Genetics and Plant Physiology – 2014, Volume 4 (1–2), pp. 101–109
Special Issue (Part 1) – Conference "Plant Physiology and Genetics – Achievements and Challenges"
24 - 26 September 2014 – Sofia, Bulgaria

Available online at http://www.ifrg-bg.com

ASSESSMENT OF SEED HETEROGENEITY IN VEGETABLE PLANTS BY X-RAY DIFFRACTION

Musaev F. B.

All-Russian scientifically research institute of selection and seed production of vegetable cultures of Federal Agency of Scientific Organizations, 143080, Moscow Region, Odintsovo disctrict, p. VNIISSOK, Selektsionnaya St., 14, Russian Federation

Accepted: *21 October 2014*

Summary: The application of microfocus X-ray diffraction spectroscopy in the study of seed quality was described. This technique has been shown to be extremely useful in the evaluation the seed quality. This fast non-destructive method for characterization of seed quality with preservation of seed integrity enhances the process of vegetable seed management, which is important when small samples of valuable seeds are analyzed. The X-ray diffraction technique allows revealing the defects of the inner structure of seeds, such as underdevelopment of endosperm or embryo, seed damage, invisible insect contamination, inner germination, etc. The X-ray method is an important approach for estimation of the quality of freshly harvested dormant seeds when germination is impossible. The application of this method gives the possibility to choose the optimal way of seed utilization. Updating the database of vegetable seed traits revealed by X-ray diffraction may be used as a basis for fast X-ray seed separation.

Citation: Musaev F. B., 2014. Assessment of seed heterogeneity in vegetable plants by X-ray diffraction. *Genetics and Plant Physiology*, Conference "Plant Physiology and Genetics – Achievements and Challenges", 24-26 September 2014, Sofia, Bulgaria, Special Issue (Part 1), 4(1–2): 101–109.

Keywords: Defects; embryo; germination; seeds quality; seedlings; X-ray diffraction.

INTRODUCTION

To estimate the quality of seeds has always been a complex and challenging task as all the information about their quality is hidden in the molecular and anatomical structure of the seed. Many methods can detect it only partially and the final result can be seen only at the end of the growing season from the already developed plant. The urgency is that getting information although incomplete about the quality of the seeds in advance, one can choose the best way to use them when time for analysis will be compensated (Arkhipov et al., 2013).

A modern level of research requires automated methods for quality seed estimation allowing to get more

*Corresponding author: musayev@bk.ru

information in a shorter time.

Currently in the literature a number of biophysical methods for evaluation of seed quality have been described (Veselov et al., 1995; Priyatkin et al., 2006; Vilkova et al., 2006; Kapustkina, 2009; Vinogradova et al., 2010). The X-ray method differs greatly due to easy use, expressivity and non-destructive character, which can be also complemented by other morphometric methods for a complex analysis of seed quality. The method is standardized (OST 56-94-88, ISO 6639/4 -87). It was developed by the team of the Agrophysics Research Institute for Agricultural Seeds (AFI) (Savin and etc., 1981; Arkhipov and etc., 2001; Arkhipov and etc., 2008).

Comprehesive studies on X-ray diffraction of vegetable seeds have been conducted in the joint work of scientists from the Research Agrophysics Institute (AFI), St. Petersburg State Electrotechnical University (ETU) and All-Russian Research Institute of Vegetable Breeding and Seed Production of Vegetables Crops (VNIISSOK) since 2006. Nowadays seeds of 11 vegetable crops belonging to 8 families have been analyzed. The results of these analyses have been partially published (Arkhipov et al., 2008; Velicanov et al., 2008; Arkhipov et al., 2011; Musaev et al., 2011; Musayev et al., 2013).

MATERIALS AND METHODS

Eleven seeds from vegetable crops of different varieties were used in the study.

Analysis of the internal structure of seeds from various vegetable crops was conducted using a microfocus X-ray method (Arkhipov et al., 2001). Radiographic seed recordings were done on a movable roentgen diagnostic installation PRDU-2. Fifty seeds of each sample were filmed at the same time on the card with an adhesive film (5 rows with 10 pieces) (Fig. 1).

The shooting mode was the following: voltage 18 kV, amperage 105 mA, 3-5 sec and 7-8 sec exposure for small and large seeds, respectively. Microfocus shooting with a direct X-ray exposure in contrast to the contact ones gives contrast X-ray images with high magnification without quality loss. X-ray projection of the seeds got on a sensitive plate of the spectrum to this region was transformed into a digital form in a special scanner «DIGORA», where the image was transferred to the computer screen for editing, analysis and archiving (Fig. 2).

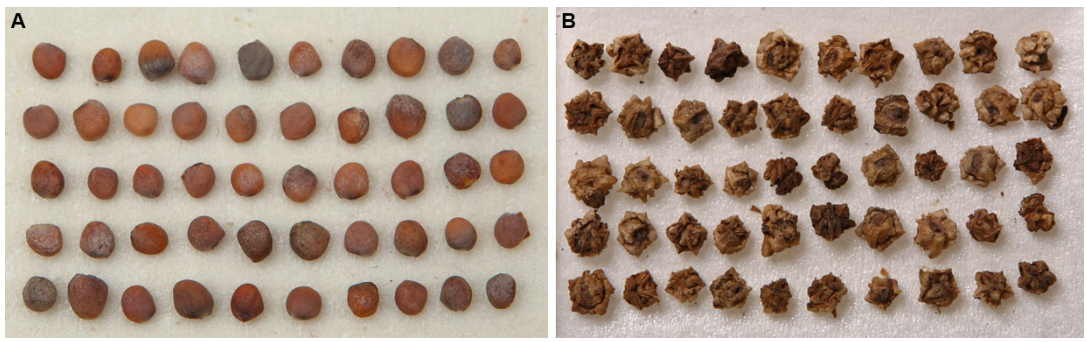

Figure 1. Preparation of seeds for X-ray filming: A) radish, B) beet.

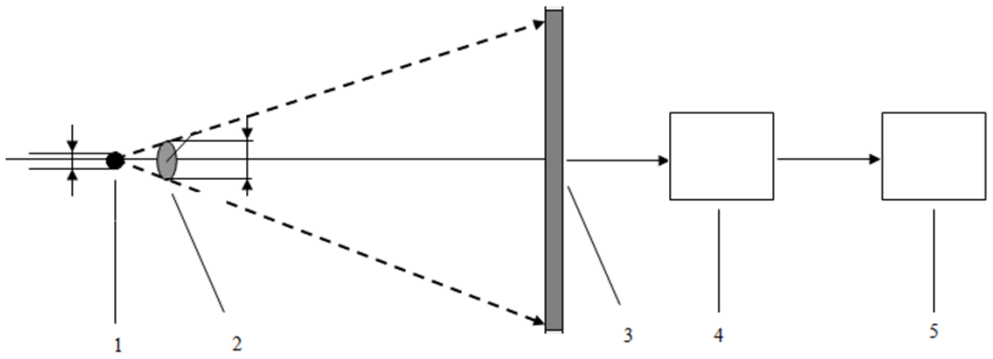

Figure 2. Functional diagram to get and process X-ray images: 1 - focal spot, 2 - object to study, 3 – image receiver, 4 - signal processing unit, 5 - personal computer.

RESULTS AND DISCUSSION

A series of experiments demonstrated the possibilities of the X-ray method in the study of the internal structure of vegetable seeds.

The analysis of a large number of radiographs of different accessions from eleven vegetable crops made it possible to identify and classify the X-ray features reflecting the state of the internal structure of seeds, damages and abnormal development of the structure.

The X-ray image of a seed is a shadow of its projection in the X-rays. As the absorption of the rays depends on the thickness and density of the material, it is possible to detect structural details on a shadow projection including shape, size and density. All deviations from the standard in a normal seed can be interpreted as damages or abnormalities (Fig. 3.1A, B, C, D). Complete (empty) or partial (underdeveloped embryo) darkening of the localization of the nucleus in the seeds was usual (Fig. 3.2). Mechanical damages and injuries occurring during harvesting, transportation or threshing were visualized on radiographs as thin black lines as in cracking of bean cotyledons (Fig. 3.3B) or carrot seed endosperm (Fig. 3.3C); or as an extended light abrupt interruption parts of embryo (spine) because of its breakage as the root breaking of embryo onion seeds (Fig. 3.3A). The method allows detection of defects and injuries even in the smallest seed marjoram cotyledons (Fig. 3.3D).

The X-ray method allows detection of damages and occupation by seed pests. One can see dark oval chambers or two long dark passages eaten by insect larvae, or right circular holes in the seeds of peas and beans (Fig. 3.4A, B) or damaged seeds of carrot and salad (Fig. 3.4C, D).

The method allows making a rapid assessment of the seed viability on the basis of X-ray analysis of seed internal structure. For example, two different batches (A; B) of pea seeds from Sovinter variety were analyzed. Visually homogeneous seeds of different parties (Fig. 4A, B) were markedly different in the radiographs. Seed radiographs of the “a” party contained more seeds with uniform optical density (Fig. 5A). In contrast, the X-ray projection of the “b” party was characterized by frequent regular and irregular opacity. The non-uniform optical density gave some kind

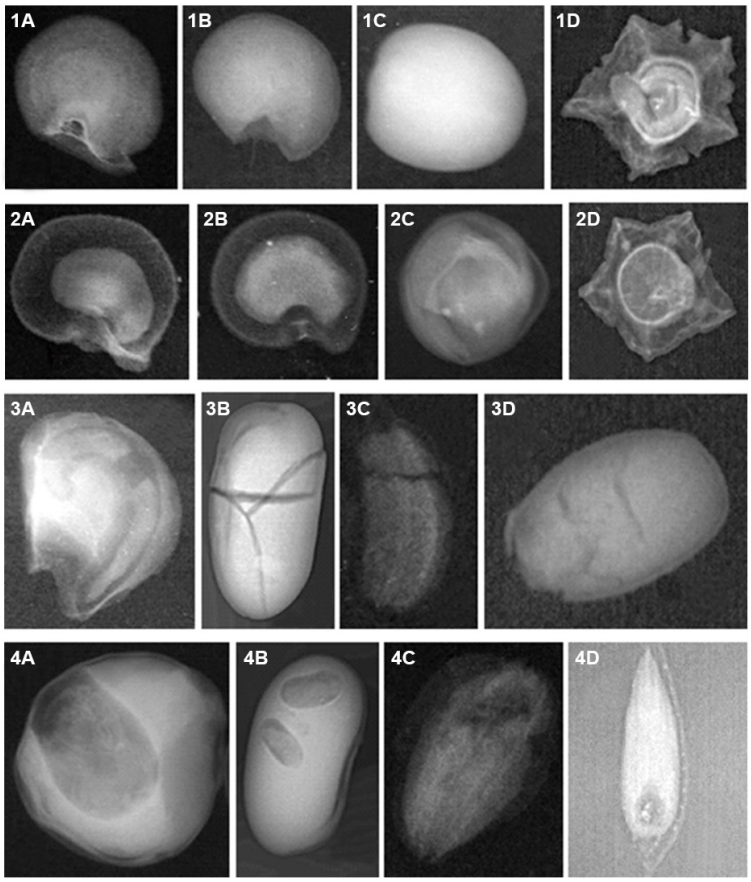

Figure 3. X-ray signs of seeds from various vegetable crops: 1) normal seeds: A) pepper, B) eggplant, C) cabbage, D) beets; 2) not filled seeds: A) pepper, B) eggplant, C) cabbage, D) beets; 3) the injured seeds: A) onion, B) beans, C) carrots, D) marjoram; 4) seeds damaged by pests; A) peas, B) beans, C) carrots, D) salad.

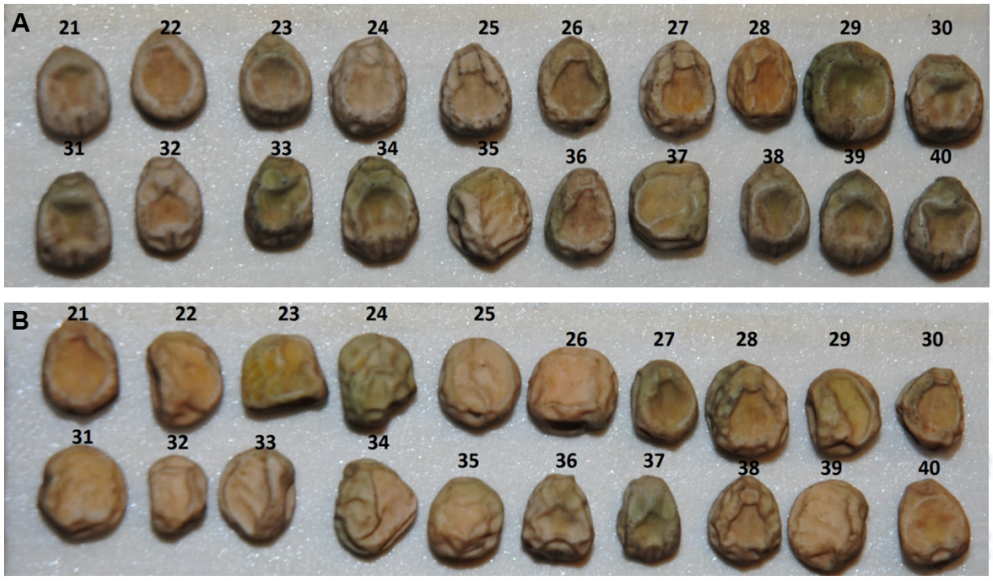

Figure 4. Fragments of photographs of two seed parties (A, B) with different quality from the pea variety Sovinter.

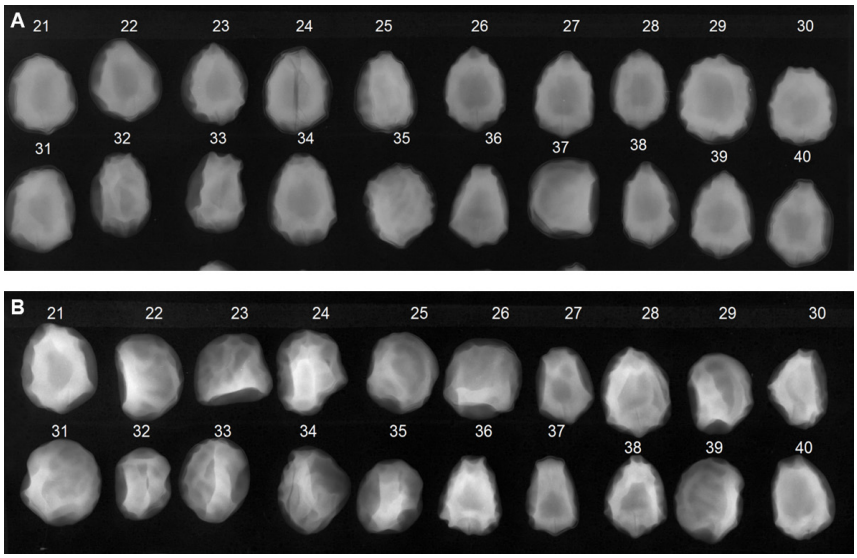

Figure 5. Fragments of radiographs of two seed parties (A, B) with different quality from the pea variety Sovinter.

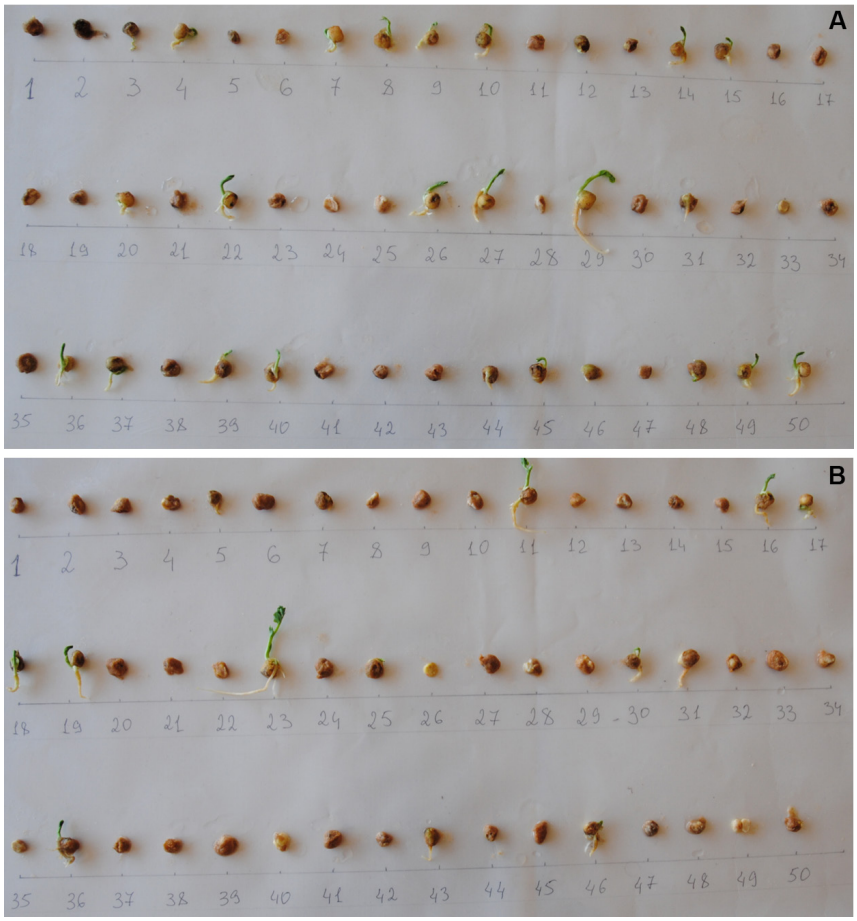

Figure 6. Fragments of pictures of seedlings from two different parties of pea seeds (A, B) from the pea variety Sovinter.

of "patchiness" that may be an indication of immature seeds (Fig. 5B).

Analyzing the results on individual germination we found different degrees of viability of seed lots (Fig. 6) that differed from each other more than 2 times: party "a" - 56%, party "b" - 26%. Additionally, germination of a seed lot conducted in accordance with GOST 12038-84 revealed 70% and 30% germination of party "a" and "b", respectively. This result was similar to the individual germination (Table 1).

X-ray analysis of the seeds is an indication of their sowing qualities. At the same time, some genotypic differences between the samples based on the internal structure of the seeds can be detected. Four radish varieties with varying degrees of viability and marketability of roots were analyzed (Table 2).

The results on the internal structure of radish seeds clearly showed that the conditioning seeds of variety *Aria* appeared on the radiographs uniformly dense and gave mature roots during seedling growth (Fig. 7). In contrast, the X-ray projection of seeds from an inbred line showed colorful seeds with clear or blurry spots that either failed to form roots during seedling growth (seeds №34, 35, 36) or did not sprout (seed №37) (Fig. 8).

The application of the X-ray method for seed quality estimation is not limited to the above examples. Further improvement of the method will successfully lead to identify hidden (internal) germination, desiccation, seed overwatering, shell peeling, separated cotyledons and other economically important traits affecting seed quality.

Investigations of other types of vegetable plants as well as attempts to improve the X-ray equipment are in progress.

Table 1. Quality of pea seeds from different varieties (2013).

Number	Crop	Variety	Year	Germination (mass analysis), %	Germination (individual analysis), %
1	Peas	Darunok	2013	93	58
2	Peas	Maksdon	2013	87	72
3	Peas	Sovinter (a)	2013	70	56
4	Peas	Sovinter (b)	2013	30	26

Table 2. Quality of radish seeds from different varieties (2013)

Number	Crop	Variety	Seed germination, %	Standard roots, %
1	Radish	Aria	88	72
2	Radish	Rozovo-krasniy s belim konchikom	72	40
3	Radish	Teplichhiy Gribovsky	90	70
4	Radish	Line 14	74	6

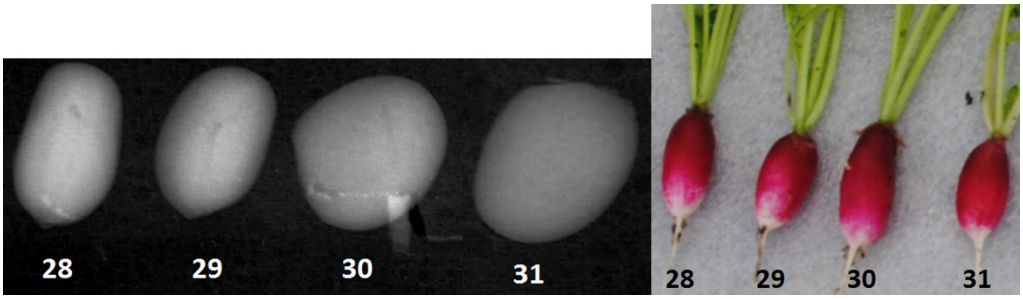

Figure 7. X-ray images of seeds from the radish variety *Aria* and their germination.

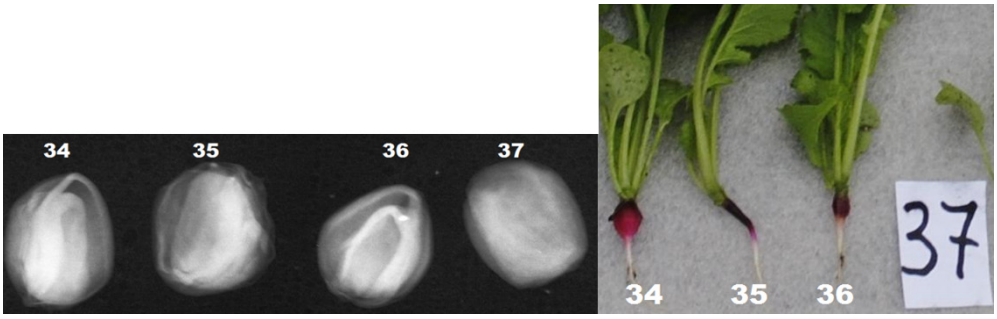

Figure 8. X-ray images of inbred radish seeds and their germination.

CONCLUSIONS

1. The X-ray method for seed quality estimation allows to identify defects and abnormalities of the internal seed structure for a number of economically important traits (Musaev et al., 2012; Musaev, 2014).
2. Seed defects and malformations correlated well with their viability which increases the relevance of the method. The method allows to estimate the viability of the analyzed seeds as well as to identify the causes of viability disturbances.
3. The advantage of this method is that it is time consuming. It allows to keep the integrity and security of the test material, which is particularly important when working with small batches of breeding material. By archiving the results of the analyses it is possible to monitor the changes in the quality of seeds during the entire period of storage.
4. Updating the database of vegetable seed traits revealed by X-ray diffraction may be used as a basis for fast X-ray seed separation.

REFERENCES

Arkhipov M.V., Alekseev D.I., Batygin N.F., Velikanov L.P., Gusakova L.P., Derunov I.V., Zheludkov A.G., Nicolenco V.F., Nikitina L.I., Savin V.N., Ponomorenko E.N., Yakushev V.P., 2001. Methods of radiography in agriculture and plant growth. M, RAAS, p. 93.

Arkhipov M.V., Potrakhov N.N., 2008. Microfocal X-ray analysis of plants. St. Petersburg. Publishing house "TECHNOLIT", p. 192.

Arkhipov M.V., Gusakova L.P., Alferov D.V., 2011. X-ray analysis of plants at the solution of problems of seed farming. Proceedings of the St. Petersburg State Agrarian University, 22: 336–341.

Arkhipov M.V., Gusakova L.P., Velikanov L.P., Vilichko A.K., Zheludkov A.G., Alferov V.B., 2013. Methodology of comprehensive assessment of the biological and economic suitability of seed material. St. Petersburg, p. 54.

Veselov T.V., Veselovskii V.A., Kartashov E.R. et al., 1995. Quantification of loss of viability of pine seeds under different storage methods. Plant Physiology, 42(4): 616–621.

Vilkova N.A., Nefedov L.I., 2006. Ways to diagnose damage grain sucking pests. Patent for invention №2278502 (RF), St. Petersburg.

Vinogradova I.S., Falaleev O.V., 2010. Application of magnetic resonance microtomography in the study of the internal structure of seeds of legumes. Bulletin of Russian Academy of Agrarian Sciences, 6: p. 10.

Kapustkin A.V., 2009. Morphological and physiological characteristics of seed germination of winter wheat when they are damaged harmful bug. Plant Protection News, №4: 39–47.

Musayev F.B., Kurbakova O.V., Kurbakov E.L., Arkhipov M.V., Velikanov L.P., Potrakhov N.N., 2011. The use of X-ray diffraction method of seed vegetables. Journal "Gavrish.", 1: 44–46.

Musayev F.B., Prozorova O.A., Arkhipov M.V., Velikanov L.P., Potrakhov E.N., Bessonov V.B., 2012. X-ray analysis of the quality of vegetable seeds. Vegetable Crops of Russia, 4: 43–47.

Musayev F.B., 2014. X-ray analysis of the quality of vegetable seeds: possibilities and perspective. Potatoes and Vegetables, 4: 32-33.

Priyatkin N.N., Korotkov K.G., Kuzemkin V.A., Dorofeyeva T.B., 2006. Investigation of the influence of the environment on the condition of the plants on the basis of GDV. Math. Universities. Instrument, 49(2): 67–72.

Savin V.N., Arkhipov M.V., Badenko A.L., Joffe J.K., Gruhn L.B., 1981. Radiography to detect internal damage and their influence on the quality of the seed harvest. Bulletin of Agricultural Science, 10: 99–104.

Musayev F.B., Arkhipov M.V., Velikanov L.P., Sokolova D.V., Bessonov V.B., 2013. Non-distractive X-ray diffraction method for studying seed anatomy in relation to seed heterogeneity. Proceedings of the International Conference "Functional Plant Anatomy". Lomonosov Moscow State University, M., pp 115–121.

Velikanov L.P., Grundas S., Archipov M.V., Demianichuk A.M., Gusakova L.P., 2008. Agrophisical direction of further development and application of X-ray method . New Trends in Agrophysics. Jubilee of the 40th anniversary of B. Dobizansky Institute of Agrophysics pas., Lublin 10-11.06.2008, pp 147-148.

OST 56-94-88. Seeds of trees. Methods

of X-ray analysis, M., 1988.

ISO 6639-4/87. Cereals and Pulses. Determination of hidden insect infestation. Part 4: Rapid Methods, 1987.

GOST 12038-84 20. State Standard Crop Seeds. Methods for determination of germination. M., 1984.