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EVALUATION OF HETEROGENEITY AND HIDDEN DEFECTS OF WHEAT (*Triticum aestivum* L.) SEEDS BY INSTRUMENTAL PHYSICAL METHODS

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Abstract

For quality control of seed material, there are a number of standard tests adopted by ISTA (International Seed Testing Association, Switzerland) as well as promising instrumental methods evaluating the characteristics of seed surface, structural integrity and integral electrophysical parameters. The aim of the study was to evaluate the efficiency of instrumental physical methods in detection of latent defects of ecologically heterogeneous wheat seeds of various genetic origin. Diversity and latent defectiveness of wheat seeds (*Triticum aestivum* L.) were evaluated using optical imaging, microfocus radiography, and electrophotography. It was found that the optical imaging method combined with automatic analysis of digital scanned images is statistically reliable to distinguish wheat seeds of different varieties and genetic lines by color characteristics of the RGB (red, green, blue) model, e.g., Hue and Saturation. Differences were also found between the seeds of the same variety and genetic line grown under field and regulated conditions. E.g., the Hue values varied from 0.081±0.0005 to 0.090±0.0006 for regulated conditions (the phytopolygon of the Agrophysical Research Institute) and from 0.084±0.0005 to 0.088±0.0005 for field conditions, the Saturation values — from 0.326±0.0005 to 0.419±0.0006 and from 0.371±0.0005 to 0.444±0.0005, respectively. With an increase in the number of cracks in the X-ray projections of wheat grains, their sowing qualities decrease. Microfocus radiography combined with automatic analysis of digital X-ray images successfully detects the damage to wheat seeds by the corn bug, and with the increase of the damage score the sowing quality of seeds in general decreases. Parameters of the digital X-ray images of seeds (Average Intensity, Shape Coefficient, and Entropy) differed between wheat varieties. The Average Intensity varied from 53.30±1.00 to 60.60±1.17, the Form coefficient from 6.67±0.35 to 8.28±0.48, and Entropy from 1.84±0.06 to 1.98±0.03. The research data indicate the effectiveness of the approaches we propose based on instrumental physical methods in the assessment of different quality and latent defectiveness of wheat seeds. Our findings make a background for the functional non-invasive diagnosis of seed quality based on the complex evaluation of external and internal anomalies and defects, significantly affecting both the biological quality of seeds and their economic suitability. This is a methodologically new tool to be used in breeding and controlled seed production.

Keywords: *Triticum aestivum* L., wheat, seed quality, optical imaging, microfocus X-ray imaging, electrophotography, image analysis

A necessary condition for obtaining a high yield of wheat and improving its quality is the use of fully formed and healthy grain for sowing [1-3]. It should be noted that at present in the Russian Federation the share of substandard seeds of grain crops can reach up to 40% [4]. To control the quality of seed material, there are a number of standard tests regulated by the ISTA (International Seed

Testing Association, Switzerland), as well as promising instrumental methods [5-8].

Progress in seed science is closely related to technical innovations and their availability for application. In addition, new methods for assessing seed quality for testing are being developed based on technological advances [9].

Instrumental methods potentially suitable for non-invasive evaluation of seed quality can be divided into three groups. The group of optical methods includes digital optical imaging [10], multispectral imaging [11], chlorophyll fluorescence measurement [12]; introscopic methods include microfocus radiography [13, 14], computed microtomography [15] and magnetic resonance imaging [16]; the third group consists of electrophysical methods (electrophotography) [17]. The method of digital optical imaging based on scanning images of seeds with obtaining various numerical characteristics, including color, is used to determine the varietal purity of rice seeds [18], describe and classify seeds of flax varieties [19]. The method of soft-beam microfocus radiography has been successfully used for many years both in Russia [13, 20] and abroad [21-24] and is included in international [25] and Russian [26] standards. The method of electrophotography [17], which makes it possible to register and quantify the glow that occurs near the surface of an object (seed) placed in a high-intensity electromagnetic field, has a certain prospect, supplementing the generally accepted methods of instrumental evaluation of seed quality.

In addition, with the advent of modern technical and software tools in seed science, computer analysis of seed images is actively used all over the world [27, 28].

Of these methods, only microfocus radiography has been standardized in Russia. Since 2022, it has the status of the current national standard GOST R 59603-2021 (developed by the Agrophysical Institute).

In the presented work, for the first time, using three non-invasive instrumental physical methods (optical imaging, microfocus X-ray diffraction and electrophotography), the characteristics of seeds were obtained and their significant differences were revealed in lines and varieties of wheat (genetic heterogeneity) when grown in field and controlled conditions (ecological heterogeneity) and post-harvest technological part-time job. It has also been shown that damage to wheat seeds by the turtle bug can be successfully detected using microfocus radiography in combination with automatic analysis of digital x-ray images.

The purpose of the study was to evaluate the effectiveness of instrumental physical methods in the study of latent defectiveness in ecologically different quality wheat seeds of different genetic origin.

Materials and methods. Seeds of common wheat (*Triticum aestivum* L.) spring varieties Zlata, Radmira, Yubileinaya 58 and hybrid lines API 1 (Agata × ITMI29) F_5, API 2 (Agata × ITMI47) F_5, API 7 (Liza × ITMI10) F_4, h2788 (Zlata × Moskovskaya) F_{12}, h3021 (Biora × Zlata) F_4 were obtained under strictly controlled growing conditions (agricultural biopolygon AFI, Leningrad Province, 2022) and in the field (FRC Nemchinovka, Moscow Province, 2020) (seeds from the AFI collection were served for sowing). The sample sizes are 100 seeds of each sample. Seeds obtained from crops were analyzed by optical digital scanning.

We also used seeds of winter common wheat cv. Svetoch, damaged to varying degrees by a harmful bug (provided by A.V. Kapustkina, Laboratory of Agricultural Entomology, VIZR, St. Petersburg; the degree of damage was assessed visually on a 4-point scale from 0 to 4 points) [29] and six samples of wheat seeds from industrial crops subjected to mechanical post-harvest processing (provided by N.I. Zhukov, OOO Strong Semen, Krasnodar Territory, harvest of 2020). The sample sizes are 100 seeds. The samples were analyzed by microfocus X-ray diffraction.

In addition, five samples of seeds of spring wheat varieties MiS, Zlata, Lyubava, Esther, Liza were received from the collection of the Federal Research Center Nemchinovka (provided by Prof. B.I. Sandukhadze, Laboratory of Breeding and Primary Seed Production of Winter Wheat). Sample volumes were 50 seeds of each variety. The obtained samples were analyzed by electrophotography.

Scanned images of seeds were obtained using a digital flatbed scanner HP ScanJet 200 (Hewlett-Packard, USA), the format of the saved file is *.tiff. Software processing of digital scanned images of seeds was carried out using the VideoTesT-Morphology program (OOO ArgusSoft, St. Petersburg, Russia). Parameters of digital scanned images, the color ratio according to the RGB model (red, green, blue, Brightness, units of brightness); Hue (relative units); Saturation (relative units) were analyzed. The technique for obtaining and processing scanned images has been described previously [30].

To control the quality of seeds, microfocus shooting of seeds was performed (a hardware-software complex of a mobile X-ray diagnostic unit PRDU-02, developed by the AFI and Ulyanov-Lenin St. Petersburg State Electrotechnical University LETI, manuvactured by ELTECH-Med, St. Petersburg, Russia). The X-ray image magnification factor was 3.0×. Processing of digital X-ray images of wheat seeds was carried out in the VideoTesT-Morphology program. Two analyzed image parameters were the seed projection area (mm^2) and the calculated average brightness normalized to the projection area (relative units). The technique for obtaining and processing digital X-ray images has been described previously [31]. Electrophotographic (gas-discharge) images of wheat seeds were obtained using the GDV-Camera Pro software and hardware complex (developed and manufactured by OOO Biotechprogress, St. Petersburg, Russia). The analyzed parameters were Average intensity (brightness units), Shape coefficient (relative units), Entropy (relative units). The technique for obtaining and processing images has been described previously [32].

The sowing quality of seeds was assessed according to GOST-12038-84 (Moscow, 2011), including seedling length (mm) and growth vigor (proportion of strong seedlings, %) as additional parameters [33].

Statistical processing, including the construction of regression models, was performed using the MS Excel program (Microsoft Corp., USA). The text and tables show the arithmetic mean values of the parameters (*M*) and their confidence intervals at the 95% probability level according to Student's *t*-test ($t_{0.05}$ × SEM).

Results. The results of automatic analysis of digital scanned images of wheat seeds are shown in Figures 1 and 2.

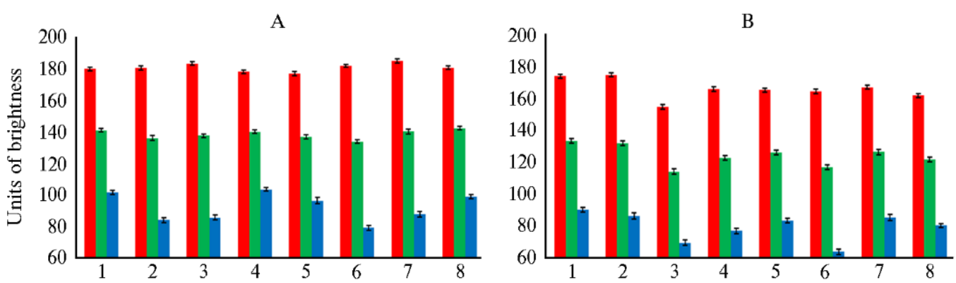


Fig. 1. Color models RGB (red, green, blue) **of seeds in wheat (*Triticum aestivum* L.) varieties grown under regulated conditions (agro-biopolygon AFI, Leningrad Provine, 2022)** (A) **and in field (FRC Nemchinovka, Moscow Province, 2020)** (B): 1 — API 1 (Agata × ITMI29) F_5, 2 — API 2 (Agata × ITMI47) F_5, **3** — API 7 (Liza × ITMI10) F_4, 4 — Zlata, 5 — Radmira, 6 — Yubileinaya 58, 7— h2788 (Zlata × Moskovskaya) F_{12}, 8 — h3021 (Biora × Zlata) F_4 (*n* = 100 eaach). Means and confidence intervals are shown, *M*±($t_{0.05}$ × SEM). HP ScanJet 200 digital flatbed scanner (Hewlett-Packard, USA); software for processing digital scanned images VideoTesT-Morphology (OOO ArgusSoft, St. Petersburg, Russia).

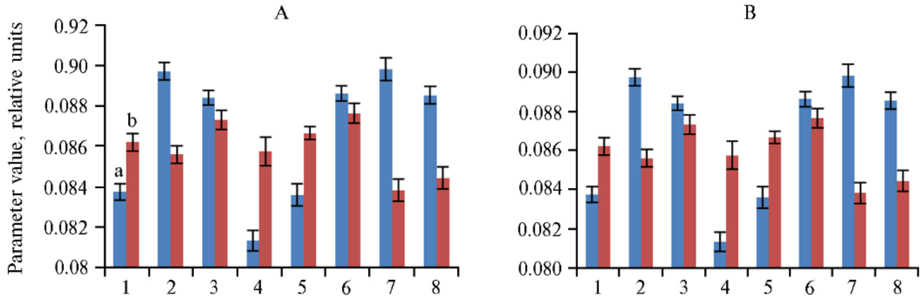


Fig. 2. Hue (A) **and Saturation** (B) **of RGB models of seeds in wheat (*Triticum aestivum* L.) varieties grown under regulated conditions (agro-biopolygon AFI, Leningrad Provine, 2022)** (A) **and in field (FRC Nemchinovka, Moscow Province, 2020)** (B)**:** 1 — API 1 (Agata × ITMI29) F_5, 2 — API 2 (Agata × ITMI47) F_5, **3** — API 7 (Liza × ITMI10) F_4, 4 — Zlata, 5 — Radmira, 6 — Yubileinaya 58, 7— h2788 (Zlata × Moskovskaya) F_{12}, 8 — h3021 (Biora × Zlata) F_4 (n = 100 eaach). Means and confidence intervals are shown, $M \pm (t_{0.05} \times SEM)$. HP ScanJet 200 digital flatbed scanner (Hewlett-Packard, USA); software for processing digital scanned images VideoTesT-Morphology (OOO ArgusSoft, St. Petersburg, Russia).

It was found that the seeds of varieties and varieties of wheat differ among themselves according to the RGB model - in the ratio of colors (Fig. 1, A, B), tone, and color Saturation (Fig. 2, A, B). In addition, we found that the seeds of the same varieties and varieties of wheat, obtained under field and controlled conditions, also differ significantly in the same parameters of the RGB model (color ratio, Hue, and Saturation) (see Fig. 1, A, B , Fig. 2, A, B).

The presented data suggest that the differences in the color characteristics of the surface of the seeds are associated with their varietal characteristics and the genetic origin of the studied material. In addition, it is important to note that growing conditions also affect the color characteristics of the resulting seeds (see Fig. 1, 2).

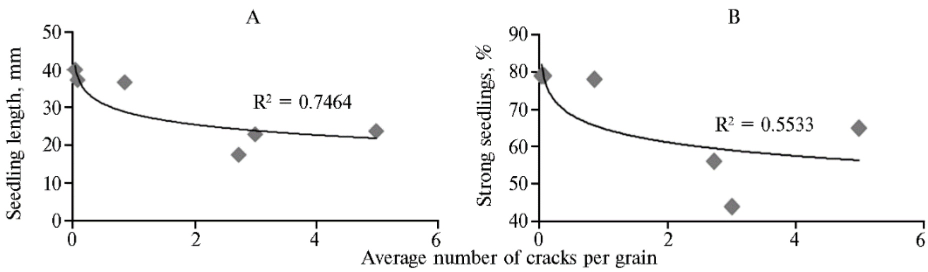
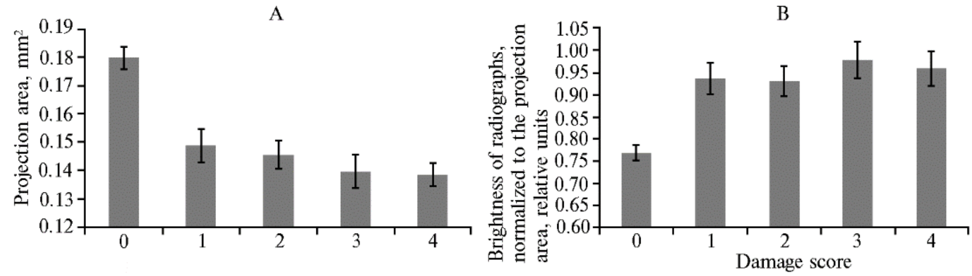


Fig. 3. Sowing quality indicators for six seed samples from industrial wheat (*Triticum aestivum* L.) crops (LLC Strong Seeds, Krasnodar Territory, harvest of 2020, for each sample n = 100**) depending on the number of cracks detected on the X-ray projection of the grain.** Device-software complex based on a mobile X-ray diagnostic unit PRDU-02 (ELTECH-Med, St. Petersburg, Russia), software for processing digital scanned images VideoTesT-Morphology (OOO ArgusSoft, St. Petersburg, Russia).

When visually analyzing digital X-ray images of six seed samples obtained in industrial wheat crops in the Krasnodar Territory (Fig. 3, A, B), we found that an increase in the number of cracks that are detected on the x-ray projection of the grain reduces the sowing quality of seeds. We attribute this to an increase in the probability of penetration of fungal and bacterial infection into the grain with an increase in the number of cracks in the endosperm. In addition, the presence of cracks near the embryo disrupts the supply of nutrients to it from the endosperm. Such a seed may either be unsimilar or have underestimated growth rates.

Evaluation of the characteristics of digital X-ray images of wheat seeds damaged by the pest showed that the projection area of digital X-ray images significantly ($p < 0.05$) decreased compared to the control and depended on the degree of damage, assessed visually in points (Fig. 4, A). The calculated average brightness of grain digital radiographs, normalized to the projection area, increased significantly ($p < 0.05$) compared to the control at any degree of damage by the harmful turtle. The maximum increase in this indicator was noted with a 3-point lesion (see Fig. 4, B). When evaluating the growth parameters, it was found that the length of the sprout on the 7th day decreased when the grain was damaged by a harmful turtle, while the maximum decrease was also noted with a 3-point damage to the grain (see Fig. 4, C). Thus, bug damage by a harmful turtle was detected using automatic analysis of digital X-ray images of caryopses. At the same time, as it was established by our studies, the sowing qualities of seeds decreased with an increase in the damage score.



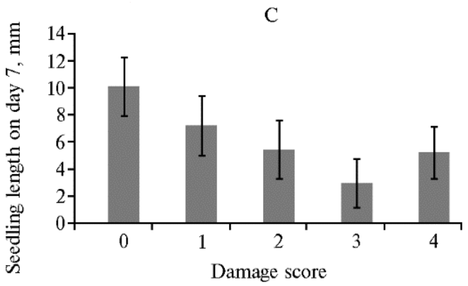


Fig. 4. X-ray (A, B) **and growth** (C) **parameters of seeds** (n = 50) **of winter soft wheat (*Triticum aestivum* L.) cv. Svetoch with varying degrees of damage by bug turtle**. Means and confidence intervals are shown, $M \pm (t_{0.05} \times \text{SEM})$. Device and software complex based on a mobile X-ray diagnostic unit PRDU-02 (ELTECH-Med, St. Petersburg, Russia), a program for processing digital x-ray images VideoTesT-Morphology (OOO ArgusSoft, St. Petersburg, Russia).

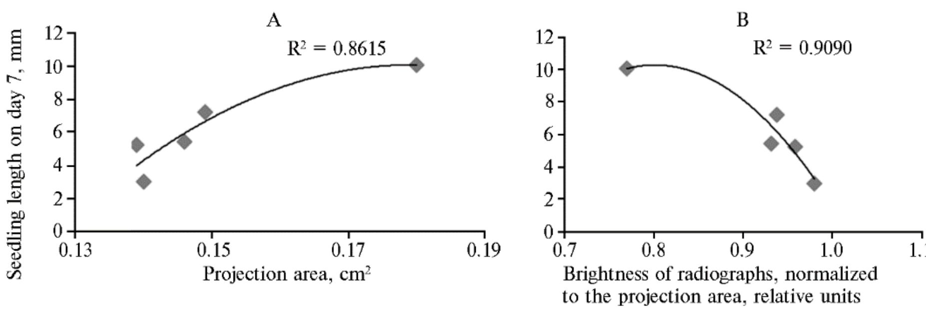


Fig. 5. Quality of seeds (n = 50) **of winter soft wheat (*Triticum aestivum* L.) cv. Svetoch with varying degrees of damage by bug turtle** depending on the **X-ray projection area of the grain** (A) and brightness of the radiographs, normalized to the grain projection area (the points on the graphs correspond to damage scores). Device and software complex based on a mobile X-ray diagnostic unit PRDU-02 (ELTECH-Med, St. Petersburg, Russia), a program for processing digital x-ray images VideoTesT-Morphology (OOO ArgusSoft, St. Petersburg, Russia).

The results of the electrophotographic analysis of digital gas-discharge images of seeds in five wheat varieties of different genetic origin (Table) showed that the Zlata variety was characterized by the minimum average image intensity, the Liza variety has the maximum intensity. The Liza variety has the minimum Shape coefficient value, the MiS variety the maximum Shape coefficient, MiS has the minimum Entropy of digital gas-discharge images, Lyubava the maximum Entropy. These differences, apparently, reflect the unequal genetic origin of the studied accessions, and not the influence of ecological and geographical factors (the seeds were obtained in the crops of the Nemchinovka in the Moscow region in 2015). The reasons for the revealed differences in the characteristics of gas-discharge luminescence in wheat seeds of different varieties require further study.

Electrophotographic Analysis of digital gas discharge images of seeds in different varieties of spring wheat (*Triticum aestivum* L.) (samples from the FRC Nemchinovka collection, *n* = 50 each)

Parameter	MiS	Zlata	Lyubava	Ester	Liza
Average intensity, units of brightness	56,26±0,73	53,30±1,00	55,74±0,97	57,75±0,84	60,60±1,17
Shape coefficient, rel. units	8,28±0,48	8,25±0,38	7,78±0,44	7,12±0,30	6,67±0,35
Entropy, rel. units	1,84±0,06	1,94±0,04	1,87±0,04	1,98±0,03	1,97±0,02

N o t e. Mean values and confidence intervals are presented, $M\pm(t_{0,05} \times \text{SEM})$.

Previously, we revealed a relationship between the electrophotographic characteristics of seeds and their viability [34], as well as plant bioproductivity [35]. With the data obtained [32], the Average intensity of digital gas-discharge images (a measure of brightness) negatively correlates with the length of the sprout and the area of the flag leaf. The Shape coefficient (a measure of the irregularity of the image contour) has a positive correlation with the weight of 1000 seeds (the indicator may reflect grain frailty caused by enzyme depletion).

Figure 6 shows an examples of images of wheat seeds obtained using various instrumental methods.

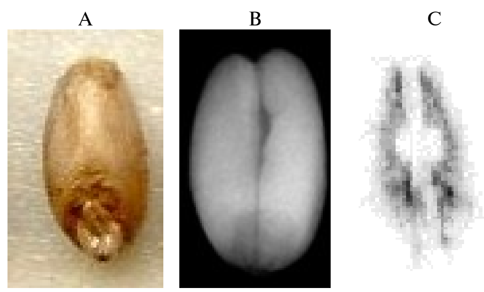


Рис. 6. Examples of digital images of wheat (*Triticum aestivum* L.) seeds obtained with various instrumental methods: A —_digital scanned image (HP ScanJet 200, Hewlett-Packard, USA), B —_digital X-ray image (mobile X-ray diagnostic unit PRDU-02, Russia), B — digital gas-discharge image (hardware-software complex GDV-Camera Pro, Russia).

When analyzing results, obtained using non-invasive techniques and reflect external features and internal structural disorders in caryopses associated with morphophysiological parameters of seedlings, it should be noted that the available publications on the problem are mainly devoted to purely methodological aspects [14, 15]. These papers concern the features of the analysis of caryopsis images depending on the topography of the defect, the degree of its severity, as well as the creation of adequate methods for digital processing of caryopter images for assessment. Therefore, we can compare our findings only with those obtained in our previous studies of the relationship between the X-ray images of the seed and the seed growth potential. In the world literature on seed science and seed production, there is practically no information about the effects of biotic and abiotic factors (plant growing conditions) in breeding and production sowings of grain crops.

The results we submit are pioneering in terms of assessing the influence of ecogenic and technogenic factors on the indicators of the grain structural integrity, both under the conditions of precision experiments and in mass crops. Here, we propose the principles of a new functional non-invasive diagnostics of seed quality. From a practical point of view, the method we propose provides an assessment of the economic suitability of commercial lots of seeds, in contrast to the characteriяфешщт of the biological quality of an individual grain. Currently, the approach has no analogues in the world. It opens up wide opportunities in studying the fundamental aspects of seed control and in developing fundamentally new ways to determine the commercial value of seeds based on a comprehensive non-invasive assessment. In general, this technique can bring seed quality control in

Russia to the highest level in the world. Further fundamental research will be devoted to a detailed analysis of the relationship between physical and growth parameters. In addition, in the near future, we plan to submit the worlds's first data on the relationship of the grain physical characteristics instrumentally measured by a non-invasive method with a plant reproductive function, quantitative and qualitative traits and the seed lot yield and quality.

Thus, the color characteristics of the seed surface, estimated using the digital scanned images, differ statistically significantly between different wheat varieties and genetic lines and in one variety grown under different (field or regulated) environmental conditions. The Hue values of seed images in different wheat varieties varied under controlled growing (phytopolygon of the Agrophysical Institute) from 0.081±0.0005 to 0.090±0.0006, in field from 0.084±0.0005 to 0.088±0.0005, the Saturation values from 0.326±0.0005 to 0.419±0.0006 and from 0.371±0.0005 to 0.444±0.0005, respectively. This may serve both to distinguish between batches of seeds and to determine seed maturity. Hidden defects, e.g., cracked endosperm assessed by the microfocus radiography method with visual interpretation, reduces seed sowing qualities. The severity of the defect affects germination and further growth parameters. It has been confirmed that damage to grain by the corn bug can be effectively detected using automatic analysis of digital X-ray images. The sowing quality of seeds decreases with increasing damage score. Statistically significant ($p < 0.05$) differences in the characteristics of gas-discharge luminescence of seed images were revealed between wheat varieties of different genetic origin. E.g., the Average intensity of luminescence varied from 53.30±1.00 to 60.60±1.17, the Shape coefficient from 6.67±0.35 to 8.28±0.48, Entropy from 1.84 ±0.06 to 1.98±0.03. Our approach to assessing the heterogeneity and latent defectiveness of seeds based on non-invasive physical methods currently has no analogues in the world and allows us to advance from biological characterization of an individual grain to assessment of the economic suitability of seed lots in industrial seed production.

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