues after 25 h imbibition (data not shown).

Germination of grains where imbibition was restricted to the proximal or distal end

Grains germinated irrespective of the point of access to water. However, it was evident that the lag phase lasted longer in the grains imbibed through the distal compared to the proximal end, especially in the dormant genotype. For Hartog grains imbibed from the distal end, the lag phase was extended by 1 d compared with those grains imbibed from the proximal end, whilst for SUN325B the delay was approximately 2 d (Fig. 10). After 3 d and 6 d for Hartog and SUN325B, respectively, the germination curves for distal imbibed grains approached the proximal imbibed samples.

Discussion

Magnetic Resonance Micro-Imaging (MRMI) provides a unique opportunity to visualize the time-course of water

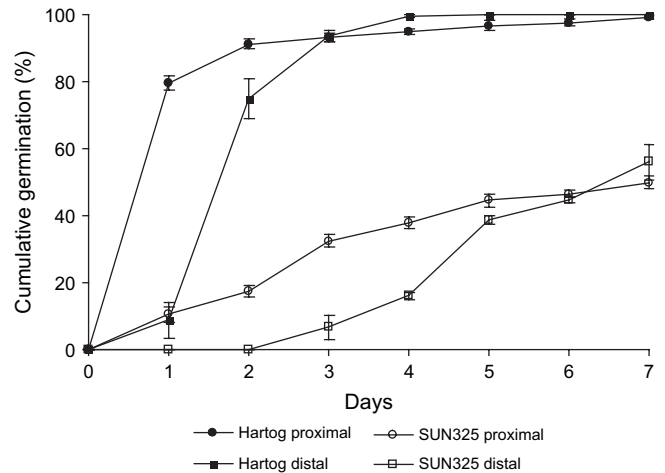


Fig. 10. Cumulative germination (%) of grains imbibed proximally (Hartog enclosed circles, SUN325 circles) and distally (Hartog enclosed squares, SUN325 squares) for 7 d. Grains were incubated on 1% agarose at 22 °C. Each point is an average of five replicates of 50 grains with bars indicating standard error.

uptake by wheat grains non-destructively and with high resolution. MRMI provides an insight not only into mechanisms of water uptake, but also the pattern of water distribution within the grain. Images taken at regular intervals over a period of 18 h clearly suggest that the rapid entry of water into the embryo and scutellum is through the micropyle, and that at any stage the germ is substantially more hydrated than any other grain tissue. The capacity of germ tissue to absorb water increases with time and has the characteristics of a purely physical process since it also occurs in heat-killed wheat (Studdert *et al.*, 1994) and non-viable barley grains (Gruwel *et al.*, 2002). Barley seeds were also found to hydrate rapidly during very early imbibition (Gruwel *et al.*, 2001) with the imbibed water also mainly confined to the germ tissue (Gruwel *et al.*, 2002), indicating that the mechanism of water uptake in barley is also through the micropyle.

The reliance on the micropyle for water uptake into the embryo, and possibly the aleurone and starchy endosperm, suggests that movement of water into the grain is an orderly process and may possibly regulate the types of compounds that may enter this sensitive tissue. Micropylar tissue can represent the remnants of the pollen tube in many species (Tran and Cavanagh, 1984). In wheat, the micropyle is located within the dorsal side of the area of attachment of the grain to the receptacle and is present as a pore or opening in the testa (Bhatnagar and John, 1972). This pore has been reported to be a channel through heavily pigmented testa cells leading almost directly to the embryo, but may contain a thin cuticular layer covering the channel (Bradbury *et al.*, 1956). It is likely therefore, that microbes or solutes dissolved in water would be restricted from accessing the embryo by this cuticle depending on molecular size. Forcing water to enter through this tiny area may be an important mechanism for protecting the embryo and internal tissues of the grain during germination.

Hydration of grain structures

The MRMI images also provided insight into water distribution within the embryo and provided a highly resolved picture of hydration of the structures in quiescent embryos. Embryo structures become visible after around 3 h of imbibition, with the radicle showing relatively early after hydration whilst the scutellum also became rapidly hydrated and continued to hydrate as water uptake progressed. This rapid initial water uptake is not surprising, considering there is a large water potential gradient in a dry wheat grain (Black *et al.*, 2006). Water is restricted to within the scutellum and specific embryo structures during early imbibition, but becomes more generalized throughout the embryo as water uptake progresses. This corresponds with Phases I and II of embryo water uptake, where the initial rapid uptake reaches a plateau that continues until germination creates another phase of rapid water uptake. This pattern of water uptake was also observed in durum grains (Abenavoli *et al.*, 2006) as well as seeds of other species such as tobacco (Manz *et al.*, 2005).

By comparison with the germ, the endosperm appears to hydrate at a much slower rate and, at least for the initial 18 h, the concentration was below the detection limits of the MRMI instrument. The inability of MRMI to provide a clear picture of early water uptake by the endosperm is presumably due to the relatively low water content of this tissue compared to the embryo and coat. The high density of starch and protein in the endosperm is likely to prohibit the concentration of significant amounts of water and hence its detection by MRMI. Water content increased slowly and over time and did not follow the rapid hydration pattern of water uptake observed with the embryo. An earlier MRI study of wheat imbibition showed that the endosperm only appears clearly hydrated after germination has occurred (Yoshida *et al.*, 1995).

Pathway of water uptake

Although the coat hydrates relatively rapidly during imbibition, neither the MRI images nor the I₂/KI uptake experiment provided evidence of direct permeation of water across the coat and into the underlying endosperm. Earlier work has reported evidence that the testa layer of the true seed coat is resistant to water penetration in wheat (Hinton, 1955; Moss, 1973) and legume species (Bewley and Black, 1982b). The wheat grain testa comprises a corky pigmented layer which is enclosed by a suberized inner and outer cuticle (Bradbury *et al.*, 1956; Parker *et al.*, 2005; Black *et al.*, 2006). In combination with the underlying nucellar layer, these tissues provide the greatest resistance to water diffusion into the endosperm (Hinton, 1955; Moss, 1973).

Other studies have suggested that water does eventually penetrate through the seed coat/testa (Butcher and Stenvert, 1973; Stenvert and Kingswood, 1976). Autoradiography of grains imbibed with tritiated water have been interpreted to suggest that penetration across the seed coat of wheat is

a possible pathway of hydration, even in the early stages of imbibition (Butcher and Stenvert, 1973; Stenvert and Kingswood, 1976). However, this technique is generally considered to have low resolution, particularly when dealing with small, mobile compounds such as water. In addition, the freezing and sectioning techniques used to produce autoradiograph images are highly likely to interfere with actual water distribution in the grain.

MRMI investigations of water movement into seeds of other species, however, have also given rise to conflicting interpretation. In pine, Terskikh *et al.* (2005) concluded that water penetrates the seed coat before hydrating the megagametophyte, cotyledons, and, finally, the radicle. Another study involving tobacco showed that water only enters the embryo through the micropyle and that there is no permeation across the seed coat (Manz *et al.*, 2005). Both studies provide evidence, however, that the micropyle region is an important point of water entry, indicating that this pattern of water uptake is not species specific.

It is well documented that the outer layers of the coat have wick-like properties and, due to the porous pericarp, are able to conduct water around the surface of the seed (Hinton, 1955; Briggs and MacDonald, 1983). The MRMI images suggest that, in grain where the only access to water was at the brush region, the water was able to flow around the grain through the capillary action of the outer pericarp and enter the seed through the micropyle. The ability of these grains to germinate, albeit at a slower rate, is consistent with this suggestion. Furthermore, the MRMI images provide evidence that the water in the coat is located between the outer pericarp and the underlying testa layer (Fig. 5, image 4; Fig. 6, image 4), indicating that this may be the zone of water movement through the coat.

Visualization of water uptake by the endosperm was achieved with I₂/KI solution. Assuming that the movement of iodine matched the water in which it was dissolved, stained tissue indicated that water had started to enter the endosperm by 7 h after the start imbibition. Use of this marker suggested that water entered the endosperm around the edge of the scutellum and then moved progressively in a distal direction through the sub-aleurone endosperm. Measurements collected by weighing the grain showed that the endosperm continues to hydrate slowly during germination and several days of imbibition.

Grain water uptake as a function of dormancy

There are many species, for example *Arabidopsis*, where the prevention of water uptake is a factor in dormancy and where secondary cell walls in the seed coat prevent the exchange of moisture and gases with the environment (Haughn and Chaudhury, 2005). In wheat, however, it is clear from the present study that there is no difference in the amount of water uptake either between dormant and non-dormant varieties or between harvest-ripe (dormant) and after-ripe (non-dormant) samples of a dormant variety. The failure to detect a difference between dormant and

non-dormant wheat, despite the apparent impermeability of the true seed coat, can be explained by (i) the ease of water transfer within the pericarp, and (ii) the absence of a strong barrier to water infiltration through the micropyle and into the germ. Dormancy in some soybean seeds appears to involve an impermeable, tough cuticle with the absence of cracks, rather than being due to a thick and waxy seed coat (Ma *et al.*, 2004). Furthermore, unlike soybeans, where water uptake is a two-component system with embryo hydration dependent on seed coat water uptake (Ma *et al.*, 2004), water uptake into wheat embryos was comparatively rapid and not related to the hydration of the coat. This means that dormancy in wheat can be defined as 'physiological dormancy', in contrast to soybean where the seed coat prevents water uptake, which would be classified as 'physical dormancy' (Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006).

The testa layer of the coat contains pigments and other hydrophobic compounds that may contribute to the impermeable nature of this tissue (Evers and Bechtel, 1988; Parker *et al.*, 2005). In red-grained wheat, flavonoids compounds present in the testa layer are polymerized into the characteristic, dark coat pigment (Miyamoto and Everson, 1958). It is possible that precursors of these compound(s), for example, the catechin tannin-like compounds described by Stoy and Sundin (1976), or other inhibitory compounds in the coat layers may be dissolved during hydration of the seed coat and delivered directly to the embryo (Black *et al.*, 2006).

Depending on the point of contact between the seed and water, it seems likely that some of the water entering the embryo via the micropyle would have already travelled through part of the coat, between the outer pericarp and the pigmented testa layer, possibly infiltrating the outer cuticle of the testa through pores, and picking up water-soluble compounds along the way. The significant extension of the lag phase in the germination of the dormant genotype SUN325, relative to the non-dormant genotype, when grains were imbibed only through the distal compared with the proximal end, is consistent with the hypothesis that there may be a chemical inhibitor to germination in the coat of the dormant genotype. This longer extension of the lag phase in the dormant compared to the non-dormant genotype, is particularly significant in view of the MRMI data which suggested that water uptake was similar in dormant and non-dormant genotypes.

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