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Assessment of Brassicaceae Seeds Quality by X-ray Analysis

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Abstract: A serious problem of vegetable production is the quality of sown seeds. In this regard, assessment of seed quality before sowing and storage is of great practical interest. The modern level of scientific research requires the use of instrumental automated methods of seed quality evaluation, allowing to obtain more information and in a shorter time. The material for the study was a variety of samples from the collection of *Brassica oleracea* L., var. *capitata*, *Raphanus sativus* L., var. *radicula*, and *Lepidium sativum* L. seeds from the Federal Scientific Center of Vegetable Breeding and the Timofeev Selection Station. Digital X-ray images of seeds were obtained using a mobile X-ray diagnostic device PRDU-02. Automatic analysis of digital X-ray images was performed in the software "VideoTesT-Morphology 5.2." The following latent defects of cabbage seeds of economic importance were revealed and identified: irregular darkening, significant "patterning" with deep separation of embryo parts, "angularity of seeds" leading to the loss of their viability. Automatic analysis of digital X-ray images of seeds confirmed the informativeness of brightness indices of digital X-ray images, as well as shape indices. Their connection with sowing qualities of the studied seeds was established.

Keywords: *Brassica oleracea* L.; var. *capitata*; *Raphanus sativus* L.; var. *radicula*; *Lepidium sativum* L.; microfocus X-ray; seed quality; seed image analysis

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1. Introduction

Poor seed quality sometimes delays the development of domestic crop production. Modern technologies that provide direct and precise sowing of seeds in the ground, eliminating the thinning of crops, as well as methods for adjusting plant stands, require uniform seeds with high field germination.

During the period of generative development, plants are particularly demanding to the conditions of light, heat and moisture supply [1]. However, climatic conditions in most regions of our country are unfavorable for seed production, poor technical equipment of seed production and post-harvest treatment has a negative impact.

The situation in seed production of vegetable crops is particularly acute. Large morphobiological diversity, increased heat demand of the majority of species requires a careful approach to the organization of seed production.

In practice, seed growers constantly have to deal with the heterogeneity of seeds. On the one hand, seed variety, or heterogeneity is a biologically useful phenomenon, developed in the process of evolution, which ensures the stability and reliability of the population, necessary for the survival of species [2,3]. At the same time, seed variability is often undesirable for the practice of agricultural production. Unevenness of seedlings, different productivity of plants and heterogeneity of products in terms of quality is largely determined by the poor quality of seeds.

Cabbage vegetable crops, white cabbage in particular, play a leading role in vegetable growing in Russia, both traditionally and in modern conditions. More than 20% of the sown areas under vegetables are occupied by cabbage in Russia [4,5]. Cabbage accounts for one fourth of the vegetable ration of the Russian population. The demand for white cabbage seeds in the country is more than 50 tons annually. Both domestic and foreign companies compete for the market. Seed production is carried out all over the world, where there are optimal natural and economic conditions.

The seeds of cruciferous crops (*Brassicaceae* family) are high in fat oil (33–40%) and protein (25–36%). They are the main nutrients of the seed [1]. At the same time, seeds have a thin, hydrophilic shell, which is easily broken in a humid atmosphere, as well as in case of indelicate drying, which does not contribute to their "durability". Fats become accessible to oxidation, the seed becomes open to fungal infection, its seeding and productive properties are reduced. In this regard, it is of great practical interest to assess the quality of seeds before sowing and putting in storage.

Traditional methods of seed quality control are mostly labor-intensive, time-consuming and lead to destruction of the analyzed sample. Modern scientific research enables the use of automated methods of seed quality evaluation, allowing to obtain more information and in a shorter time.

The method of radiography of seeds favorably differs from others simplicity of use, expeditiousness and non-destructive nature, which allows applying other, morphometric methods for the analysis of seeds quality. The method is standardized: ISO 6639/4-87; GOST 28666.4-90; GOST R 59603-2021 [6–8].

In recent years, in a joint work of the staff of the Federal Scientific Vegetable Center (FSVC), Agrophysical Research Institute (ARI) and St. Petersburg Electrotechnical University (SPbETU), "Methodological guidelines for radiographic analysis of vegetable crops seed quality" were developed based on a large-scale experiment on the seeds of 25 species of vegetable crops belonging to 10 botanical families [9–11].

Large-scale studies on the radiographic analysis of seeds, including vegetable crops, are also conducted abroad. Various radiographic characteristics of seeds of cucumber [12], watermelon [13], tomato [14,15], *Capsicum annuum* L. [16] and broccoli cabbage [17], using automated digital X-ray image analysis techniques [15,16,18].

Research objective: study of latent deficiency of *Brassicaceae* seeds and its relation with sowing qualities.

2. Materials and Methods

For the radiographic analysis, the collection of seeds from Federal Scientific Vegetable Center and Timofeev Breeding Station of N.N. Timofeev of Timiryazev Russian State Agrarian Academy (RSAA) was used (Table 1).

Analysis of the internal structure of seeds was performed according to the "Methodology of radiography in agriculture and crop production" [19] and "Methodological guidelines for radiographic analysis of vegetable seeds quality" [9] at the Department of Electronic Instruments and Devices, SPbETU. Radiographic imaging of seeds was performed on a mobile X-ray diagnostic device PRDU-02 and an X-ray microscope RM-1, produced by JSC "ELTECH-Med", (Russia). Seed sampling: 50 seeds (5 rows of 10 seeds) of each analyzed sample (Figure 1).

Table 1. Seed collection for radiographic analysis.

Sample	Variety	Maintainer	Production Area
Brassica oleracea L., var. *capitata*	Amager 611	FSVC	Derbent, Russia
Brassica oleracea L., var. *capitata*	Belorusskaya 455	FSVC	Moscow Region, Russia
Brassica oleracea L., var. *capitata*	Moskovskaya pozdnyaya 15	FSVC	Moscow Region, Russia
Brassica oleracea L., var. *capitata*	Podarok 2500	FSVC	Derbent, Russia
Brassica oleracea L., var. *capitata*	F1 Malakhit	RSAA	Italy
Brassica oleracea L., var. *capitata*	F1 Transfer	RSAA	Italy
Brassica oleracea L., var. *capitata*	F1 Valentina	RSAA	Italy
Brassica oleracea L., var. *capitata*	F1 Valentina	RSAA	Australia
Raphanus sativus L., var. *radicula*	Ariya	FSVC	Moscow Region, Russia
Raphanus sativus L., var. *radicula*	Pink-red with a white tip	FSVC	Moscow Region, Russia
Raphanus sativus L., var. *radicula*	Niger, population I_3	FSVC	Moscow Region, Russia
Raphanus sativus L., var. *radicula*	Inbred line I_4	FSVC	Moscow Region, Russia
Lepidium sativum L.,	Prestige	FSVC	Moscow Region, Russia
Lepidium sativum L.,	Flagman	FSVC	Moscow Region, Russia
Lepidium sativum L.,	Mechta Derbenta	RSAA	Derbent, Russia

(**a**) (**b**)

Figure 1. Preparation of seeds for radiographic study: (**a**) white cabbage, (**b**) radish.

Shooting mode is set depending on the size of the seeds: voltage 16–20 kV, current strength 98–105 µA, exposure 3–5 s. Microfocus imaging, unlike contact imaging, allows obtaining sharp, contrast X-ray images with high magnification, without loss of quality. The latent image on the plate is digitized in a special scanner "DIGORA PCT" (SOREDEX, Finland), from where the image is transmitted to the computer screen for editing, analyzing and archiving (Figure 2).

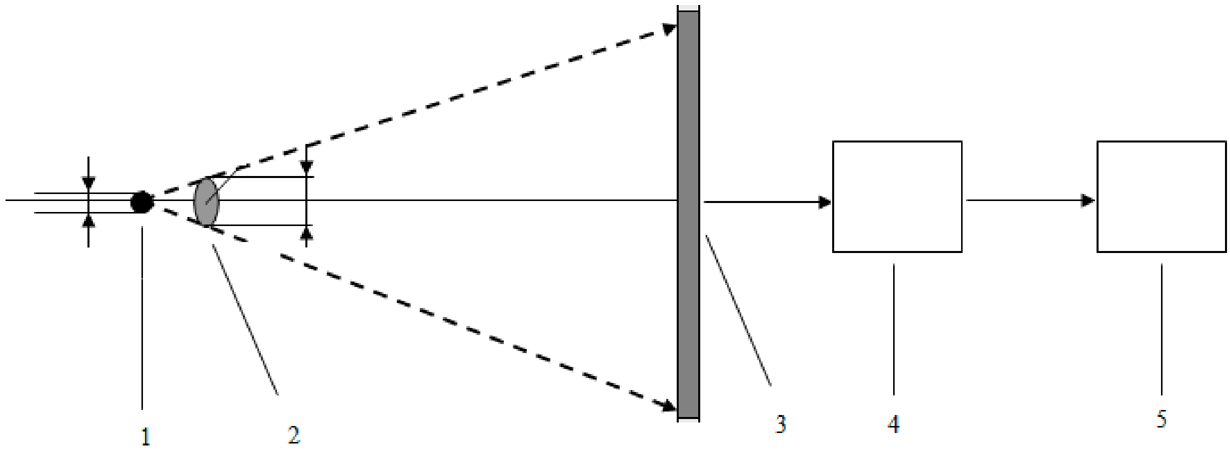


Figure 2. Functional scheme of receiving and processing of X-ray images: 1—radiation source (X-ray tube), 2—object of irradiation (seed), 3—receiver (CCD), 4—signal processing unit (special scanner), 5—personal computer.

Identification and classification of hidden seed defects was performed by visual transcription by an operator radiographer. The following parameters were used in the visual transcription of seed X-rays: normal, empty, germ partitioning of germ parts with soft partitioning, patterned, angulated, with irregular shading.

Additionally, software processing of digital X-ray images of seeds was carried out using morphometry software "VideoTesT-Morphology 5.2. ("Argus-BIO"), produced by "ArgusSoft", Ltd., Saint-Petersburg, Russia. The following parameters were analyzed: roundness, (dimensionless value) (1), circle factor (dimensionless value) (2), average brightness (brightness units) and brightness deviation (brightness units).

$$F_r = \frac{4A}{f^2\pi}, \quad (1)$$

where A—Area, f—maximal Feret diameter

$$F_c = \frac{4\pi A}{P^2}, \quad (2)$$

where A—Area, P—perimeter

Then, individual germination of seeds was assessed using filter paper and Petri dishes under controlled temperature according to GOST 12038-84 [4]. Seeds were germinated on moistened filter paper at 25 °C in the light. The emergence rate of white cabbage seeds was determined after three days, seed germination—8 days; the emergence rate of garden cress seeds was determined after 3 days, seed germination—5 days. We took pictures of seeds, seedlings and plants with a professional camera CANON-5D with a macro lens CANON-100 with a resolution of 12–24 megapixels in the FSVC photo laboratory, combined with digital morphometry of seedlings (root and sprout length), carried out with the use of morphometry software "VideoTesT-Morphology 5.2. ("Argus-BIO").

Statistical analysis (calculation of Spearman correlation coefficients) was performed in Statistica 10, TIBCO, Palo Alto, CA, USA.

3. Results

3.1. Inspection of Brassica oleracea L., var. Capitata Seeds

The results of radiographic analysis of seeds of eight samples of white cabbage of different origin and production sites, revealed a great heterogeneity of their internal structure, (Figure 3). Even a general glance at the radiograph is able to determine the heterogeneity of seeds. The details of the internal structure of the seeds are differently arranged and give out different drawings in the image.

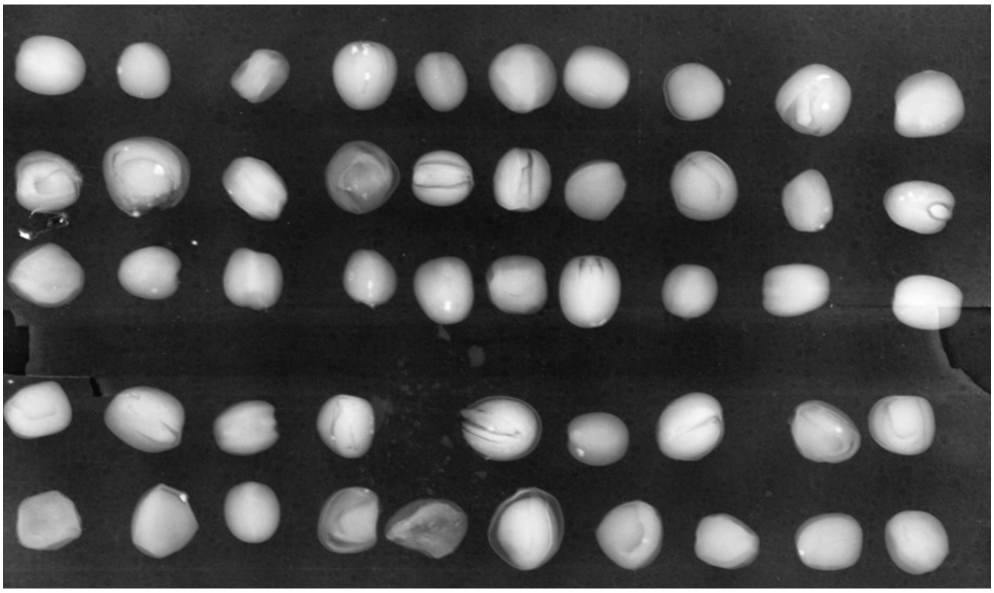


Figure 3. Radiographic image of *Brassica oleracea* L., var. *capitata* seeds.

Analysis of Table 2 allows us to indicate a general tendency of connection between X-ray features and seed viability. It consists in the following: as a rule, seeds, which look uniformly light or with insignificant detailing on the images, sprout well. Basically, the number of normal seeds from the point of view of radiographic analysis coincides with the number of germinated seeds. Seeds from the group "germ partitioning of germ parts with soft partitioning" germinated (Table 2).

Table 2. Comparison of the results of radiographic analysis and laboratory germination of *Brassica oleracea* L., var. *capitata* seeds.

Parameters	**Varieties**			
	Amager	**Belorusskaya**	**Moskovskaya Pozdnyaya**	**Podarok**
	Trait according to the results of radiographic analysis, %			
Normal	45	41	42	56
Empty	0	0	14	0
Germ partitioning of germ parts with soft partitioning	11	8	8	9
Patterned	17	16	5	22
Angulated	13	23	22	10
With irregular shading	14	12	9	3
	Germination, %			
Germinated	54	52	48	62
Sprouting	16	14	11	18
Not germinated	30	34	39	20

X-ray seed quality indices were established visual analysis of numerous of different varieties of cabbage seeds and their comparison with the results of laboratory germination.

We present a selection of radiographic images of seeds and photos of sprouts, where the X-ray image of a particular seed and its behavior under conditions favorable for germination are compared.

The obvious separation of the embryo parts and irregular shadows on the projection of seed #1 were reflected in its delayed germination and in the abnormality of the seedling (Figure 4).

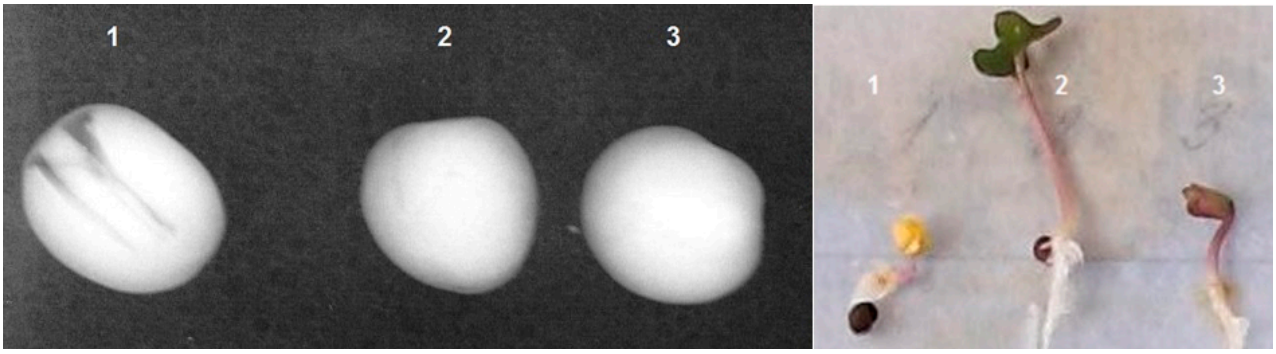


Figure 4. Radiography of seeds and photograph of white cabbage sprouts with "separation of embryo parts" clearly visible in seed number 1.

A softer separation of the embryo parts of seed #8 without additional shadows does not lead to a deterioration in the quality of the seedling (Figure 5).

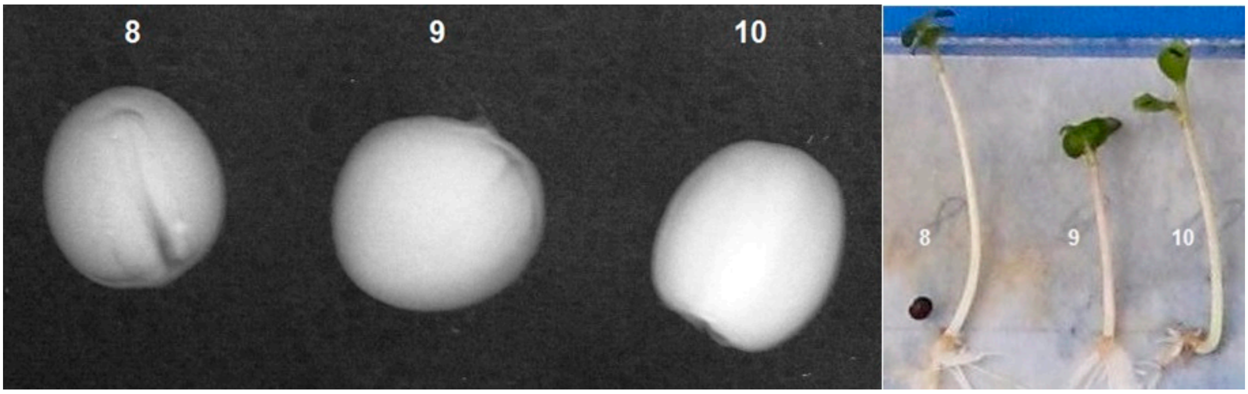


Figure 5. Radiography of seeds and photograph of white cabbage seedlings "soft separation of embryo elements".

The pronounced "patterning" with deep separation of seed embryo parts and reduced values of the index "average brightness" # 38, 40 indicate their non-viability (Figure 6, Table 3).

Table 3. Analysis of brightness parameters of digital X-ray images of *Brassica oleracea* L., var. *capitata* seeds with the trait "patterning of internal structure".

Parameter	Seed ID		
	38	**39**	**40**
Average Brightness, brightness units	162	184	162
Brightness Deviation, brightness units	39	42	45

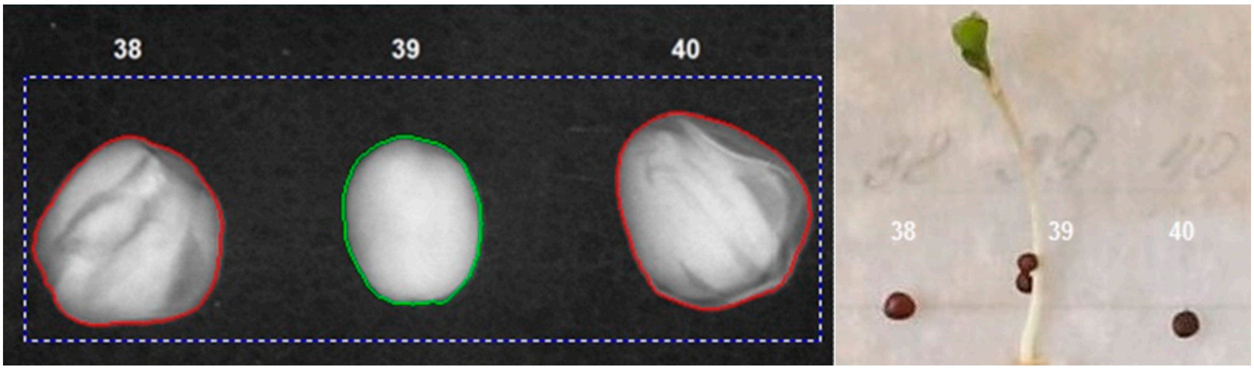


Figure 6. Radiography of seeds and photograph of white cabbage sprouts with "patterning of internal structure" (seed number 38 and 40 only).

Irregular shading on the projection of seed # 46, confirmed by low values of the indices "average brightness" and "standard deviation of brightness" are also evidence of its non-viability (Figure 7, Table 4).

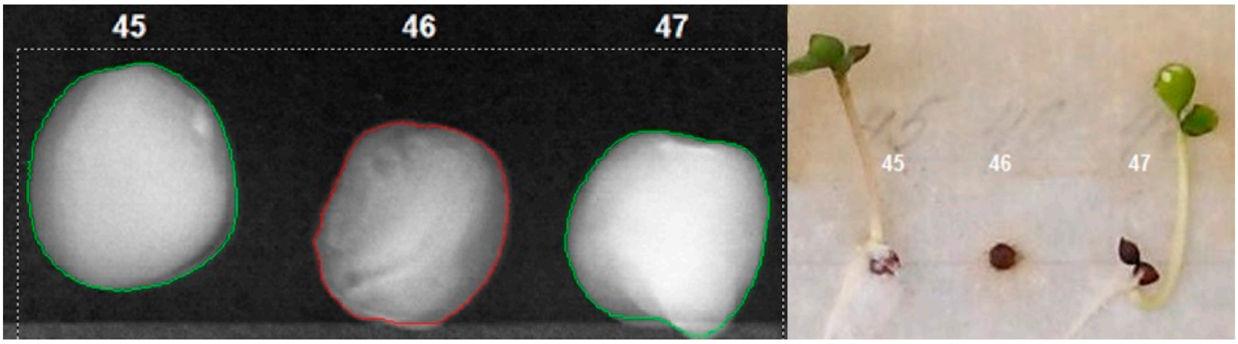


Figure 7. Radiography of seeds and photograph of *Brassica oleracea* L., var. *capitata* seedlings with "irregular shading" trait.

Table 4. Analysis of brightness parameters of digital X-ray images of *Brassica oleracea* L., var. *capitata* seeds with the trait "irregular shading".

Parameter	Seed ID		
	45	**46**	**47**
Average Brightness, brightness units	165	142	193
Brightness Deviation, brightness units	42	35	47

Angular shape of a seed in most cases indicates its non-viability (Figure 8). As a result of automatic analysis of digital X-ray images of white cabbage seeds, it was found that the trait "angularity" of the seed can be described by such indicators as roundness and circle factor.

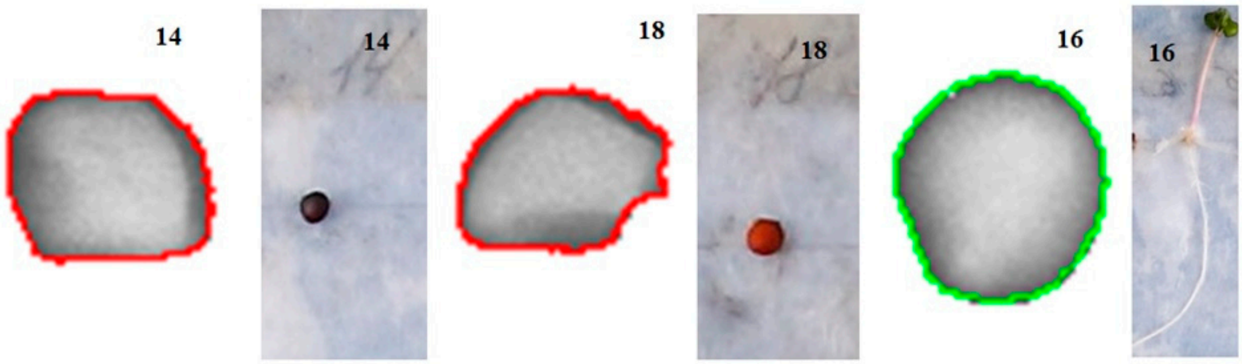


Figure 8. Radiography of seeds and photograph of *Brassica oleracea* L., var. *capitata* seedlings with angular seed shape.

In seeds with the trait "angularity" the values of these indicators are significantly lower than in normally formed seeds (Table 5). Seeding qualities of angular seeds in most cases are impaired (Figure 8).

Table 5. Analysis of the shape of digital X-ray images of angular *Brassica oleracea* L., var. *capitata* seeds.

Parameter	Seed ID		
	14	18	16
Roundness, nondimensional value	0.734	0.647	0.890
Circle factor, nondimensional value	0.927	0.907	0.991

Surface mycota of seeds are clearly distinguished on radiographs due to their dense consistency (Figure 9). Although they do not particularly affect seed germination, their negative effect may appear in the further development of the plant.

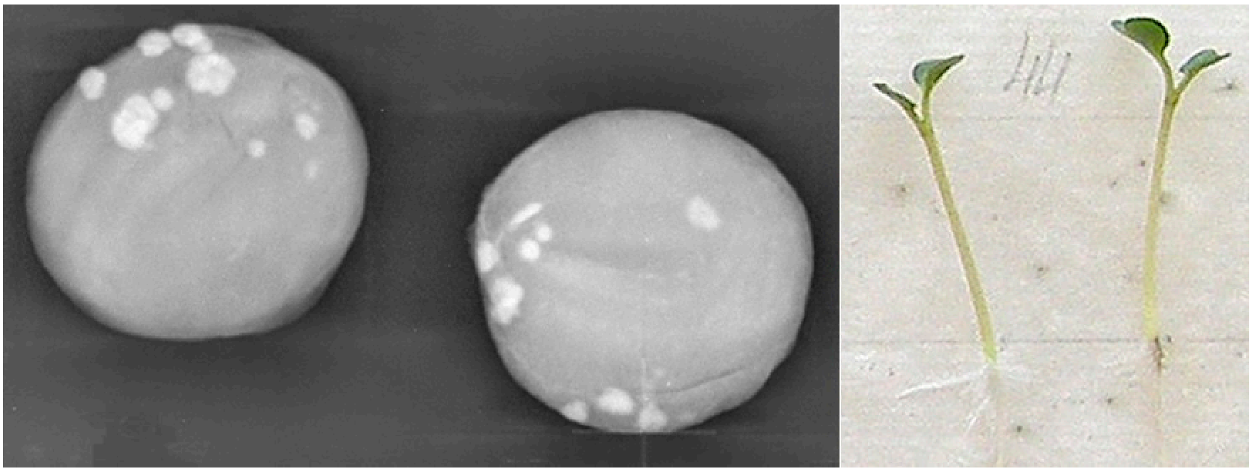


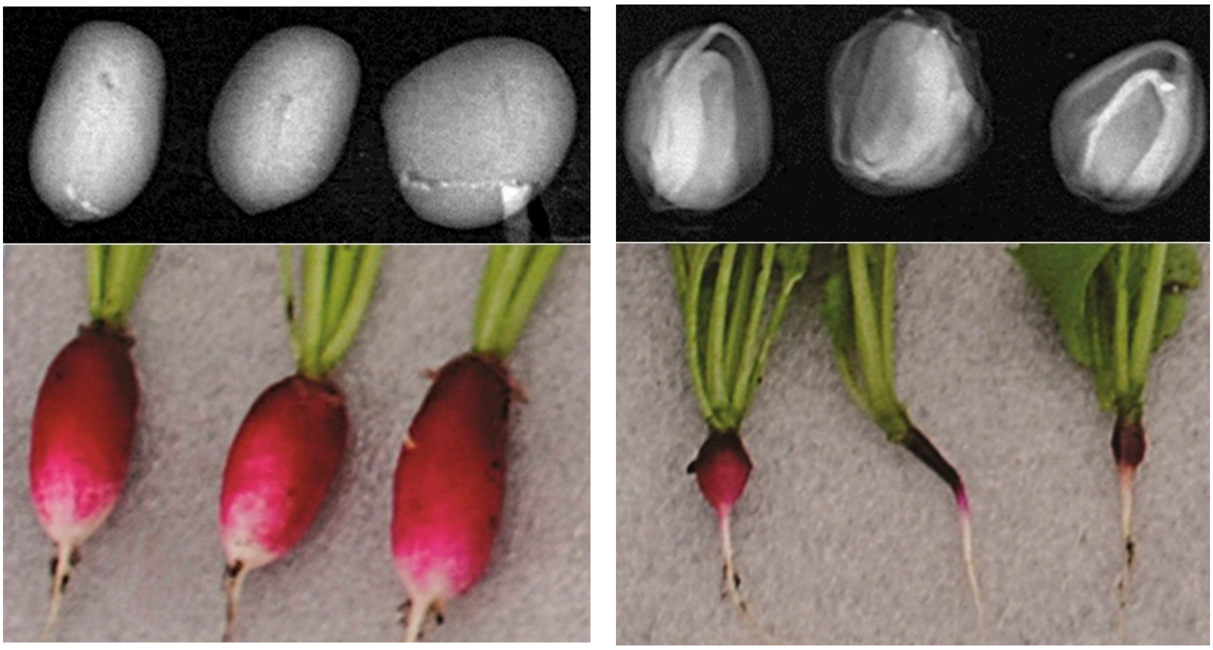
Figure 9. Radiography of infected seeds and photograph of *Brassica oleracea* L., var. *capitata* seedlings.

Thus, the main X-ray traits of cabbage seeds, which can be used to judge about their seeding qualities, were revealed. They are as follows: clear separation of embryo details indicates reduced seed viability (Figure 4). Irregular darkening (Figure 7) in the images also indicates poor seed quality. Expressed "patterning" with deep separation of seed embryo parts (Figure 6) as an indicator of loss or reduction of nutritive tissue, respectively, leads to seed death. The images often show angular seeds (Figure 8). They look as such only on the X-ray images, mainly due to some desiccation of the cotyledons, while outwards they have almost regular round shape. In our experiment, about 75% of such seeds do not germinate. The patterns revealed are probabilistic. Further research is required to find out the reasons for this relationship.

The high information value of the method of seed radiography provides an opportunity for multidimensional analysis. Radiography of seeds as a "non-destructive" method is especially convenient in breeding practice, when working with small lots of breeding or collection material.

3.2. Inspection of *Raphanus sativus* L., var. *Radicula* Seeds

Populations and homogeneous radish seeds were analyzed and fully preserved for further work. As a rule, seeds of inbred lines are obtained piecemeal and it is not allowed to waste them for analysis. In such cases, the method of radiography is indispensable, as it provides complete preservation of the analyzed seed sample. Seeds of variety Aria (I_0) in the X-ray image (Figure 10a) have low, but sufficiently uniform optical density of projection and during germination gave complete root crops. On the contrary, the internal structure of seeds of inbred generation I_3 looks more "patterned," with pronounced darkening revealing voids and loss of embryo body density (Figure 10b). When germinated, such seeds were unable to form complete root crops, an inbred depression expressed by reproductive dysfunction.



(**a**) (**b**)

Figure 10. Reproductive dysfunction of *Raphanus sativus* L., var. *radicula* plants due to defects in internal seed structure: (**a**) population seeds (I_0) and their complete crop; (**b**) seeds of inbred line (I_3) and unformed product swelling.

3.3. Inspection of *Lepidium sativum* L., Seeds

The manifestation of defects, anomalies, and flaws on the X-ray projection of seeds can be very diverse. Some of them are even species-specific. Here is an example of one of them. Seeds of cress varieties Prestige, Flagman of FSVC selection and "Mechta Derbenta" obtained at the Breeding Station named after N.N. Timofeev were taken for radiographic analysis. Seeds of Prestige and Flagman varieties were multiplied in the FSVC experimental field (Moscow Region), and "Mechta Derbenta" in Derbent district of the Republic of Dagestan.

Visual analysis of X-ray radiographs of cress seeds showed the following. Seeds of Prestige and Flagman varieties propagated in conditions of temperate zone (Moscow region) look mostly light on X-ray projections, indicating their completeness and fullness (Figure 11). Regular longitudinal shadows are associated with the anatomical structure of the seed. In laboratory germination, they showed a high degree of germination.

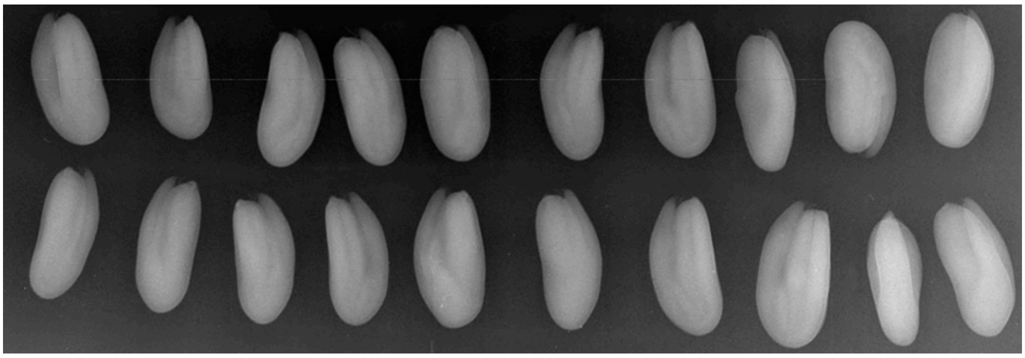


Figure 11. Fragment of X-ray image of *Lepidium sativum* L. seeds of Prestige variety.

A scanned sample of cress seeds is shown in Figure 12. Analysis of X-ray images of seeds of variety "Mechta Derbenta" revealed unusual "patterning" of images not revealed on seeds of other crops. In the X-ray image presented, 10 of 50 analyzed seeds were found (Figure 13). As a result of laboratory germination, 12 seeds did not germinate (Figure 14). All seeds with "patterned projections" (# 9, 14, 16, 18, 19, 22, 27, 42, 43, 45) failed to germinate. Two seeds (# 11 and 34) with normal dense projection but relatively small size did not germinate yet. It is noteworthy that such significant internal injuries are not reflected in the external appearance of the seeds (Figure 12), hence, have a hidden nature.

Figure 12. Photo of *Lepidium sativum* L. seeds of Mechta Derbenta variety.

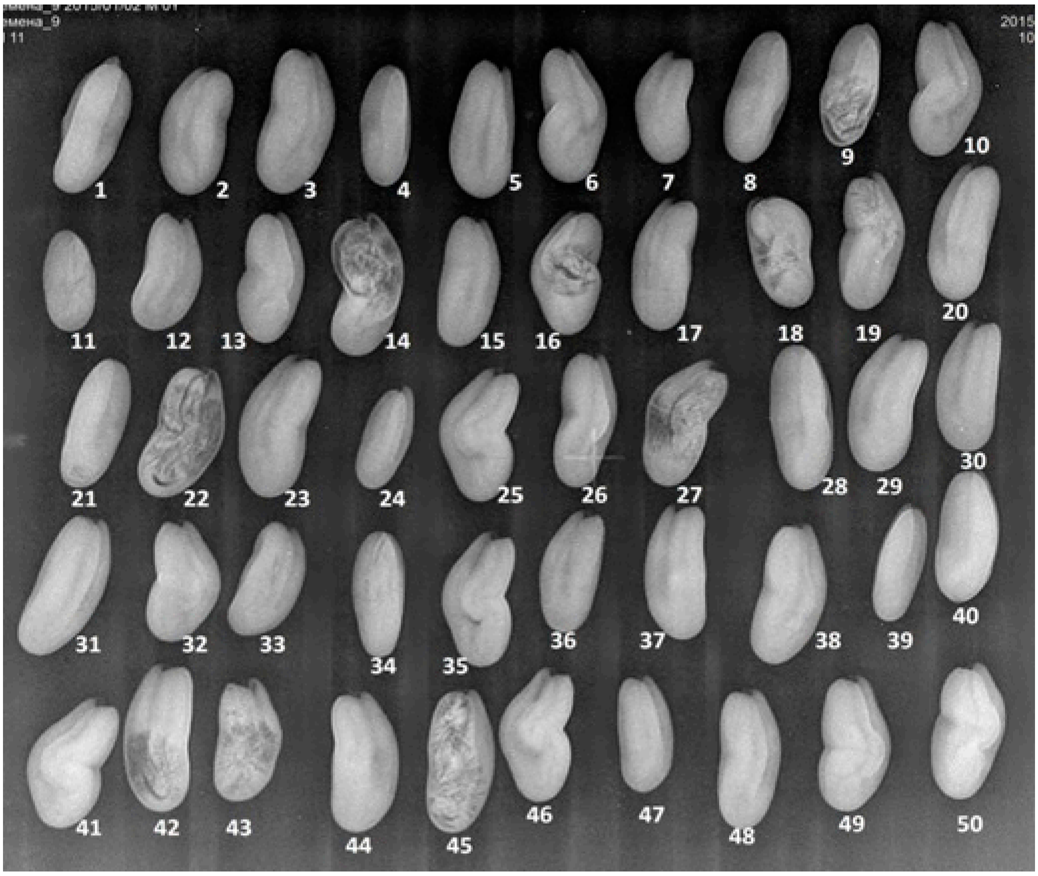


Figure 13. X-ray image of *Lepidium sativum* L. seeds of Mechta Derbenta variety.

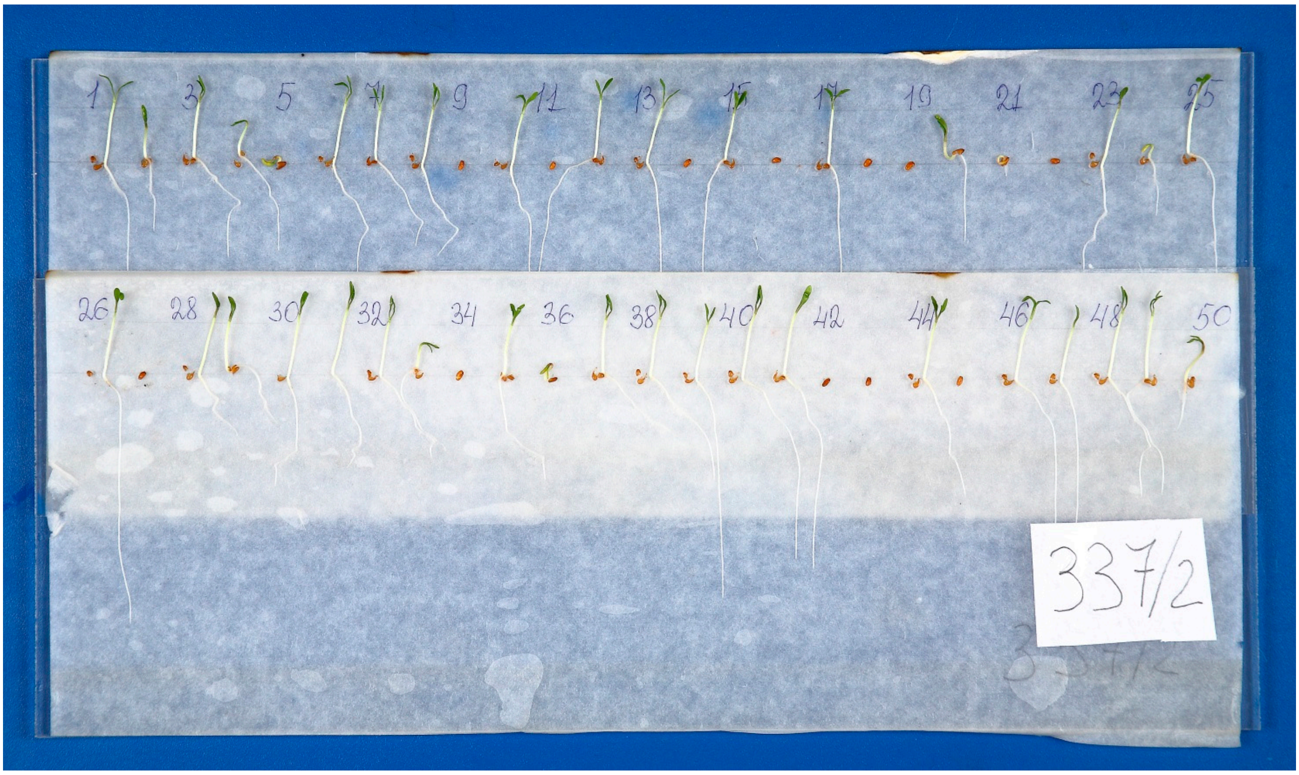


Figure 14. Photo of *Lepidium sativum* L. seedlings of Mechta Derbenta variety.

The results of automatic analysis of digital X-ray images of garden cress seeds revealed that the indicator "average brightness" is quite informative with respect to the trait "patterning of internal structure" of seeds. With the increase of "patterning" the average brightness of digital X-ray images decreases (Tables 6 and S1), and the sowing quality of seeds (Figure 15) deteriorates. The green border shows the automatic classification of the seeds as the best in brightness of the digital X-ray images, the yellow is the intermediate class.

Table 6. Results of automatic analysis of digital X-ray images of *Lepidium sativum* L. seeds Mechta Derbenta variety.

Parameters of X-ray Pattern of Seed	**Seed ID**									
	41	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**
										
Average Brightness, Brightness Units	184	182	175	185	168	184	184	188	187	184
Brightness Standard Deviation, Brightness Units	25	33	23	26	27	28	28	26	26	30

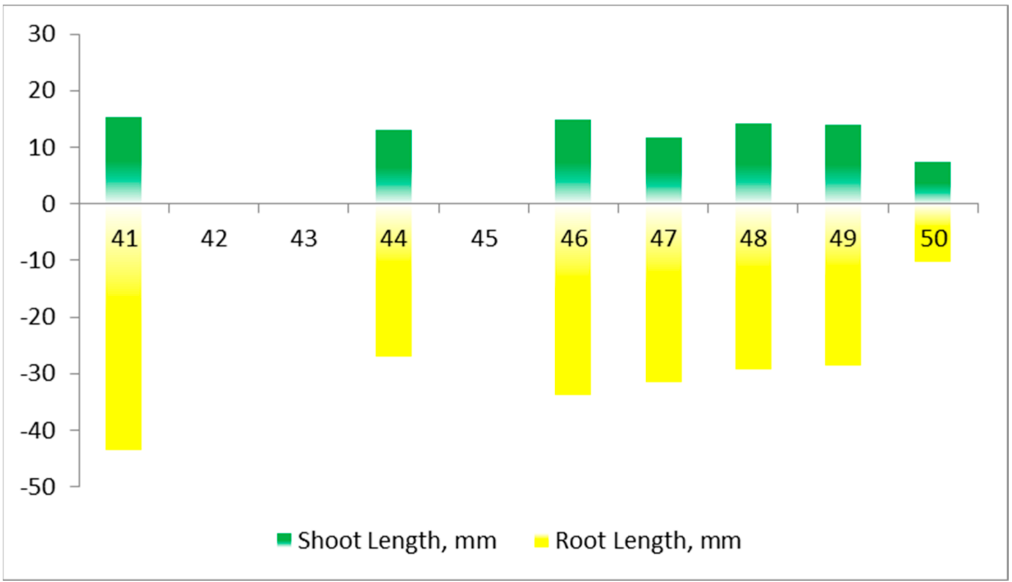


Figure 15. Results of digital morphometry of *Lepidium sativum* L. seedlings Mechta Derbenta variety, preliminary analyzed by X-ray technique.

Spearman correlation coefficients ($p < 0.05$) were, respectively: between average brightness of digital X-ray images and shoot length r = 0.34; standard deviation of brightness and shoot length r = −0.38; average brightness of digital X-ray images and root length r = 0.38; standard deviation of brightness and root length r = −0.40.

So, according to the results of analysis of a large number of X-ray images, X-ray trait directly related to the viability of seeds was established. Regular "patterning" of the internal structure of garden cress seeds on the X-ray projection is evidence of their non-viability. By means of X-ray quality analysis, it is possible to quickly and without loss of the seeds themselves to give a conclusion about the viability of a batch of damaged seeds. According to Table 7, the results of radiographic analysis of seeds almost coincide with the results of their laboratory germination: 74 and 77% of germination, respectively. In this case, the informative and fast performance of the radiographic method is obvious.

Table 7. Comparison of methods for analyzing the quality of *Lepidium sativum* L. seeds.

Replications	X-ray Analysis, %			Laboratory Germination, %
	Normal	Insects Damaged	Irregular Shadings	
1	76	14	10	80
2	72	20	8	74
3	64	36	0	72
4	85	12	3	82
Mean	74.0	20.5	5.0	77.0

It is noteworthy that this X-ray trait has so far only been detected on cress seeds and only on the lot of southern reproduction. This is the result of the work of insect pests. The rapeseed moth (*Meligethes aeneus* (Fabricius, 1775) pests on the seeds of cultivated cruciferous plants [20,21]. During bud formation, they invade plants and feed on the inner parts of buds and flowers, eating stamens, pistils, petals and pollen. As a result, incomplete seeds develop and seed production decreases.

4. Discussion

The possibilities of radiographic analysis of seeds are not limited to the above examples. With the help of the method, it is possible to determine the degree of seed fullness, injuries, the presence of internal (hidden) germination and other defects and abnormalities of the internal structure.

The experimental data obtained demonstrate the effectiveness of the method of microfocus X-ray radiography for the analysis of the germination of cabbage seeds related to the peculiarities of their internal structure. The radiographic analysis of the internal structure of wheat and barley grain [22], seeds of ornamental apple tree forms [23], tomato [15], pepper [16], eggplant [24] tomato and melon [18] showed high information value of the method and speed of its execution.

Analysis of images of seed material, in particular, evaluation of various shape indices for classification of seed species diversity, is widely enough applied in seed science [25–27]. Regarding the application of the X-ray method, it should be noted that the works of other researchers are mainly devoted to the visualization and identification of individual, but quite common types of hidden defects, for example—incomplete seed [28], hidden insect infestation [29] and embryo defects [14].

At the same time, cabbage seeds have specific X-ray traits, such as, "angularity of seeds", indicating the incompleteness of hoarding tissue. Just as specific is the trait of "regular patterning" of the internal structure of cress seeds, a consequence of damage by insect pests, directly related to their viability. It should be noted that earlier the study of the features of the internal structure of cabbage seeds was of a trial nature [30]. Thus, in the work of Gusakova (1997) [31], Derunov (2004) [30] the sign of "unfulfilled" cabbage seeds, determined by the radiographic method, indicating the non-viability of seeds, was shown. From the few modern works we can cite the example of the study of Abud et al. (2018) [17], which shows the influence of features and defects of the internal structure of broccoli seeds on their vigor. We tried to give the work a more systematic character and analyzed the defectiveness of cabbage seeds not only visually, but also using the means of computer morphometry. Defects and deficiencies in the internal structure of cabbage seeds were identified and classified by us, the degree of their impact on seed viability was shown [10]. This approach allows us to objectify the express-analysis of cabbage seeds quality when they are fully preserved, to extract information about defects and disadvantages of internal structure, which significantly complements the traditional methods used in seed control.

5. Conclusions

The method of seed radiography makes it possible to identify and record defects and abnormalities of the internal structure of the seeds of vegetable cruciferous crops according to a number of economically significant features.

The detected defects and abnormalities of seed development are well correlated with their viability, which increases the practicality of the method. The high informative value of the method makes it possible not only to establish the degree of viability of the analyzed seeds, but also to identify the causes of its reduction.

Automatic analysis of digital X-ray images of seeds is an effective tool in the objectification of such traits of their hidden defects as irregular darkening, angularity and patterning of the internal structure.

The advantage of the method is its rapid application, integrity and safety of the studied material, which is especially important when working with small lots of seeds of collection and breeding material. By recording and archiving the results of analysis, it is possible to trace the change in the quality of seeds over the entire period of their storage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8010029/s1, Table S1. Results of automatic analysis of digital X-ray images of *Lepidium sativum* L. seeds Mechta Derbenta variety.

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