INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-4, Issue-4, Oct-Dec-2014

ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 14th June-2014 *Revised: 9th July-2014* *Accepted: 10th Aug-2014*

Research article

A X-RAY IMAGE ANALYSIS FOR ASSESSMENT OF FORAGE SEED QUALITY

Krishna P. Panchal, Neeta R. Pandya, Susy Albert, Dhara J. Gandhi

The Maharaja Sayajirao University of Baroda, Faculty of Science, Department of Botany, Vadodara-390 002, Gujarat, India.

*Corresponding author: drsusyalbert@rediffmail.com

ABSTRACT: Seed quality, an important factor in stand establishment is found to vary greatly among seed lots, especially during seed harvesting and processing. X- ray technique was used to discriminate between filled, partially filled and empty chaffy grass caryopsis, establishing relationship between internal structures and seed viability. The relationship between x ray image pattern and germination has been analysed. The potential use of x ray image analysis as a rapid and non destructive means to successfully screen minute forage seeds could be established.
Key words: forage grasses, germination, mammography, seed quality, X-ray radiography

INTRODUCTION

A weed free seed lot is the most efficient strategy to achieve established market standard. Separation of good quality seeds, if done with right equipment and appropriate methods, can increase purity and germination. It can also help in discarding the number of diseased seeds and can improve the visual, commercial and planting quality of seed lot. Seed quality is essential for maintaining the seed viability for extended periods of time. Knowledge of the seed's physical characteristics may offer an insight on germination levels. An x-ray technique allows for a non-destructive evaluation of seed fill, mechanical damage and potential insect injuries. Radiography has been found to be very useful in assessment of seed such as maize [1], cotton [2], forest tree species [3, 4] and industrial crop germplasm accessions [5].

X-ray inspection has a distinct advantage over most of the other detection technologies as it allows non-destructive imaging of interior features of a sample to detect hidden defects or contaminants. However, it also has a number of disadvantages, including a relatively high cost, the need for radiation shielding and the dangers inherent in using radiation, and the need for high voltage power supplies to generate the x-rays. For these reasons, x-ray inspection is generally considered as the last alternative option. Even though x rays are considered to be potentially harmful to seeds, the low rate that is absorbed during the test is not sufficient to induce genetic mutations and does not affect their germination [6, 7] and so, its use continues to expand in the 3 different industries. Till the date, x-rays have found extensive use in manufacturing industries for quality control inspection. But there is no record of x-ray radiography being used in assessing quality of forage grass seeds especially minute seeds. The traditional way to determine viability of seed lot is by performing a germination test. Though effective, germination tests are tedious and time consuming, taking anywhere from a couple of weeks to a couple of years, depending on the seed dormancies involved.

In the present study, a new attempt is made for the screening of seeds, especially minute seeds of forage grasses through x-ray radiography. Efforts have been made to use soft x ray technique generally used for mammography in the field of medicine, to assess the quality of the forage grass seeds.

MATERIALS AND METHODS

Seed samples

Forty five different species of grass florets were collected from Bandheli grassland (Godhra Forest Division) and Rampur grassland (Baria Forest Division), in Gujarat, India. The samples were manually cleaned to remove all foreign matter, dust and visibly broken and immature florets and was subjected to x ray analysis and germination studies. The x-ray studies were carried out at the Department of Radiology and Imaging, The Gujarat Cancer Research Institute, Ahmedabad, Gujarat, India.

X-ray studies

The first and foremost step is to draw a true representative sample from the seed lot. To obtain a random sample for testing it is always best to take samples from different parts of the bag or container.
As we required both purity and germination tests:
1. Seeds for germination tests were taken from the pure seed fraction, after conducting the physical purity analysis which was done manually.
2. The counting of the seed was done without discrimination as to the size and appearance.
The seeds were placed between the x-ray source and a photosensitive film or paper. When the seeds were exposed to the x-rays of low energy (longer wavelength, approximately 1 nanometer), an image (radiograph) is created on the film. Photographic processing converts the radiograph into a visible picture.

The protocol followed was as mentioned below:

- Fresh untreated 100 florets were linearly arranged on a white paper, numbered and sealed with cellophane tape.
- A very slow industrial x-ray film with ultra-fine grain and mammographic radiation (soft x-rays) was used to prevent excessive damage to the live tissue of the seed.
- The exposure conditions applied were as follow:
  kV = 22, ffd = 58 cms for all florets, while mAs were different for different species (depending upon their size).
- The films were processed with x-ray developer.
- After the radiographs were taken, these seeds were physically observed by dissecting and compared with the image developed.
- Ratios of filled, empty or damaged florets were calculated.
- The germination test was then carried out to detect possible relationships between seed physiology and resulting seedling/seed ratio, as revealed by radiographs.

After radiography, seeds were germinated and evaluated according to the International Rules for Seed Testing (ISTA) to establish the relationship between the type of seed damage and its consequence in the germination.

Germination studies

Two seed lots were used for germination study the exposed and the unexposed one. Two sets of germination experiments were laid down.
100 seeds from each seed lot were placed under moist conditions (100 ml of water) on filter papers placed on plastic trays (35 x 23 x 6 cms.) for 15 days under room temperature. Subsequently watering was done to maintain relative humidity.
Germination results were expressed as percentage. Germination rate is the average number of seeds that germinate over the 15 days period.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated x 100}}{\text{Number of seeds on tray}}$$

The percentage germination of treated and untreated samples were compared and represented graphically.

RESULTS

X-rays have found extensive use in the forage seed quality assessment. The observations of radiographic films showed full or empty seeds, good or bad seed morphology, damaged or destroyed seeds. The applications of x-ray radiography shows a promising future in pasture development by obtaining good quality seed collection, seed processing, nursery practice, seed trade and plant quarantines, and for research etc. The ratio or percentage of different classes along with the possible germination percent are given in the table 1. While physiological features like filled and empty seeds, insect damaged seeds, healthy and unhealthy seeds (on the basis of seed size), etc. are evaluated with the x-ray films (Figs. 1 and 2). The x – ray test, introduced by Simak & Gustafsson [6] to assess seed quality of some conifers, within the scope of seed technology, consist of the radiographic analysis of internal seed structures and is, at present, utilized for several purposes. The IDX (Incubation = I, Drying = D, X – raying = X) method has been used to separate viable and non viable seeds of *Pinus sylvestris.* Seeds with low optical density (a light image) were considered viable. X – ray research is known to examine seed tissue to assess desiccation tolerance [8]. When a beam of x – rays passes through seeds, a permanent image of them is formed on a film. The images can present a higher or lower degree of radiopacity (light) and radioluminescence (dark) depending on the absorption level of x – rays by the seeds, which is determined by factors such as composition, thickness and density of the tissues, and wavelength of the ionizing radiation [9, 10].

Table 1. X-ray analysis and germination performance of selected forage grasses caryopsis.

S.No.	Botanical name	mAs	Seed Size (LxTxW)	Mature caryopses %	Immature caryopses %	Injured seeds	Germination %	
							Treated seeds	Untreated seeds
Sp. 1	*Andropogon pumilus*	10	2.7x0.4x0.2	34	44	0	38	40
Sp. 2	*Apluda mutica*	2	2.1x0.6x0.9	36	34	0	85	90
Sp. 3	*Aristida funiculata*	2	1.8x0.8x0.5	36	50	0	35	35
Sp. 4	*Arthraxon lanceolatus*	2.2	1.8x0.3x0.3	32	48	0	75	90
Sp. 5	*Bothriochloa pertusa*	2.2	1.8x0.7x0.4	0	1	0	0	0
Sp. 6	*Brachiaria reptans*	5	1.9x1.4x0.2	28	56	0	38	40
Sp. 7	*Capillipedium huegellii*	2.2	1.4x0.6x0.3	24	49	0	30	40
Sp. 8	*Cenchrus biflorus*	36	1.5x1.0x0.7	11	31	0	0	20
Sp. 9	*Cenchrus ciliaris*	22	1.9x0.8x0.5	41	5	0	75	95
Sp. 10	*Cenchrus setigerus*	36	1.8x1.0x0.7	79	9	0	0	70
Sp. 11	*Chionachne koenigii*	45	3.4x2.1x1.3	0	2	0	0	0
Sp. 12	*Chloris barbata*	2.8	1.4x0.5x0.4	57	25	0	75	75
Sp. 13	*Chloris virgata*	2.8	1.5x0.4x0.5	41	36	0	24	30
Sp. 14	*Chrysopogon fulvus*	22	4.6x0.5x0.9	9	33	0	42	50
Sp. 15	*Coix lachryma-jobi*	2	4.4x3.6x2.6	41	24	0	55	60
Sp. 16	*Dactyaloctenium aegyptium*	36	0.9x0.9x0.5	35	19	0	8	10
Sp. 17	*Dichanthium annulatum*	2.2	2.5x0.8x0.5	47	12	0	88	90
Sp. 18	*Dichanthium caricosum*	36	2.3x0.9x0.4	27	35	0	0	45
Sp. 19	*Digitaria adscendens*	5	2.2x0.8x0.4	46	27	0	78	85
Sp. 20	*Echinochloa colonum*	36	1.6x1.2x0.7	18	20	0	0	75
Sp. 21	*Echinochloa crus-galli*	36	1.8x1.7x0.9	52	9	0	0	65
Sp. 22	*Echinochloa stagnina*	2.8	2.7x2.3x1.7	95	5	0	100	100
Sp. 23	*Eleusine indica*	11	1.2x0.6x0.5	57	28	0	4	5
Sp. 24	*Hackelochloa granularis*	11	-	0	0	0	0	0
Sp. 25	*Heteropogon contortus*	2.2	3.9x0.4x0.3	46	15	0	80	80
Sp. 26	*Imperata cylindrical*	22	-	0	0	0	0	0
Sp. 27	*Ischaemum indicum*	36	1.5x0.7x0.5	17	30	0	0	40
Sp. 28	*Ischaemum pilosum*	36	2.4x0.8x0.6	5	2	0	0	50
Sp. 29	*Ischaemum rugosum*	22	2.2x0.9x0.8	39	24	0	75	75
Sp. 30	*Melanocenchris jacquemontii*	16	-	0	0	0	0	0
Sp. 31	*Ophiorus exaltatus*	36	-	0	0	0	0	0
Sp. 32	*Panicum antidotale*	36	1.6x0.9x0.7	42	27	0	12	15
Sp. 33	*Panicum trypheron*	5	1.6x1.2x0.4	23	35	0	20	20
Sp. 34	*Paspalidium flavidum*	5	1.2x1.2x0.4	61	32	0	24	25
Sp. 35	*Pennisetum setosum*	22	-	0	0	0	0	0
Sp. 36	*Schoenefeldia gracilis*	2.8	2.0x0.5x0.4	59	33	0	94	95
Sp. 37	*Sehima ischaemoides*	15	1.8x0.4x0.5	35	44	0	45	45
Sp. 38	*Sehima nervosum*	2	4.3x1.0x0.3	55	20	0	8	10
Sp. 39	*Sehima sulcatum*	36	2.6x0.9x0.6	11	8	0	0	5
Sp. 40	*Setaria glauca*	11	1.5x0.9x0.5	23	31	0	15	25
Sp. 41	*Setaria tomentosa*	5	1.6x1.2x0.3	35	39	0	10	45
Sp. 42	*Sorghum halepense*	36	2.8x1.7x1.1	47	16	0	0	60
Sp. 43	*Thelepogon elegans*	36	3.6x1.4x0.5	28	28	0	0	85
Sp. 44	*Themeda cymbaria*	10	2.3x0.9x0.3	89	7	0	55	60
Sp. 45	*Themeda triandra*	2.2	3.2x0.9x0.3	60	21	0	87	95

Results obtained from the germination test correspond to the expected germination based on the classes obtained with the radiography of the seeds (Table 1). The germination test revealed that seeds which are healthy enough and are exposed to the minimum dosage of soft x-rays are able to germinate and their germinability correlate with that of untreated seeds of the same species. Results indicate that x ray technique for assessment of seed quality does not change its germination potentiality much after x raying. The higher energy of 100 kV as well as high exposure time which is generally used in hard x ray is not suitable for radiography of food products. It damages the seeds being used for assement, Mammography is a specific type of imaging that uses a low-energy (usually around 30 kV) x-ray system to examine biological material. It is a specific type of imaging that uses a low dose x-ray system and high contrast, high-resolution film for examination of biological material. In the field of medicine it is used to detect breast cancer. X-raying with the same kV and different mAs showed that the seeds x-rayed with lowest mAs were not affected with the radiations and showed normal germination when compared to the untreated seeds.

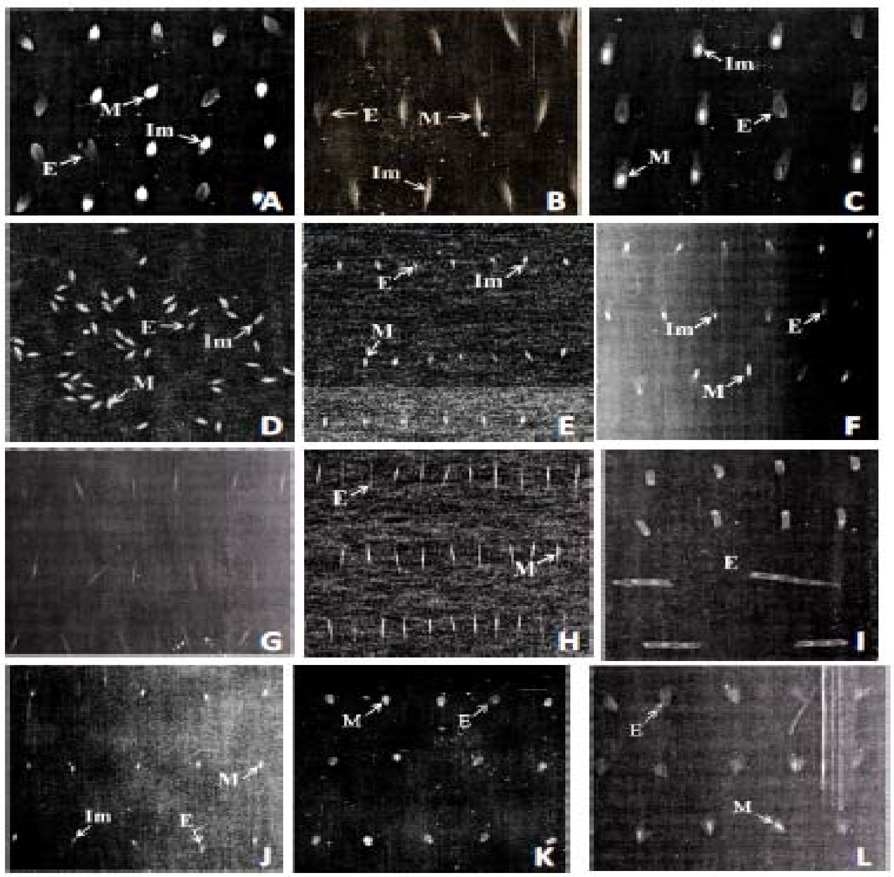

FIG.1

FIG. 1: X- ray images of caryopsis
A-*Sorghum halepense* B-*Chrysopogon fulvus* C- *Thelepogon elegans*
D- *Digitaria adscendens* E- *Dichanthium annulatum* F- *Dichanthium caricosum*
G- *Heteropogon contortus* var. *typicus* H- *Heteropogon contortus* var. *genuinus*
I- *Ophiorus exaltatus* J- Eleusine indica K-*Hackelochloa granularis*
L-*Melanocenchris jacquemontii*
Im-Immature, E-Empty, M-Mature

X-ray analysis can be used to determine the quality of seeds, showing the cause of bad germination. X-ray images provide information on the internal structure and morphology of seeds, mechanical damage, percentage of empty and filled seeds, micro fractures, possible embryo deformations and insect infestation. These techniques have been developed in Cuba for different X-ray sets. Morphological features of seed embryo and seed coat can be determined by a combination of x-ray image, optical microscopy and micro hardness determination methods on seed coats [11]. In the present study seeds subjected to x-ray analysis for quality has been further used in germination study. The results of x-ray image analysis have been conducted with its germination capacity. This has also been compared with the germination capacity of seeds which were not subjected to x – ray analysis to determine any damage occurred due to the x – rays.

In majority of the studied species the percentage germination of both treated and untreated seeds appeared to be almost same except for a few species in which the x ray imaged seeds showed zero percent germination. Untreated *Cenchrus setigerus, Echinochloa colonum, Echinochloa crus-galli, Sorghum halepense* and *Thelepogon elegans* showed 70, 75, 65, 60 and 80 percent germination respectively. But the x ray imaged seeds showed zero percent germination indicating x ray imaging to have caused physiological damage to the seeds inhibiting/ stopping germination. Untreated *Dichanthium caricosum, Ischaemum indicum* and *Ischaemum pilosum s*howed 40-50 % germination but the treated seeds showed no germination. Out of 45 species studied five of them *Hackelochloa granularis*, *Imperata cylindrica*, *Melanocenchris jacquemontii*, *Ophiorus exaltatus* and *Pennisetum setosum* did not show seed setting at all (Table 1.). Two species *Chionachne koenigii* and *Bothriochloa pertusa* showed immature caryopsis (2 and 1 % respectively).
The seed containing fully developed embryos or 100 % filled with storage food material germinated to a large extent (table 1). The empty or fungus attacked seeds did not germinate at all. Nevertheless the germination rate of the best material amounted to around 90 %. Part of this, inferior germination may be due to embryo damage caused during seed processing.

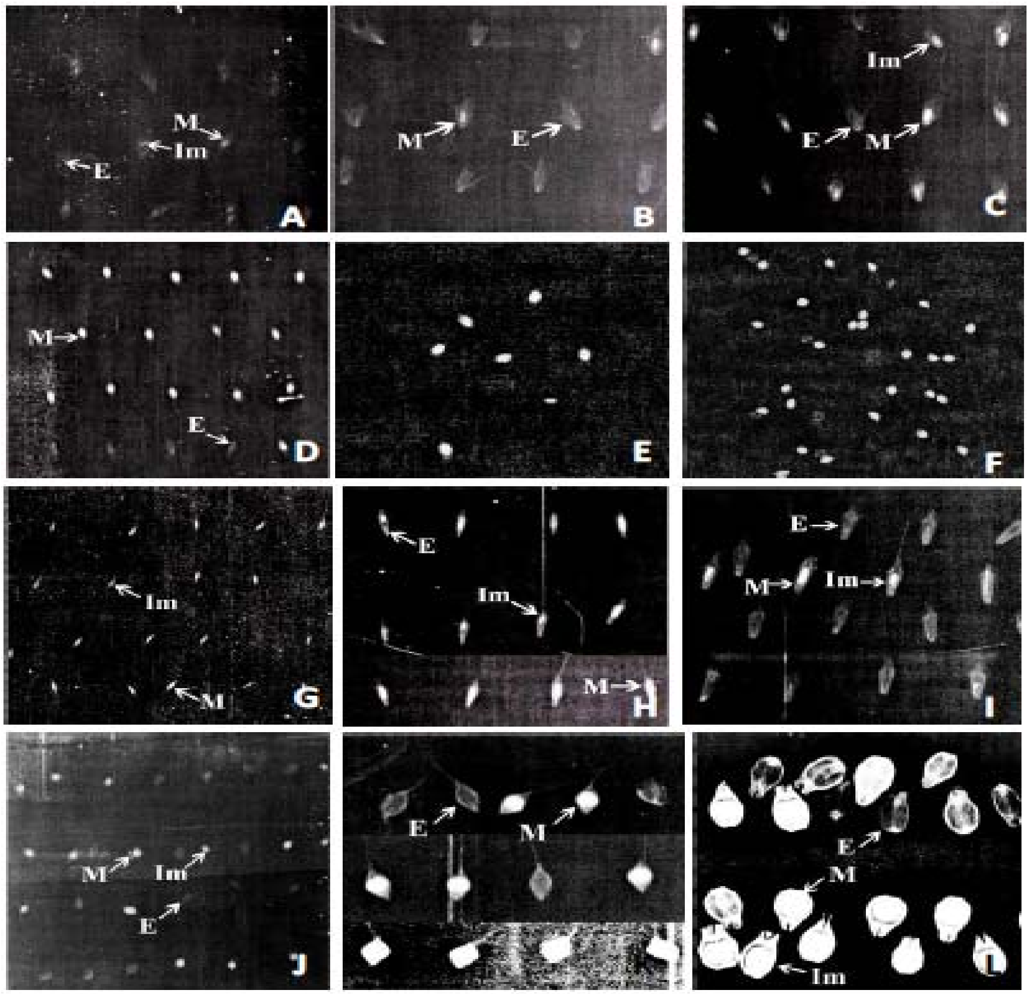

FIG.2

FIG.2: X- ray images of caryopsis
A-*Ischaemum indicum* B-*Ischaemum pilosum* C-*Ischaemum rugosum*
D- *Panicum antidotale* E-*Panicum trypheron* F-*Paspalidium flavidum*
G-*Schoenefeldia gracilis* H-*Sehima ischaemoides* I-*Sehima sulcatum*
J- *Echinochloa colonum* K-*Echinochloa crus-galli* L-*Coix lachryma-jobi*
Im-Immature, E-Empty, M-Mature

DISCUSSION

This paper focused on using mammography technique to observe internal seed characteristics and its relativity with the germination. Features that were observed using this technique included the mature, immature, healthy seeds; filled and empty seeds, cavities due to insect infestation in the seeds, etc. were also observed.

The range of light and dark shades observed in radiographic images of seeds (Figures 1 and 2) is defined as a function of the level of absorption of x-rays in distinct regions of the seed, which is determined by the thickness, density and composition of the tissues. Seed radiography can be of help to evaluate seed viability also. Therefore, it is necessary to establish a relationship between the internal structures of the seeds and the corresponding seedlings that are produced, which was done by the comparison of germinating both the x-ray treated and non-treated seeds (Table 1). Conventional x-ray imaging has been used to reveal insect infestation [12, 13, 14] and seed damage [1, 15, 16] and to indicate seed quality [17, 18, 19]; the mammography technique is similar to that but one step advanced in detecting such minute forage grass seeds along with more clearest images. The x-ray image analysis technique is a precise method which enables examination of regions that are damaged and their location. It is a non-destructive method allowing the x-ray treated seed to be submitted to quality physiological tests. Machado [3] studied the abnormalities in aroeira-branca embryos by radiography. Seeds with embryo / embryo cavity proportion lower than 100%, as determined by x-ray test did not germinate and those with abnormal embryos produce abnormal seedlings or did not germinate, providing evidence that the x-ray test is effective to assess seed quality.

Even though x-rays are potentially harmful to seeds, the low rate that is absorbed during the test is not sufficient to induce genetic mutations and does not affect their germination [7]. But an alternative option to avoid the risk of mutation is mammographic x rays which were used in the present study. The mammograms are generated with x-rays of lower energy (soft x-rays), much less harmful to the live tissues. In addition, it is a test that also does not require previous seed treatment, with the advantages of being nondestructive, quick and easy-to-perform.

In the present study seeds subjected to x-ray analysis for quality has been further used in germination study. The results of x-ray image analysis have been conducted with its germination capacity. This has also been compared with the germination capacity of seeds which were not subjected to x – ray analysis to determine any damage occurred due to the x – rays. The X-ray technique is efficient for the detection of damages and abnormalities in forage seeds having a very smaller size, sometimes detrimental to germination. Discarding damaged or abnormal seeds, as shown in this study, can improve the germination performance of seed lots for pasture development. This procedure, when carried out before sowing, gives more satisfactory results for grass species. Germination studies carried out also indicates that no physiological damage has occurred due to x ray technique used to assess the quality of the seeds in most of the species studied.

CONCLUSION

In the present study, x-ray imaging of such smaller sized grass caryopses was done for seed lot screening and for such a small sized seeds study with normal characterization was not possible. For these efforts were done with modifying the specifications, successful results could be obtained and results stands as one of the major achievement in the field of pasture development. X ray technology is a powerful tool for rapid and efficient selection of pure seed fraction.

Acknowledgment

Authors are thankful to the Forest Department, Working Plan Circle, Vadodara, Gujarat for providing financial support to conduct the present study. Authors are also thankful to the Head, Department of Botany, The Maharaja Sayajirao University of Baroda, for providing the laboratory facilities.

REFERENCES

[1] Carvalho, M.L.M., Van Aelst, A.C., Van Eck, J.W., Hoekstra, F.A. 1999. Pre-harvest stress cracks in maize (*Zea mays* L.) kernels as characterized by visual, X-ray and low temperature scanning electron microscopical analysis: Effect on kernel quality. Seed Science Research, 9, pp. 227-236.

[2] Ferguson, D., Turner, J.H. 1971. Influence of unfilled cotton seed upon emergence and vigor. Crop Science, 11, pp. 713-715.

[3] Machado, C.F. 2002. Metodologia para a condução do teste de germinação e utilização de raios-X para a avaliação da qualidade de sementes de aroeira-branca (Lithraea molleoides (Vell.) Engl.). Piracicaba, 51f. Dissertação (Mestrado em Fitotecnia). Escola Superior de Agricultura Luiz de Queiroz.

[4] Skrzyszewska, K., Chłanda, J. 2009. A study on the variation of morphological characteristics of silver fir (*Abies alba* Mill.) seeds and their internal structure determined by X-ray radiography in the Beskid Sądecki and Beskid Niski mountain ranges of the Carpathians (southern Poland). Journal of Forest Science, 55 (9), pp. 403–414.
[5] Jenderek, M.M., Longwell, J.M., Ellis, D.D., Dierig, D.A. 2010. Non-destructive seed evaluation of selected industrial crop germplasm accessions. Meeting Abstract. AAIC 22nd Annual Meeting, Fort Collins, Colorado, September 19-22, pp: 4.
[6] Simak, M., Gustafsson, A. 1953. X-ray photography and sensitivity in forest tree species. Hereditas, 39, pp. 458-468.
[7] Bino, R.J., Aartse, J.W., Van der Burg, W.J. 1993. Non-destructive X-ray analysis of *Arabidopsis* embryo mutants. Seed Science Research, 3, pp. 167-170.
[8] Goodman, R.C., Jacobs, D.F., Karrfalt, R.P. 2005. Evaluating desiccation sensitivity of Quercus rubra acorns using X-ray. Canadian Journal of forest Research, 35, pp. 2823-2831.
[9] Simak, M. 1980. X-radiography in research and testing of forest tree seeds. The University of Agricultural Sciences, Department of Siliviculture. Rapporter, 3, pp: 34.
[10] International Seed Testing Association. ISTA. 1993. International rules for seed testing. Seed Science and Technology, 21. pp: 363.
[11] Paiziev, A.A., Krakhmalev, V.A. 2006. Microstructure of Dormant Cotton Seeds. Asian Journal of Plant Sciences, 5, pp. 492-497.
[12] Schatzki, T.T., Fine, T.A. 1988. Analysis of radiograms of wheat kernels for quality control. Cereal Chemistry, 65, pp. 233–239.
[13] Karunakaran, C., Jayas, D.S., White, N.D.G. 2003. X-ray image analysis to detect infestations caused by insects in grain. Cereal Chemistry, 80, pp. 553–557.
[14] Haff, R.P., Slaughter, D.C. 2004. Real-time X-ray inspection of wheat for infestation by the granary weevil. *Sitophilus granarius* (L.). Transactions of the ASAE, 47, pp. 531–537.
[15] Milner, M., Lee, M.R., Katz, R. 1952a. Radiography applied to grain and seeds. Food Technology, 6, pp. 44–45.
[16] Létang, J.M., Peix, G., Droulez, L. 2002. Automatic selection of seeds using pattern recognition techniques in high resolution x-ray images. NDT.net, 7, http://www.ndt.net/article/ecndt02/412/412.htm.
[17] Simak, K., Sahlén, K. 1981. Comparison between the x-radiography and cutting tests used in seed quality analysis. Seed Science and Technology, 9, pp. 205–227.
[18] Foucat, L., Chavagnat, A., Renou, J.P. 1993. Nuclear magnetic resonance micro-imaging and X-radiography as possible techniques to study seed germination. Scientia Agricola, 55, pp. 323–331.
[19] Downie, B., Gurusinghe, S., Bradford, K.J. 1999. Internal anatomy of individual tomato seeds: relationship to abscisic acid and germination physiology. Seed Science Research, 9, pp. 117–128.