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Autor: Brereton, Ian / Lenk, Rudolf / Zelaya, Fernando O.

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GERMINATION OF THE SEEDS OF *CASTANOSPERMUM AUSTRALE* (BLACK BEAN) AS STUDIED BY THE HIGH-FIELD NMR MICROIMAGING

BY

Ian BRERETON*, Rudolf LENK & Fernando O. ZELAYA***

ABSTRACT

Germination of the seeds of *Castanospermum australe* (black bean) as studied by the high-field NMR Microimaging. – The germination of the seeds from the Chesnut tree (*Castanospermum australe*) has been investigated by the NMR Microimaging at 190 MHz. Conventional ^1H spin-echo and T_1 images reveals some details of black bean seeds vascular structure: a system of small spherical holes and curvelinear pathways.

Key-words: *Castanospermum australe*, NMR Microimaging, Biological water content & dynamics, Germination of seeds.

INTRODUCTION

The usefulness of Nuclear Magnetic Resonance (NMR) [18] and Magnetic Resonance Imaging (MRI) [16] in Biology and Medicine has been well established. One of the most important characteristics of these methods is that they are non-invasive and non-destructive and supply us the *full information* of matter, including structure, order and Brownian dynamics [18].

In Biology and Medicine repeated measurements can be made of the same tissue over time without causing injury or interference with growth. This is particularly valuable in Botany, Horticulture and Agriculture, researching water and oil relations of living plant organs such as stems, roots and seeds and mobilization of seed reserves during their germination. Generally it was difficult to observe plant tissue development unless the plant is sectionned and prepared for microscopy. This may result in a loss of valuable anatomical and physiological information, i.e. water status (concentration & mobility), cell volume, organelle location and orientation. The application of NMR Spectroscopy and Imaging avoids these difficulties.

*Centre for Magnetic Resonance, University of Queensland, St. Lucia, QLD 4072, Australia.

**Laboratoire de Biochimie & Physiologie Végétales, Pavillon des Isotopes, Université de Genève, CH-1211 Genève 4, Switzerland.

The relationship between spin-lattice relaxation and water content during the storage of maize seeds was reported by RATKOVIC [25]. In some seeds NMR can readily detect other mobile components, for example oil molecules [19]. The study of spin-lattice relaxation, measured during development of the Bean (*Phaseolus vulgaris*, L) seed, has shown an important variation of the T_1 relaxation times of biological water protons [9].

We have used the spin-relaxation method to study the germination process of oat and barley seeds *in-situ* in a home-made relaxometer, working at 25 MHz [17, 18]. The seeds, suspended in the standard 7 mm NMR tube and supplied by water were investigated continuously *in-vivo* by pulse NMR of water protons at the level of the seeds. In these seeds it was found that the germination process is concomitant with the increase of water content and molecular mobility. This processus is characterized by a mobilization and transformation of reserve substances (starch) and their transfer to growing tissues. The results show that the T_1 relaxation times in a seed yield the physiological information, as in the germinating (living) seeds the T_1's are higher than in the heat-treated (killed) seeds.

The NMR Microimaging method [2, 4, 22] was applied to investigations of several topics in Botany, Horticulture and Agriculture [1, 3, 5, 7, 8, 10, 14, 15, 24, 26–28, 31–34].

In the present work we have applied the NMR Microimaging to the study the germination of the seeds from the *Castanospermum australe* (Australian Chesnut tree). This tree, native to rainforest of eastern Australia (Moreton Bay) [29] has leaves dark green, 30–60 cm long, flowers in spring-summer, pods usually 12–18 cm long, with seeds brown, 3–5 per pod. The seeds, ca 3 cm in diameter, are known to be toxic [6].

It has been proven that the seeds from *C. australe* are a fruitful source of alkaloids, such a castanospermine [11, 12], having novel biochemical properties, namely the highly potent inhibitory effects on a variety of enzymes, human immunodefficiency virus (HIV), retroviruses [30] and tumor cells [21]. Compounds of this type are chemopreventive and therapic agents in the treatment of AIDS.

MATERIAL & INSTRUMENTATION

Preparation and germination of seeds: Seeds for hydratation and germination were wrapped in tissue-paper and kept moist at 26°C. Germination occurs within 3–4 days as evidence by the appearance of a small root at one hand of the seed. Hydrated seeds were imaged after 2–3 days exposure to moisture. The dry seeds were selected directly from a seed pod which had not come into contact with water for at least three weeks.

Non destructive assessment: Images collected from seeds were acquired using a Spectrospin (Zürich-Fällanden, Switzerland) 40 cm horizontal bore magnet, operating at 190 MHz and interfaced to a Bruker (Karlsruhe, Germany) MSL console. An actively

shielded 8.0 cm internal diameter gradient set, with a maximum field-gradient of 100 Gauss/cm at 100 A, was employed. The RF probe was a home-built, in the Centre for Magnetic Resonance, 64 mm internal diameter low-pass birdcage resonator. The T_1-weighted images were acquired by selective inversion of the water resonance followed by an inversion-recovery delay (IR) prior to a standard single-slice spin-echo image sequence. The IR delay was set to the delay determined for a null signal following inversion, acquired from the whole seed. Image acquisition time for T_1-weighted images was 190 min. The slice thickness in most cases was 2 mm and the image in-plane pixel resolution was 0.23 mm. The 4.1 ms Hermite shaped pulses were used for slice selection. The recycle delay (TR) was 1.2 s.

RESULTS & DISCUSSION

Fig. 1 shows a T_1-weighted image of a dry black bean seed with the 2 mm slice, located approximately at the midpoint of the seed's lenght. The striking feature of this image is the presence of small circular spots, dispersed regularly below the surface within a bisected seed with otherwise essentially homogenous flesh and further striations extending into the centre of the seed. These spots most likely arise from the vascular

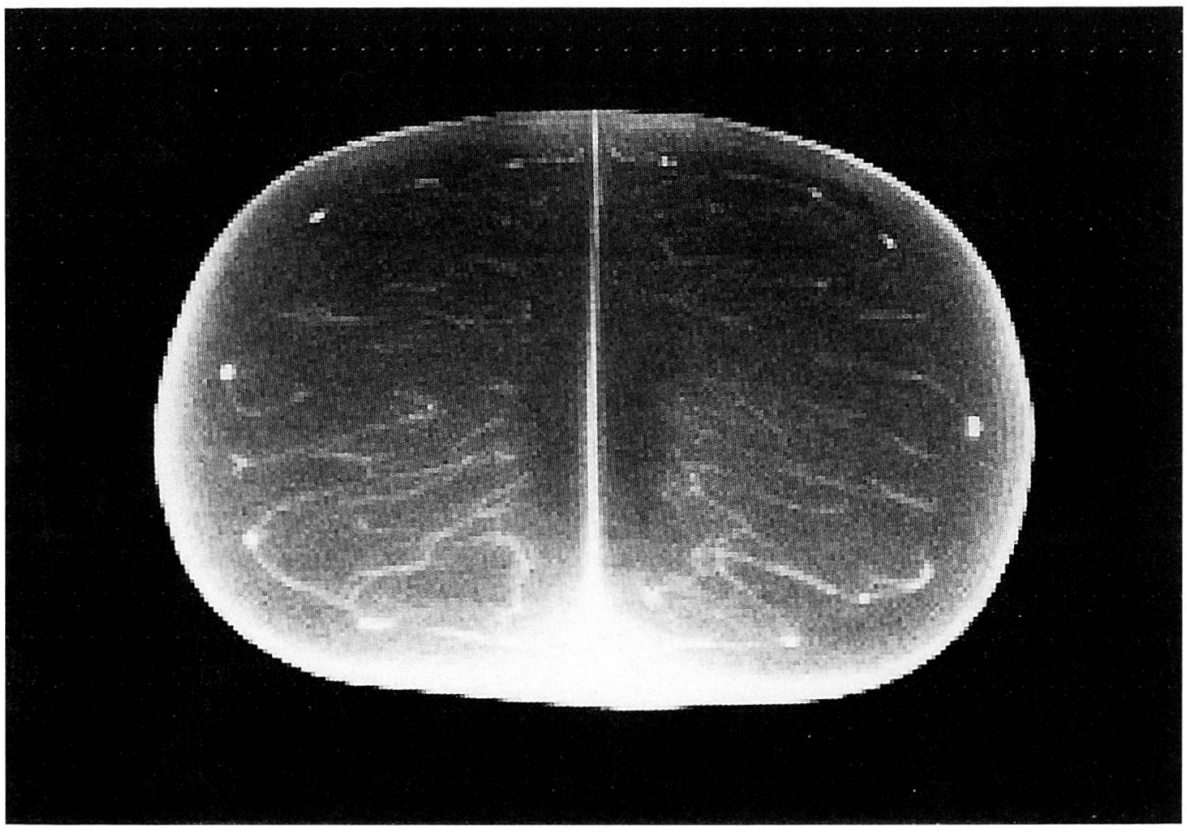

FIG. 1

T_1-weighted image of a dry black bean seed (2 mm slice is located at the midpoint of the seed's length).

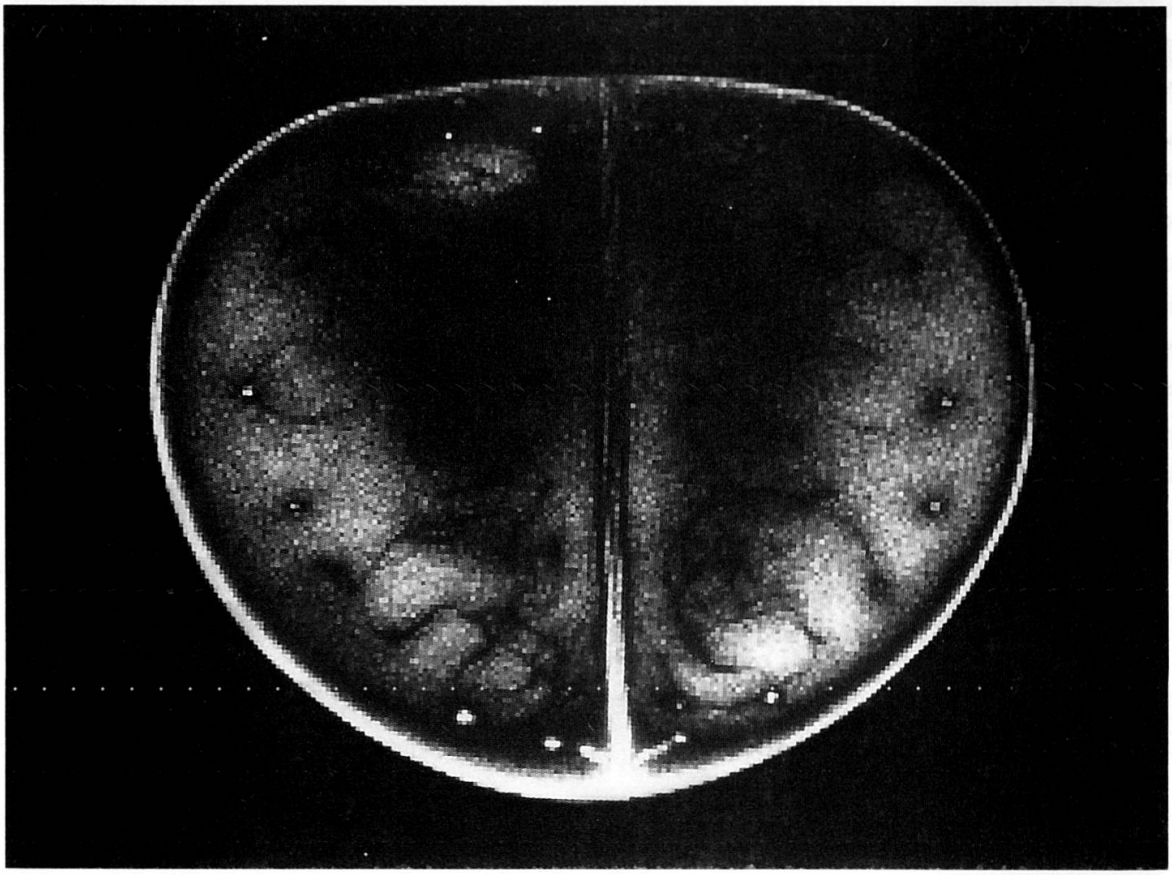

FIG. 2

T_1-weighted image of a black bean seed after hydration for 4 days.

bundles which extend from one end of the seed to the other and are responsible for the delivery of assimilates (sugars, amini acids, mineral ions) during its development.

Hydrated seeds, which are exposed to moisture for several days showed a significant increase in image intensity around the circumference of each half of the seed, decreasing towards the centre. Fig. 2 shows the T_1-weighted image (IR 170 ms) of a seed after hydration for 4 days, indicating a progressive change in T_1 relaxation time across the seed during germination, which is given by the creation of faster molecules by hydrolysis of stored substances in the germinating seed and their transport to growing parts of the system. The increase of the T_1 relaxation is less important as that found in the oat and barley seeds [17, 18]. This is given by the fact that the water content in the oat and barley seeds is less important than that in the black bean.

The same seed was imaged under identical conditions following a further 7 days by which time germination had occurred and a root approximately 2 cm in lenght had developed (data not shown).

Further, in an T_1-weighted sagitall image (IR 170 ms) of a hydrated seed with the slice bisecting the left half of the seed, depicted in Fig. 2, the vascular bundles running the length of the seed are clearly visible.

In conclusion, this study has illustrated the potential of T_1-weighted NMR imaging for observing physico-chemical changes in seeds during the germination process. In the T_1-weighted images, the image intensity provides a measure of the spatial variation in water-proton T_1 relaxation times. This may be directly correlated to biophysical activity of the germinating seeds. The "structure", presented in Figs 1 and 2 can be obtained by the NMR Imaging only.

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