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Effect of electric field on physical states of cell-associated water in germinating morning glory seeds observed by ^{1}H-NMR

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Abstract

Morning glory seeds in dry conditions (0.099 g H_2O/dry wt.) were exposed to electric fields and germinated. The physical state of water in the germinating seeds of both control and exposed groups were examined using ^{1}H-NMR spectroscopy and NMR microscopy. Three water fractions were observed which were characterized by different relaxation times (T_1) and chemical shifts. The average region containing long T_1 fractions was approximately 50 μm in diameter and consisted of half-permeable barriers. The maximum intracellular water transport rate was 2.3×10^{-5} cm^2/s. The treatment with electric field (500 kV/m for 60 min) increased the fraction with the shortest T_1 and decreased that with the longest T_1. Because the total water content in the treated seeds (3.4 g H_2O/dry wt.) was similar to that in the untreated seeds (3.9 g H_2O/dry wt.), the treated seeds held more water in a condition in restricted motion than the untreated seeds. It is thought that the membrane systems were affected by the electric polarization which led to an unusual accumulation of water and the hydration of stored macromolecules during the imbibition process. This set of events led to excessive swelling of stored macromolecules, resulting in the disruption of membrane systems and irregular organization of tissue structures.

Keywords: Germination; Physical state of water; Electric field; NMR; Morning glory (*Pharbitis nil*) seed

1. Introduction

Electric fields abound in our natural environment, in the living spaces of developed society, in industrial factories and in medical diagnostics as well as medical therapeutics. The impact of these electrical fields may be positive or negative on biological systems, depending upon the prevailing conditions where the event occurs. The electric field sometimes perturbs the structural organization of cells, involving enzymes and membranes, and resulting in unusual metabolism and functions. Knowledge of the mechanisms of the action of the electric field on various biological systems like cells, tissues and organs, may be effectively used as a means regulating biological activity and removing undesirable substances from the system. Comprehensive work has been done with plants on the stimulation or retardation of growth by electric fields [1–7] and associated air

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ions [8–10]. Plant damage due to the electric field was observed in some cases [2–5,11]. An increase in plant respiration was reported by applying a 5–10 kV/m electric field [12] which was ascribed to cytochrome contents. Prolonged ripening was achieved by suppressing the climacteric rise in respiration and retarding ATP production using an electric field [13]. A unique use of an electric field is electroporation to fuse protoplasts or to introduce a foreign gene into plant cells [14,15] which is a useful technology for cell manipulation. Moreover, control of microorganisms using an electric field has been tested [16–18]. Responding to demands from agricultural technology, the effects of electric fields on various hard and dormant seeds have been studied with the expectation of improved germination of seeds [19–24]. However, no significant trend was obtained; in some cases, the electric field stimulated germination of a particular type of seed, but in other cases, it either had no effect or it suppressed germination. The data gathered in these studies seem to indicate, that the moisture content of the seeds, and the strength, direction and type of electric field all effect the results.

Of the alfalfa, lettuce, radish, carrot and morning glory seeds tested in preliminary experiments [25], only morning glory seeds were found to be sensitive to an electric field. When dry morning glory seeds were treated with a static electric field in the range of 300–600 kV/m between two air-spaced electrodes for 60 min, germination was suppressed. Hence, we set out to elucidate the molecular mechanism of the action of electric fields preventing germination.

Mobile water within cells serves to transport substrates and O_2 required for metabolism to reaction sites and removes products and CO_2 from those reaction sites. Heat generated by metabolic reactions is considered to be released from cells by way of mobile water. The movement of cell-associated water, water within cells and between cells, although in near thermodynamic equilibrium, is influenced by local heat production by metabolism and by functional movement of cell structures using ATP [26]. Hence, the mobility of cell-associated water controls the rate of metabolism and vice versa [27–30]. This is similar to the concept of Wheatley and Clegg [31]. Besides, movement of water is controlled by cellular organization, such as compartmentalization by membrane structure [32,33], and water is ordered by macromolecules, such as proteins and polymers of organic compounds [34,35]. Thus, the dynamics of water molecules in cells closely correlates with the organic properties of macromolecular structures. All physiological events are considered to be summarized in the conditions of cell-associated water as mentioned above which are able to be examined by ^{1}H-NMR [36–41] and also by ^{1}H-NMR imaging [42].

In this investigation, we attempted to examine the effect of the electric fields on dry morning glory seeds based on the subsequent changes in the physical states of cell-associated water during germination. Small molecular perturbations induced by the electric field on the dry seeds in a resting state may be multiplied and clearly observed by transferring the seeds to an active state utilizing the metabolic energy. ^{1}H-NMR spectrometry and ^{1}H-NMR microscopy which detect and map mobile water in tissues were employed. ^{1}H-NMR microscopy including diffusion measurements [35,43,44] using pulse magnetic field gradients [45,46] is a useful new technique to examine the physiological condition of tissues. It is anticipated that these techniques will be helpful in elucidating the effects of the electric field on living systems.

2. Materials and methods

2.1. Sample preparation

Dry morning glory (*Pharbitis nil*) seeds were treated with static electric fields of various strengths up to 500 kV/m for 60 min. The water content of the seeds was 0.099 ± 0.012 g H_2O/g dry wt. and the dry weight of a seed was 0.037 ± 0.005 g. The outline of the electric field generator is shown in Fig. 1. Seeds were placed on a glass dishes and set between the electrodes with a total of 3.0 cm air-space. An electric field of magnitude 500 kV/m for 60 min had been known to suppress the germination rate of morning glory by 50% in preliminary experiments [25]. After treatment, the seeds were imbibed and germinated on wet filter papers in a temperature-controlled chamber at 25°C for 48 h under fluorescent lamps (400–600 nm, 30 W/m^2: 12 h illumination/day). The water content increased to 3.88 ± 0.53 g H_2O/g dry wt. for the untreated seeds, and to 3.42 ± 0.43 g H_2O/g

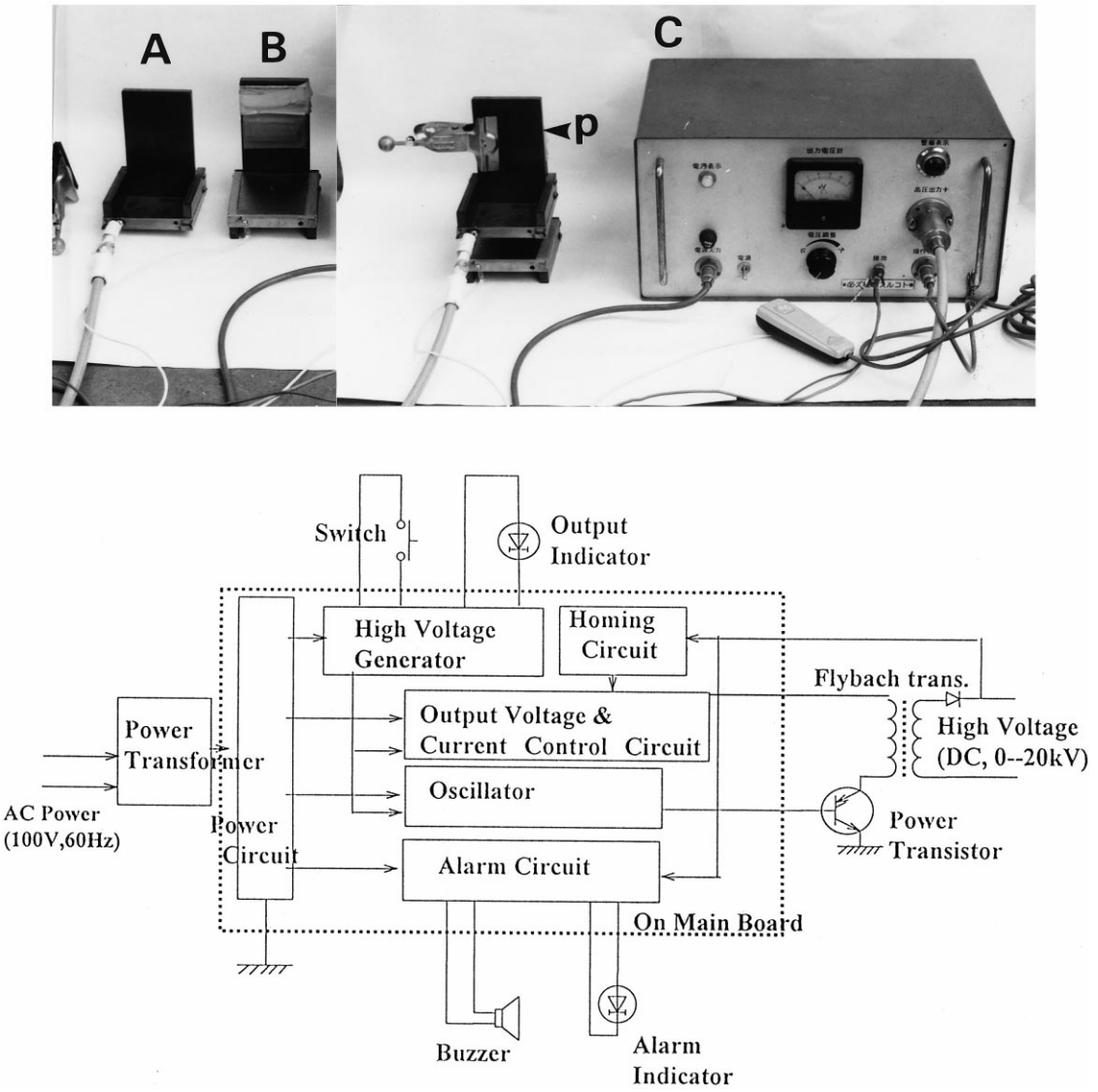

Fig. 1. Apparatus used for the electric field treatment. Top: photograph of the apparatus. A cathode (A) and an anode (B) of the chamber for the treatment are shown. The gap of the two electrodes was adjusted using the upright back-panels (p). (C) An electric field generator. Bottom: diagram of the electric field generator circuit.

dry wt. for the seeds treated with 500 kV/m for 60 min.

2.2. NMR measurements

An NMR spectrometer (JEOL GSX-270, JEOL, Akishima, Tokyo) with a superconducting magnet operating at 270 MHz for ^{1}H was used for measurements. An intact morning glory seed was placed in a 10-mm NMR tube and spin-lattice relaxation times were measured by the inversion recovery method, varying the interval between the 180° and the 90° pulses [47].

NMR images were measured using the microimaging probe devised by Ishida et al. [48]. Morning glory seeds were placed in a 10-mm NMR tube and inserted into the center of the probe. Images were measured by the two-dimensional Fourier-transform method. Sinc-function modulated pulses (2 ms) were applied with 58 mT/m z-axis magnetic field gradient to determine the slice thickness and 256 points of data with 128 encoding steps were acquired with

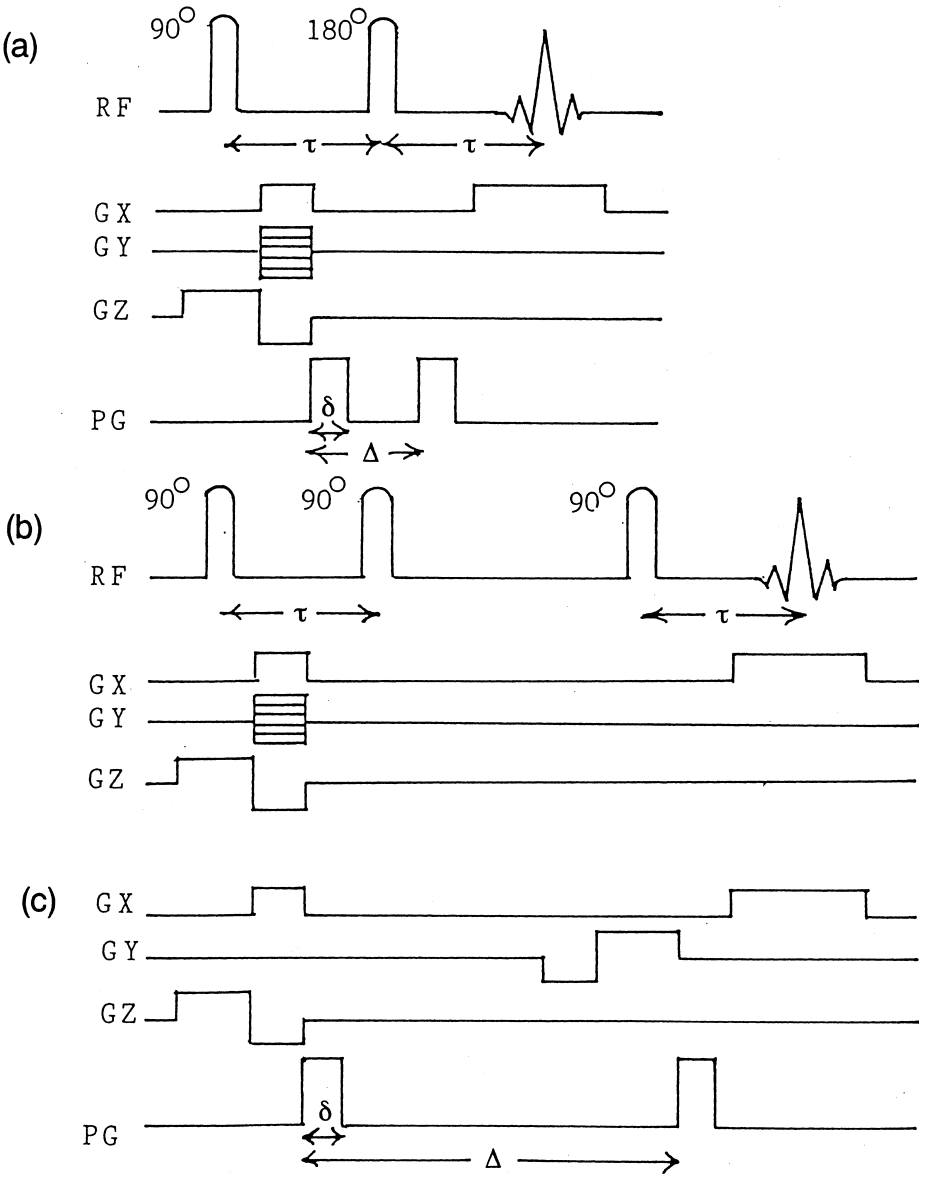

Fig. 2. Pulse sequences for diffusion measurements. Pulse gradient spin-echo method (a), pulse gradient stimulated-echo method (b) and one-dimensional profile measurement of restricted diffusion by the pulse gradient stimulated-echo method (c; RF sequence is the same as b). RF indicates radiofrequency; τ, pulse interval; GX, GY and GZ, gradients for imaging; Δ, diffusion time; δ, duration of pulse magnetic field gradients. The echo time (*TE*) is given by two τ.

x-axis and *y*-axis magnetic field gradients of 58 mT/m. Four transient acquisitions were accumulated. Images were recreated on a 256×256 matrix by an image processor (JEOL GIM-270, JEOL, Akishima, Tokyo) and displayed on a black and white television monitor, resulting in a resolution of 0.05×0.05 mm and a 1.6-mm slice thickness (0.004 mm^3). Strong pulse magnetic field gradients (PG; 278 mT/m for 3 ms) were applied for measurements of diffusion weighted images using pulse gradient spin-echo methods with a 12-ms diffusion period (a) or the pulse gradient stimulated-echo method with varying diffusion period (b) as shown in Fig. 2 [43]. The echo time (*TE*) was 27 ms and the repetition time (*TR*) was 5.0 s. Image data of four acquisition transients were accumulated. All measurements were carried out at 25±1°C. After Fourier-transformation and data processing, the image data was transferred to

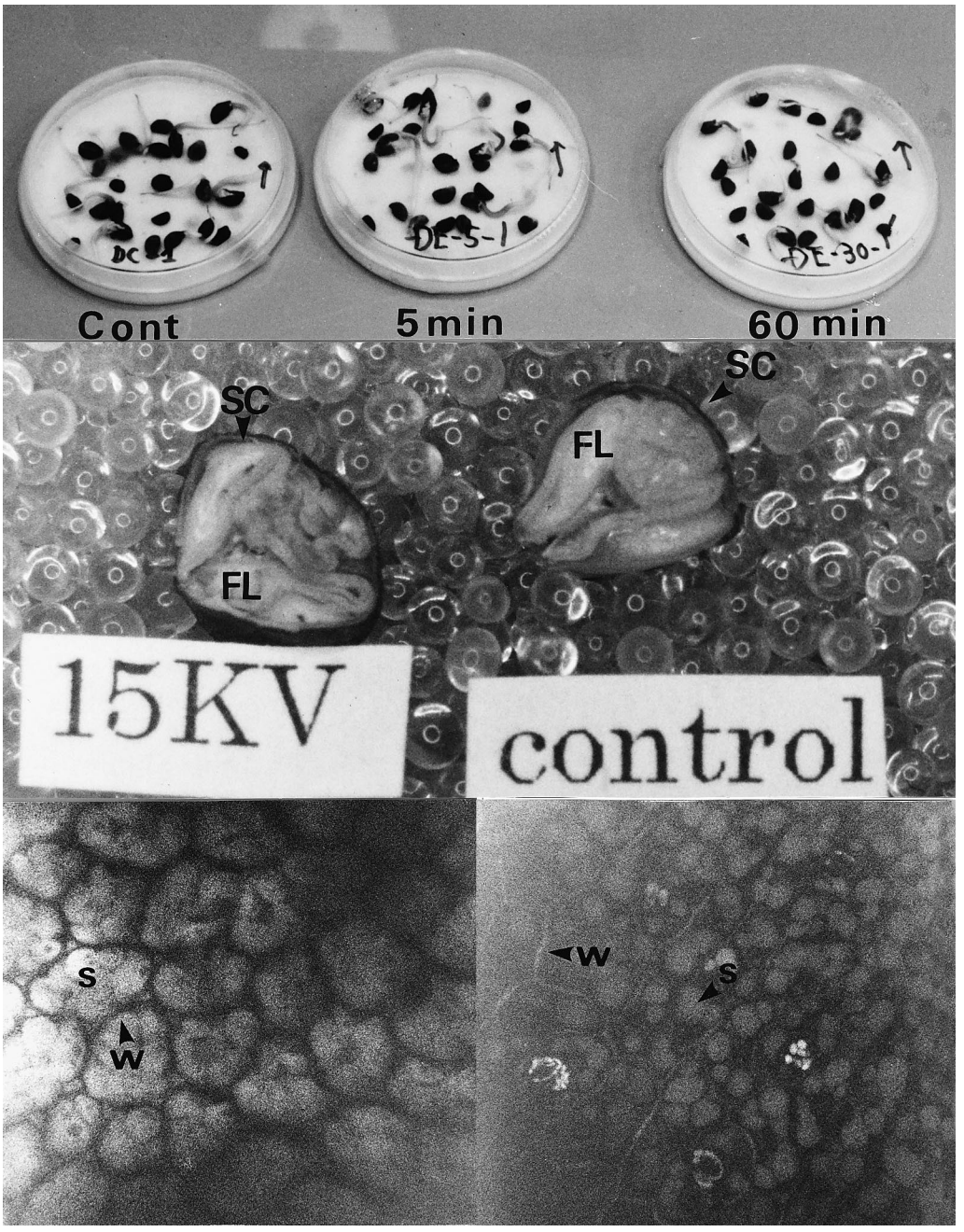

Fig. 3. Germination test of morning glory seeds treated with an electric field with 500 kV/m for 5 min and 60 min (top). Cont, untreated seeds; 5 min, treated for 5 min; 60 min, treated for 60 min. Middle: pictures of the seed treated with 500 kV/m for 60 min (left; 15 kV) and the untreated seed (right; control). SC, seed coat; FL, folded leaves. Bottom: micrographs of the treated seed with 500 kV/m for 60 min (left) and the untreated seed (right) observed by a laser confocal microscope. Lines indicated by w are cell walls and areas by s are starch granules.

a microcomputer (NEC PC9801, NEC, Tokyo), diffusion coefficients were calculated with the formula of Stejskal and Tanner [45], and diffusion coefficient images were displayed by an image analyzer (AVIO SPICCA II, Nippon Avionics, Tokyo). One-dimensional profiles along the x-axis with a fixed position in the y-axis on an ^{1}H-NMR image were measured by the pulse gradient stimulated-echo method [49]. A

1-mm area at a certain position on the *y*-axis was selectively excited along the *x*-axis (Fig. 2c) using 89 mT/m of a *y*-axis magnetic field gradient at the 90° rf pulse. After Fourier-transformation, profile data were transferred to a microcomputer (NEC PC9821, NEC, Tokyo) and analyzed using Meerwall–Ferguson's modification [50] of the model of Tanner [32].

3. Results

The effect of a static electric field of 500 kV/m which was treated for dry morning glory seeds, on the germination is shown in Fig. 3 (top). The effect was slight when the seeds were treated for 5 min, but treatment for 60 min significantly decreased the germination rate. The germination rate of seeds treated for 60 min in triple replications was 44 ± 4%, while that of the untreated seeds was 84 ± 8% (Table 1). The germination rate decreased to 50% of that of the untreated seeds. Sectional pictures and micrographs of tissues for the untreated (right) and the treated (left) seeds are shown in Fig. 3, middle and bottom, respectively. The degradation of starch was suppressed in the treated seed compared to the untreated seed (bottom), although significant effect was not seen in morphology (middle) of the seeds.

Table 1
Effect of a 500 kV/m electric field treatment for 60 min on the germination of morning glory seeds

	Sown seeds	Untreated	Treated
1	25	23	10
2	25	19	12
3	25	21	11
Average	25	21 ± 2	11 ± 1
%	100	84 ± 8	44 ± 4

The treatment was carried out on dry seeds (water content, 0.099 ± 0.012 g H_2O/dry wt.), then seeds were imbibed and germinated at 25°C for 48 h. Water increased to 3.88 ± 0.53 g H_2O/dry wt. for the untreated seeds and 3.42 ± 0.43 g H_2O/dry wt. for the treated seeds during germination.

^{1}H-NMR images of morning glory seeds treated by electric fields of various strength are shown in Fig. 4. In the image of the untreated seed, folded leaf tissues and a primary vein were observed. These tissues were observed in the seed treated with 133 and 267 kV/m electric fields, although images grew weak in this order. The signal of tissues was no longer observed in the seeds treated with an electric field of more than 400 kV/m (the images of seeds treated

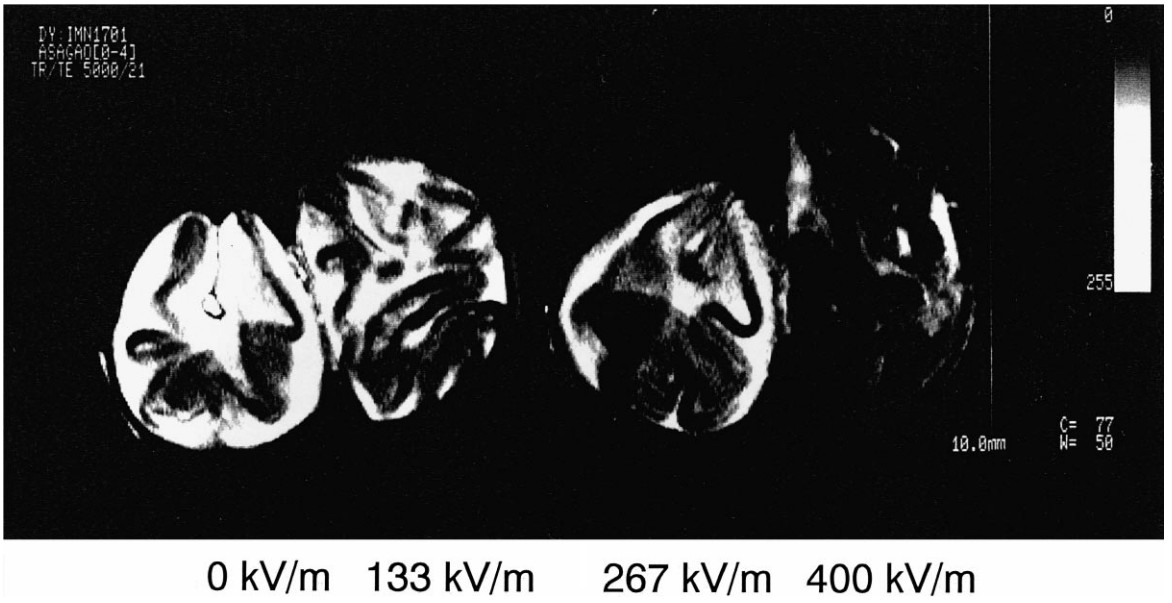

Fig. 4. ^{1}H-NMR images of morning glory seeds treated with various magnitudes (0, 133, 267 and 400 kV/m) of the electric field. Dry seeds were treated with the electric fields then germinated for 48 h on wet filter papers.

Table 2
Water fractions detected on semi-logarithmic plots of ^{1}H-NMR signal recovery for the untreated and the treated seeds

Fractions	Untreated		Treated	
	T_1 (ms)	Ratio (%)	T_1 (ms)	Ratio (%)
I	422 ± 69	12.9 ± 6.2	383 ± 73	29.5 ± 9.8
II	920 ± 65	29.2 ± 6.2	1029 ± 40	19.8 ± 4.6
III	1617 ± 381	59.3 ± 0.9	1391 ± 200	50.7 ± 5.3

with 500 kV/m are seen in Figs. 6 and 7). Despite this, the water content was not greatly reduced compared with the untreated seeds (3.88 ± 0.53 g H_2O/g dry wt. for the untreated seeds and 3.35 ± 0.91 g H_2O/g dry wt. for seeds treated with 400 kV/m). Based on the principle that NMR imaging detects mobile water, the results indicate that the most of cell-associated water in the seeds treated with more than 400 kV/m was restricted in motion.

The dry morning glory seeds were treated with 500 kV/m static electric field for 60 min, then the status of water was examined further in the following experiments. At least three water fractions with different spin-lattice relaxation times (T_1), approximately 400, 1000 and 1400–1600 ms, were detected in the semi-logarithmic plot of the recovery of the ^{1}H-NMR signal of the water proton. The relaxation times and estimated amounts of the individual water fractions from the plots are listed in Table 2. The shortest relaxation time was reduced to 383 ± 73 ms and the amount of this fraction was increased by exposure to the electric field. The treatment reduced the amount of water associated with the long fraction.

Fig. 5 shows the spectrum of water in the untreated seed (Fig. 5a), which is not symmetrical, indicating that the peak consists of components with various chemical shifts. The spectrum obtained with a 400 ms interval (Fig. 5b) between the 180° and 90° pulses of the inversion recovery method, showed the presence of peaks with different chemical shifts and various recovery times. One is a broad peak already having recovered and there are two sharp peaks which have not yet recovered. The chemical shifts show that water molecules which belonged to the individual peak components, each experienced slightly different static magnetic fields in the NMR apparatus. The mobility of the water molecules in each of the component was not identical to each other as demonstrated by the different recovery times. These components can be divided into three groups in regard to recovery time. The spin-lattice relaxation times (T_1) of each group, estimated from the recovery times, [47] are listed in Table 3. Comparing the spectral recovery of the treated seeds with that of the untreated seeds, the estimated T_1 of the shortest group became even shorter after treatment.

The state of the water in the seeds was examined by NMR microscopic techniques. Untreated (Fig. 6, right) and treated (Fig. 6, left) seeds were placed together in a 10-mm sample tube, and diffusion-weighted images were measured by the pulse gradient stimulated-echo method with varying diffusion times

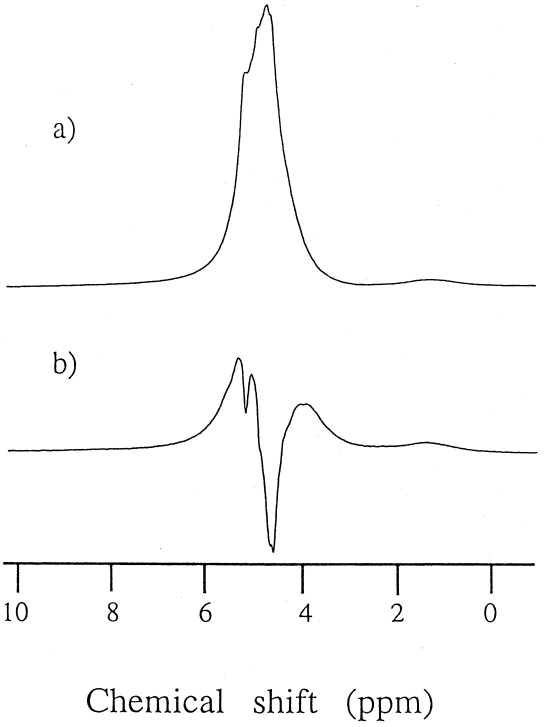

Fig. 5. Spectra of water signal of the untreated seed. (a) Spectrum of water signal and (b) that measured by inversion recovery method with pulse interval (PI) of 400 ms.

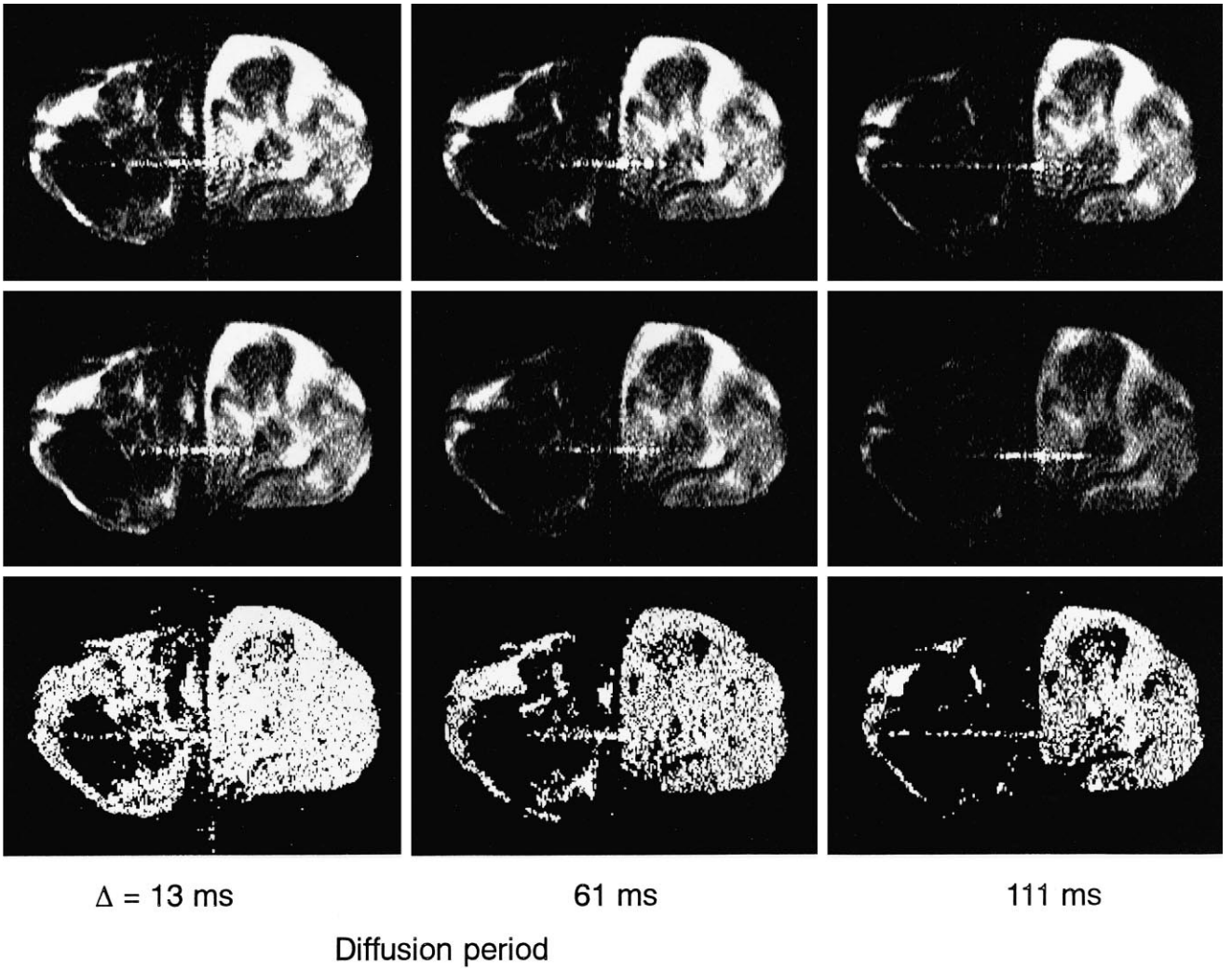

Fig. 6. ^{1}H-NMR images (top), diffusion-weighted images (middle) and diffusion coefficient images (bottom) of untreated (right) and treated (left) seeds measured by the pulse gradient stimulated-echo method (Fig. 2b) with 13, 61 and 111 ms of diffusion period (Δ). The treatment was carried out on dry seeds with 500 kV/m for 60 min and then germinated on wet paper.

to 13, 61 and 111 ms. ^{1}H-NMR images (top), diffusion-weighted images (middle) and calculated diffusion-coefficient images (bottom) are shown in Fig. 6. Self-diffusion coefficients were calculated using the formula of Stejskal and Tanner [45],

$$\ln(A/A_o) = -\gamma^2 D\delta^2(\Delta-\delta/3)G^2 \quad (1)$$

where A is the signal intensity of the diffusion-weighted image, A_o that of the corresponding ^{1}H-NMR image, γ is the gyromagnetic ratio, D the self-diffusion coefficient, δ the length of the pulse magnetic field gradients, Δ the interval between the two pulse magnetic field gradients, and G is the magnitude of the pulse magnetic field gradients.

In the ^{1}H-NMR image, the water signal intensity fluctuated to show an anatomical image of folded cotyledons in the untreated seed, while in the treated seed, the signal intensity was weak, except for several regions in the cotyledon tissues and veins (Fig. 6, top). Signal intensity was attenuated in all tissues in the diffusion-weighted images for the untreated seed. The tendency was similar in the treated seed, although the signal intensity was originally weak and not detected in some places (Fig. 6, middle). The diffusion coefficient with 13 ms of diffusion period was high at all places where the water signal, even a weak one, was detected. The diffusion coefficient declined differently depending upon tissue type by

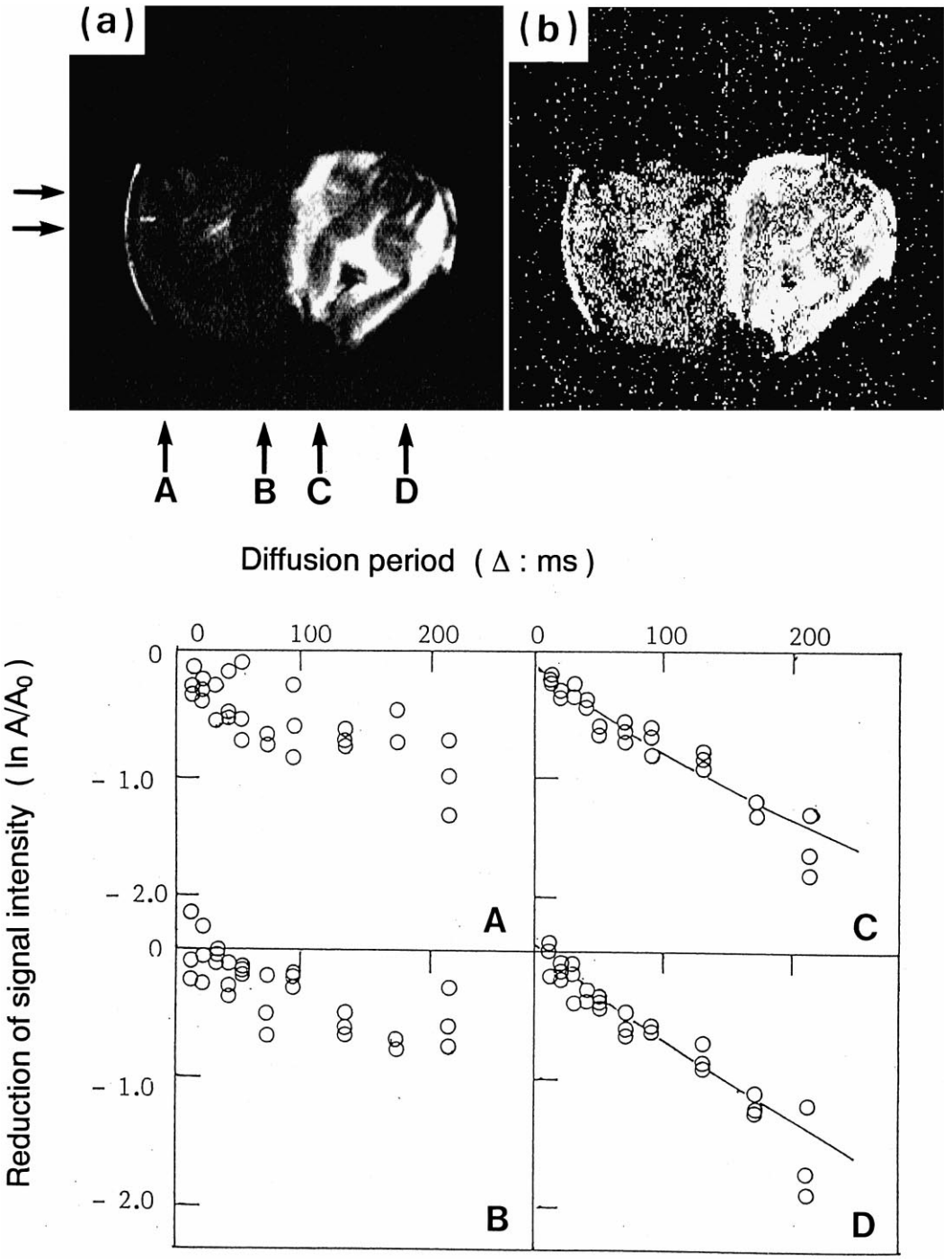

Fig. 7. ^{1}H-NMR image (a) and diffusion coefficient image (b) of the untreated (right) and the treated (left) seeds measured by the pulse gradient spin-echo method (Fig. 2a) (top). Curve fitting analysis of one-dimensional profiles measured by the pulse gradient stimulated-echo method (Fig. 2c) combined with selective excitation along the *x*-axis between the arrows on the *y*-axis using various diffusion times (bottom). The narrow horizontal area between the arrows on the *y*-axis (left side of images) was selectively excited. The sampling points indicated by the arrows A, B, C and D under the ^{1}H-NMR image (top). The conditions of the treatment were the same as Fig. 6.

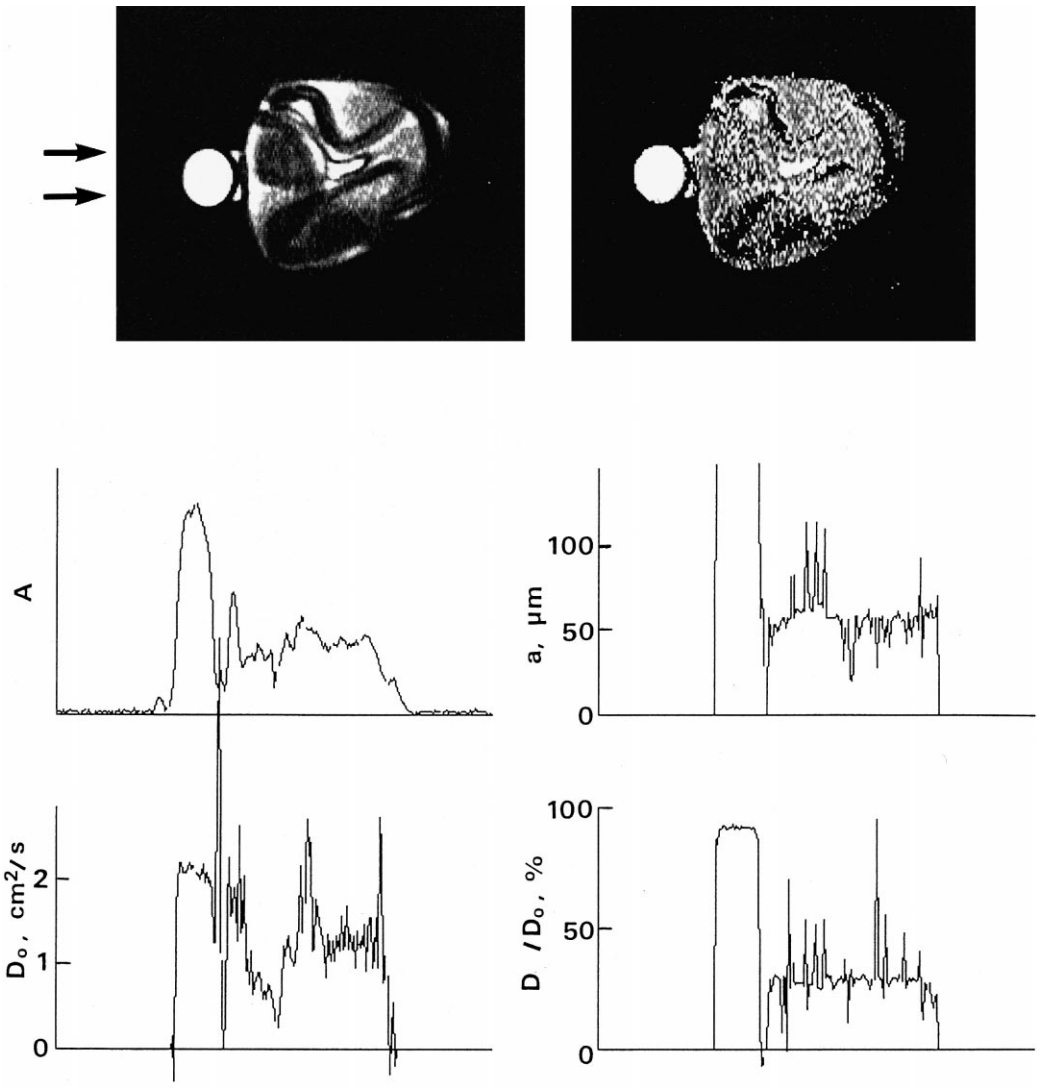

Fig. 8. ^{1}H-NMR image (left) and diffusion-coefficient image (right) of an untreated morning glory seed with distilled water in a capillary tube (top), and one-dimensional profiles of amount of water (A), initial diffusion coefficient (D_o), barrier spacing (a) and barrier permeability (D/D_o) (bottom). The measurements were carried out using the same method as Fig. 7. The narrow horizontal area between the arrows on the y-axis (left side of images) was selectively excited. The conditions of the treatment were the same as Fig. 6.

increasing the diffusion period up to 111 ms, indicating that water movement was restricted by the cell and tissue organization (Fig. 6, bottom). Therefore, we then examined how water movement was restricted.

Area-selective diffusion measurement was employed. First, the ^{1}H-NMR image (a), and a diffusion-coefficient image (b) of the untreated (right) and the treated (left) seeds were measured by the pulse gradient spin-echo method with 12 ms of diffusion period, in order to obtain an overall view (Fig. 7, top). In treated seeds, the epidermis of the cotyledon and the vein were emphasized on the diffusion-coefficient images. The seed coat did not contain much free water. Next, a narrow central area with 1.0 mm width of the y-axis was selectively excited (Fig. 7, between arrows) and a set of one-dimensional profile data (A and A_o) along with the x-axis was obtained with varying diffusion periods (16 points) by the pulse gradient stimulated-echo method. Signal attenuation curves plotted against diffusion period at positions indicated by arrows A, B, C and D on the ^{1}H-NMR image are shown in Fig. 7 (bottom). In untreated seeds (Fig. 7C,D), the attenuation rate

Table 3
Relaxation of the three component groups of proton signals with various chemical shifts of the untreated and the treated seeds

Component groups	Untreated		Treated	
	Recovery (ms)	T_1 (ms)	Recovery (ms)	T_1 (ms)
I	211 ± 32	304 ± 41	165 ± 12	238 ± 17
II	512 ± 85	737 ± 122	475 ± 87	684 ± 125
III	912 ± 25	1313 ± 36	700 ± 71	1008 ± 102

The components I, II, and III correspond to the components observed in Fig. 5. The figures are approximate values because PI (the interval between 180° and 90° RF pulses) are very coarse.

(A/A_o) declined with increasing diffusion period. The fall in attenuation rate was not linear, an effect also observed with growing barley seeds [51]. This may indicate that water molecules move through semi-permeable barriers [32]. Solid lines were fitted curves to Meerwall-Ferguson's modification [50] of the model of Tanner [32]. The D_o value is approximately 2×10^{-5} cm^2/s, being comparable to that of pure water (2.14×10^{-5} cm^2/s at 25 ± 1°C) [44]. In general, the model was not successful in the treated seeds because the attenuation of the signal fluctuated irregularly. Diffusion coefficients, obtained from the diffusion-coefficient image measured by pulse gradient spin-echo method (Fig. 7, right), show that cell-associated water is strongly restricted in treated seeds.

To confirm the results of Fig. 7 that cell-associated water in the control plant tissue showed a high diffusion rate compared to animal cells [29], the experiment was repeated by comparing pure water in a capillary tube as shown in Fig. 8. An ^{1}H-NMR image (left) and a diffusion-coefficient image (right) measured by the pulse gradient spin-echo method are shown (Fig. 8, top). One-dimensional profiles of restricted diffusion of water between arrows (left side of the image) were measured with varying diffusion periods (16 points) using the selective diffusion measurement of Fig. 7. The data were analyzed by the curve-fitting method for Meerwall–Ferguson's [50] modification of the model of Tanner [32] and the profiles of the analyzed values of the initial diffusion rate (D_o), the spacing between the barriers (a) and the permeability of the barriers (D/D_o) are shown in Fig. 8 (bottom). The D_o value ranged from 0.3×10^{-5} to 2.3×10^{-5} cm^2/s with position. The primary vein and epidermal tissues showed high D_o values comparable to those of pure water. The D_o value of most tissues fluctuated around 1.0×10^{-5} cm^2/s. The apparent barrier spacing fluctuated around 50 μm, whereas, the permeability was maintained at approximately 35% in all areas, despite the fact that the diffusion rate fluctuated considerably according to position.

4. Discussion

Promotive effects of electric fields on the germination rate have been reported for hard seeds and dormant seeds, such as corn [22,23], carrot [21], lettuce, brassica, radish [20], alfalfa [52] and oil-bearing seeds [19]. This is important since it can save costs by increasing the germination rate and preventing failure in seeding in agriculture. However, this could not be realized in our preliminary experiments using strong static electric fields [25]. Morning glory seeds were found to be sensitive to the treatment of the electric fields. Application of a 500-kV/m static electric field for 60 min for the dry seeds reproducibly reduced the germination rate to 50% of that of the untreated seeds (Fig. 3, Table 1). ^{1}H-NMR imaging was used to study the effect because it is a useful tool to investigate physiological conditions of plant tissues, especially relating to morphology [42]. The image signal grew weaker with the increasing strength of the treatment (Fig. 4). This indicates that water molecules in the cells are more strongly restricted in motion in treated seeds than in untreated seeds, considering that NMR detects mobile water in the tissues. Mobile water sustains fast metabolism [42] and supply of nutrients [31]. Therefore, it is present in physiologically active cells. The treatment with the electric field reduced the mobile water associated with active cells in tissues based on the above consideration.

Water status in the seeds was examined further. Water in living tissues is known to consist of several components in regards to the relaxation of the magnetized proton [29,53]. For example, there is water which is restricted in motion with a T_2 of less than 1 ms (approximately 8%) and free water with T_2 of 45 ms (approximately 82%) in cytoplasm, together with another component having a longer T_2 ascribed to extracellular water in muscle tissues [40]. In germinating morning glory seeds, three components with different T_1 values were distinguished on the semilogarithmic plots of the recovery of the ^{1}H-NMR signal: approximately 400, 1000 and 1400–1600 ms (Table 2). Multicomponent water fractions are similar to those reported for other plant tissues [54,55]. Although the shortest T_1 values are comparable to those in muscle, liver, stomach, kidney and brain [38], the situation is different in plant cells, which have large vacuoles, many vesicles and extracellular walls. Referring to the line width of ^{31}P-NMR signals [42,56,57], the longest component is ascribed to that of vacuole containing small molecules, such as metabolic intermediates, secondary products and inorganic ions, and the middle component to that of cytoplasm in plant tissues. The water with the shortest T_1 is considered to be exchangeable water around macromolecules, such as starches, proteins and strings of macromolecules in vesicles or between cell walls [58].

The asymmetric water signal seen in Fig. 5 consists of peaks with various chemical shifts. Relaxation of the peaks is divided into three groups (Table 3). This is similar to green cherry tomatoes [59]. Although T_1 values in Table 2 are longer compared to those in Table 3, the water fractions detected on the semilogarithmic plots correspond to the three components groups in the spectrum. Kasturi et al. [60,61] reported a water signal with a different chemical shift in *Artemia* cysts from that of pure water and stated that specialized organizations which order water, such as strings of macromolecules, are responsible for the chemical shift. The interaction of water molecules with macrosubstances may also cause the chemical shift in the morning glory seeds. Similar asymmetry of the water signal from components with various chemical shifts were also reported for many leaves by McCain [62]; the chemical shift of the water signal is reported to be susceptible to the orientation of the samples in the static magnetic field in the NMR apparatus, and is ascribed to the anisotropy of aligned chloroplast membranes which are pressed to the cell wall and thus order the water molecules. Morning glory seeds do not contain many chloroplasts, but have many oriented membrane structures and cell walls, which might cause such anisotropy. A similar result was reported for red osier dogwood stem by Burke et al. [63]. These coincide to the above idea of Kasturi et al. [60,61] that intracellular water forms polarized multilayers.

Water molecules in the individual peaks of the morning glory seeds experienced different magnitudes of magnetic field. Hence, the chemical shift of the water signal led to the concept of water compartments [62], containing specialized macromolecular structures [58,60]. Plant materials have several compartments which may correspond to component groups observed in the current investigation, whereas the compartmentalization is not clear from the unit peak of *Artemia* cysts [60,61]. The spectral discrepancy between the plant materials and the brine shrimp can be partly accounted for by the difference in water content. Water greatly exceeded solid cell constituents by a factor 3.5 in the morning glory seeds (3.3–3.9 g H_2O/g dry wt.) while the water content of fully hydrated *Artemia* cysts is 1.4 times the amount of dry matter [64]. In a similar way, the diffusion coefficient is more than 3 times higher in the plant materials (Fig. 8) [49] than in the brine shrimp [33,35]. Excessive amounts of water in plant materials constitutes fractions characterized with long T_1 values and represented by the sharp peaks (Fig. 5), which are surmised to be cytoplasm and vacuoles. Water in the *Artemia* cysts corresponds to the broad and faster recovering component group identified in the morning glory seeds, based on the comparison of the line widths of the proton signals. However, the fraction is water between macromolecules in vesicles or cell walls in plant materials.

The diffusion-weighted images indicate the distribution of high mobility water due to its principle of measurement. Thus the diffusion measurement is useful to map areas with active metabolism because high mobility water relates to active metabolism [42], and rapid nutrient supply [31]. The tissues of the untreated seeds, water transport rate (D_o) ranged from 0.3×10^{-5} to 2.3×10^{-5} cm^2/s (Fig. 8). The bar-

rier spacing which is calculated assuming that high mobility water molecules were surrounded by a spherical membrane, was approximately 50 μm and the permeability of the barrier was 35% (Fig. 8). The values are similar to those for growing barley seeds [49]. Note that the intracellular diffusion coefficient (D_o) was as high as that of pure water [44,49] in spite of the fact that cells have many structures and constituents ordering water. Water movement in cells is considered not to obey simple self-diffusion law, but to relate to active cell functions, e.g. heat generated by metabolism and structural movement may elevate water movements against the ordering by the cell structures and constituents.

The treatment in the electric field increased the amount of the fraction with the shortest relaxation time and further reduced the T_1 of that fraction (Tables 2 and 3). This resulted in the intensity of the signal being weakened in most places on the ^{1}H-NMR image (Figs. 4, 6 and 7). Since the water content after imbibition is similar in both the untreated and the treated seeds, the treatment has the effect of capturing water in restricted motion and undetectable by NMR imaging [65]. In the restricted diffusion measurements, signal attenuation with an elongating diffusion period varied irregularly in the treated seeds (Fig. 7). This may indicate that the susceptibility for proton magnetization varied even in the small volume of the imaging voxel due to the irregular organization of tissue structures. Since the proton motion in the dry or frozen *Artemia* cysts is reported [66], the electric field polarizes molecules and macromolecular complexes in membranes of the dry seeds during treatment. The perturbation of the membrane systems forces a small reorientation and disarrangement of fatty acids, proteins and complex macromolecules which prevents systematic incorporation of water and normal recovery of membrane functions, random space connectivity of ionic pathway, and formation of enzyme–enzyme and enzyme–cytomatrix association [67] by hydration during imbibition. Water absorbed irregularly in vesicles may intrude into and contact with stored materials, which are usually separated from excess amount of water in cells, resulting in the unexpected swelling of the stored macromolecules. The expanding pressure of macromolecules caused by hydration is strong enough to disrupt the membrane system severely [68]. Part of the damage may be repaired by self-restoration ability of the living system, but a part of the damage may be accelerated by receiving metabolic energy and force due to physiological function of the system at the expense of ATP. Thus the small effects on the dry seeds were multiplied through the crossover process of germination. The resulting irregular organization of hydration and recovery of the system may induce inhomogeneity in the magnetic field in the NMR apparatus as shown in Fig. 7A,B. This may correspond with the result of Fig. 3 (bottom) that the starch degradation is suppressed and non-uniform water distribution is taken place in the treated seed.

Summing up, the treatment with the static electric field above 267 kV/m for 60 min induces reorientation and disarrangement of the membrane components in dry seeds. This prevents normal recovery of membrane systems, resulting in the disorder and the disruption of membrane organizations and functions due to irregular absorption of water with stored macromolecules during germination (Figs. 3 and 7). The above events increase the volume of restricted water in motion (Figs. 4 and 7) and leads to retardation of the differentiation and the development of water fractions with the longest T_1 values (Tables 2 and 3), which is attributed to water in cytoplasm and vacuoles. Therefore, the electric field treatment on dry morning glory seeds reduces intact cells in germinating seedlings. The mechanism may be similar to that of the soaking effect observed in severely dried soybean seeds at germination [68].

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