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# Trends of the development of methods for determining the viability of crop seeds

Maksim Moskovskiy
*Department of technology and equipment for breeding*
*Federal Research Agroengineering Center*
Moscow, Russia
sky74@yandex.ru

Viktor Khamuev
*Laboratory of post-harvest processing, drying and storage of grain and seeds*
*Research Agroengineering Center*
Moscow, Russia
vic-tor250476@yandex.ru

Kirill Lepeshkin
*Laboratory of post-harvest processing, drying and storage of grain and seeds*
*Federal Research Agroengineering Center*
Moscow, Russia
lepeshkin_kirill@mail.ru

***Abstract*—Scientific research to determine the viability of the system of cleaning and sorting of seeds. An analysis of the current state of determining seed viability has shown that, despite many studies, the main task of promising research projects is the development of a high-precision X-ray laser, a device that uses stimulated radiation to generate or amplify electromagnetic radiation in the near-X-ray or ultraviolet region of the spectrum, that is, as a rule, about several tens of nanometers (nm) wavelength. The main results of the use of a set of technical tools based on Raman spectroscopy laser is the determination of seed viability in a short period of time and obtaining information about the ability of seeds to germinate. Substantiate the need to use laser Raman spectroscopy to identify a complex of biomarkers of crop seeds as signs of their viability, as well as for computer (intelligent) electron-optical cleaning and sorting of seeds. The biological properties of seeds must be considered when developing the process of sowing seed. Among the basic biological properties of seeds include viability. Existing traditional technical means for determining the viability of crop seeds do not fully cope with the task.**

***Keywords - viability, seeds, photoseparator, multi spectroscopy, Raman spectroscopy, selection, hyperspectral visualization.***

## I. Introduction

Under the viability of seeds understand the vitality of their embryos. Vi-ability is defined as the ratio of the number of seeds with live embryos to the total number of seeds in a sample, expressed in percent. Timely receiption of information on the viability of seeds will provide an opportunity to make such conclusion: processing the material as seed or as food. Regular assessment of the viability of freshly harvested seeds would save considerable resources spent on post-harvest seed treatment.

## II. Experimental part

The purpose of research is to substantiate the need to use laser Raman spectroscopy to identify a complex of biomarkers of crop seeds as signs of their viability, as well as for computer (intellectual) electronic optical cleaning and sorting of seeds.

Materials and methods. Existing traditional technical tools for determining the viability of crop seeds do not fully cope with the task.

The biological properties of seeds must be considered when developing the process of sorting seed material. Among the basic biological properties of seeds include viability.

Various theories have been proposed to explain the loss of viability of seeds during storage. Basically, it can be divided into two groups: theories connecting loss of viability with an internal factor resulting from seed me-tabolism, and theories suggest that reasons of these factors are unusual for seeds associated with microorganisms that develop on seeds. There are huge number of articles which attribute the loss of viability to both factors [1]. It is usually difficult to make a distinction between cause and effect.

The viability of cereal seeds is determined by temperature and humidity during storage. Bakeke and Noker [2] written that the viability of raw oats (humidity is 26.4%) decreased to 6% after storage for three days at 40 °C. Symptoms of wheat damage ("sick" wheat) appeared at the humidity 12.2% at 40 ° C after 279 days of storage, but they were not observed at lower temperatures. Tjut and Christensen [3] stored barley with a humidity of 10 to 18% at room temperature for 30 days and found a slight decrease in germination even at high humidity, if there were no mold fungi.

The literature is full of legendary stories about the preserved viability of wheat from ancient Egyptian burial grounds. Ewart (1908) [4] collected a lot of data about seed viability over the past years, but Crocker (1938, 1948) [5] re-viewed the literature about seed viability in recent years. Ewart tried to divide the seeds into 3 groups: 1) short-live, or microbiotic seeds, the time of live does not exceed 3 years; these include seeds with a lot of water, such as maple, elm, willow, acorns, and most nuts; 2) mesobiotic seeds with average life expectancy from a few to 15 years, and 3) macrobiotic seeds are living for more than 15 years. This classification is suitable for natural conditions, but in the laboratory it is violated.

Seed quality is very important for optimizing plant growth and increasing yields on farms. Rapid, uniform germination, subsequent development of seeds, formation of crops are important factors affecting to the yield, because the plant has a limited ability to compensate for the low density of plants. Fac-tors affecting viability before harvesting are particular interest to seed growers, and problems arising after sowing are of great importance to farmers, agrono-mists and gardeners.

Methods for determining the viability are:

- germination at low or sharply variable temperatures;
- germination under normal conditions established for the determination of germination;
- biochemical methods;
- physiological methods.

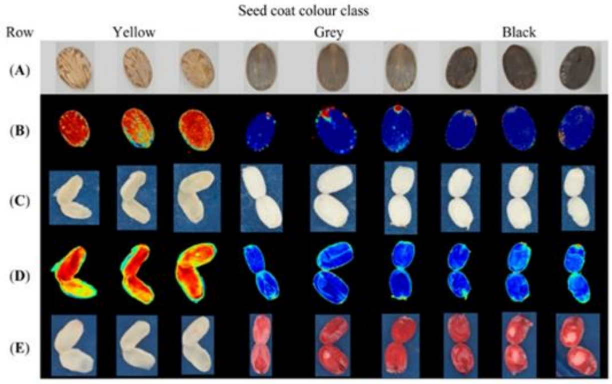
During the germination of seeds in the usual way after a calculation of germination it was calculated a significant number of fresh, swollen, but non germinating seeds, it can be assumed that the conditions do not ensure the normal course of germination and give rise to germs as the germination conditions change. For this, the method of germination is used at low, sharply variable and elevated temperatures. Thus, in such cases, the seeds of wheat, rye, barley, oats, and flax are kept at a temperature of 8–12 ° for the first 3–4 days, and then give a generally accepted temperature of 20 °. Sharply variable temperatures are used for cereal grass seeds: 6 hours at 30–32 ° and 18 hours at 8–10 °.

Another way for determining viability if there are many fresh swollen seeds during evaluating of the germination of seeds, so they are treated differently before being put on germination: dried or heated; prick; remove the shell; scarified or treated with sulfuric acid. However, not everyone can make the right choice of a hydroponic system without special knowledge and skills.

Determining the viability of seeds by physiological methods is related to the ability of their embryos to colouring (the method of D.N. Nelyubov), as well as the plasmolytic method A.V. Doroshenko [6].

The biochemical methods for determining the viability of seeds include the following: method A.A. Gurevich [7] using dinitrobenzene, tetrazole meth-od proposed by Lacon [8], widely distributed throughout the world. When tetrazole. The indicator serves as a colorless solution of tetrazole salt, which is absorbed by the seeds. In seed tissues, it interferes with the flow of reduction processes in living cells and removes hydrogen from dehydrogenases. As a result of the hydrogenation of 2,3,5-triphenyltetrazole-chloride or bromide in living cells, triphenyl formazan is formed — a persistent, red, and diffusible compound. This makes it possible to distinguish the living parts of the seeds, which turn red, from the unpainted dead. In addition to fully stained viable seeds and completely unstained non-viable, seeds that are only partially colored can be found. Different parts of partially stained seeds contain a different percentage of dead tissue. The assignment of such seeds to the category of viable or non-viable is determined, not by the intensity of staining, but by the localization and distribution of necrosis in the embryo and (or) in the endosperm.

The investigating methods for determining the viability are invasive and long-term, and in some cases dangerous for health, which indicates the impossibility of their use as signs of applicable computer (intellectual) sorting of seeds.




The using of less time-consuming, high-speed, non-destructive methods that automate the control process, today may be laser multi- or hyperspectral installations.

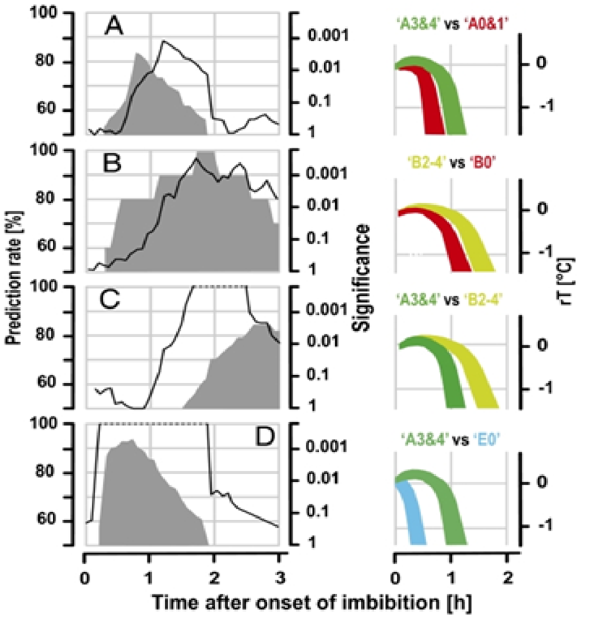
Row (A) shows RGB images of the intact seeds; (B) is images transformed by nCDA to divide dead and viable seeds (intact seeds); (C) is RGB images of cut seeds; (D) is images transformed by nCDA to divide dead and viable seeds (based on cut seeds) and (E) is RGB images taken after the cut seeds has been immersed in tetrazolium.

Fig. 1. Overview of seeds divided into three classes based on visual colour of seed coat: yellow, grey and black.

Studies have been conducted in Iran, the aim of these studies was to emphasize the use of multispectral imaging for testing the quality of castor beans [9]. Visually, 120 seeds were divided into three classes: yellow, gray and black seeds. After that, images were obtained on all seeds (Fig.1) at 19 different wavelengths in the range of 375–970 nm. The average intensity for each individual seed was extracted from the images, and there was a significant difference between the three color classes with the best separation in the near infrared wavelength range. The results indicate that castor bean reflection data may be valuable in predicting seed viability. All seeds, which were visually sorted as yellow, could be defined as dead in the tetrazolium test, and viability variations were observed in gray and black seeds.

In a joint work, scientists from England and Austria investigated the possibility of using infrared thermography to predict whether the calmed pea seed grows or dies upon water absorption [10]. Image analysis of 22,000 images per individual seed showed that infrared thermography can detect biophysical and biochemical changes associated with absorption and germination. These “thermal footprints” differ depending on the viability of this species. As a result, it was shown (Fig.2) that infrared thermography can diagnose the stage of development of germinating pea seeds noninvasively and in real time, associated non-invasively registered thermal pro-absorbing pea seeds with biochemical parameters , which were previously studied using invasive methods, visualized in real time the earliest physicochemical events during the germination of pea seeds and diagnosed the viability of seeds in the first phase long before phenomena of radicules, after which the seeds can be dried out and restored.

Scientists from China have developed a new micro-prototype technique (a highly sensitive and selective method for measuring the concentration and flux of oxygen on the cell surface) [11] for a quick and non-invasive measurement of seed viability by measuring the oxygen supply to

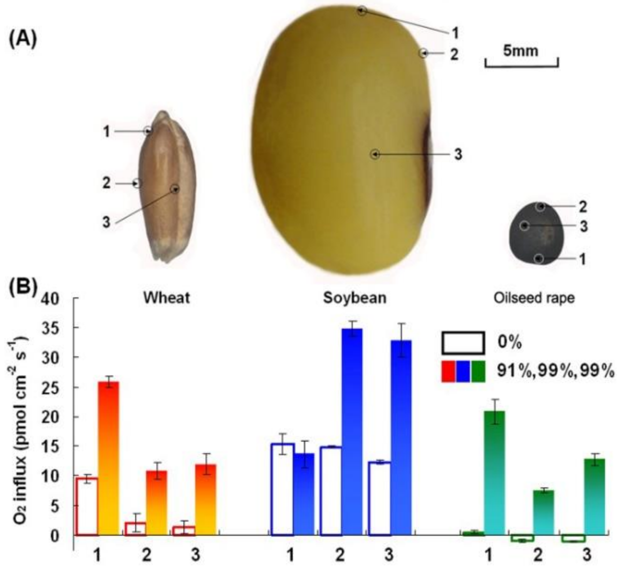



rT, relative temperature; (Left y axes and gray time windows) Rate with which the viability of an unknown seed was successfully predicted. (Middle y axes and black lines) significance levels with which the mean values of two seed types differed over time. The schematics on the far right indicate the seed types tested. (A) Viable and dead seeds can be separated in untreated A seeds with a success rate of up to 85% after 50 min of imbibition. (B) Untreated A and aged B seed lots both produced viable seeds that differed in the third hour of imbibition. (C) Highly viable A seeds can be separated from heat-killed E seeds with a success rate of more than 90% after less than 30 min of imbibition and (D) highly viable A seeds can be separated from heat-killed E seeds, with a success rate of more than 90% after less than 30 min of imbibition.

Fig. 2. Time windows in which pea seed viability can be predicted in the first 3 h of imbibition.

intact seeds in about 10 seconds. kund, for screening a single seed (Fig.3). Soybean, wheat and oilseed rape were used as models for testing this method. After 3 hours absorption, the oxygen flow was recorded in real time with a total measurement of less than 5 minutes. The results showed a significant positive correlation between oxygen influx and viability in all 3 seed types. A linear equation has also been established between oxygen supply and seed viability for each seed type. For measurements, the seeds were in the early stage of absorption without germination.

In the Chinese Agrarian University studies have been conducted. The purpose of this study was to study the possibility of using hyperspectral imaging technology to determine the viability of a single wheat seed. Specific objectives were: (1) to study whether spectrum pre-processing methods can improve the accuracy of classification models; (2) determine the most effective wavelengths associated with seed viability; and (3) investigate the efficiency of the classification of the PLS-DA models (partial least squares discriminant analysis) and SVM (support vector machines) in order to find a satisfactory combination of the spectral data set, pretreatment method, wavelengths and classifier for accurate seed viability. Both sides of individual seeds were subjected to hyperspectral imaging (400–1000 nm) to obtain spectral data on reflectivity.




(A) Three seeds and detect sites. For wheat seed, site1, site2 and site3 represents the center of the embryo, the center of side with embryo of the whole seed, and the center of the ventral groove, respectively. For soybean seeds, the sites were located at the top of the embryonic axis (site1), the centre of the embryonic axis (site2), and the centre of one cotyledon (site3). For oilseed rape, site1, site2 and site3 represents the center of the embryonic axis, the opposite side of the embryonic axis, and the center of one of the cotyledons, respectively. The bar represent is 5 mm. (B) Oxygen influx at different sites of wheat seeds (red) with 91% and 0% viability, soybean seeds (blue) with 99% and 0% viability, and oilseed rape (green) with 99% and 0% viability. Positive value indicates oxygen influx. Bars represent standard errors.

Fig. 3. Detection sites of seeds and oxygen fluxes in wheat, soybean, and oilseed rape.

To build the models, four sets of spectral data were used, including the side of the ventral groove, the reverse side, the average value (average value of two-sided spectra of each seed), and the mixed data sets (double-sided spectra of each seed). The classification models, the least-squares partial discriminant analysis (PLS-DA) and support vector machines (SVM) in combination with some pre-processing methods and a sequential projection algorithm (SPA) were created to identify viable and non-viable seeds. The results showed that the standard normal-grade (SNV) -SPA-PLS-DA model had high classification accuracy for whole seeds (> 85.2%) and viable seeds (> 89.5%). The prediction set was based on a mixed spectral dataset using only 16 wavelength ranges.

A methodology for obtaining hyperspectral images, preprocessing, as well as wavelength and classifier selection was established for the classification of viable and non-viable wheat seeds. Pre-processing of SNV (standard normal variation) [13] and SG (Savitsky-Golay) [14] is more appropriate than pre-processing of MSC (multiplicative correction of dispersion) [15]. Similarly, the

SPA method, which extracted the most effective wavelengths and optimized models, was suitable for determining the characteristics of wheat seeds depending on viability. Both the SVM and PLS-DA algorithms can be used for simulation to predict the viability of wheat seeds. Our results showed that the SNV-SPA-PLS-DA model, based on a mixture data set, has a large classification capability, with an overall accuracy of more than 85.2%, with a viability accuracy and a F-measure of more than 89.5%, in the set forecasts. In addition, after screening for this model, the average final percentage of seed lot germination may exceed 89.5%. Finally, we conclude that hyperspectral spectroscopy has great potential for effective differentiation of the viability of wheat seeds in the VIS / NIR range (400–1000 nm) (Fig. 4).

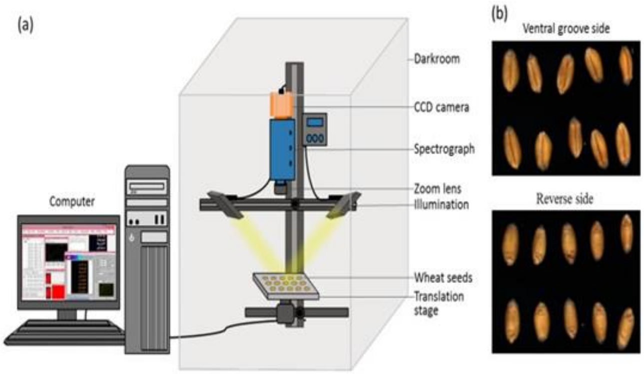



Fig. 4. (a) Schematic diagram of the hyperspectral imaging system. (b) Hyperspectral images of the ventral groove and reverse side of wheat seeds.

## III. Results and discussion

Analysis of the current state of technical equipment of determining the viability showed that the main task of promising research projects is the development of a laser hyperspectral installation analyzing the properties of seeds.

For automated selection of seed viability, it is necessary to have a bank of spectral lines of biomarker complexes (proteins, fats, amino acids, etc.) deter-mining the viability of seeds of different varieties for each crop, as well as technical means for identifying images of each seed and removal method defective seed from the stream. As a result, the creation of computer (intellectual) electron-optical sorters will allow selecting, by the presence in the seed, of a living embryo (for sowing seeds with high sowing and productive properties) from the seeds that lost the embryo, which will be used in as food grains. One of the most promising of these areas is the raster infrared thermography of multi- or hyperspectral visualization of images of Raman scattering (Raman) induced electromagnetic radiation.

## IV. Conclusion

The main result of the use of the complex of technical means of laser hyperspectral visualization of determining the viability of seeds in the selection and seed production of grain crops is the regular assessment of seeds, which makes it possible to give a guaranteed increase in yield of at least 10 centners per hectare during one harvesting season.

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