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X-RAY: CHARACTERIZATION OF G*inkgo biloba* L. SEEDS USING DIGITAL AND MANUAL MEASUREMENTS[1]

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ABSTRACT - The aim of this paper was to: a) verify if digital radiographic image measurements of G. biloba seeds could replace those obtained with a manual caliper; b) determine the degree of seed development through digitally measuring the air chamber of the seed and, c) make a radiographic pattern to characterize the seed species according to its anatomical and morphological structure, identify physical damage and characterize the species according to physical variables. In order to draw the radiographic patter, the seeds were submitted to different treatments: seed imbibition in rolled paper at 20 ºC for 48 hours; artificial damage by puncturing dry and imbibed seeds; artificial damage by fracturing dry and imbibed seeds. Seed anatomy structures were measured with a digital caliper and a manual caliper. The digital radiographic measurements could: a) replace the measurements taken with a manual caliper; b) obtain measurements that a manual caliper cannot supply; c) measure the air chamber in order to determine the level of seed filling; d) characterize the species by its anatomical and morphological structures, detect insect damage or fracture and make a radiographic pattern of G. biloba seeds.

Keywords: X-ray. Forest Seed. Anatomical and Morphological Structures.

RAIOS X: CARACTERIZAÇÃO DE SEMENTES DE *Ginkgo biloba* L.

RESUMO - Os objetivos deste trabalho foram: a) verificar se as medições das imagens radiográficas digitais de sementes de *G. biloba* poderiam se relacionar com aquelas obtidas pelo paquímetro manual; b) determinar o grau de desenvolvimento das sementes através de medições digitais da câmara do ar das sementes e c) realizar um padrão radiográfico para caracterizar as espécies de sementes de acordo a suas estruturas anatómicas e morfológicas, identificar danos físicos e caracterizar as espécies de sementes de acordo com suas estruturas anatômicas e morfológicas. Com o objeto de realizar o padrão radiográfico, as sementes foram submetidas a diferentes tratamentos: embebição em rolos de papel a 20 ºC por 48 horas; dano artificial por puncionamento das sementes secas e embebidas; dano aritificial por fratura das sementes secas e embebidas. As estruturas anatômicas das sementes foram medidas com paquímetro digital e manual. As medições radiográficas digitais permitiram: a) substituir as medições realizadas com o paquímetro manual; b) realizar medições que o paquímetro manual não pode realizar; c) medir a câmara do ar para determinar o grau de enchimento da semente; d) caracterizar as espécies por suas características anatômicas e morfológicas de suas estruturas, detectar danos por insetos ou fraturas e realizar um padrão radiográfico de sementes de *G. biloba*.

Palavras-chave: Raios X. Sementes florestais. Estruturas anatômicas e morfológicas.

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INTRODUCTION

The only living species of the ginkgopsida (Ginkgoaceae), *G. biloba* L. (Teillier, 2000), presents the appearance of a tree with strong monopodial growth, reaching up to 25-30 m (80-100 ft) in height.

Strasburger (1872) described *Ginkgo* embryology, and Dimitri and Parodi (1972) described *G. biloba* as a dioecious tree of great dimensions, with branches extending laterally and falling leaves. It presents yellowish flowers, the male are amentaceous and the female reduced to two bare ovules, supported by a long common stem. It has drupaceous seeds, yellow, 1.5 to 2.5 cm (0.5 to 1 in) long. It's an ornamental species that flourishes in spring and fructifies at the end of summer and in autumn. It reproduces through seeds, roots and shoots. The leaves of this tree exhibit a beautiful yellow color –golden in autumn- and its fleshy seeds are malodorous.

One of the ways of studying the quality of the seeds of vegetal species is through X-rays, widely utilized in Medicine, Biology and different industries (ISTA, 2003). On the other hand, the Association of Official Seed Analysts published an X-ray manual on the use of this technique in seeds of agricultural and forest species (BELCHER; VOZZO, 1979). ISTA (1991) also published a manual on seeds of trees and shrubs.

Several researchers have worked with seeds of different species applying the X-ray technique SIMAK; SATTLEEN, 1981; MARINVAL; CHAVAGNAT, 1983; CHAVAGNAT, 1984; SAUTON et al., 1988; FOLMER, 1988; CHAVAGNAT, 1988; SALINAS et al. (2008a y b); SALINAS et al. (2009a y b). The advantages observed in the use of this methodology were related with seed quality aspects such as: level of filling in seed and fruit; presence of foreign agents within seminal structures; different types of physical damage, and integrity of the different seminal structures (CHAVAGNAT; LE LEZEC, 1984; SCHATZKI; FINE, 1988; CHAVAGNAT, 1990; CAUBEL; CHAVAGNAT, 1992; BOUVIER et al., 1992).

Forest tree seeds have been analyzed through X-rays for a long time, as Stockwell (1942) demonstrated. He worked with *Pinus* species seeds in order to compare the embryo size with its growth rate. Later, when the X-ray technique was more accesible and there was a growing demand for tree seeds, it became a quick routine test in laboratories (SOCIETY OF AMERICAN FORESTERS, 1966). Some of the studies mentioned were conducted employing X-ray equipment used in the human health area. However, the present work employed X SEMAX equipment, specifically designed for seed analysis (CRAVIOTTO et al., 2004).

The objective of the research was: a) verifying if digital measurements of the size of the *G. biloba* mesotesta (Font Quer, 1970) and the megagametophyte (BEWLEY & BLACK, 1994) taken with the Visualix software were able to replace the measurements obtained with a manual caliper; b) determining the level of seed development by digitally measuring the air chamber, and c) establishing a radiographic pattern with its corresponding photographic images to characterize the species according to the anatomical and morphological features of the seeds and d) identifying physical damage caused by insects.

MATERIAL AND METHODS

A SEMAX (INTA-TEXEL, Argentina) X-ray equipment was used with a power of 35 kW, an intensity of 10 mA and an exposition time of .65 seconds. The system for capturing and digitalizing images is Visualix (2000). It provides immediate digitalization of the radiographic images obtained, which are sent to the computer and may be displayed on the computer monitor with the VixWin (2000) software.

A sample of 20 seeds was used and the length (mm) and width (mm) of the mesotesta were measured with a manual caliper and with VixWin software. The digital radiographic images also allowed us to determine the presence or absence of embryo.

The air chambers of a sample of 11 seeds were measured taking into consideration the thickness of the chamber in the: upper, lower, left and right areas.

In order to describe the size, the level of seed filling and the physical features considering size and embryo presence, we employed a multivariate analysis of Principal Components and an Analysis of Conglomerates, which according to the measured variables allowed us to observe how they were distributed in reference to their size.

To make a pattern of digital radiographic images, we took corresponding digital photographic images.

Before taking the digital radiographic and photographic images, the seeds were submitted to different treatments: a) imbibition in rolls of paper at room temperature (20 ºC) for 48 hours; b) artificial damage by puncture, simulating insect attack on dry seeds; c) artificial damage by fracturing dry seeds; d) artificial damage by puncture, simulating insect attack on imbibed seeds, and e) artificial damage by fracturing imbibed seeds.

The data submitted to the multivariate analysis of principal components, allowed us to describe the physical characteristics of the studied seeds through variables obtained by this method, which are linear combinations of the original variables.

RESULTS AND DISCUSSION

SEMAX equipment has been employed to perform X-ray analysis of forest species seeds, among others, and to obtain digital radiographic images.

The VixWin software allowed us to digitally measure the length (mm) and width (mm) of the mesotesta, the length and width of the megagametophyte, the presence and length of embryos and the thickness of the air chamber found between the endotesta that surrounds the megagametophyte and the mesotesta. The endotesta is very thin and it is attached to the megagametophyte. With a manual caliper we measured the length and width of the mesotesta and verified that the digital measurements on the radiographic image were able to replace the ones obtained with a manual caliper. In addition, the digital measurements allowed us to measure the internal parts of the seed and the air chamber.

Table 1 shows that the first principal explained 57% of the total data variability and the 3 first principal components together explained 97%.

Table 1. Variance proportion explained for each principal components (PC).

PC	Value	Proportion	Accumulated proportion
1	4.55	0.57	0.57
2	1.63	0.20	0.77
3	1.14	0.14	0.97

Table 2 shows the correlations of the original variables with the three first axes of principal components.

Each PC is a linear combination of the variables (coefficient x variable). First PC explain the greater percentage variation and the others PC explain the lesser percentage. The PC analysis is a multivariable descriptor.

Table 2. Correlation of the three first principal components with the original variables.

Variable	PC 1	PC 2	PC 3
Digital length of the mesotesta	-0.86	0.24	-0.27
Digital width of the mesotesta	-0.83	0.36	-0.26
Digital length of the embryo	-0.53	-0.83	-0.05
Digital length of the megagametophyte	-0.71	0.05	0.68
Digital width of the megagametophyte	-0.72	0.24	0.63
Caliper length of the mesotesta	-0.89	0.07	-0.26
Caliper width of the mesotesta	-0.86	0.15	-0.27
Embryo presence	-0.54	-0.82	0.01

Cophenetic correlation = 0.988

Correlations between the variables of length and width of the mesotesta measured by both methods were also calculated. The correlation coefficient between the length of the mesotesta measured digitally and with a manual caliper was r = 0.93; ($P < 0.01$). Likewise, the width of the mesotesta by both methods showed a high correlation r = 0.89; $P<0.01$, which would indicate that digital measurement is able to replace manual measurements.

The first Principal Component separated the seeds with greater mesotesta and megagametophyte length and width from those that showed lesser mesotesta and megagametophyte length and width (Figure 1).

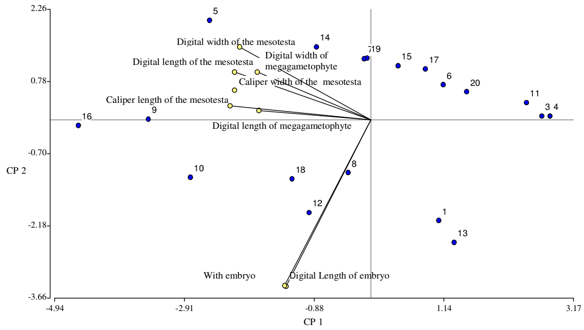


Figure 1. Biplot of the first and second principal components in *G. biloba* seeds

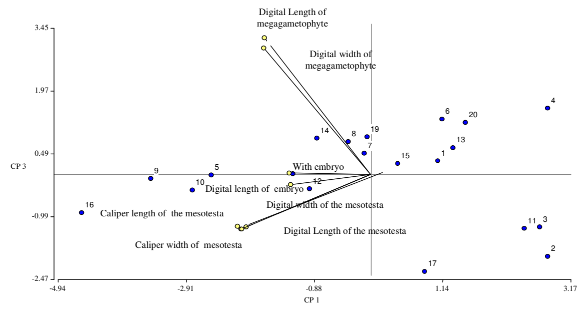


Figure 2. Biplot of the first and third principal components in *G. biloba* seeds.

The second Principal Component separated the seeds with embryo presence from those with none and the digital length of the embryo, and the third Principal Component separated the seeds of greater length and width measured digitally from those that showed less length and width (Figure 2).

The dendrogram obtained through the Analysis of Conglomerates (Ward Method) (Figure 3) showed two main groups of seeds: with embryo and without embryo. Within each of them, 3 subgroups may be identified. The seeds with embryo were

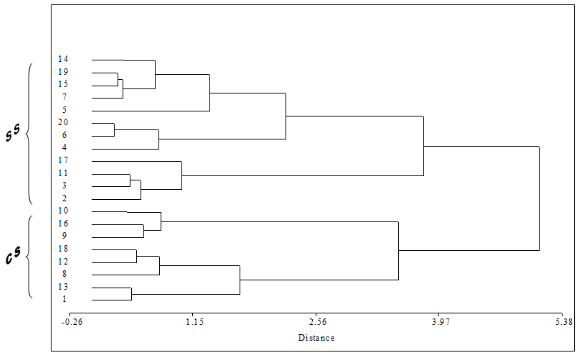


Figure 3. Dendrogram obtained through the analysis of conglomerates (Ward Method) of *G. biloba* seeds with and without embryo. SS: without embryo; CS: with embryo

grouped in the following subgroups: bigger seeds (16, 9 and 10), medium seeds (8, 12 and 18), and smaller seeds (1 and 13). The seeds without embryo were smaller than those with embryo, divided in the following subgroups: bigger seeds (5, 15, 14, 7 and 19), medium seeds (20, 6 and 4) and smaller seeds (2, 3, 11 and 17).

The analysis of the Principal Components in measuring air chamber thickness showed that the first Principal Component explained 75% of the total variability in the observations and the three first Principal Component together explained 97% (Table 3).

Table 4 shows the correlations of the original variables with the three first Principal Components axes.

The first Principal Component (Figure 4) separated the seeds according to their size, the ones with more filling on the right of the graph and the smaller ones on the left, and according to thickness top and left of the image; the second Principal Component separated them mainly according to air chamber thickness in the lower part and the third Principal Component according to the thickness of the right.

Table 3. Variance proportion of each Principal Component for seed air chamber.

PC	Value	Proportion	Accumulated Proportion
1	2.99	0.75	0.75
2	0.52	0.13	0.88
3	0.37	0.09	0.97

Table 4. Correlation of the first three Principal Components with the original variables for the seed air chamber.

Variable	PC 1	PC 2	PC 3	
Upper area		-0.90	0.35	-0.10
Lower area		-0.79	-0.57	-0.23
Left area	-0.92	0.24	-0.19	
Right area		-0.85	-0.10	0.52

Cophenetic correlation = 0.997

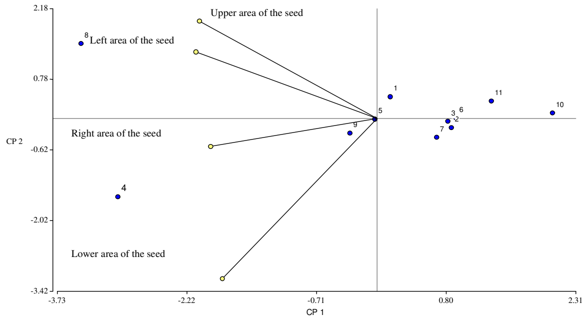


Figura 4. Biplot of the variables distribution and the *G. biloba* seeds according to the principal components analysis.

The dendrogram (Figure 5) obtained through the analysis of conglomerates (overlap average) using the Ward Method showed four groups defined by size: completely filled seeds (11; 10 and 6), filled seeds (3; 7 and 2), not completely filled seeds (9; 5 and 1), and scarcely filled seeds (8 and 4).

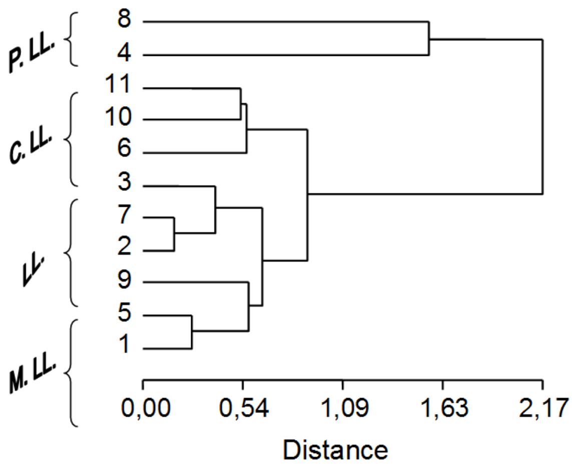
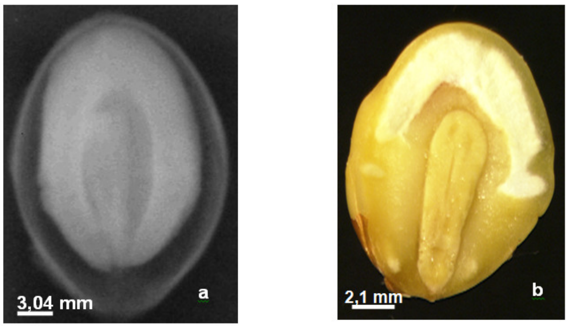


Figura 5. Classification of *G. biloba* seeds according to the size obtained through an analysis of conglomerates (overlap average). P. LL.: poorly filled seeds; C. LL.: completely filled seeds; LL.: filled seeds; M. LL.: not completely filled seeds

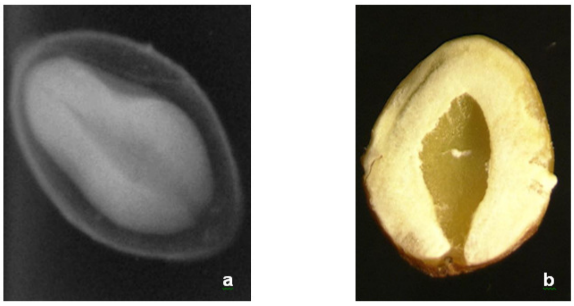
Multivariate statistical analyses of the air chamber measurements between the mesotesta and the endotesta of the seed allowed us to perform a good evaluation of the level of seed filling in the studied species. In addition, measurements of the air chamber and internal parts of the seeds may be taken exclusively with digital tools, since the manual caliper is not able to measure the air chamber. On the other hand, measurements of the internal parts of the seeds taken with a manual caliper would imply using a destructive method.

The multi-varied analysis employed allowed us to characterize the seed according to its anatomical constitution and the presence or absence of embryo.

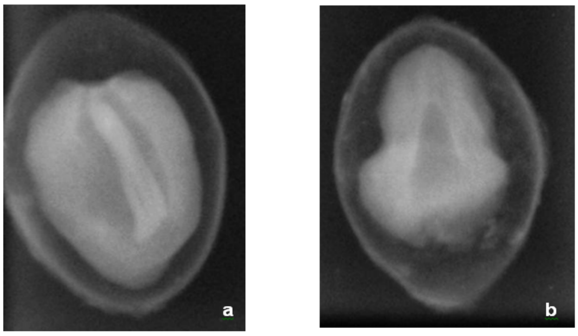
By making a pattern of digital radiographic and photographic images (Figure 6), we were able to



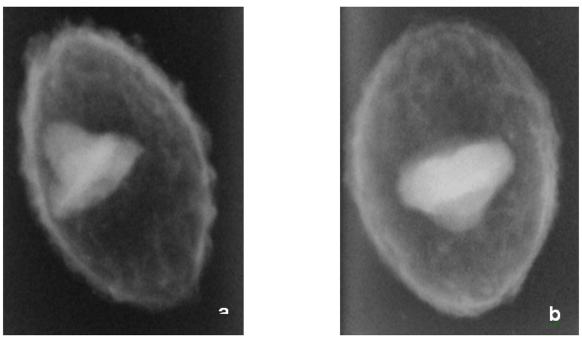
a) Digital radiographic and photographic image of *G. biloba* filled seed.
a: radiographic image; b: photographic image.



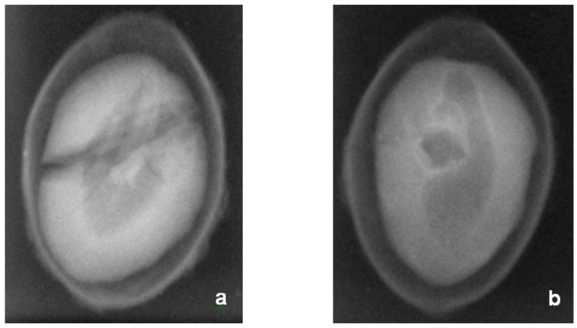
b) *G. biloba* seed without embryo.
a: radiographic image; b: photographic image.



c) *G. biloba* seeds with development 50% above their size.
a; b: radiographic images.



d) *G. biloba* seeds with development 50% below their size.
a; b: radiographic images.



e) *G. biloba s*eeds artificially fracturing and punctured.
a; b: radiographic images.

Figure 6. Pattern of digital radiographic and photographic images of *G. biloba* seeds.
a) Digital radiographic and photographic image of *G. biloba* filled seed; b) *G. biloba* seed without embryo; c) *G. biloba* seeds with development 50% above their size; d) *G. biloba* seeds with development 50% below their size; e) *G. biloba s*eeds artificially punctured.

observe a clear difference between seeds with different levels of filling, seeds with one or two embryos and without embryo, seeds with atrophied embryos, seeds with aborted embryos, seeds with artificial damage simulating insect attack and fractures. Additionally, the seeds submitted to different treatments showed the same level of image sharpness.

Physical characterization of seeds was determined by de degree of development and by the injuries made artificially by puncturing and fracturing. These injuries simulated those produced by insects, harvest and/or conditioning of seeds and they were observed by radiographic images.

Digital radiographic images obtained by different treatments for the realization of the radiographic pattern of species in study, did not show differences in the degree of clarity. Nevertheless, the radiographic images allowed to make a good differentiation between poorly filled seeds and completed filled seeds, and seeds with artificial damage simulating the attack of insects and fracturing. The use of X-ray technique was a good tool to characterize *G. biloba* seed lots by its anatomy and visualization of the damages simulating that produced by insects. The results of this work agreed with the works made by Yates (1974) and Havel (1974), who made studies of the damages produced by insects in seeds by the use of the X-ray technique. Kamra (1976) considered that insects can damage seeds and fruits of different ways. Damage produced by insects is generally made by a deposition of their eggs inside fruits or seeds and then when larvae develop feed on the content of them. Another damage produced by insects is the introduction of their sucking bucal apparatus inserting enzymes that degrade their cell contents producing empty seeds inside cones in some forest species like *Pinus elliottii* (DEBARR, 1970; DEBARR; EBEL, 1973). Seeds and fruits can suffer mechanical damages during harvest and processing and they are shown by fractures and fissure, etc. (KAMRA, 1963).

Seeds can be observed inside fruits without open them with the help of the X- rays. So, the number of seeds and their anatomy and empty fruits can be distinguished from fruits containing seeds without opening them (KAMRA, 1976). Salinas et al. (2008 a, b) and Salinas et al. (2009 a, b) made the following radiographic patterns of forest species: *P. elliottii, Taxodium distichum, Acacia longifolia and Albizzia julibrissin*.

Making a digital radiographic pattern with its corresponding digital photographs allowed us to characterize species, which complemented the research with the use of digital measurements.

CONCLUSIONS

The X-ray technique allows us to characterize *G. Biloba* seeds according to their anatomical-morphological features, detect insect damage or fractures and establish a pattern of radiographic images;

Digital measurements by radiographic images are able to replace those taken with a manual caliper;

Digital measurement of the seed air chamber determines their level of filling.

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