

A FRACTAL SCALING LAW FOR SEED HYDRATION KINETICS

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Abstract. The paper reports results of a study of the water sorption rate, PGSE NMR (*Pulsed Field Gradient Spin Echo Nuclear Magnetic Resonance*) measurements of water diffusion coefficient and photosensitivity by difference spectroscopy *in vivo* of tobacco (c.v. Książnicki) and pea (c.v. Finalle) seeds. The probability of water uptake upon seed hydration and the spin-echo amplitude function of seed water proton, both related to the long-lasting evolution, take the form of power law. These dependencies were used to demonstrate that the random deposition of water molecules into the seeds matrix during hydration process leads to filling up the seed volume with characteristic clusters created by diffusion limited by aggregation (DLA). The hydration process starts with the creation of small clusters at and near macromolecules and finishes along with a big percolation one, spreading throughout the seed. Using the fractal model for hydration process it was shown that the PhyA (phytochrome A) heterodimer was a photoreceptor for water flow regulation, activated by a very low fluence reaction (VLFR).

Keywords: seeds, sorption half-time, fractal dimension, phytochrome

INTRODUCTION

It is believed that seed hydration is a 'physical' first order process related to the interactions between water and solid state matrix of seeds [5,21]. Unfortunately, the first order kinetics of the water uptake, predicted by steady state thermodynamics, does not satisfy experimental data.

Firstly, the time constants calculated on the basis of mass or volume changes are different, proving density evolution upon seed hydration [10]. Secondly, these values are too high to explain the water content change measured throughout the range of the hydration process.

The high degree complexity of the seed matrix, composed of solid state constituents and inter-cell free space, is responsible for this discrepancy. Adequately to such structure properties, the flux rate is limited by water aggregation around the hydrophilic group of biopolymers and inside intercellular free space. Consequently, a lattice composed with of irregular curves being a trace of water flow fills the whole seed volume at the end of the hydration process. It was predicted by the theory of stochastic processes [6,13] and illustrated by means of computer simulations [23] that diffusion limited by aggregation (DLA) creates fractal forms and networks.

The experimental data led to an attractive hypothesis that the fractal lattice formed by a water flow trace is involved in the seed hydration progress [15]. Recently, a fractal model for seed hydration has been introduced for the first time [16] allowing fractal dimension calculation for underlying geometry. On the basis of this model with gravimetry, pulsed $^1\text{H-NMR}$, *in vivo* difference spectroscopy and rape seed germination data, a coherent explanation of the chemical stimuli impact and light control of water flux rate was proposed [14].

Seeds are able to modulate uptake of water by the light absorbed by the photoreceptor of germination – phytochrome. The former is a red/far-red (R/FR) light reversible pigment which exists in two forms: a physiologically inactive red light absorbing form P_r , which can be transformed by red light (R) into the physiological active far red light absorbing form P_{fr} , which in turn can be transformed by far red light (FR) into P_r . Therefore, the detection of phytochrome was made possible in plant tissue by means of dual wavelength spectrophotometry [19], following alternate irradiation of plant material with red and far red light.

In natural environment many responses induced by brief red irradiation occur in response to two distinct fluency ranges. The two response types were termed the very low fluence response (VLFR) and the low fluence response (LFR). One kind of phytochrome (PhyA) is responsible for VLFR reaction, another one (PhyB) controls the LFR reaction, both with different spectroscopic and biochemical properties [18,20]. By using the fractal model for seeds hydration, the activity of PhyA heterodimer interacting with plasma membrane in control of water uptake in viable rape seeds was proved [16].

Although the fractal model for seed hydration seems to be useful in many fields, further testing on different seed species is required before it can be commonly applied. This is the purpose of this study.

MATERIALS AND METHODS

Tobacco seeds c.v. Księżnicki or pea seeds c.v. Finalle were used in all measurements. In order to equalise the water potential, prior to every experiment

seeds were gently shaken (0.5 hour) in darkness with deionised water and again dehydrated. Groups of seeds screened for uniformity of size and layered on Petri dishes were placed in a desiccator with bidistilled water. The desiccator was left in a germination chamber at 293 K. The uptake of saturated water vapor in darkness was interrupted daily by irradiation with monochromatic light. Eight samples were irradiated simultaneously in a desiccator. Back sides of the samples were protected from the light by black paper. The light was transmitted only through the surfaces of the samples covered by appropriate interference filters (Carl Zeiss Jena). The reference, dark control sample, was covered by filter of $\lambda = 1100$ nm. Radiant photon flux densities were measured with a LII meter showing maximum sensitivity for 633 nm. Every other flux density was calculated from the calibration curves for light sources and filter transmission. During irradiation, light was transmitted through water filter in order to exclude misinformation resulting from heating effects. Sample mass was measured as quickly as possible, under dim green light.

The difference spectra for phytochrome phototransformation $P_{fr} \rightarrow P_r$ were measured using dual-wavelength spectrophotometer operating with the reference beams set at 800 nm. Measurements were made at 276 K with 60 pea hypocotyle segments in one sample. The difference spectra were determined by irradiating pea samples with modified projector lamp assembly equipped with 250 W quartz iodine lamp (Philips, Eindhoven, The Netherlands) and suitable interference filters (B40, Balzers, Lichtenstein). The total reversible phytochrome was indicated by the change in the optical density reading $\Delta(\Delta A)$ following alternating irradiation of the sample with actinic sources of red (660 nm) and far-red light (720 nm), calculated as $\Delta(\Delta A) = (\Delta A)_{660} - (\Delta A)_{730}$.

The H-NMR experiments were carried out at 299 K using a 400MHz spectrometer Bruker Spectrospin400. The diffusion coefficients were measured by ^1H -PGSE NMR (*Proton Pulsed Field Gradient Spin Echo Nuclear Magnetic Resonance*) method. The spin-echo amplitude was measured as a function of the impulse gradient width δ whereas the interval Δ between impulse gradient and the gradient impulse amplitude were constant.

Calculations

(a) In order to qualify the seed hydration process in the whole range of water content the universal form of kinetic equation was followed up in the form:

$$m(t) = m_{\infty} (1 - e^{-(dt)^{\alpha}}) \quad (1)$$

where $m(t)$ is the water content on a dry mass basis at time t , m_{∞} is maximum water content on dry mass basis theoretically predicted at the equilibrium with external water potential (maximal water activity) when $t \rightarrow \infty$, d is the diffusional

time constant depending on the kind of seeds, α is the dimensionless parameter (stretch exponent). All these parameters combine into the equation:

$$\tau = \left(\frac{\ln 2}{d} \right)^{1/\alpha} \quad (2)$$

describing half-time of the water uptake related to the sorption time of approximately 63% saturated water content.

(b) The relative effectiveness of different light treatment was compared as the relative water uptake rate U , given as a function of the half-times of water uptake $\tau(\lambda)$ and τ_0 in seeds which were irradiated at wavelength λ or kept in darkness continuously, respectively:

$$U = \frac{\tau_0 - \tau(\lambda)}{\tau_0} \quad (3)$$

(c) In order to analyse the probability of water sorption the equation (1) can be transformed into the form:

$$1 - \frac{m(t)}{m_\infty} = e^{-(dt)^\alpha} = P(m) \quad (4)$$

where $P(m)$ is the water flux propagation probability function related to hydration time (probability function of the water deposit into seed matrix). It was proved that if water deposition into seed matrix is conditioned on restricted diffusion the equation (4) takes the form of power law for long-lasting evolution of hydration process. Therefore, these dependencies should be used to demonstrate that the random deposition of water molecules into the seeds matrix during hydration process leads to filling up the seed volume with characteristic fractal clusters created by diffusion limited by aggregation (DLA) at and near hydrophyl groups of macromolecules and inter cell space. The fractal model of the seed hydration [16] based on the theory of stochastic processes and Lévy statistics [22] shows that:

$$\alpha = \frac{1}{d_f} \quad (5)$$

where d_f is the fractal dimension, which is generally not a whole number but a fraction.

(e) The basic property of every fractal object is self-similarity [11, 12]. Otherwise, if the water flow trace has fractal geometry, the α parameter value is not dependent on the observation time scale: the same goes for macrostate (sorption experiment) and microstate ($^1\text{H-NMR}$ experiment). Therefore the fractal scaling

of lengths determines the form of the probability density of the water flow, both in the scale of sorption experiment and over the range of evolutionary time scale, accessible to the NMR measurement (20-500 ms).

The normalized spin-echo amplitude function takes the form:

$$E = \frac{S(q, \Delta)}{S(0)} = e^{-4\pi^2 q^2 \Delta D} \quad (6)$$

where Δ – diffusion time, D – diffusion coefficient, $q = \gamma\delta G/2\pi$ – wave vector, γ – gyromagnetic ratio G – field gradient, δ – impulse time.

According to the method applied [8,9], one can observe either the dynamic evolution of the probability density in time or its spatial form at fixed time. The former is best described by the so-called KWW (Kohlrausch-Williams-Watts) function [3] $E = \exp(-Db)^\alpha$ (where α is the stretch exponent, D is the diffusion coefficient), the latter by the power law $E \sim (q)^{-d_f}$ for long evolutionary times. The quantity b includes the time scale of measurement (T), the experimentally given evolutionary time, and a length scale, given by the wave vector q with the interrelation $b = 4\pi^2 q^2 T$. The exponent d_f is the fractal dimension of the underlying geometry. Therefore, if the spin-carrier environment has a fractal geometry, the experimental data fits a power law, allowing fractal dimension calculation.

RESULTS AND DISCUSSION

Tobacco seeds with an initial water content of 0.094 g water per g dry mass basis were allowed to imbibe water by keeping them in humid air. Kinetics of the water sorption for these seeds is shown in Figure 1.

On the basis of this data, in agreement with equation (1), the parameter $\alpha = 0.55$ (stretch exponent) was calculated. The water sorption probability function in these seeds changes (Fig. 2) from the exponential form for short time to the power law for long time. The double logarithmic plot for water uptake probability function reveals linear dependence for long time evolution of seed hydration process, allowing the calculation of the fractal dimension $d_f = 1.83$ on the basis of linear regression analysis. As one can see the equation (5) is confirmed by the water sorption data.

Tobacco seeds, typically used as a model to study the light control of germination, were used to compare the effects of different light treatments on the sorption rate.

The light control of the water flux taken up by tobacco seeds was analysed in terms of water uptake half-time (Eq. 2) in the seeds irradiated by different doses of far-red light. The former was calculated on the basis of kinetic data by using the curve fitting procedure. Dependence of the water sorption half-time on the photon fluence of far-red light applied daily for tobacco seed irradiation reveals

(Fig. 3) a decrease and increase in the water sorption half-time compared to the water sorption half-time in the seeds kept in darkness continuously.

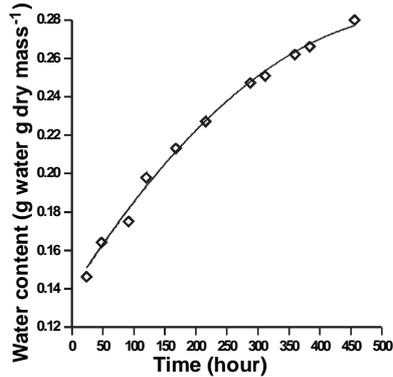


Fig. 1. Time course of seed water content in tobacco seeds kept in darkness

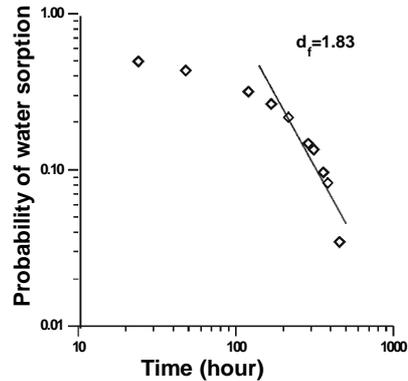


Fig. 2. Probability of water sorption in tobacco seeds related to time

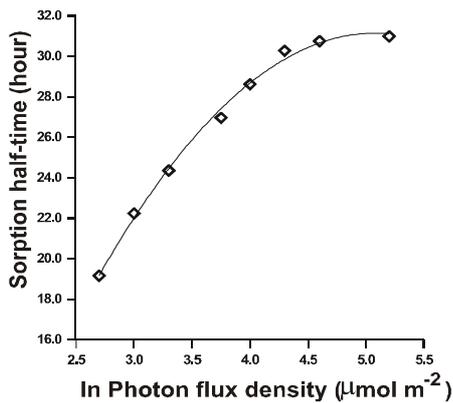


Fig. 3. Dependence of the water sorption half-time on the photon flux density of far-red light used daily for tobacco seed irradiation. Half-time of water sorption in the seeds kept in darkness continuously was 25 hours

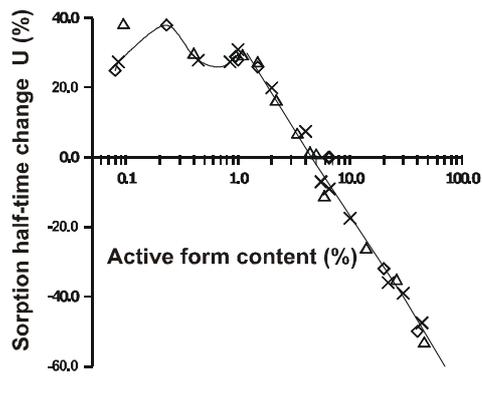


Fig. 4. Relationship between the half-time of water sorption relative change U and the $[P_{fr}]$ form fraction in relation to the total phytochrome $[P]=[P_{fr}+P_r]$ generated daily in tobacco seeds by far red irradiation

All light induced effects related to the sample kept in darkness were quantitatively characterised by the water uptake rate U (Eq. 3). On the basis of the spectral characteristics of both the light source and features of the photoreversible phytochrome, the relative content of the biologically active, far-red absorbing form of phytochrome P_{fr} compared to the total photoreversible phytochrome P_{tot} was calculated [4]. Figure 4 shows that the response of water uptake rate to the

phytochrome active far-red absorbing form in tobacco seeds is distinctly bi-phasic in accordance with the VLFR and LFR reactions. The maximum water uptake (40% increase comparing to the dark control) was observed in the case of very low irradiation which created approximately 0.2% of P_{fr} . Responses to fluence creating about 1.5% of P_{fr} appeared to be the cumulative result of activities underlying both VLFR and LFR. For photon flux creating more than 5% of P_{fr} , far-red light inhibits the water uptake rate.

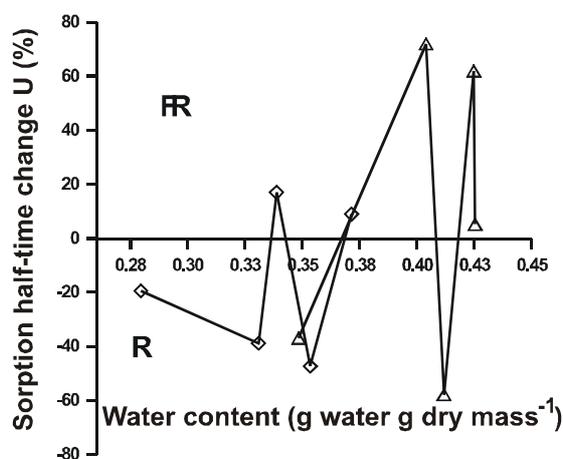


Fig. 5. The red (R) and far red (FR) light reversibility effect on tobacco seeds water flux photo-control. The uptake of saturated water vapor in darkness was interrupted daily by alternative irradiation by red (R) or far-red (FR) light. Photon fluence was $15 \mu\text{mol m}^{-2}$

Established operating criteria for the involvement of the phytochrome in a light-mediated response require an effect by brief irradiation (pulse) with red light to be fully reversed by a subsequent pulse of far-red light. In order to determine the reversible red/far-red (R/FR) effect on the water uptake rate the seed sample mass content was measured as a function of the daily consecutive R-FR-R-FR irradiation by $15 \mu\text{mol m}^{-2}$ photon fluence.

Figure 5 shows that FR irradiation induces an increase in the water uptake rate, whereas R irradiation induces an opposite effect. Both effects are reversible. This is a clear proof for the involvement of a photomorphogenic receptor phytochrome in the regulation of water uptake in tobacco seeds. The fluence range used in this experiment is too low for PhyB activation. The detailed calculation performed in the same way as for rape seeds [16] proved biological activity of the phytochrome heterodimer composed of P_r and P_{fr} monomers of phytochrome type A. Moreover, only P_{fr} monomer is biologically active.

The R/FR effect on pea seed hydration was published earlier [17]. On the basis of the range of photon fluence used to irradiate seeds in these measurement it is clear that PhyA heterodimer composed with one monomer in the P_{fr} form is active in the regulation of the seed hydration, similarly to rape and tobacco seeds.

The fully R/FR reversible phytochrome PhyA which is responsible for light control of water flux regulation during pea seed imbibitions persists throughout in the whole range of seed hydration and is detected (Fig. 6) in pea hypocotyle segments

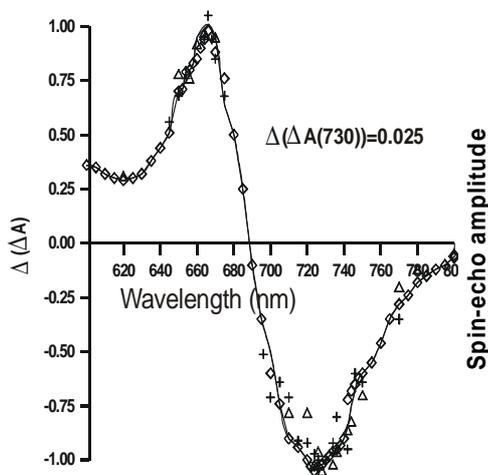


Fig. 6. Difference spectrum ($P_{fr} \rightarrow P_r$) of phytochrome phototransformation in hypocotyle segments of pea seedlings

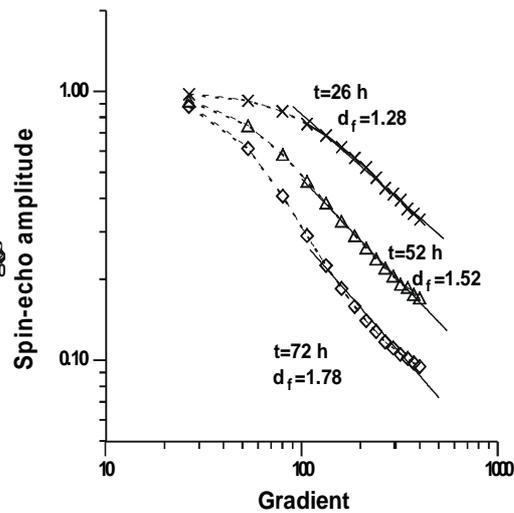


Fig. 7. Power dependence of spin-echo function vs. separation time between gradient impulse in pea seeds

Figure 7 shows the power dependence of the normalized spin-echo amplitude function vs. field gradient in pea seeds measured after 26, 52 and 72 hours of hydration. The fractal dimensions calculated on the basis of linear parts of these dependencies by means of linear regression analysis were 1.28 (26 h), 1.52 (52 h) and 1.78 (72 h). By comparing value obtained with the former value predicted by computer simulation one can see that the hydration process starts with the creation of small clusters at and near macromolecules and finishes along with a big percolation cluster, spreading throughout the seed. The fractal dimension value $1 < d_f < 2$ predicts one-dimensional water flow lines that cover the two-dimensional surfaces immersed in three-dimensional seed volume at the end of hydration.

SUMMARY

Over the last years there has been growing interest in the application of the fractal concept [11,12] in chemistry, biophysics and medicine [1,9,22]. This is because these idea exhibits [2,7] a surprising range of dynamic behaviour of complex systems.

This study focuses on fractal forms and networks with regard to the water flux rate measured in time scales of the water sorption process and $^1\text{H-NMR}$ PGSE experiment. For tobacco and pea, like for rape seeds, the hydration process is influenced by restricted diffusion. Because DLA structures persist as a trace of the water flow, the diffusion is not as effective as in free space. Moreover, these DLA structures probably create a functional network allowing an effective flow of energy and information. Consequently, a very low fluence rate absorbed by PhyA heterodimer controls the water uptake of the germinating seeds.

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ZASTOSOWANIE ZASADY SKALOWANIA FRAKTALNEGO DO KINETYKI HYDRATACJI NASION

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Streszczenie. Praca przedstawia wyniki badań szybkości sorpcji i współczynników dyfuzji wody metodami PGSE (*echo spinowe z impulsowym gradientem pola magnetycznego*) oraz fotoczułości metodą spektroskopii różnicowej *in vivo* nasion tytoniu (odm. Książnicki) oraz grochu (odm. Finale). Zarówno prawdopodobieństwo pobierania wody podczas hydratacji nasion jak i amplituda echa spinowego protonów wody nasiennej dla długiego czasu ewolucji mają postać praw potęgowych. Zależności te zastosowano do wykazania, że przypadkowe umiejscawianie molekuł wody w matrycy nasiennej w czasie hydratacji prowadzi do wypełniania objętości nasion charakterystycznymi gronami powstającymi w wyniku dyfuzji ograniczonej przez agregację (DLA). Proces hydratacji rozpoczyna się od powstania małych gron w sąsiedztwie makromolekuł a kończy się wraz z utworzeniem dużego grona perkolacyjnego obejmującego całe nasiono. Stosując model fraktalny do procesu hydratacji nasion ujawniono, że heterodimer PhyA (*fitochromu typu A*) aktywowany przez bardzo małą dawkę światła w reakcji typu VLFR jest fotoreceptorem dla regulacji przepływu wody.

Słowa kluczowe: nasiona, półokres sorpcji, wymiar fraktalny, fitochrom