Article **ECORFAN Journal-Republic of Nicaragua**
December 2020, Vol.6 No.11 5-9

X-ray analysis of seed of *Pinus devoniana*

Análisis de semilla de *Pinus devoniana* con rayos X

AVENDAÑO-LÓPEZ, A.*†`, QUINTANA-CAMARGO, M.``, PADILLA-GARCÍA, J.` and ARRIAGA- RUÍZ, M.`

Universidad Tecnológica de la Sierra Hidalguense. Mexico-Tampico Highway, Km. 100 Colonia. Pachuca-Huejutla section. Zacualtipán de Ángeles, Hidalgo, México, C.P. 43200. Mexico.

ID 1st Author: *A., Avendaño-López*

ID 1st Co-author: *M., Quintana-Camargo*

ID 2nd Co-author: *J., Padilla-García*

ID 3rd Co-author: *M., Arriaga-Ruíz*

DOI: 10.35429/EJRN.2020.11.6.5.9 Received June 15, 2020; Accepted December 30, 2020

Abstract

The aim of this study was to establish the relationship between a system based on X-ray and behavior seed viability test with tetrazolium and standard germination analysis. From radiographic classification was established relating seed physiological response; seeds that produce normal seedlings, abnormal seedlings, and dead seeds: 3 classes were established. Also, internal morphological characteristics were established: the embryonic cavity filling, developing embryo structures, defects, density and appearance, and even invasion and damage from pests. Correlation coefficients of .70 ** presented with seedling length; 796 ** for tetrazolium viability; ** 818 and between the filling cavity and classified as normal. Concluded that X-ray analysis, in addition to being useful in determining the physical quality of the seed, leads to the estimate of the physiological response in *Pinus devonian* seeds, as it is possible to estimate the relationship between the response variables and morphological functional, without being a destructive technique.

Analysis seeds X-Ray, *Pinus Devonian*

Resumen

El objetivo del presente trabajo fue establecer la relación entre un análisis basado en Rayos X y el comportamiento de la semilla con el ensayo de viabilidad con tetrazolio y de germinación estándar. A partir de imágenes radiográficas se estableció una clasificación de semillas relacionando la respuesta fisiológica; se establecieron 3 clases: semillas que generan plántulas normales, plántulas anormales y semillas muertas. Así mismo se establecieron características morfológicas internas: el llenado de la cavidad embrionaria, el desarrollo de estructuras de embrión, malformaciones, densidad y aspecto, e incluso invasión y daños causados por plagas. Se presentaron coeficientes de correlación de .70** con longitud de plántula; .796** para viabilidad con tetrazolio; y de .818** entre el llenado de cavidad y las clasificadas como normales. Concluyendo que un análisis de Rayos X, además de ser útil en la determinación de la calidad física de la semilla, conlleva a la estimación de la respuesta fisiológica en semillas de *Pinus devoniana*, ya que es posible estimar la relación entre variables morfológicas y la respuesta funcional, sin ser una técnica destructiva.

Analisis semillas, Rayos X, *Pinus devoniana*

Citation: AVENDAÑO-LÓPEZ, A., QUINTANA-CAMARGO, M., PADILLA-GARCÍA, J. and ARRIAGA- RUÍZ, M. X-ray analysis of seed of *Pinus devoniana*. ECORFAN Journal-Republic of Nicaragua. 2020. 6-11:5-9.

* Correspondence to Author (Email: aavedan@cucba.udg.mx)
† Researcher contributing first author.

Introduction

Seed quality is a concept made up of four main components: physical, physiological, genetic, and sanitary characteristics, the objective of which is to know the seed's potential during germination, development in the field and particular requirements during storage; the analyses are carried out in the laboratory and/or greenhouse, using standardised techniques, which ensure uniform and repeatable results.

The X-ray technique in seed analysis is a non-destructive method, useful in internal studies such as anatomy, imperfections, physiological changes that occur during maturation and insect attack, among others, based on digitalised radiographic images that, through software, allow measurements and observations of the embryonic cavity of the seed (Altzugaray, et al., 2006). This technique has been used in the analysis of grass seeds, establishing a density profile of specific regions in embryonic structures that caused problems during germination (Pérez-Talavera, 1999). Iglesias, et al (2006) evaluated the morphometry, viability, and variability of *Pinus hartwegii* seed from Perote, Veracruz based on X-ray irradiated seed plates, which allowed the characterisation of seed lots from different populations.

However, its use in seed analysis laboratories in Mexico is not common, largely due to the lack of established protocols, as international protocols have been designed for seed of species. *Pinus devoniana* Lildl is native to Mexico.

General objective of the work

To establish a protocol for seed quality analysis of *Pinus devoniana* based on seed classification through digitised X-ray images.

Methodology

The work was carried out in the seed analysis laboratory of the Germplasm Bank "El Centinela" of the National Forestry Commission (CONAFOR). It was also carried out at the Seed Analysis Laboratory of the Institute of Science and Technology (INCITES) of the Agricultural Production Department of the CUCBA.

A sample of a batch of seeds of *Pinus devoniana* Lindl. was used.

A FAXITRON MX-20 seed X-ray machine was used. Using 400 seeds distributed in 4 replicates of 100. Each seed was identified to follow its physiological condition. Once the images were digitised, the external characteristics were obtained, such as length, width and thickness, and internal characteristics: embryo cavity, embryo length and width of each seed. It was necessary to carry out tests with different numbers of seeds to establish the appropriate calibration of the equipment and the optimum sample size; in subsequent tests of radiographic plates, exposure time and distance between the X-ray emitting tube and the seed were determined with an automatic calibration that varied between 1.3 and 2.7, which allowed a clearer visualisation of the internal structures of the seed, as can be seen in figure 1. It was thus established that, according to the characteristics of the equipment used, the sample size per plate or image is 10 seeds.

Figure 1 X-ray plate image of *Pinus devoniana* seed using a sample of 10 seeds at and speed of 20 seconds

According to ISTA (1996), the regulation of the X-ray equipment depends on several factors such as thickness, density, and composition of the seed.

Viability test with Tetrazolium

The viability test gives a relatively quick indication of the germination potential of a batch of seeds by reacting the tetrazolium salt and the hydrogen released during seed respiration, generating a red staining of living cells. Seeds stained red in all their structures are considered live seeds (figure 2).

ISSN-On line: 2414-8830

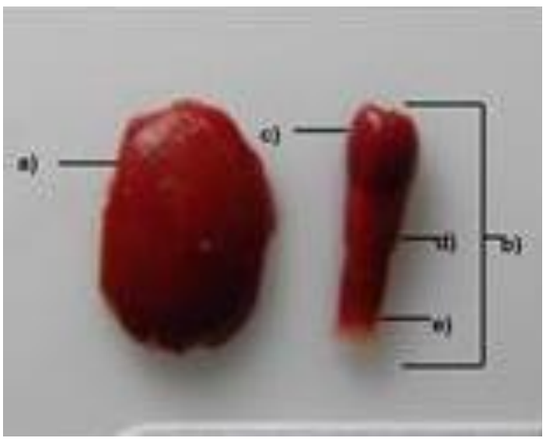


Figure 2 Seed completely stained red in all its structures. a) Endosperm, b) Embryo, consisting of c) Cotyledonary leaves, d) Hypocotyl and e) Radicle.

Figure 3 Dead seeds

Germination analysis

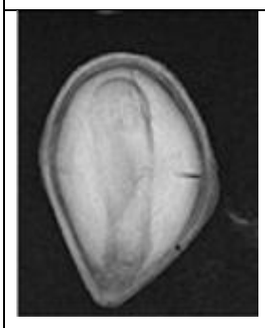
The standard germination analysis was carried out by incubating the seed at 25°C for 14 days using germination paper as substrate. 14 days using germination paper as substrate, the seedlings obtained were classified as normal, abnormal, in addition to counting the dead seeds, figure 4 shows the irradiated seedlings obtained in this test.

Figure 4 Seedlings of *P. devoniana* from the St. George germination test

From the X-ray images and the results obtained in the initial standard germination test, a seed classification was established by relating the physiological response of the seed and internal morphological characteristics such as: the percentage of filling of the embryo cavity, the number of seeds in the embryo cavity, the percentage of seeds in the embryo cavity and the percentage of seeds in the embryo cavity.

Visualisation of development of embryo structures, malformations, density and glassy or hyaline appearance, embryo detachment and even invasion and pest damage. This classification of irradiated seed was the basis for seed classification used in the vigour analyses carried out.

Seed grading pattern

	Visually healthy seed, with defined internal structures. Cover without damage or cracks. Entire endosperm does not present dark, vitreous areas or fissures. Embryonic cavity with more than 90% filled by the embryonic axis. Embryo attached to the basal area of the endosperm, without fissures or alterations. It develops a normal seedling. Viable seed in tetrazolium analysis.
	Seed without external damage. Endosperm with dark, opaque vitreous areas or fissures. Vitreous embryo, with dark areas, without defined embryonic axis structures. Cotiledoeas illegible leaves, have dark or vitreous areas. Visible deterioration due to physical damage. It does not germinate. Seed NOT viable in tetrazolium analysis.
	Damaged endosperm. Embryo attached the basal area of the endosperm shows alterations, the cotyledonary axis is vitreous. Cotyledonary leaves may or may not be legible with dark or vitreous areas. It develops abnormal or low vigor seedlings. Seed NOT viable in tetrazolium analysis.

ISSN-On line: 2414-8830

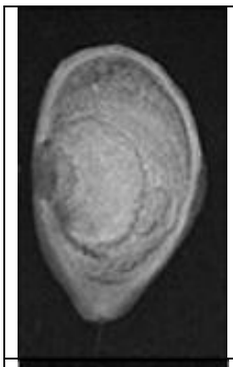
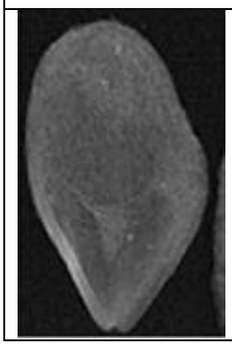
	Seed with hardly visible external damage. Sweeps for plague. Vain seed.
	Seeds without external damage. Dark and vitreous colorations in embryo and endosperm. Not filling of embryonic cavity and embryo thinning. Abnormal seedling.
	Seed without external damage. Intact endosperm with no fissures or vitreous, opaque or dark areas. It does not present defined embryonic axis structures, amorphous, vitreous tissue. It does not germinate, it is classified as dead seed. NON-viable seed in tetrazolium assay.
	Seed without external dali. Vana.

Table 1

Results

Seeds without visual damage showed up to 26.4% and 31.6% physiological damage, respectively, when analysed radiographically, which indicates the usefulness of the X-ray method in the evaluation of internal damage in relation to visual analysis. Similar results were observed by Carvalho et al (1999) in the analysis of pre-harvest internal damage in maize seeds.

Correlation coefficients of .70** with the seedling length test; .796** for viability with tetrazolium; .37NS with accelerated ageing and .818** between cavity filling and those classified as more vigorous.

Seed viability was also found to be affected by storage time, reflected in a lower percentage of stained seeds in the Tz viability test between the first and second samples.

According to McDonald (1994), although the X-ray test is a physical test, it provides information that can assist in viability assessments and can reveal morphological deficiencies that indicate the structural potential for viability.

Acknowledgements

Thanks to CONAFOR. Delegación Jalisco for the facilities provided for the use of the X-ray equipment.

Conclusions

The X-ray analysis test gives a quick and useful insight into the quality of the seed lot of *Pinus devoniana*, as it shows clear and accurate images of the physical condition of the seeds without the need to destroy the sample.

In general, X-ray analysis of pine seed was effective in detecting damage and abnormalities and provides comprehensive information about the association of seed morphology and seedling development and should be used as a supplementary procedure to assess the physiological potential of the seed.

References

Alzugaray, C.; Salinas, A.; Carnevale, N.2006. Aplicación de la técnica de rayos x en la evaluación de calidad de semillas forestales nativas: Schinopsis balansae engl. y Aspidosperma quebracho-blanco schlecht.

Agromensajes. Facultad de Ciencias Agrarias Universidad Nacional de Rosario, Argentina

Carbalho, M.L.M. et al. Charac-terization of pre-harvest stree cracks in corn seed by visual, X-ray and LTSEM analysis: effect on seed quality. 1999.Utilizar sistema APA

ISSN-On line: 2414-8830

McDonald, M. B. 1994. Seed germination and seedling establishment Physiology and Determination of Crop Sci. Soc. Amer., Madison, WI. USA. p. 37-60.

Gomes-Junior, F.G.; Yagushi, J.T.; Belini, U.L.; Cicero, S.M.; Tomazello-Filho, M. 2012. X-ray Densitometry to assess Internal Seed Morphology and Quality. Seed Science and Technology; volume 40: 1, (102-107)

Pérez Talavera, S.; Caballero Torres, I.; Micó Infanzón, M.; Pérez Lezcano, A.; Guerra, M. 1999. El estrés radiacional en las semillas M1 de trigo estudiado a través de la densitometría de las radiografías de rayos X. Revista de Tecnología e higiene de los alimentos. Volumen 36 (302): 113-115.

ISSN-On line: 2414-8830