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To the 80th anniversary of Prof. S. P. Gabuda

FREE WATER IN BEAN SEEDS: THE IMBIBITION PROCESS OBSERVED BY MICRO-MAGNETIC RESONANCE IMAGING

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Water uptake by dry Lima beans (Phaseolus limensis L.) was traced using 1H micro-magnetic resonance imaging.The studies are performed using two-dimensional longitudinal images. Channels through which water comes in an imbibing bean seed are determined; the water distribution inside the seed is shown to be heterogeneous.

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Water being one of their main components of seeds, plays a special role in their life; moreover, together with proteins, fats, and carbohydrates, water is the basis of seed cells and defines the extent and level of their activity. What matters for the functioning of a living organism is the state in which water exists in the organism rather than its total water content. There are several models describing the state of water in biological systems, which have been changing with the accumulation of experimental data through the use of modern physicochemical methods. The classical monographs on plant physiology consider all the water in a plant as a single ordered system bound with the structural elements of the cell [1]. Further research fruited into an idea about the prevalence of liquid, unbound (free) water in plants. According to these models, the percentage of bound water can be 1% to 3% of the total content, and its molecules are arranged as one or more layers around biological molecules [2]. At present, it is believed that water in all biological objects is a complex heterophase structure comprising several modifications with different types of interactions with biological molecules [3-5].

The imbibition of exogenously dormant seeds when they get in contact with water is studied by various methods, i.e., by measuring the changes in seed weight, water potential, humidity, respiration rate, and other properties. These studies have concluded that the flowing of water into seeds is not a smooth, but a stepwise process, which goes through several phases. In the first phase, which the literature refers to as *physical swelling*, there is an exponential increase in the seed weight. When water gets inside the seeds, it fills the space between the tissues and cell components. However, the question of how water gets into the seeds – through the seed coat or through individual areas on its surface – is controversial [6, 7].

In addition to the traditional methods, a substantial contribution to our understanding of seed imbibition comes from the use of nuclear magnetic resonance imaging (NMI). It allows continuous monitoring of the water uptake into a seed and its movement inside the seed, without cutting the latter [8-10].

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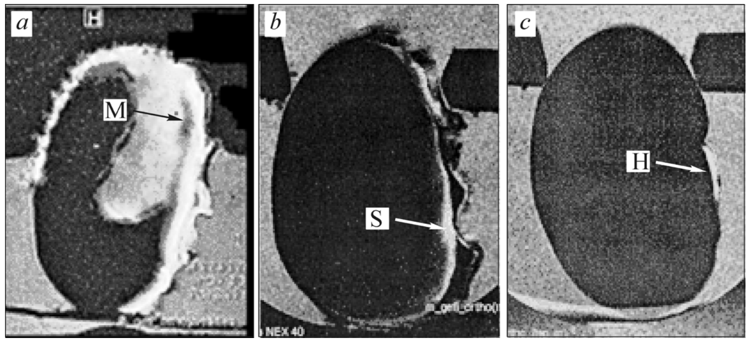

Images of longitudinal sections in imbibing Lima bean seeds: water comes through the micropyle M (hilum and strophiole are sealed); the time of imbibition is 5 h (*a*); water comes through the strophiole S (hilum and micropyle are sealed; the time of imbibition is 6 h (*b*); the micropyle and strophiole are sealed; the hilum is left open; the time of imbibition is 22 h (*c*).

The aim of our work was to investigate the possible channels through which water gets inside imbibing seeds, using Lima bean samples. The imbibition of air-dry bean seeds was studied using changes in the seed weight and by NMI. In the latter case, an NMR spectrometer (Bruker, Germany) with an imaging accessory and a gradient echo technique were used. [11]. A bean seed was placed into a test tube intended for MRI experiments so that the longest dimension of the seed was directed vertically; then distilled water was added in an amount based on the calculated amount of absorbed fluid according to the weight measurements. At regular time intervals during the imbibition, NMR images were recorded for longitudinal sections. There were 25 to 36 such sections in one seed. The NMR images were reconstructed with a 512×512-pixel matrix by using a field of view of 4.0 cm. The repetition time of the pulse sequence was 300 ms to 400 ms; the echo signal time T_E was 4 ms; and the magnetization rotation angle by the exciting RF pulse was 30°. A 40-fold accumulation was used to increase the signal-to-noise ratio.

The images obtained as a result of the experiments have light and dark areas; the light areas correspond to higher local concentrations of mobile water molecules.

Air-dry bean seeds contain 7-11% of water, which is in a bound state; therefore, in the images they look dark against the background of water in the test tube. When water gets into the seeds, they begin to have white ares. Literature sources identify three possible channels of water inflow: the micropyle (a narrow channel in the integuments of the ovule [12]), the hilum (a scar left by the attachment to the funiculus) [8, 10], and the lens beneath the hilum (strophiole) [9, 13]. In our experiments, only one of the above channels was left for water uptake; the other ones were sealed up with glue.

According to our data, the physical swelling phase ends within 10-15 h. The seeds absorb approximately 100% water relative to their original weight. These imbibition data were used in planning the imaging experiment. The results are shown in the figure. Water gets inside through the micropyle, moves along the perimeter, and fills in the air gap between the cotyledons (see the figure, *a*). Then, the water gets into the cotyledons from two sides – from the gap and from the perimeter – until the entire seed is imbibed. The figure, *b* shows the imbibition of a seed with a sealed micropyle and hilum. Water gets inside the seed through the strophiole, moves along the perimeter to the radicle, watering the embryonic axis, and then moves further along the seed perimeter. The figure, *c* shows a seed with a sealed micropyle and strophiole; the hilum is left open. During the observations, water fills the hilum tissue, but does not get inside the seed.

Thus, the channels for the inflow of water into an imbibing bean seed are the micropyle and the strophiole. Note that it is also necessary to conduct research of the seed drying process, which affects the preservation of seeds.

REFERENCES

1. D. A. Sabinin, *Physiological Bases of Plant Nutrition* [in Russian], AN SSSR, Moscow (1955).
2. L. A. Abetsedarskaya, F. G. Mikhtafutdinova, and V. D. Fedotov, *Biofizika*, **13**, 630-636 (1968).

3. S. P. Gabuda, *Bound Water. Facts and Hypotheses* [in Russian], Nauka, Novosibirsk (1982).
4. S. P. Gabuda, A. A. Gaidash, V. A. Drebushchak, and S. G. Kozlova, *Pis'ma Zh. Eksp. Teor. Fiz*., **82**, No. 9, 697-700 (2005).
5. S. P. Gabuda, A. A. Gaidash, V. A. Drebushchak, and S. G. Kozlova, *J. Struct. Chem*., **46**, No. 6, 1131-1133 (2005).
6. K. E. Ovcharov, *Physiology of Formation and Germination of Seeds* [in Russian], Kolos, Moscow (1976).
7. A. S. Ginsburg, V. P. Dubrovskii, E. D. Kazakov, G. S. Okun', and V. A. Rezchikov, *Moisture in Grain* [in Russian], Kolos, Moscow (1969).
8. L. N. Pietrzak, J. Fregeau-Reid, B. Chatson, and B. Blackwell, *Can. J. Plant Sci*., **82**, 513-519 (2002).
9. K. Kikuchi, M. Koizumi, N. Ishida, and H. Kano, *Ann. Bot*., **98**, 545-553 (2006).
10. M. Garnczarska, T. Zalewski, and M. Kempka, *Physiol. Plant*., **130**, 23-32 (2007).
11. M. L. Gyngell, *Magn. Reson. Imaging*, **6**, 415-419 (1988).
12. *Pyl'tsevkhod (Micropyle)*, Encyclopedic Dictionary by Brokgauz and Efron: in 86 vol. [in Russian], St. Petersburg (1890-1907).
13. J. C. Manning and J. Van Staden, *Ann. Bot. (New Series)*, **59**, 705-713 (1987).