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Research Article
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 10-25°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 (43.5 and 47.5 kJ mol^{-1}) 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.

Key words: Wheat seeds, kinetics, germination, magnetic field, water properties

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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 (p) and specific thermal capacity at constant pressure (C_p) of water for 4, 25 and 35°C (Kholmanskiy, 2015a) on the Temperature Dependences (TD) have predetermined the temperature range of vital functions for the majority of organisms. Seeds of various plants get activated in moist environment at 3-5°C (stratification) and have maximal values of germination rate (VG) in the range of 20-30°C. In this case, TD for VG can be approximated by polynomial of 2nd order for many plant species (Balkaya, 2004). It has been found in Kholmanskiy (2014) that, in proximity to points T_{ex} = 4, 25 and 35°C, TDs for p and C_p of water can be approximated by quadratic functions of $\Delta T = (T-T_{ex})$. Obviously, parabolic shape of TD for VG in the range from 4-40°C is an expression of thermodynamics of transitions between ice-like (W_6) and spiral (W_α) water clusters (Kholmanskiy, 2015b) 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 2-25°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 (Kholmanskiy, 2015c). 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 20-40°C. Owing to these transitions, W_α-clusters build long-lived macrostructures with correlated spin states of water molecules (Kholmanskiy, 2015d). 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) (Tverdislov *et al*., 2007; Kholmanskiy, 2010, 2015a). In Kholmanskiy and Strebkov (2007) and Kholmanskiy (2010, 2015b) the physical-chemical mechanism of stratification was attributed to CF effects and the assumption was made that critical phenomena in water at 20-30°C limit the thermodynamics of plant seed sprouting.

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 growing medium and oxygen concentration, temperature and humidity, lighting and various geo-cosmic 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, 2015d; 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 and 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 and 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 and 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; Tien and Wang, 2009). The germination rate for wheat seeds increased by 20% with the magnetic induction (B) growth from 10^{-4}-10^4 Gs while for B = 0.01-0.03 Gs and higher (up to 10^4 Gs) it did not change. In review (Galland and Pazur, 2005), it is recorded that 15-25 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 15-20% 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 10-25°C in constant MF of various configurations in the rage of B~50-10^4 Gs has been investigated in this work to study the mechanism of plant adaptation to external impacts.

MATERIALS AND METHODS

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:

$$V_S = \frac{\Delta m}{\Delta t} = K_S\left(M_o - m\right) \quad (1)$$

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 Eq. 1:

$$m = M_o[1\text{- } \exp(\text{-}K_S t)] \text{ and } \ln\frac{\left(M_o - m\right)}{M_o} = \text{-}K_S t \quad (2)$$

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

$$V_G = \frac{\Delta M}{\Delta t} = K_G M \quad (3)$$

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 Eq. 3 yields the following dependence:

$$M = M_o \exp(K_G t) \quad (4)$$

By applying dependences Eq. 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 MF 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.

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) and 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 0.5-1.0 cm. Seeds in the course of sprouting were illuminated using PL11WG23 luminescent lamps. The MM-3 magnetic mixer was used having rotation rate w~1000 min^{-1}. Rectangular ~10^4 Gs magnets made of neodymium alloy were applied having two layouts: B_1 ($3\times2\times1$ cm^3) and B_2 ($4\times1\times1$ cm^3). Induction axis of B_1 magnet was oriented along edge 1 cm while that of B_2 magnet was oriented along 4 cm edge. It was found with the help of a compass that the value of magnetic induction of B_1 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 B_2 was mounted on the mixer magnet (WB_1), so that its induction axis could rotate in the horizontal plain. Two B_1 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 (ZB_1), while in the other the induction vector was oriented along H component of GMF (HB_1).

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 species, 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 20-30°C (Kurt, 2012; Balkaya, 2004). It has been found in Kholmanskiy (2014) that TDs of p and C_p for water near values of T_{ex} = 4, 25 and 35°C can be approximated by quadratic functions of ΔT = (T-T_{ex}). Based on these data, the assumption can be made that parabolic form of TD for VG in the proximity of 4 and 40°C is determined by thermodynamics of transitions between W_6- and W_a-clusters in water that play the role of limiting factor affecting seed germination metabolism (Yakuschkina and Bakhtenko, 2004; Watt *et al*., 2011). 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 of electromagnetic nature can make a contribution to the seed stratification mechanism (Maronek, 1975).

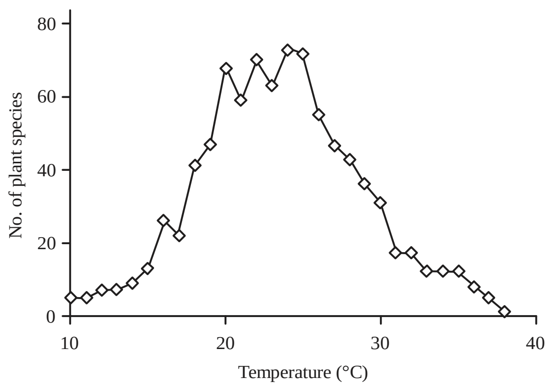

Fig. 1: Distribution of optimal plant seed germination temperatures (within brackets, number of plant species is given): Grain crops (19), vegetables (47), flowers (20), trees and bushes (21) and weeds (47)

Table 1: Parameters of wheat seed swelling and germination kinetics at different temperatures

Temperature (°C)	K_S 10^2 (mg h^{-1})	Δt = 0-t_1 (h)
10	1.9	40
15	2.5	32
24	4.0	18
	4.1	20
	5.5	12
25	4.6	14.5
26	5.4	8
	5.7	8
	5.9	9.3
27	6.2	7
27.5	6.5	7
Es (kJ mol^{-1})	47.5	

Values of K_S and K_G for 10, 14, 21 and 25°C have been obtained from kinetic dependences shown in Fig. 2 with the use of Eq. 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 Table 1 and 2.

The analysis of swelling and germination processe's 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 (Skirukhin and Volgarev, 1987).

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.

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 and Rogozhkin, 2013). Corcule is separated from endosperm by scutellum (Fig. 4) that performs secretory and transport functions (Rogozhkina and Rogozhkin, 2013). The plane of scutellum makes an angle of φ ~30°-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^+ (Lednev, 1991; 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 (Bogatina *et al*., 1979).

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

	K 10^3 (mg h^{-1})					
Temperature (°C)	K_o (r^2)	K_H (r^2)	K_Z (r^2)	K_W (r^2)	$\Delta t = t_1$-t_2 (h)	E_G (kJ mol^{-1})
10	1.7 (0.9278)				40-215	43.5
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	

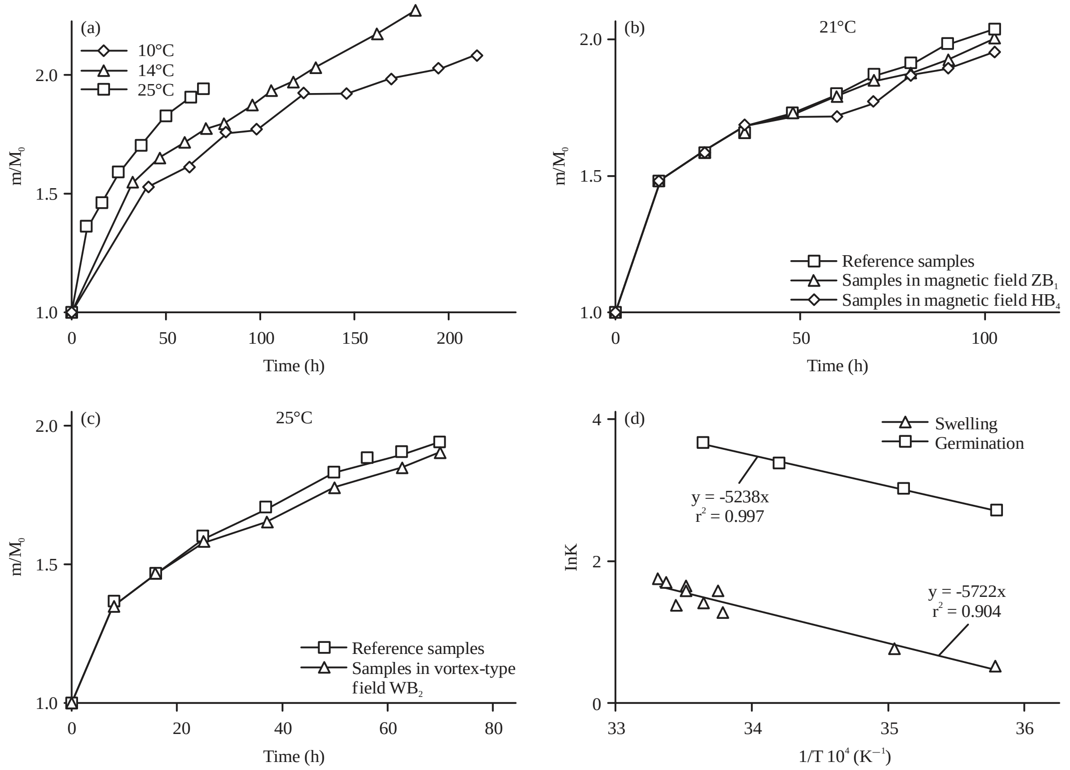

Fig. 2(a-d): Kinetics curves of swelling and germination for wheat seeds at temperatures (a) 10°C, 14°C, (b) 21°C, (c) 25°C and (d) their arrhenius approximation

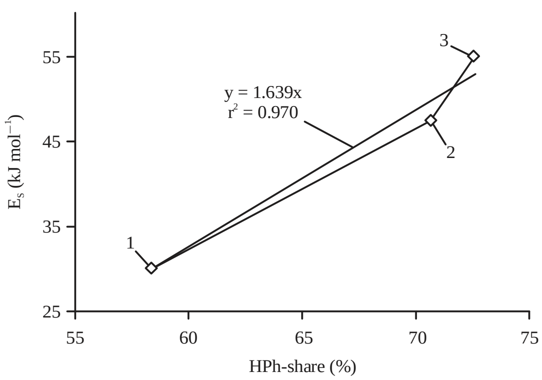

Fig. 3: Dependence of effective activation energy (E_s) for seeds of oat (1), wheat (2) and bean (3) on the share of hydrophilic substances (HPh) within them

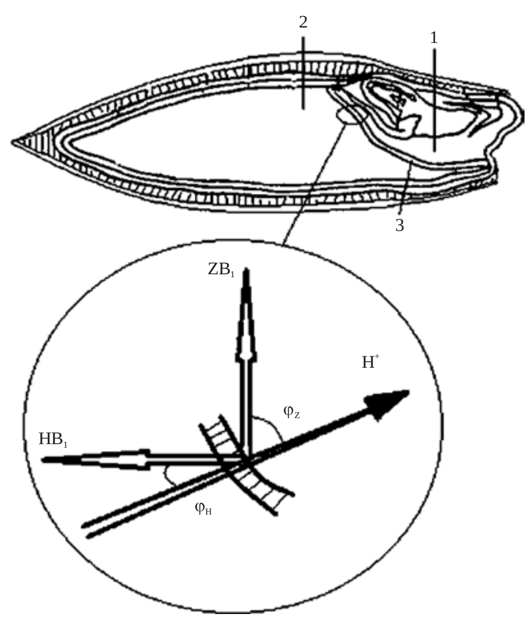

Fig. 4: Geometry of wheat grain with its axis oriented along the vector of H-component of GMF and diagram presenting orientations of MF induction vectors and those of proton flux in scutellum plasmalemma. 1-corcule, 2-endosperm and 3-scutellum plasmalemma

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 Fig. 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 ZB_1 will act on all seeds while field HB_1 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 ZB_1 configuration of MF and it is what has been observed in experiments. Obviously, effect of WB_2 field will be substantially weaker than that of HB_1, due to the induction vector rotation.

We will note that unlike wheat seed energy of germination of a seed of tobacco slightly increases in a magnetic field (Aladjadjiyan and Ylieva, 2003). It indicates dependence of the mechanism of influence of a magnetic field on speed germination of seeds of plants from their structure.

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 (20-30°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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