MRI Contrast in the Examination of Early Somatic Embryos

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Abstract. *The article deals with the MRI-based in vivo examination of early somatic embryos. The volume of water in the embryos was measured using two different methods. The first technique applied was proton density (PD) measurement of the sample via MRI (realized at the ISI, ASCR Brno). The second approach consisted in the dehydration and subsequent weighing of the sample (realized at MENDELU, Brno). Both the methods were compared. The correlation coefficient of the volume of water measured by these techniques is 0.816. The aspects of major interest for the investigation of the related biological processes are the various image contrasts and the change of the relaxation times T_1 and T_2 on the boundary between the embryo and the substrate.*

Keywords: MRI Contrast, Somatic Embryos, Plants, Water Changes, Relaxation T_1 and T_2.

1. Introduction

Magnetic resonance imaging (MRI) is a non-invasive tool applied by many researchers to study molecules. The MRI approach is frequently used not only in medicine, but also in biological, biochemical, and chemical research. In plant biology, MRI is utilized to support the research of water and mineral compounds transported within a plant [1, 2], the determination of plant metabolites [3, 4], the investigation of cellular processes [5], and the examination of the growth and development of plants [6]. MRI is also instrumental towards monitoring water changes in early somatic embryos of spruce (ESEs) [7]. These embryos constitute a unique plant model system applicable for the study of various types of environmental stresses (including metal ions) under well-controlled experimental conditions [8-10].

2. Method

The general aim of the experiment was to perform in vivo measurement of the ESEs using MRI techniques, and the entire process comprised several stages. Within the first step, a comparison was carried out of the volumes of water in the cultures (proton density measurement using a 4.7 T system) with the volumes of water determined upon the weight changes observed after desiccation of the sample. Subsequently, we compared the acquired MR images weighted by proton density (PD) and relaxation times T_1, T_2, and the last stage of the experiment consisted in determining the changes of the basic magnetic field B_0 caused by the magnetic susceptibility of ESEs. The acquired results will be used for the examination of growth focused on ESEs contaminated with heavy metals. To measure the T_2 relaxation, we applied the spin echo (SE) method; the measurement of the T_1 relaxation was realized using inversion recovery (IR) and rapid acquisition with relaxation enhancement (RARE). All the described experiments were performed on the 4.7 T (Magnex) and 9.4 T (Bruker) MRI systems operated by the Institute of Scientific Instruments, Brno. The MAREVISI (8.2) and MATLAB (7.11.0) programs were used for the processing.

3. Plant Material and Cultivation Conditions

A clone of early somatic embryos of the Norway spruce (Picea abies/L./ Karst.) marked as 2/32 and a clone of the Blue spruce (Picea pungens Engelm.) designated as PE 14 were used in our experiments. The cultures were maintained on a semisolid (Gelrite Gellan Gum, Merck, Germany) half-strength LP medium [11] with modifications [12]. The concentration of 2,4-dichlorofenoxyacetic acid and N^6-benzyladenine was 4.4 and 9 µM, respectively [13]. The pH value was adjusted to 5.7 - 5.8 before autoclaving (121°C, 100 kPa, 20 min). The organic part of the medium, excluding saccharose, was sterilized by filtration through a 0.2 µm polyethylensulfone membrane (Whatman, Puradisc 25 AS). The cultivation was carried out in Petri dishes (diameter 50 mm). The sub-cultivation of stock cultures was carried out in 2-week intervals; the stock and experimental cultures were maintained at the temperature of 23±2°C in a cultivation box kept in a dark place. The experiment started with colonies of early somatic embryos which weight was about 3 mg. Ten colonies per one Petri dish were cultivated. After 2 weeks the colonies of early somatic embryos were harvested and after fresh weight determination they were dried at 105°C to stable weight. Thirty colonies were used for weighing and statistical analysis.

4. Results

A comparison of the volumes of water in the embryos measured via the above-discussed methods can be seen in Figs. 1 and 2. While the measurement using desiccation and subsequent weighting was conducted at MENDELU, the proton density measurement with the SE technique was performed at the ISI Brno.

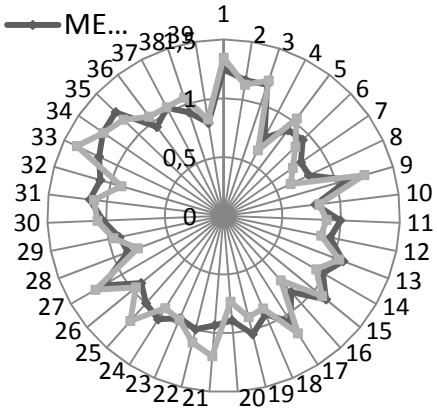

Fig. 1. Comparison of the normalized values of the samples 1-39 measured at the ISI and MENDELU.

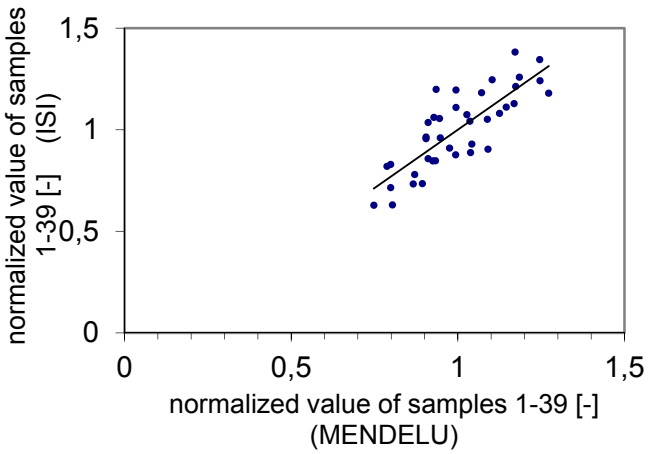

Fig. 2. Normalized value of the samples measured at the ISI and MENDELU.

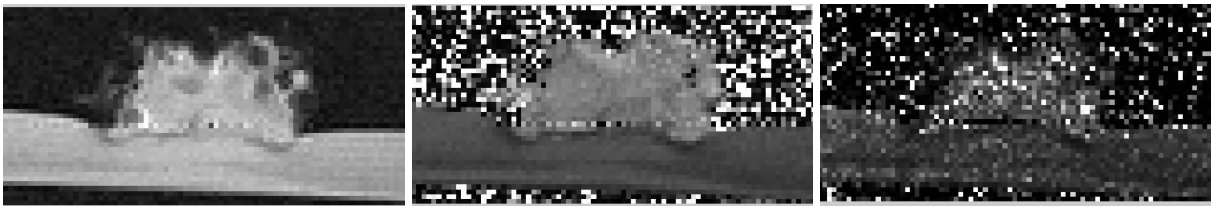

Fig. 3. Images of the ESEs measured with the 4.7 T magnet. Left: image of the PD; centre: the T_1 map; right: the T_2 map.

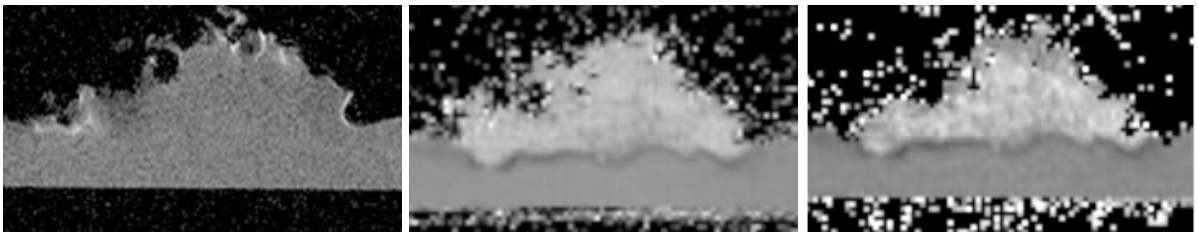

Fig. 4. Images of the ESEs measured with the 9.4 T magnet. Left: image of the PD; centre: the T_1 map; right: the T_2 map.

The data from MENDELU and the ISI were normalized to the mean value for all the samples examined. The correlation coefficient of the volumes of water measured by both methods is 0.816. The proton density, relaxation times T_1 and T_2, and B_0 maps of the embryos measured using the 4.7 T and 9.4 T MRI systems are shown in Figs. 3, 4, and 5.

The difference of the magnetic field between the embryos and the medium ($\Delta B_0 = 16{,}92\mu T$) was established from the map of the magnetic field B_0. To measure the magnetic field B_0 map, we applied the gradient echo method ($\Delta T_E = 5$ ms, 9.4 T MRI system). Susceptibility difference ($\Delta\chi = 1.8 \cdot 10^{-6}$) is calculated from ΔB_0. Susceptibility difference is difference between susceptibility of embryos and substrate. A comparison of the relaxation times T_1 and T_2 in the embryos and the substrate can be seen in Table 1. The measurement was performed with the 4.7 T and 9.4 T MRI systems.

Fig. 5. Map of the magnetic field ΔB_0 in a measured sample; the 9.4T MRI system.

Table 1. Table of the relaxation times T_1 and T_2 in the ESEs and the substrate.

B_0 field	T_1 [ms]	T_2 [ms]	Method
ESEs			
4.7 T	748	64	IR/SE
9.4 T	1540	90	RARE/SE
ESEs			
4.7 T	492	36	IR/SE
9.4 T	988	51	RARE/SE

5. Discussion and Conclusion

The authors utilized two methods to carry out a comparison of the volumes of water in early somatic embryos. The first technique consists in MRI-based proton density (PD) measurement of the sample. The second method requires double weighting of the embryos before and after desiccation. The difference in weight is equal to the volume of water in the embryos. The correlation coefficient between the normalized results measured via both

methods was 0.816; the results after the normalization of the mean value of the determined volumes shown in Figs. 1 and 2 exhibit differences. Generally, MRI may be used as an effective approach to non-invasive measurement of water in organic structures.

The above-mentioned differences between the volumes are caused by several factors: a) the volume of water is also evaluated for the area affecting the substrate (nutrient medium), b) low sensitivity of the RF coil, c) imperfect image segmentation. With the second method, the removal of embryo from the substrate can accompanied by accidental withdrawal of a larger amount of material than is necessary for the subsequent desiccation and weighing. The use of a higher magnetic field in combination with a small RF coil having high sensitivity can improve the signal-to-noise ratio (SNR) and, consequently, provide higher resolution and thinner slices. Table 1 shows the relaxation times T_1 and T_2 of the samples in different magnetic fields. The change of the relaxation times T_1 and T_2 on the boundary between the embryo and the substrate are of interest for the investigation of biological processes.

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References

[1] Scheenen TWJ., Vergeldt FJ., Heemskerk AM., Van As H. Intact plant magnetic resonance imaging to study dynamics in long-distance sap flow and flow-conducting surface area. *Plant Physiol*., 144(2): 1157-1165, 2007.

[2] Ionenko I. F., Anisimov A. V. and Dautova N. R., Early changes of water diffusional transfer in maize roots under the influence of water stress, Biol. Plant., 54 (2010) 488.

[3] Y. Q. Pu, F. Chen, A. Ziebell, B. H. Davison and A. J. Ragauskas, *BioEnergy Res*., 2 (2009) 198.

[4] K. G. Zulak, A. M. Weljie, H. J. Vogel and P. J. Facchini, BMC Plant Biol., 8 (2008)

[5] J. Lambert, P. Lampen, A. von Bohlen and R. Hergenroder, Two- and three-dimensional mapping of the iron distribution in the apoplastic fluid of plant leaf tissue by means of magnetic resonance imaging, *Anal. Bioanal. Chem*., 84 (2006) 231.

[6] S. M. Glidewell, M. Moller, G. Duncan, R. R. Mill, D. Masson and B. Williamson, *New Phytol.*, 154 (2002) 197.

[7] V. Supalkova, J. Petrek, J. Baloun, V. Adam, K.Bartusek, L. Trnkova, M. Beklova, V. Diopan, L. Havel and R. Kizek, Multi-instrumental Investigation of Affecting of Early Somatic Embryos of Spruce by Cadmium(II) and Lead(II) Ions, *Sensors*, 7 (2007) 743.

[8] R. Mikelova, J. Baloun, J. Petrlova, V. Adam, L. Havel, H. Petrek, A. Horna and R. Kizek, *Bioelectrochemistry*, 70 (2007) 508.

[9] J. Petrek, J. Vitecek, H. Vlasinova, R. Kizek, K. J. Kramer, V. Adam, B. Klejdus and L. Havel, *Anal. Bioanal. Chem*., 383 (2005) 576.

[10] Víteček, J., Adam, V., Petřek, J., Vacek, J., Kizek, R., Havel, L.: Esterases as a marker for growth of BY-2 tobacco cells and early somatic embryos of the Norway spruce, *Plant Cell Tissue Organ Cult.* 79, (2004) 195-201.

[11] von Arnold, S.J. Improved efficiency of somatic embryogenesis in mature embryos of Picea abies (L.). *Plant Phys.* 1987, 128, 233-244.

[12] Havel, L.; Durzan, D.J. Apoptosis during diploid parthenogenesis and early somatic embryogenesis of Norway spruce. *Int. J. Plant Sci*. 1996, 157, 8-16.

[13] Vlasinova, H.; Mikulecky, M.; Havel, L. The mitotic activity of Norway spruce polyembryonic culture oscillates during the synodic lunar cycle. *Biol. Plant*. 2003, 47, 475-476.