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Dependence of wheat seed germination kinetics on temperature and magnetic field

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Abstract. Temperature dependence of wheat seed swelling and sprouting kinetics in water in the range of 10° C to 25°C for constant magnetic field (~ 10^{4} Gs) has been studied. Grain weight growth was chosen to serve the parameter under control. Activation energies for swelling and germination processes have been estimated (43.5kJ/mol and 47.5kJ/mol) of which the latter depended linearly on the share of hydrophilic substances in seeds. The assumption has been made that the quantum-cooperative phenomena in hydrate shells of biomolecules and clusters with correlated states of water spin-isomers define the adaptive physiology. Optimal temperatures of seed stratification and sprouting has been attributed to transitions between ice-like and spiral clusters. Magnetic field did not effect the kinetics of swelling but it slightly inhibited the rate of sprouting. Negative influence of magnetic field was explained by effect of Lorenz force on protons in the plain perpendicular to their motion in scutellum plasmalemma.

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Keywords: wheat seeds, kinetics, germination, magnetic field, water properties.

Highlights

- Ice-like and spiral clusters of water play an important role in seed physiology.
- Activation energies for swelling depended on hydrophilic substances in seeds.
- Magnetic field slightly inhibited the rate of germination.

1. Introduction

Water plays the role of both matrix and principal metabolite in physiology of plants. In the course of phylogeny, abnormal water's physical properties have influenced the thermodynamics of living organisms, as well as that of the adoptive physiology mechanisms. Extremal points (T_{ex}) for volumetric density (ρ) and specific thermal capacity at constant pressure (CP) of water for 4 °C, 25 °C and 35 °C (Kholmanskiy, 2015) the temperature dependences (TD) have on predetermined the temperature range of vital functions for the majority of organisms. Seeds of various plants get activated in moist environment at 3 °C to 5 °C (stratification) and have maximal values of germination rate (V_G) in the range of 20 °C to 30 °C. In this case, TD for V_G can be approximated by polynomial of 2nd order for many plant spices (Balkaya, 2004). It has been found in (Kholmanskiy, 2014) that, in proximity to points $T_{ex} = 4$ °C, 25 °C and 35 °C, TDs for ρ and C_P of water can be approximated by quadratic functions of $\Delta T = (T - T_{ex})$. Obviously, parabolic shape of TD for V_G in the range from 4 °C to 40 °C is an expression of thermodynamics of transitions between ice-like (W₆) and spiral (W_{α}) water clusters (Kholmanskiy, 2015) in the metabolism of seed germination.

Formation of W_{α} -clusters in biologically active liquids is supported by chiral metabolites and hydrate shells of bio-molecules comprising chiral fragments. In the range of 2 °C to 25 °C, the value of reaction activation energy (E_A) for cooperation of W_{α} -clusters to form macro-clusters is comparable with the energy of the lower rotational levels of water spin-isomers (Kholmanskiv, 2015). As a result, quantum-cooperative effects determined by resonance transitions among spin-isomers play an essential role in thermodynamics of water, in the range of 20 °C to 40 °C. Owing to these W_{α} -clusters transitions, build long-lived macrostructures with correlated spin states of water molecules (Kholmanskiy, 2015). Abnormal chirality fluctuations of sugar solutions at T<15 °C were attributed to the dynamics of these states (Kholmanskiy, 2015a). They also may sensitize living systems in relation to week physical factors including chiral ones (CF) (Tverdislav et al., 2007; Kholmanskiy, 2010; 2015). In (Kholmanskiy & Strebkov, 2007; 2010; Kholmanskiy, Kholmanskiy, 2015b) the physical-chemical mechanism of stratification was attributed to CF effects, and the assumption was made that critical phenomena in water at 20 °C to 30 °C limit

the thermodynamics of plant seed sprouting.

Nomenclature	M current value of grain mass (mg)
B magnetic induction (Gs)	Mo initial mass of grain (mg)
CF chiral factor	m mass of water (mg)
C _P specific thermal capacity of water at constant pressure (J kg ⁻¹ K ⁻¹)	ρ volumetric density of water (g cm ⁻³)
GMF geomagnetic field (Gs)	V_G germination rate (mg h ⁻¹)
HB_1 B in the direction of H-component of GMF (Gs)	V_S swelling rate (mg h ⁻¹)
ZB ₁ B in the direction of Z-component of GMF (Gs)	T temperature (°C)
E_A activation energy (kJ Mol ⁻¹)	T _{ex} Extremal temperature (°C)
HPh share of hydrophilic substances (%)	TD temperature dependence
E_G , K_G activation energy (kJ Mol ⁻¹)	ΔT temperature range
and kinetic constant (h^{-1}) for germination	t time (h)
E_s , K_s activation energy (kJ Mol ⁻¹)	W_6 , W_α ice-like and spiral water clusters
and kinetic constant (h ⁻¹) for swelling	
MF magnetic field	

Anatomy and physiology of seeds reproduce in miniature the key elements and stages of living organisms' ontogenesis (Prokofyev, 1982; Polevoy, 1989). Seed's chemical composition for higher plants represents adequately the metabolic synergism of right-type and left-type compounds (e.g., sugars and amino acids). With regard to the appearance of water's T_{ex} specificity in physiology of seeds they can serve a model to study sensitivity mechanisms of living organisms in relation to the impact of external physical factors.

Metabolism rate of seed is sensitive to the origin of medium oxygen concentration, growing and temperature and humidity, lighting and various geocosmic factors. Therefore, the mechanism of adaptive physiology of seeds has to be studied based on an adequately chosen characteristic of metabolism, and the values of E_A for the key stages of germination process have to be determined from the TD of the chosen characteristic which can be the growth rate of mass of seed, roots and green sprouts in the stages of swilling and germinating (Kholmanskiy, 2015; (Kholmanskiy et al., 2015). Faraday effect, Lorenz force and spiral trajectories of electric charges in magnetic field (MF), generally, make it helpful to apply MF effects to the kinetics of seed sprouting process for modeling the mechanism of effects produced by external chiral factors on organisms.

Physical-chemical mechanism of constant and alternating MF impact on the physiology of plants is not yet perfectly clear (Bingi, 2002; Galland & Pazur, 2005). A great number of studies in magnetobiology were related to modeling the influence of geomagnetic field (GMF) on the growth rate of roots and underground seedlings (Galland & Pazur, 2005; Bogatina et al., 1978, 1979). Low reliability of data obtained using such methods for evaluation of metabolism intensity, as well as the weakness of MF effects, impel to apply probabilistic-statistical computations for data processing in with case feebly marked dependences of seed physiology on external conditions get obscured, as a rule (Kholmanskiy, 2015b). Incongruent results are sometimes recorded on the influence of MF on seed sprouting (Galland & Pazur, 2005). For example, a threshold-like dependence of the growth rate of wheat roots and coleoptiles on the MF intensity has been observed (Bogatina et al., 1979; Pai-Tsun & Show-Ran, 2009). The germination rate for wheat seeds increased by 20% with the magnetic induction (B) growth from 10^{-4} Gs to 10⁴ Gs while for B=0.01Gs to 0.03Gs and higher (up to 10⁴ Gs) it did not change. In review (Galland & Pazur, 2005), it is recorded that 15 Gs to 25 Gs MF accelerates rice grain germination while the sprouting rate of wheat, soya beans and sunflower increases in 50 Gs MF. It has been found in (Kholmanskiy, 2015c) that water evaporation rate from the sand, as well as seed swelling in sand and in water, do not depend on the intensity and direction of MF. Weight growth rate of seeds in the course of germination, as well as that of plant herbage, increase by 15% to 20% in a MF whose intensity exceeds that of GMF by two to four orders of magnitude.

With regard to the above data, the dependence of wheat seed germination kinetics on temperature in the range of 10 °C to 25 °C in constant MF of various configurations in the rage of $B{\sim}50$ Gs to 10^4 Gs has been investigated in this work to study the mechanism of plant adaptation to external impacts.

2. Mathematical model

The rates of seed swelling and germination were determined on the single-seed basis. Therefore, the aggregate weight of seeds in the course of swelling was divided on the initial number of seeds while in the course of sprouting only live seeds were taken into account. The share of live seeds was the measure of germination ability, and its value was close to ~90%. Swelling rate of grain (V_S) in water in the initial stage will be proportional to the weight of conditionally dry matter of grain:

$$V_{\rm S} = dm/dt = K_{\rm S}(M_{\rm o}-m), \qquad (1)$$

where K_S is swelling kinetic constant, M_o is initial mass of seed, m is mass of water within damped seed. The following dependence of water mass within grains on time can be defined from (1):

$$m = M_o[1 - exp(-K_S t)]$$
 and $ln[(M_o - m)/M_o] = -K_S t$
(2)

Grain germination rate (V_G) , in the initial stage, can be described with the use of the following equation:

$$V_G = dM/dt = K_G M, \qquad (3)$$

(4)

where K_G is germination kinetic constant and M is instantaneous value of the mass of grain and sprouts including that of grain substance, water and carbon dioxide. Solving (3) yields the following dependence:

 $M = M_0 \exp(K_G t).$

By applying dependences (2) and (4) to the experimental curves of swelling and sprouting we can obtain the values of kinetic constants at various temperatures and in MF. With the use of Arrhenius approximations of temperature dependence for kinetic constants effective values of EA for swelling and sprouting process were estimated. Average values of germination rates were taken for uniform MF configurations (constant and vortex-type). Experimental error calculated based on these values did not exceed 10%. Program Microsoft Office Excel was used to draw, approximate and process the data on kinetic dependences.

3. Materials and methods

Data on optimal temperatures for seed germination were found in various agricultural reference books and materials published on the Internet. Data on the following plant species, mainly those of the Northern hemisphere, have been analyzed (within brackets, number of plant species is given): grain crops (19), vegetables (47), flowers (20), trees and bushes (21), weeds (47).

Wheat seeds harvested in 2014 were used in experiments. Grains (~6 g, ~150 pieces) were

distributed inside Petri dishes with random axis orientations. In the stage of swilling, seeds were immersed under water surface while in the stage of sprouting they were covered with wet lab wipes. Tap water was kept in normal environment for, at least, 24 h prior to usage. Grains were weighed with the help of laboratory balance with an accuracy of 20 mg. Petri dishes were installed above a magnet with a gap of 0.5 cm to 1.0 cm. Seeds in the course of sprouting were illuminated using PL11WG23 luminescent lamps. MM-3 magnetic mixer was used having rotation rate w~1000 min⁻¹. Rectangular ~10⁴ Gs magnets made of neodymium alloy were applied having two layouts: B₁ $(3 \times 2 \times 1 \text{ cm}^3)$ and B₂ $(4 \times 1 \times 1 \text{ cm}^3)$. Induction axis of B₁ magnet was oriented along edge 1 cm while that of B₂ magnet was oriented along 4 cm edge. It was found with the help of a compass that the value of magnetic induction of B₁ at a distance exceeding 1 m is comparable that of GMF (0.5 Gs). Therefore, reference samples were located as far as ~3 m away from both magnets. Magnet B₂ was mounted on the mixer magnet (WB_1) so that its induction axis could rotate in the horizontal plain. Two B1 magnets were mounted one upon the other above the samples in two ways of which one refers to the induction vector orientation along Z component of GMF (ZB1) while in the other the induction vector was oriented along H component of GMF (HB₁).

4. Results and discussion

Figure 1 shows the distribution of temperatures for which V_G of seeds attains its maximum over number of plant spices. For a large number of plant spices, TD of V_G can be approximated by polynomial of 2nd order with an acceptable level of accuracy, and they have a maximum in the range of 20 °C to 30 °C (Balkaya, 2004). It has been found in (Kholmanskiy, 2014) that TDs of ρ and C_P for water near values of T_{ex} = 4 °C, 25 °C and 35 °C can be approximated by quadratic functions of $\Delta T = (T - T_{ex})$. Based on these data, the assumption can be made that parabolic form of TD for V_G in the proximity of 4 °C and 40 °C is determined by thermodynamics of transitions between W_{6} - and W_{α} clusters in water that play the role of limiting factor affecting seed germination metabolism. The data on water clusters restructuring, as well as those on abnormal chirality fluctuations in sugar solutions at temperatures below 15 °C (Kholmanskiy, 2015b) support the assumption that CF can make a contribution to the seed stratification mechanism.

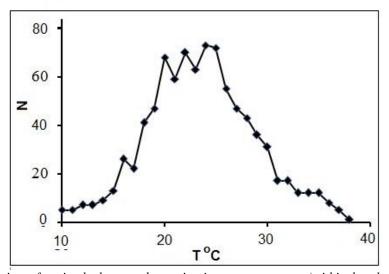


Figure 1. Distribution of optimal plant seed germination temperatures (within brackets, number of plant species is given): grain crops (19), vegetables (47), flowers (20), trees and bushes (21), weeds (47).

Values of K_S and K_G for 10 °C, 14 °C, 21 °C and 25 °C have been obtained from kinetic dependences shown in Figure 2 with the use of equations (2) and (4). By application of Arrhenius approximation to TDs of K_S and K_G values of E_A for seed swelling and sprouting process have been estimated. Calculated E_A values are presented in Tables 1 and 2.

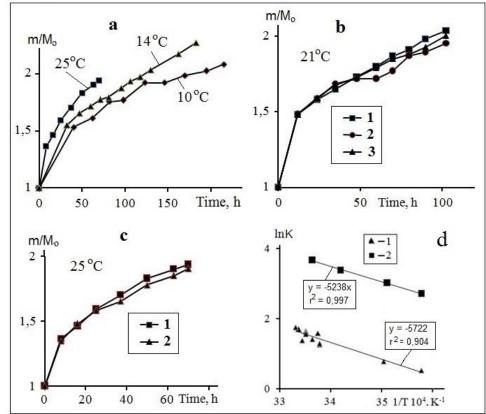


Figure 2. Kinetics curves of swelling and germination for wheat seeds at temperatures 10 °C, 14 °C, 21 °C, 25 °C (a, b, c) and their Arrhenius approximations (d): 1 - swelling, 2 - germination. Figure 2 (b): 1 - reference samples, 2, 3 - samples in magnetic fields ZB₁ and HB₁, respectively. Figure 2 (c): 1 - reference samples, 2 - sample in vortex-type magnetic field WB₂.

The analysis of swelling and germination processes' kinetics has shown the following. Kinetics of seed swelling was insensitive to external magnetic fields which was coherent with (Kholmanskiy, 2015c). Values of E_S for wheat seeds were comparable with those for seeds of oat (30 kJ Mol⁻¹) and beans (55 kJ Mol⁻¹) (Kholmanskiy et al., 2013). It has been reported that E_A of drying process for various fruit and

vegetables depends linearly on the share of hydrophilic substances (HPh share) in them. While the share of water in grains of oat, wheat and beans is practically the same (~14%) the aggregate content of starch, proteins and sugars (HPh share) is 58.3%, 70.6% and 72.5%, respectively (Chemical composition of foodstuff, 1987).

T (°C)	10	15		24		25		26		27	27,5	Es
												(kJ Mol ⁻¹)
K _s 10 ²	1,9	2,5	4,0	4,1	5,5	4,6	5,4	5,7	5,9	6,2	6,5	47,5
$(mg h^{-1})$												
$\Delta t = 0 - t_1 (h)$	40	32	18	20	12	14,5	8	8	9,3	7	7	

Table 2. Parameters of wheat seed	germination	kinetics at different	temperatures in	n magnetic fields.

T (°C)		K 10 ³ (r	$\Delta t = t_1 - t_2 (h)$	EG		
	$K_o(r^2)$	$K_{\rm H}$ (r ²)	$K_Z (r^2)$	$K_W(r^2)$		(kJ Mol ⁻¹)
10	1,7 (0,9278)				40-215	
14	2,3 (0,9961)				47-183	
21	3,3 (0,9943)	2,8 (0,9903)	2,5 (0,9524)		24-102	
25	4,4 (0,9782)			4,2 (0,9912)	25-70	43,5

Figure 3 shows the dependence of E_A for swilling on HPh share, linear behavior of which proves the essentiality of the role that hydration plays not only in drying process dynamics but also in swilling.

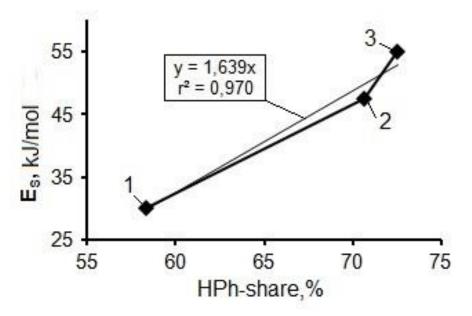


Figure 3. Dependence of effective activation energy (E_s) for seeds of oat (1), wheat (2) and bean (3) on the share of hydrophilic substances (HPh) within them.

The values of E_S and E_G are close to each other which can be explained in the assumption that kinetics of biochemical reactions that drive both the development of corcule and sprouting of wheat roots and coleoptile is, to a substantial extent, limited by the dynamics of water in grains, as well. This is because water is a source of protons that play a principal role in processes of breathing and enzyme activation (Rogozhkina & Rogozhkin, 2013). Corcule is separated from endosperm by scutellum (see Figure 4) that performs secretory and transport functions (Rogojkina & Rogojkin, 2013). The plane of scutellum makes an angle of $\varphi \sim 30^{\circ}$ to 90° with the grain axis. In plasmalemma of epithelial scutellum cells, an H⁺-pump functions that delivers protons to endosperm. Nutriments are transferred through plasmalemma in symport with H⁺ (Polevoy,1989). These facts can be employed to explain the reduction of seed swelling rate, in the initial stage, by ~15% and ~25% in magnetic fields directed along H- and Z-components of GMF, respectively, and by 4% in vortex-type magnetic field.

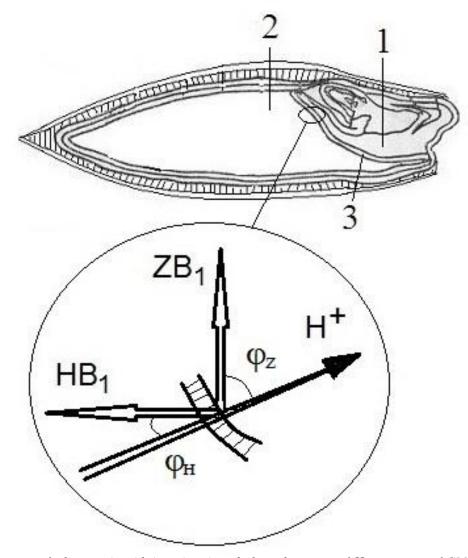


Figure 4. Geometry of wheat grain with its axis oriented along the vector of H-component of GMF and diagram presenting orientations of MF induction vectors and those of proton flux in scutellum plasmalemma. 1 - corcule, 2 - endosperm, 3 - scutellum plasmalemma.

Lorenz force acting on protons in the direction perpendicular to their motion in plasmalemma is proportional to the product of induction, velocity of H⁺ and sine of angle φ between them, as shown in Figure 4. For a random distribution of grains on a plane, only approx. one half of them will have a value of angle of scutellum plane with the grain axis, and therefore that of angle ϕ_{H} , close to 90° while that of angle ϕ_z will be close to 90° for any grain orientation. That is why field ZB₁ will act on all seeds while field HB₁ will produce effect only on, approximately, one half of them. As a result, an averaged inhibitive effect of MF on all seeds will be twice as strong in the case of ZB₁ configuration of MF, and it is what has been observed in experiments. Obviously, effect of WB₂ field will be substantially weaker than that of HB₁, due to the induction vector rotation.

5. Conclusion

Studies of temperature effect on the rate of seed germination have shown that water, as a matrix and donor of protons, limits the rate of both swelling and sprouting of plant seeds. Along with hydration of hydrophilic substances within seeds, correlated states of water spin-isomers in its supramolecular structures built from ice-like and spiral clusters play an important role in the mechanism of metabolism's sensitivity to the impact of external factors (temperature, magnetic fields). Transitions between these two cluster types define quantum-cooperative effects of water and thus the values of optimal stratification temperatures of stratification (~4°C) and sprouting (20 °C to 30 °C) for seeds of the majority of plant species. Weak negative effect of magnetic fields on seed sprouting kinetics was attributed to the action of Lorenz force on protons in the plain perpendicular to their motion in scutellum plasmalemma.

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