

Measurement of Tissue Cultures of Early Somatic Embryos of Norway spruce

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Abstract— The objective of this study was the monitoring of the growth and development of early somatic embryos in a magnetic field strength of 4.7 T. The samples were exposed to radiofrequency (RF) field, gradient (G) field and combination of both fields. To measure longitudinal (T_1) and transverse relaxation times (T_2), the spin echo based techniques were used. 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. Control measurements of changes in the size of the investigated tissue of early somatic embryos was done using camera and subsequently evaluated in MATLAB. All measurements were realized at the Institute of Scientific Instruments in Brno.

1. INTRODUCTION

Nuclear magnetic resonance (NMR) is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation. NMR is also routinely used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI). The 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, the determination of plant metabolites, the investigation of cellular processes, and the examination of the growth and development of plants. MRI is also instrumental towards monitoring water changes in early somatic embryos of spruce (ESEs) [1]. 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 [2–4]. The earlier experimental results show that the effect of gradient magnetic fields on ESEs varies considerably [5]. Thus, it is not easy to answer the question of whether magnetic fields accelerate or suppress the growth of plant cultures but some other measurements for different settings of the external magnetic field can prove that the external magnetic field can change the dynamics of the model of matter [6].

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, we compared the acquired T_1 - and T_2 -weighted MR images, the next stage of the experiment consisted in determining the changes of the size of ESEs. To measure the T_2 relaxation, we applied spin-echo (SE) technique (echo times (TEs): 18, 30, 50, 100 and 200 ms); the measurement of the T_1 relaxation was realized using inversion recovery spin echo (IRSE) technique (inversion times (TIs): 10, 100, 300, 1000 and 3000 ms). Other samples of ESEs were “measured” by the same techniques, but in the first case, the only gradients were switched off and in the second case, the only RF field was switched off. All the described experiments were performed on the 4.7 T (Magnex) MRI system at Institute of Scientific Instruments in Brno. The MAREVISI (8.2) and MATLAB (R2014b) programs were used for the processing. The progress of growth was evaluated from 2D images of clusters in the Petri dishes, and the area of the ESEs was calculated. The acquired images of the ESEs were processed to provide the required data. For that reason, we created an application in MATLAB, which allows for the recognition and subsequent calculation of the area occupied by the ESEs [7]. Table 1 shows the distribution of the Petri dishes during the experiment.

3. PLANT MATERIAL AND CULTIVATION CONDITIONS

A clone of early somatic embryos of the Norway spruce (*Picea abies*/L./Karst.) marked as 2/32 were used in our experiments. The cultures were maintained on a semisolid (Gelrite Gellan

Table 1: Distribution of measured samples.

Sample n.	1	2	3	4	5	6	7	8
Method	IRSE, SE	RF	G	Control/without external field	IRSE, SE	RF	G	Control/without external field
Date of measurement	2014/10/20–2014/11/06				2014/10/21–2014/11/07			

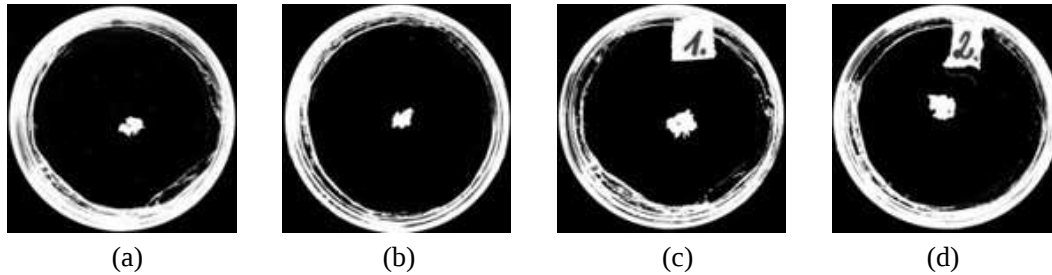


Figure 1: Petri dishes with ESEs prepared to evaluate the size of the tissue: (a), (b) images of sample 1 and 2 from the first day of experiment; (c), (d) corresponding images from the 13th day.

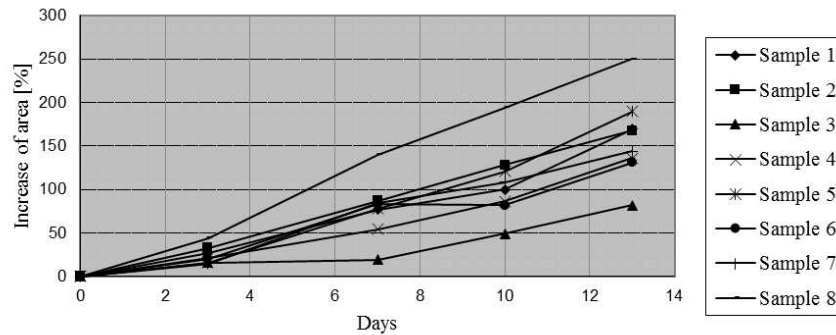


Figure 2: The growth of individual samples during the experiment.

Gum, Merck, Germany) half-strength LP medium with modifications. The concentration of 2, 4-dichlorophenoxyacetic acid and N6-benzyladenine was 4.4 and 9 μm , respectively. 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 polyethylenesulfone 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-weeks intervals; the stock and experimental cultures were maintained at the temperature of $23 \pm 2^\circ\text{C}$ in a cultivation box kept in a dark place. The experiment started with colonies of early somatic embryos which weight was about 3 mg. Only one cluster per one Petri dish was cultivated.

4. RESULTS

Figure 1 shows the images of the pre-processed Petri dishes. From these images was evaluated increase tissue during the experiment.

Figure 2 shows the changes of the size of ESEs tissues. These results show that the most stable and suitable conditions for tissue growth were provided during the measurement of T_1 and T_2 . If the increase in these two samples expressed as a percentage, these values are 189.7% (sample 1) and 169.5% (sample 5). On the other side was very different growth and development of control samples that were exposed only to the influence of the magnetic field of the earth. If the increase in these two samples expressed as a percentage, these values are 136.4% (sample 4) and 250% (sample 8). Values which reflect an increase in the area may be dependent on the error, which is caused for example by bad evaluating the size of the surface tissue.

The other aspects of interest for the investigation of the related biological processes is 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. Figure 3 shows the T_1 and T_2 maps and the changes in development of

sample 1 at first and last day of experiment. A comparison of the mean of the relaxation times T_1

Table 2: The relaxation times (mean value) T_1 and T_2 of the ESEs and the substrate.

B_0 field	T_1 [ms]	T_2 [ms]	Method
first day of experiment (day 0)			
ESEs	398	61	IRSE/SE
Substrate	634	36	
last day of experiment (day 13)			
ESEs	761	86	IRSE/SE
Substrate	386	46	

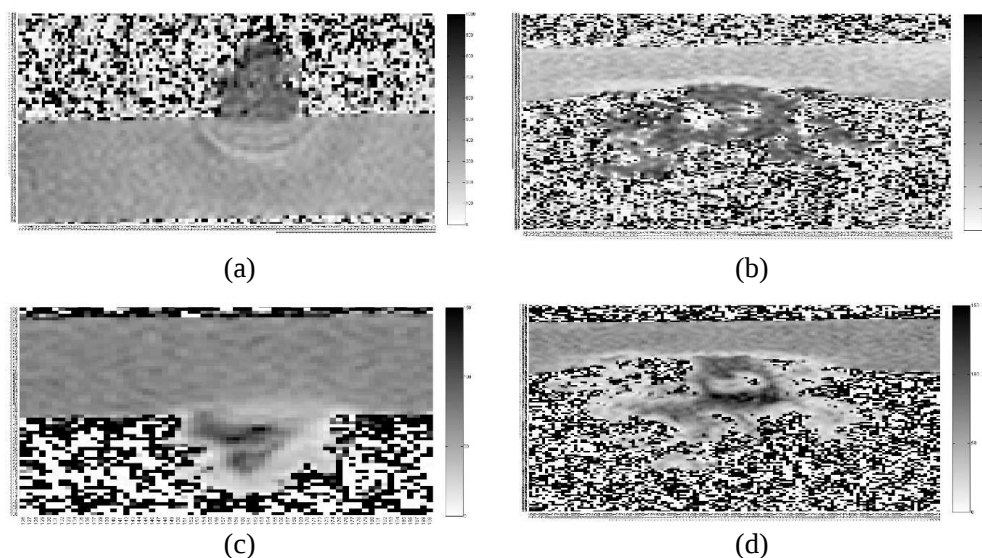


Figure 3: The changes in development of sample 1 during the experiment: (a), (c) T_1 and T_2 maps (ms) in first day of the experiment (0 day); (b), (d) T_1 and T_2 maps (ms) in 13th day.

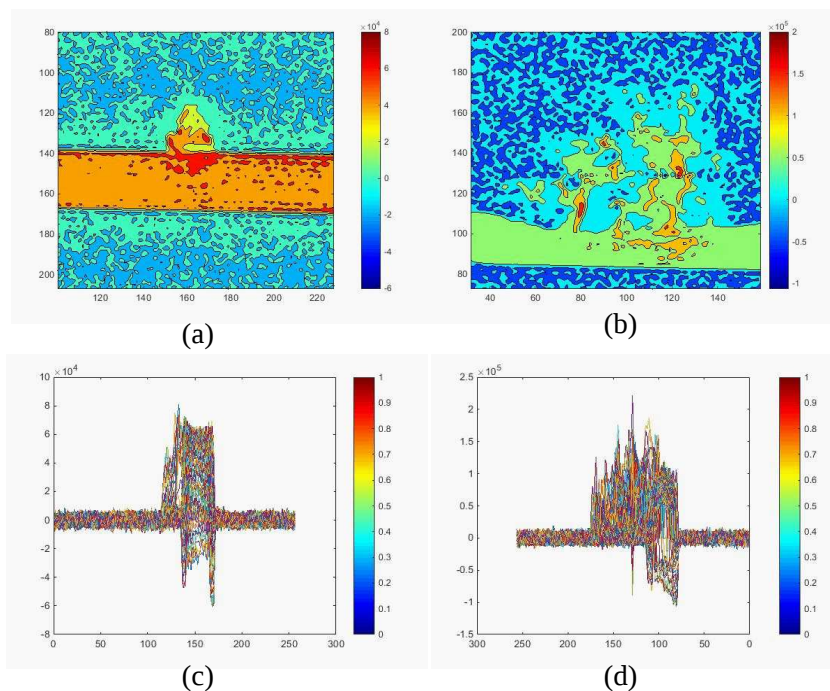


Figure 4: Changes in the development of sample 1 during the experiment: (a), (c) distribution of ESEs growth deposits in substrate; (b), (d) the image of the location of biological changes in the substrate.

and T_2 in the ESEs (sample 1) and the substrate can be seen in Table 2.

An important aspect to assess the development of ESEs is evaluating the change between the embryo and the substrate. These change in development of ESEs are shown in Figure 4. Figures 4(a) and 4(c) show the distribution of growth ESEs deposits in substrate. These place are connected by isolines. Figures 4(a) and 4(c) also show how ESEs can growth into the substrate and use the substrate for their own development. Between the substrate and the ESE are biological changes that are seen in Figure 2(b) and Figure 2(d). This change manifests itself by changing the value of the magnetic susceptibility. We can only assume that this change represent a place where the ESEs draws nutrients from the substrate. This change is also seen in Figures 4(b) and 4(d). The biological effect of this area is not confirmed yet.

5. CONCLUSION

The experimental results show that the effect of gradient magnetic fields on ESEs varies considerably (Table 1). We are still unable to assess whether the effect of stationary magnetic fields for plant organisms is positive or not but we now that the external magnetic field changes the dynamics of the model of matter and theoretically can change the growth of organisms. Table 2 shows the relaxation times T_1 and T_2 of the samples in different magnetic fields. The changes 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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