

GENOTYPE INFLUENCES ON ACORN NUTRIENT CONCENTRATIONS (*Quercus robur* L.)

Nikolic, N*, Orlovic, S., Krstic, B.

The objective of this study was to determine macronutrient (N, P, K, Ca, Mg and Na) concentrations in different parts of English oak's acorn to estimate genotype variability of these traits. Acorns were collected during the 1998 harvest from seventeen *Quercus robur* L. genotypes. Trees originate from vegetative seed plantation Banov Brod, belonging to a Forest Estate Sremska Mitrovica (Srem, Vojvodina, Serbia). The investigation of nutritional status of acorns revealed the significant differences among the studied genotypes. Considering all genotypes, the general sequence of the nutrient amounts for cotyledon tissue was as follows: N>K>Ca>P>Mg>Na. Concentrations of Ca and Mg were higher for pericarp and seed coat than for cotyledon tissue. Pericarp and seed coat nutrient concentrations showed lower variability compared with cotyledon's, with the exception of potassium concentration.

Key words: acorn, macronutrients, N, P, K, Ca, Mg, Na, oak, *Quercus robur*

*Natasa Nikolic
Faculty of Natural Sciences
Department of Biology and Ecology
Trg Dositeja Obradovica 2
21000 Novi Sad

STORAGE OF SCARIFIED *ACACIA CYANOPHILLA* SEEDS

Beti Piotta¹, Elisabetta Falleri², Raffaello Giannini², Giuseppe Tranne³

¹Agency for the Protection of the Environment and for Technical Services, Via V. Brancati 48, 00144 Rome. piotto@apat.it

²Department of Science and Technologies of the Forest Environment (DISTAF), University of Florence, Via San Bonaventura 13, 50145 Florence. efalle@tin.it

³Sustainable Development Service, Ministry of the Environment and Territorial Protection, Via Cristoforo Colombo 44, 00147 Rome

ABSTRACT

Untreated and mechanically scarified seeds of *Acacia cyanophylla* were stored for 0, 6, or 12 months at +3°C or -3°C, in sealed containers or in vacuum packing. Scarification was performed in an *ad hoc* modified Forsbeg scarifier operated electrically. Twenty-four different treatments, studied through a multifactor design, were experienced. Considering the effect of single factors on germination, mechanically scarified seeds gave the most rapid and complete germination compared to untreated ones. No influence was recorded for storage temperature and kind of container. Highly significant differences for storage periods were found: seeds stored for 12 months attained higher germination percentages than seeds stored for 0 or 6 months. Whatever the storage duration, scarified seeds behaved much better than untreated ones. As no loss in germination was observed it seems reasonable to assume that storage for at least 12 months is highly safe for scarified seeds. These results, having practical applications for commercial seedsmen or nurserymen, express a good utilisation of simple, modern seed technology since seed can be scarified properly and then easily stored until delivery.

KEYWORDS

Acacia cyanophylla Lindl., seed scarification, seed storage, physical dormancy, hardseededness.

CORRESPONDING AUTHOR

Beti Piotta

Relationship between cone weight and seeds traits as revealed by several years study on *Cedrus atlantica* individuals at Tala-guilef (Djurdjura, Algeria).

F. KROUCHI and A. DERRIDJ

Laboratoire de Biosystematique forestière
Faculté des Sciences Agronomiques et Biologiques
Université Mouloud Mammeri (Tizi-Ouzou, Algérie)
E-Mail : krouchi@yahoo.com

Summary

Cone weight, total number of seeds per cone, amount of filled seeds, weight of filled seeds and their *in vitro germination* were examined during several years on identified individuals of *Cedrus atlantica* in a natural stand located at Tala-guilef Djurdjura north westerner.

At cone level the results revealed a positive correlation between cone weight and seed weight indicating that heavy cones bear heavy seeds, while other parameters did not show particular relationships. These results suggest that during harvesting foresters cannot rely on cone weight as a good predictor of seed amount and germination.

At tree level the results did not show any trade-offs between seeds traits (for example weight and number) and number of cones produced by a tree. The advantage for any tree of being highly fertile in terms of number of cones produced is not particularly combined to additional advantage in terms of high number of seeds contained in these cones.

Key words: *Cedrus atlantica*, cones, seeds, *in vitro* germination, correlation, Algeria.

GERMINATION CHANGES OF *PICEA ABIES* (L.) KARST. SEEDS AT WATER-BASED PRETREATMENTS

Anu Hilli¹, Eila Tillman-Sutela¹ and Anneli Kauppi²

Finnish Forest Research Institute¹, Kirkkosaarentie, FIN-91500 Muhos, Finland
University of Oulu², Department of Biology, P.B. 3000, FIN-90401 Oulu, Finland

Various pretreatments of seeds are a prerequisite for mechanized seedling production in modern forest tree nurseries. However, treatment results of Norway spruce seeds are frequently unsatisfactory. The aim of this work was to study changes in the germination indices of spruce seeds during the multi-phased pretreatment chain and the impact of seed structures on these changes. Furthermore we studied the effect of short-term storage on germination indices of the pretreated seeds. Seeds were extracted using the ordinary method by sprinkling water on the cones or restricting that amount of water sprinkled on the cones. Cleaned and dried seeds were stored at $-18\text{ }^{\circ}\text{C}$ for three months prior to IDS-treatment. The changes in germination indices were studied using germination tests and radiography. Seed structures were observed using SEM. In general the germination parameters of both seed lots increased during the pretreatment chain despite the opening of the seed coat, which had already occurred at the extraction phase. The seed coat usually opened from the micropyle almost until the edge of the nucellar cap. The IDS-treatment succeeded well and the germination parameters in the best seed fractions were 13-28 % better than in the unsorted seed batches prior to IDS-treatment. The germination indices remained nearly unchanged during one year's freeze storage. This indicates that the large nucellar cap typical for spruce seeds protected the megagametophyte and the embryo from oxidation and degradation during pretreatment phases and short-term storage.

Freezing response in Scots pine (*Pinus sylvestris* L.) seeds of different moisture contents as assessed by differential scanning calorimetry (DSC) and germination test

Gunnar Sven Pamuk*, Urban Bergsten, and Philippe Lingois

Abstract

Responses to freezing temperatures of Scots pine (*Pinus sylvestris* L.) seeds in early germination stages were examined for dry (7%), 15%, 20%, 25% and 30% moisture content using differential scanning calorimetry (DSC) to possibly identify critical moisture content at freezing. In a complementary experiment seeds of the different initial moisture contents were incubated for 2 or 5 days, and subsequently exposed to two freezing temperatures (-5°C or -20°C) during two different time periods (2 days or 5 days), and germination test (21 days, 20°C). In the DSC experiments, seeds with higher moisture content (20%, 25%, and 30%) had a significantly wider endothermic peak (-30.51 J/g, -40.36 J/g, and -47.02 J/g) than seeds with lower moisture content (15% and dry seeds; -6.94 J/g and -0.65 J/g). Germination capacity after freezing was significantly higher (81%-83%) for seeds with moisture contents from dry to 20%, compared to seeds with moisture contents between 25%-30% (68%-72%). Control seeds (no freezing exposure) had a germination capacity of 97%. A critical moisture content of Scots pine seeds seems to exist between 15%-20% moisture content according to the DSC and the germination test. The desire to identify in pine seeds the critical moisture content of mortality from freezing temperatures is required for future water uptake studies with autumn direct seeded pine seeds, and the development of seed coating that restricts water uptake under certain critical moisture content.

- Gunnar Sven Pamuk* and Urban Bergsten, Swedish University of Agricultural Sciences, Department of Silviculture, Svartberget, 922 91 Vindeln, Sweden.

- Philippe Lingois, Luleå University of Technology, Division of Polymer Engineering, 971 87 Luleå, Sweden.

* Corresponding author. Received _____.

Improvement in storability of ashwagandha (*Withania somnifera* Dunal) seeds through pre-storage treatments by triggering their physiological and biochemical properties

**V. Vakeswaran and V. Krishnasamy
Department of Seed Science and Technology
Tamil Nadu Agricultural University
Coimbatore – 641 003, Tamil Nadu, India.**

Withania somnifera is an important medicinal plant being cultivated in India and is propagated mainly through seed. Seeds lose their initial viability of 40-50% rapidly and reach 5% at the end of one year. Leaves of *Azadirachta indica* (neem), rhizome powder of *Curcuma longa* (turmeric) and *Acorus calamus* (vasambu) have been reported to possess anti-insect properties. Leaf powder of *Albizia amara* (arappu) has been reported to improve the germination of stored seeds. Acetyl salicylic acid is an antioxidant. Halogenation with iodine helps to stabilize the lipo-protein membranes. Keeping these in view, graded seeds were subjected to pre-storage treatments with leaf powders of neem and arappu, rhizome powder of turmeric and vasambu, acetyl salicylic acid and iodination, and stored in 700 gauge polyethylene bags. Bimonthly evaluations were made on seed germination, seedling growth and biochemical attributes viz., protein, enzymes (amylase and catalase), lipid peroxidation and electrical conductivity. Iodination was better in controlling seed deterioration process. The physiology and biochemistry of improvement in storability are discussed.

Effect of seed priming in Pea (*Pisum sativum* L.) seeds

V. Vakeswaran, A. Vijayakumar and R. Jerlin
Department of Seed Science and Technology
Tamil Nadu Agricultural University
Coimbatore – 641 003, Tamil Nadu, India.

The technique of hydration and dehydration was adopted to improve the germinability and vigour of 8 months old seeds of pea cv. Bonneville. The old seeds soaked in the solution of oxalic acid at 10^{-3} M for 6 hours and dried to their original weight, improved the germination percentage and vigour of the seedling. The percentage increase in germination was to the tune of 32.0% over the control and water soaked and dried seeds other chemicals like tannic acid, sodium dihydrogen phosphate (hydrated), disodium hydrogen phosphate, sodium chloride and boric acid were also tested but did not increase the germination as well as seedling vigour. Sodium chloride (2×10^{-4} M) decreased the germination percentage and vigour of the seedling in both fresh and old seeds.

Standardization of germination test procedure in Ashwagandha (*Withania somnifera* Dunal)

V. Vakeswaran and V. Krishnasamy
Department of Seed Science and Technology
Tamil Nadu Agricultural University
Coimbatore – 641 003, Tamil Nadu, India.

Ashwagandha (*Withania somnifera* Dunal), Family Solanaceae is an important cultivated medicinal plant of India, which is erect herbaceous, evergreen, tomentose shrub and grown for its roots which are stout, fleshy, cylindrical and are one to two cm thick. It is commonly known as 'Winter Cherry' in English. The seed testing procedure for conducting the germination test for most of the medicinal plants is yet to be standardised and incorporated in the seed testing manual. Therefore, standardization of optimal conditions required for germination such as, temperature and media are important for getting reproducible results in the laboratory vis-à-vis assessment of the near planting value of the seed lots.

Four replicates of 100 seeds each were subjected to the following test conditions in order to standardize the optimum temperature and suitable medium for germination and to finalize first and final count days. Temperature [T_1 : 20°C constant; T_2 : 25°C constant; T_3 : 30°C constant; T_4 : 20 & 30°C (16 h and 8h, respectively) and T_5 : 25 & 30°C (16 h and 8 h, respectively)], Substrate : Sand (S), Roll towel (RT) and Top of paper (TP).

In the present investigation, the alternate temperature of 25 & 30°C recorded the lowest days for germination initiation (4.0 days), days for first count (6.0 days) and the highest germination (63.0 per cent), rate of germination (3.91), root (2.6 cm) and shoot (2.94 cm) length of seedlings and vigour index (360). It could be concluded that seeds of ashwagandha should be exposed to alternating temperature of 25-30°C and placed on top of paper medium for conducting the laboratory germination test.

When the germination test was conducted with top of paper medium at alternating temperature of 25-30°C, the radicle emergence occurred even at fourth day (23 per cent) and the germination reached the highest at 12th day (62 per cent) and retained as such upto 17th day, thereafter mortality of seedlings occurred. On 18th day the mortality was two per cent and increased as the period advanced. Hence, the final count for germination test for ashwagandha can be made on 17th day.

Physiological and biochemical changes during seed development and maturation in Ashwagandha (*Withania somnifera* Dunal)

V. Vakeswaran and V. Krishnasamy
Department of Seed Science and Technology
Tamil Nadu Agricultural University
Coimbatore – 641 003, Tamil Nadu, India.

Ashwagandha (*Withania somnifera* Dunal), Family Solanaceae is an important cultivated medicinal plant of India, which is erect herbaceous, evergreen, tomentose shrub and grown for its roots which are stout, fleshy, cylindrical and are one to two cm thick. It is commonly known as ‘Winter Cherry’ in English. Tracing the pattern of seed development and assessing the time of maturity have great practical utility to obtain seeds with good quality characteristics. To gain an insight into the source-sink relationship and to manipulate the agronomic practices, the knowledge on morphological, physiological and biochemical changes occurring in the developing seed assumes significance.

Seed crop of *W. somnifera* was raised during March, 2000 in four beds of size 3 x 2 m² to form four replications at Tamil Nadu Agricultural University farm, Coimbatore. About 250 flowers were tagged at the time of anthesis with the detail of date of flowering in each replication. Sufficient number of fruits were collected at seven days interval from the date of tagging viz., 7, 14, 21, 28, 35 and 42 days after anthesis (DAA).

The fresh and dry weight of seed attained maximum at 35 DAA recording 5.6 and 2.3 mg 10 seeds⁻¹, respectively and decreased thereafter. Physiological maturity is normally defined as the stage at which the seed attain its maximum dry weight.

Eventhough the seeds started germinating in the earlier stages of development, it was increasing with increase in maturity of seed. Maximum germination of 63.5 per cent was reached at 42 DAA. The maximum electrical conductivity of seed leachate (0.980 dSm⁻¹) was registered at seven days after anthesis. As maturity advanced it decreased and reached the minimum (0.069 dSm⁻¹) at 42 DAA indicating that membrane integrity was maximum at this stage.

The highest activities of α -amylase and catalase occurred at seven days after anthesis in the developing seed which coincided with the highest moisture level. Thereafter, the activities of both the enzymes reduced and reached the lowest level at 42 DAA.

**Effect of boron on seed yield and resultant seed quality in pea
(*Pisum sativum* L.)**

V. Vakeswaran, A.Vijayakumar and R. Jerlin
Department of Seed Science and Technology
Tamil Nadu Agricultural University,
Coimbatore-641 003, Tamil Nadu, India.

A field experiment was conducted to study the effect of boron on seed yield and its influence on the resultant seed quality in pea (*Pisum sativum* L.). Aqueous solution of borax in different concentrations (1000, 2000, 3000, 5000 and 7000 ppm) were sprayed at flowering stage. Observations were made on number of pods plant⁻¹, pod yield plant⁻¹, number of seeds pod⁻¹, seed yield plant⁻¹, shelling outturn, and 100 seed weight. The results indicated that all the parameters were found to increase with the increasing rates of B application. Harvested seeds were then analysed in the laboratory for its physiological and biochemical characters. Percentage germination, seedling drymatter production, vigour index, electrical conductivity, protein content and free amino acids exhibited positive relationship with the increased application of B.

Quantitative and qualitative changes in seed quality during seed development and maturation in pea (*Pisum sativum* L.)

V. Vakeswaran, A. Vijayakumar and R. Jerlin
Department of Seed Science and Technology
Tamil Nadu Agricultural University
Coimbatore – 641 003, Tamil Nadu, India.

Full germination capacity and vigour cannot be attained until seeds reach physiological maturity. This study was designed to assess the quantitative and qualitative changes in pea (*Pisum sativum* L.) seed quality during development and maturation and to fix the physiological maturity stage. A field trial was conducted with pea cv. 'Bonneville' and seeds were harvested in 7 days interval from the starting of flowering. Seeds were assessed for its quality parameters in the laboratory. Seed dry weight attains maximum at 49 DAA which is being concluded as the physiological maturity stage. Seed moisture content at this stage was 30%. The onset of ability to germinate occurred at 21 DAA. Maximum seedling dry weight and vigour index occurred at 49 DAA. Electrical conductivity and free amino acid reduced as maturation of seed advanced but the seed protein content found to be increased.

**Effect of spacing and fertilizer level on growth, seed yield and resultant seed quality
in pea (*Pisum sativum* L.) cv. Bonneville**

V. Vakeswaran, A. Vijayakumar and R. Jerlin
Department of Seed Science and Technology
Tamil Nadu Agricultural University
Coimbatore – 641003, Tamil Nadu, India.

The treatments with four spacings (2.5, 5.0, 7.0 and 10 cm between plants and a uniform spacing of 40 cm between rows) and three levels of NPK fertilizers (80:60:50; 120:80:70 and 160:100:90 kg ha⁻¹) were imposed on seed crop of pea (*Pisum sativum* L.) cv. Bonneville. Observations were made on days to first flowering, plant height, number of pods plant⁻¹, pod yield plant⁻¹, number of seeds pod⁻¹, seed yield plant⁻¹, shelling outturn and 100 seed weight. All the parameters were significantly influenced by spacings and fertilizer levels. With every increased level of NPK fertilizer and spacing, generally these characters showed favourable response. Germination potential, seedling growth, vigour index, dry matter production, electrical conductivity, protein content and free amino acids of the resultant seeds also have positive association with increased fertilizer levels and spacing.

Technology for synthetic seed development in Aswagandha (*Withnia somnifera* Dunal)

V. Vakeswaran and V. Krishnasamy
Department of Seed Science and Technology,
Tamil Nadu Agricultural University,
Coimbatore -641 003, Tamil Nadu, India.

Aswagandha is one of the important medicinal plants being cultivated in India. Though seed is the primary propagating material in this crop, the germination potential of the seeds is very low. The synthetic seed technology is one of the promising biotechnological tools which is being practically utilized in major forage crops like *Medicago sativa*. Ever first attempt was carried out to bring out this viable tool to this important medicinal plant - Aswagandha. Standardization of media composition for somatic embryogenesis is the first step in synthetic seed technology. As a first step in this, several combinations of media compositions were tried with different explants for callus induction. The leaf explant in the MS medium supplemented with 2, 4-D (4 mg l^{-1}), kinetin (0.2 mg l^{-1}) and CH (500 mg l^{-1}) produced maximum callus. These calli were then transferred to several combinations of media compositions for somatic embryo production. MS medium supplemented with 2, 4-D (0.5 mg l^{-1}), kinetin (0.2 mg l^{-1}), BAP (1 mg l^{-1}) and CH (500 mg l^{-1}) resulted in maximum somatic embryos. To confirm this, microtomic studies were carried out and confirmed as somatic embryos. The next step in synthetic seed production is to standardize the optimum encapsulating material for these somatic embryos. Sodium alginate at 3% in combination with calcium chloride at 1%, produced stable beads. To maximise the germination of synthetic seeds, study on inclusion of artificial endosperm has been carried out and it revealed that synthetic seeds with already standardized encapsulating materials in combination with half strength MS medium, BAP (2 mg l^{-1}), IBA (0.2 mg l^{-1}), activated charcoal (0.1 mg l^{-1}) and bavistin (0.5 mg l^{-1}) as artificial endosperm improved the germination and conversion potential of synthetic seeds in aswagandha.

**Influence of plant growth regulators in germination of
Withania somifera Dunal seeds**

V. Vakeswaran and V. Krishnasamy
Department of Seed Science and Technology
Tamil Nadu Agricultural University
Coimbatore-641 003, Tamil Nadu, India.

The influence of plant growth regulators like gibberellic acid (GA₃), indole butyric acid (IBA) and indole acetic acid (IAA) on the ashwagandha (*Withania somnifera* Dunal.) seed germination was studied. GA₃ was found to be more effective in improving the seed germination in ashwagandha than either IBA or IAA.

Variation in cone and seed traits among provenances of *Cedrus atlantica* Manetti in Algeria.

A. DERRIDJ and F. KROUCHI

Laboratoire de Biosystematique forestière
Faculté des Sciences Agronomiques et Biologiques
Université Mouloud Mammeri (Tizi-Ouzou, Algérie)
E-Mail : aderridj@ yahoo.fr

Cones and seeds were studied on 11 provenances of *Cedrus atlantica* in Algeria during two consecutive years. During year 1986 we analysed seed dimensions, amount of filled seeds and weight of 1000 seeds. During 1987 we measured the following parameters: cone dimensions and weight, total number of seeds in 100 cones, amount of filled seeds in 100 cones, weight of the total number of seeds, weight of 1000 filled seeds, number of filled seeds per hectolitre of cones, weight of filled seeds per hectolitre of cones.

The results obtained are discussed in regard to geographical range of the provenances and their potential to provide seeds for reforestation programs.

Keys words: *Cedrus atlantica* Manetti, seed yield, cone dimensions, seed dimensions, provenances, Algeria.

ETHEPHON (2-CHLOROETHYLPHOSPHONIC ACID) COMBINED WITH
SHORT PRECHILLING IMPROVES GERMINATION IN STORED
BEECHNUTS.

Claudine Muller^{*}, Elyane Laroppe^{*}

^{*} INRA, Centre de Nancy, Unité de Recherche sur les semences forestières,
54280 CHAMPENOUX, France

Beechnuts are deeply dormant and require long cold prechilling before they germinate (one to three months). The methodology of pretreatment without medium at controlled moisture content applied before or after storage is the best method to take into account the dormancy and its heterogeneity within a seedlot (consequence of the genetic variability). It allows a very fast and grouped seedling emergence in the nursery. However, these necessary long prechilling may damage older, less vigorous seedlots. Thus, the effects of chemical treatments on dormancy breaking, in particular growth regulators, as a means of shortening the treatment have been studied. In previous experiments it has been demonstrated that ethephon strongly stimulates percentage and germination rate of freshly collected beechnuts and allows to reduce sometimes by half the duration of the cold phase in comparison with the classical pretreatment (which may need 4 to 20 weeks). In the present study ethephon + short prechilling (3 weeks) strongly stimulates germination and seedling emergence of beechnuts previously stored for 3 years at either 7 or 9% moisture content. It avoids the viability loss of less-

vigourous beech seeds during longer prechilling (5 weeks) and it allows to restore the initial potential of the seedlots. When seeds are short-prechilled with ethephon, no significant effect of the seed moisture content during storage is observed on seedling emergence in the laboratory. Used in association with short cold treatment, ethephon opens new prospects for eliminating dormancy, particularly in nurseries where short prechilling have many advantages.

Abstract of Paper 1.

Effect of Maturation on Seed Germination of *Dalbergia cochinchinensis* Pierre.

Le Quang Hung

Nong Lam University, Ho Chi Minh city, Vietnam

Germination of *Dalbergia cochinchinensis* Pierre seeds at maturity stage of green, yellowish-brown and dark brown fruits showed primary dormancy in incubator of 30°C/20°C (16h/8h). Germination percentage was highest for seeds from green fruits at 28 days, followed by yellowish-brown and dark brown fruits. After four weeks of storage, germination of seeds from yellowish-brown fruit was highest, and mean germination rate was lowest from seeds of green fruits.

Abstract of Paper 2.

Germination of *Citrus grandis* L. Seeds and Seed Greening Disease Testing by PCR (Polymerase Reaction Chain)

Le Quang Hung

Nong Lam University, Ho Chi Minh city, Vietnam

Seeds of *Citrus grandis* L. “Buoi Duong” and *Citrus grandis* L. “Buoi Thanh Tra” reached high germination percentage at 30/20°C (16/8h), germination of 97% at 43.7% moisture content (mc) and 93% at 39.1% mc, respectively. Germination percentages reduced with low moisture content by desiccation, 21% at 3.3% mc with “Buoi Duong” and 19.4% with “Buoi Thanh Tra”. The results of testing showed that these two seed lots were intermediate seed storage behavior. Seed of these lots showed negative with greening disease by testing with PCR (Polymerase Reaction Chain) and these seed lots were used as stock for grafting.

DNA Changes in Naturally and Artificially
Aged Longleaf Pine (*Pinus palustris* Mill.) Seeds

Tolentino, E. L., Jr.¹, Elam, W.W.², and Bonner, F.T.³

ABSTRACT

Total seed DNA of naturally and artificially aged longleaf pine seeds was quantified and analyzed. Natural aging consisted of storage at temperature controlled conditions (4° and 30°C) and natural forest conditions. Accelerated aging test (AAT) technique was used to artificially age seeds.

No consistent pattern of change in seed DNA of AAT-treated seeds was observed. Three lots showed increases in DNA after 90 days. Hyperchromism is the suspected cause. No definite pattern of change was recorded for seeds stored in temperature-controlled conditions especially the samples in 4°C. Seeds in 4°C conditions maintained at least 50% germination in three lots after 360 days. Storage at 30°C caused significant decline in viability after 360 days. Regression analysis revealed differences in the rate of change of DNA in the two aging regimes. The use of AAT to examine aging mechanisms may not be reliable. The high seed moisture contents and the massive fungal infection in AAT are probable reasons.

Gel electrophoresis revealed DNA fragmentation after 144 hours of AAT and 90 days of natural forest conditions. Incipient signs of fragmentation were observed in seeds stored at 30°C. No fragmentation was observed in DNA of seeds stored at 4°C even after 360 days. Big molecular weight fragments were possibly formed through the crosslinking of liquid peroxidation by-products and DNA for the following treatments: 240 or more hours of AAT, 4°C after 360 days, and all seeds at 30°C conditions. DNA fragmentation and crosslinking are presumed critical in viability loss of longleaf pine seeds.

KEYWORDS: Natural aging, artificial aging, seed aging, seed DNA, longleaf pine, accelerated aging test.

¹ Associate Professor, Silviculture & Resources Rehabilitation Division, Institute of Renewable Natural Resources, College of Forestry, University of the Philippines Los Baños, College, Laguna 4031, Philippines.

² Retired Professor, Department of Forestry, College of Forest Resources, Mississippi State University, USA.

³ Retired Principal Plant Pathologist, USDA Forest Service, Starkville, Mississippi.

Recalcitrant Behavior of Cherrybark Oak Seed: An FT-IR Study of Desiccation Sensitivity in *Quercus pagoda* Raf Acorns

Sharon Sowa¹ and Kristina F. Connor²

¹Department of Chemistry, Indiana University of Pennsylvania, Indiana, PA 15705, USA and ²U.S. Forest Service, Center for Bottomland Hardwoods Research, Box 9681, Mississippi State, MS 39762, USA

The recalcitrant behavior of cherrybark oak (*Quercus pagoda* Raf) acorns was examined in terms of effects of moisture content on seed storage longevity and (short term) seed germination. Seed samples collected over two consecutive years were fully hydrated, then subjected to drying under ambient conditions of temperature and relative humidity on the lab bench and sampled regularly for moisture determination (gravimetric analysis), and germination (greenhouse conditions). Fourier transform infrared spectroscopy (FT-IR) was used to follow changes in macromolecular structure as moisture and viability were lost. Transmission spectra were collected on dry and rehydrated samples of separate embryonic axis and cotyledon tissue. Long-term storage longevity was highly dependent on initial acorn moisture content. Germination was also highly dependent on short-term moisture content, and severely declined when seed moisture dropped below 17% (fresh weight basis). FT-IR analyses revealed significant differences in moisture and lipid profiles between embryonic axis and cotyledon tissue during short term drying. A strong absorbance near 1740 cm^{-1} in cotyledon tissue indicated a high concentration of ester carbonyl groups (storage lipids). Membrane lipid structure exhibited reversible shifts between gel and liquid crystalline phases upon drying and rehydration in both axes and cotyledons (peak frequency and bandwidth near 2850 cm^{-1}); however, reversibility declined as viability was lost. Irreversible changes in protein secondary structure, illustrated by shifts in the amide absorbance near 1650 cm^{-1} , were the most sensitive indicators of viability loss.

**Standardization of seed extraction methods in *Terminalia chebula* Retz.
(Kadukkai) and *Terminalia bellirica* Roxb. (Thandrikai)**

M. Sivaprakash¹, G. Dharmaraj², P.R. Renganayaki³ and M. Jayaprakasam⁴

Forest College and Research Institute
Tamil Nadu Agricultural University, Mettupalayam – 641 301

Terminalia chebula Retz. *T bellirica* Roxb, belongs to the family Combretaceae. The family is of great importance in forestry. The genus Terminalia is the source of a number of timbers of commercial importance and the myrobalans, which are the dried fruits of *T. chebula* and *T bellirica* and the most important among the different valuable products. The seed extraction is a problem. Hence, a study was conducted to standardize the best seed extraction method.

The better seed management practices started from extraction of the seeds. Faulty procedures if applied may reduce the germinability, physical damage and thus reduce storability. The purpose of extraction is to promote germination and to offer better seed handling practice. Series of treatments (totally 26) were tried to standardize better seed extraction method for both the species. Among the different methods tried, mechanical extraction was found to be best for both the species. In *Terminalia chebula* and *T. bellirica* mechanically extracted seeds (by seed hammering) completes it germination very early (43.00 days and 36.50 days respectively) when compare to other treatments. In *Terminalia chebula* and *T. bellirica*, the highest germination percentage and vigor index were recorded in mechanical extracted seeds (48% and 68%; 10.665 and 46.774 respectively). By visualizing all these observation, mechanical extraction is the best method of seed extraction for both the species. Mechanical extraction disrupt the barrier for the uptake of water by the seed and permits radical to occur emergence by weakening seed coat structures.

-
- 1) Ph.D. Scholar
 - 2) Associate Professor
 - 3) Assistant Professor
 - 4) Professor & Head

Dormancy breaking treatments and effect of growth regulators on germination improvement in *T. chebula* and *T. bellirica*

M. Sivaprakash¹, G. Dharmaraj², P.R. Renganayaki³ and M. Jayaprakasam⁴

Forest College and Research Institute

Tamil Nadu Agricultural University, Mettupalayam – 641 301

Seed dormancy refers to a stage in which viable seeds fail to germinate. Dormancy in seeds may be either advantageous or problematic during seed handling. Seeds, which have not been given pre-treatment to overcome dormancy, may fail to germinate altogether; germination may take place over a lengthy period. The purpose of pre-treatment is to ensure both fast and uniform germination of seeds.

The seeds of myrobalans possessed mechanical dormancy neither physical nor physiological factors are include. Simple removal of hard seed coat itself is enough to break mechanical barrier. A problem in *T. chebula* is the poor germination percentage of seed even after breaking the mechanical barrier. In *T. bellirica* the seed exhibits low germination in nature because of hard seed coat. Moreover the laboratory method of germination is not available in the International Rules for seed Testing for those species.

An experiment was conducted during 2000-2002 in Forest College and Research Institute, Mettupalayam with the objective of standardization of suitable dormancy breaking treatment for *Terminalia* spp. For this experiment, mechanically extracted seeds of *Terminalia chebula* and *T. bellirica* were used. Totally 29 various dormancy breaking treatments have been tried for *T. chebula*, and 36 treatments for *T. bellirica* have been tried to overcome the mechanical dormancy. Among the different dormancy breaking treatments, in *T. chebula* 2 days cold water soaking of mechanically extracted kernels gave better results in terms of faster rate of germination (1.45) early completion of germination (25.50 days), the highest germination per cent (84.00) and vigor index (23.465). In *T. bellirica*, mechanical extracted kernels gave better results in terms of speed of germination (1.98) highest germination percentage (95.5) and maximum vigour index (81.78) followed by KNO₃ 10% for 120 minutes. Scarification with H₂SO₄, cold water scarification also performed equally well. Hence, cold water soaking of mechanically extracted seeds of *T. bellirica* is suitable method to get good germination percentage.

Among the application of various growth regulators such as GA₃, IBA, IAA and NAA in different concentrations and different durations tried. IBA 200 ppm for 24 hours was found to be the best for the species *T. chebula* as it recorded early germination (26.50 days) and took lesser time to complete germination (40.50 days), when compare the other treatments. The germination percentage and vigour index were also higher in the same treatment (54.50 and 13.054 respectively). For *T. bellirica*, the seeds soaked in NAA for 12 hours registered higher germination percentage (96.00) and vigour index (93.028) and also it showed early initial germination (15.5 days) and took lesser days to complete

germination (32.5 days) when compared to other treatments. From the study it is inferred that *Terminalia* sp.seeds, may possessed with combinational dormancy of both mechanical and chemical that is why it performed for mechanical removal of abstraction (removal of seed coat) and for low concentration of IAA (nullifying the effect of inhibitor).

- | | |
|------------------------|------------------------|
| 1) Ph.D. Scholar | 2) Associate Professor |
| 3) Assistant Professor | 4) Professor & Head |

(Address for correspondence)

Dr.P.R.RENGANAYAKI,
Assistant Professor (Dept. of Seed Science and Technology),
Tamilnadu Agricultural University
Forest College and Research Institute,
Mettupalayam – 643103.
Tamilnadu.

Effect size and density of seeds on seed quality in *Terminalia chebula* and *Terminalia bellirica*

M. Sivaprakash¹, G. Dharmaraj², P.R. Renganayaki³ and M. Jayaprakasam⁴

Forest College and Research Institute
Tamil Nadu Agricultural University, Mettupalayam – 641 301

A number of studies in tree species showed that grading is an integral part of post harvest operations to enhance the planting value of seed lots. Grading that entitled to remove the empty, immature, broken and insect damaged seeds are done mainly to improve the physical and physiological quality of the seed lot.

In order to improve the seed quality, the studies were conducted during 2000-2002 in Forest College and Research Institute, Mettupalayam. In this study, the results of the size grading (by visual) of seeds indicated that medium and bigger size seeds performed better than the smaller size seeds in terms of early germination (28.00, 28.00), speed of germination (0.634, 0.631), germination percentage (49.25, 52.25), seedling height and vigour (14.69, 16.16) in *T. chebula* and *T. bellirica* respectively.

Density grading of Terminalias using water, 2% NaCl and 3% NaCl showed advantage rather than size grading. In all the three solutions sinkers performed better with early germination (27.00, 19.50), speed of germination (0.682, 1.375), germination percentage (50.50, 68.25), seedling height and vigour index (13.88, 53.137) in *T. chebula* and *T. bellirica* respectively. From the study it can be concluded as, by density grading using floatation techniques ill filled, air spaced and empty seeds of *T. chebula* and *T. bellirica* can be removed .

-
- 1) Ph.D. Scholar
 - 2) Associate Professor
 - 3) Assistant Professor
 - 4) Professor & Head

(Address for correspondence)

Dr.P.R.RENGANAYAKI,
Assistant Professor (Dept. of Seed Science and Technology),
Tamilnadu Agricultural University
Forest College and Research Institute,
Mettupalayam – 643103.
Tamilnadu.

Seed storage studies in *Terminalia chebula* and *Terminalia bellirica*

M. Sivaprakash¹, G. Dharmaraj², P.R. Renganayaki³ and M. Jayaprakasam⁴

Forest College and Research Institute

Tamil Nadu Agricultural University, Mettupalayam – 641 301

On account of the diverse biological activities taking place in seed during storage, seed deteriorative process in physical, physiological and biochemical attributes finally results in complete loss of viability i.e. death.

To study the viability status of the *Terminalia* seeds, an experiment was conducted during 2000-2002, in Forest College and Research Institute, Mettupalayam. The freshly fallen fruits of *T. chebula* and *T. bellirica* were collected from the ground and dried to a moisture content of 12% and packed in fresh gunny bag and stored under ambient conditions. To avoid pest and disease damage during storage the seeds were treated with Thiram and Bavistin @ 3 g/kg of seed. The results of this study revealed that the *Terminalia* species can be stored with pre-treatment of Thiram and Bavistin for a period of one year without any decline in germination, even with higher germination, seedling length and vigor index than fresh seeds. The results indicated that, when compare to the fresh seeds, the older seeds performed well in terms of early and better germination percentage, indicating the release of hard seeded ness, over the period of storage. No significant variation was observed for 100 fruit weight and moisture content irrespective of treatments and period of storage. Maintenance of viability during storage may also be due to hard seeded ness, which did not permit the external moisture to enter into the seed, to maintain the optimum moisture content throughout the storage period.

1)Ph.D. Scholar

2)Associate Professor

3)Assistant Professor and

4)Professor & Head

(Address for correspondence)

Dr.P.R.RENGANAYAKI,

Assistant Professor (Dept. of Seed Science and Technology),

Tamilnadu Agricultural University

Forest College and Research Institute,

Mettupalayam – 643103.

Tamilnadu.

**Standardization of planting ratio for hybrid seed production in sunflower
(*Helianthus annuus*, L.) hybrid KBSH-1.**

P.R.Renganayaki¹ and V.Krishnasamy²

Department of Seed Science and Technology,
Tamil Nadu Agricultural University, Coimbatore – 3.

A study was undertaken to standardize suitable planting ratio for successful hybrid seed production in sunflower (*Helianthus annuus*, L.) hybrid KBSH-1. This is most popular hybrid released from Bangalore. The hybrid seed production system in sunflower includes, pollination of CGMS line (A) with maintainer line (B) on one hand to produce 'A' line seed and with restorer line R on the other hand to produce hybrid. In majority of the cases, the pollination is very much inadequate resulting in poor seed set. To have effective pollen supply from male to female parent, the parental lines are to be planted in a definite row ratio. It varies depending upon the pollen production, pollen viability and pollinating agent.

The study revealed that among the female rows, the first three rows nearer to male parent were on par in seed set and thereafter a gradual decline in seed set was noticed up to 8th row and from 9th row onwards a moderate increase in seed set was observed up to 12th row. The higher seed set in first and last three female rows from male row could be due to the availability of sufficient pollen and closeness of pollinator to seed parent. Seeds obtained from first three rows of female parent nearer to male exhibited higher 100-seed weight and germination percent compared to seeds from the female plants farther away from the male row. Since, seed set of three rows on either side of the pollen parent were on par, a row ratio of 6:1 (female: male) could be recommended for sunflower hybrid KBSH-1 seed production. The staggered sown crop registered 20 percent higher seed yield than the crop in which male and female parents were sown simultaneously.

Key words: Sunflower, hybrid seed production and Planting ratio

- 1) Assistant Professor
- 2) Professor

(Address for correspondence)

Dr.P.R.RENGANAYAKI,
Assistant Professor (Dept. of Seed Science and Technology),
Tamilnadu Agricultural University
Forest College and Research Institute,
Mettupalayam – 643103.
Tamilnadu.

Mid Storage correction to prolong shelf life of parental and hybrid seeds of Sunflower (*Helianthus annuus*, L.) hybrid KBSH-1.

P.R.Renganayaki¹ and V.Krishnasamy²

Department of Seed Science and Technology,
Tamil Nadu Agricultural University, Coimbatore – 3.

A study was conducted at Tamil Nadu Agricultural University, Coimbatore to evaluate the effect of mid-storage correction (hydration – dehydration treatment) in prolonging the shelf life of seeds of sunflower hybrid (*Helianthus annuus* L.) KBSH-1 and its parental lines during storage. The longevity of seed in storage is largely influenced by the genotypes, history of seed taken to storage, moisture content of seed, container in which it is packed and temperature of storage environment.

The study revealed that four-month-old seeds soaked in butylated hydroxy toluene (10^{-4} M) and sodium dihydrogen phosphate (10^{-3} M) for two hours and dried to original moisture content maintained better germination and vigor during subsequent storage. Hybrid exhibited better response to the mid storage seed treatments compared to its parental lines. The rate of seed deterioration during storage was lower in hybrid than other genotypes.

Key words: Sunflower, Seed storage, Mid-storage correction, Seed vigor and viability

- 1) Assistant Professor
- 2) Professor

(Address for correspondence)

Dr.P.R.RENGANAYAKI,
Assistant Professor (Dept. of Seed Science and Technology),
Tamilnadu Agricultural University
Forest College and Research Institute,
Mettupalayam – 643103.
Tamilnadu.

Histological study on seed ageing in sunflower (*Helianthus annuus L.*) hybrid and its parental line

P.R.Renganayaki¹ and V.Krishnasamy²

Department of Seed Science and Technology,
Tamil Nadu Agricultural University, Coimbatore – 3

Biochemical and physiological aspects of seed play a major role in determining seed quality. In case of hybrid seed production involving GMS system the genetic make up of each line is different and it is inherited to the hybrid in varying degrees which decides the hybrid seed quality. Morphological and anatomical characters of seed and seed coat are of great importance in maintaining the viability during ageing. The pattern of senescence varies with parental lines.

Therefore, to determine the role of seed coat on maintaining the viability during ageing a study on histology has conducted at Tamil Nadu Agricultural University, Coimbatore. The microtoming sections of seed coat indicated that in epidermal layer of female and hybrid, more number of hard sclerenchymatous cells was observed compared to maintainer and restorer line. Such hard cells would offer resistance to leaching of internal solutes; thereby it favored the storability of seeds. Whereas the maintainer line seed coat had lot of dark cells, which are phenolics; on denaturation it may lead to rapid seed deterioration may be one of the reasons for loss of viability of 'B' line. Since the seed coat of hybrid is developed from maternal integument, the structure of female and hybrid remained same.

Key words : Sunflower –hybrid-parental lines- nature of seed coat- seed deterioration.

-
- 1) Assistant Professor
 - 2) Professor

(Address for correspondence)

Dr.P.R.RENGANAYAKI,
Assistant Professor (Dept. of Seed Science and Technology),
Tamilnadu Agricultural University
Forest College and Research Institute,
Mettupalayam – 643103.
Tamilnadu.

Seed handling practices of Jamun (*Syzygium cuminii* skeels)

A. Vijayaraghavan,¹ P.R. Ranganayaki,² G. Dharmaraj³

Forest College & Research Institute
Tamil Nadu Agricultural University, Mettupalayam

Agroforestry, Social forestry, Silvipasture, Hortisilvipasture, Urban

and recreation forestry are the few systems of a forestation programme for greening of wastelands. Jamun is an evergreen moist deciduous tree used as fuel, timber and its seeds having highly medicinal properties. They are propagated mostly through seeds. Since it is an recalcitrant seed an investigation was made to study the storage behavior of freshly collected jamun seeds of different fruit size by storing in different media at different moisture conditions viz, 25%, 30%, 35%. Of these, seeds stored in sand medium at 35% performed better up to 4 months of storage, also the big sized seeds gave four vigorous seedlings (Polyembryony) when compared to all other seed size and moisture content upto 4 months of storage.

Key words: Jamun- seeds- fruit size- polyembryony- storage-moisture content, etc.

Preliminary studies on seed longevity of *Pongamia pinnata*

Maitreyee Kundu

Rain Forest Research Institute. P.O. 136, Jorhat 785001, Assam, India

Abstract

The seeds of *Pongamia pinnata* were stored at four moisture contents of 4.5% to 11.8% and four temperatures of -20°C to 15°C . Seeds were sampled at intervals up to three years for assessment of germination capacity and viability equations were used to express the relationship between storage conditions and viability of seeds. Drying the seeds to 4.5% moisture content did not affect the germination and the seeds can best be stored at 4.5% moisture content and -20°C , therefore supporting the orthodox nature of the seeds of this species. The upper moisture limit is very low (12%). The relation between upper limit and oil content of seeds has been discussed.

LONGLEAF PINE SEED HEALTH: EFFICACY OF CHEMICAL TREATMENTS

James P. Barnett
USDA Forest Service, Southern Research Station
2500 Shreveport Hwy.
Pineville, LA 71360

Abstract—The demand for longleaf pine (*Pinus palustris* Mill.) seed has increased at least 10 fold in the last few years due to emphasis on restoring this ecosystem. One of the limitations in increasing longleaf pine seedling production has been the limited availability of quantity seeds. Longleaf pine seeds have large, thin and fibrous coats that carry significant populations of microorganisms. Many of the fungi carried on the seeds are pathogenic during germination and early seedling establishment. Seed processors and nursery managers continue to seek alternative methods to reduce losses due to infestations of seed pathogens. Container production is now the preferred method of growing seedlings and container stock is several times as costly as bareroot nursery seedlings. So, availability of high quality seed is an important consideration in minimizing seedling cost, as well as in meeting demands for planting stock.

Pathological evaluations of longleaf seeds, both earlier and current, indicate that *Fusaria* represent one of the major seedborne pathogens. Studies show that both appropriate sterilants and fungicides will reduce pathogens and result in improved germination and seedling establishment. The results from a number of chemical treatments will be presented and discussed.

Uso de phytobiopsia y creacion de imagenes en fisiologia de semillas.

Rafaela Carvajal, PhD¹; J. A. Vozzo, PhD²; Ramesh Patel, MD³; Asmita Roy, BSc³

¹ Instituto Nacional de Investigaciones Agrícolas, Maracay, Republica Bolivariana de Venezuela

² USDA-FS, Starkville, MS

³ University of Mississippi Medical Center, Jackson, Ms

La tomografía computarizada (TC) es usada para determinar biodensidades de los tejidos, lo cual puede ser útil en estudios morfológicos, la resonancia magnética (RM) compara la distribución de los protones, aplicable en estudios fisiológicos y la phytobiopsia; un procedimiento físico un poco más invasivo, es valioso para extraer muestras del tejido interno de las semillas, para ser usados en análisis químicos cuantitativos. El uso de estos procedimientos en conjunto permiten una identificación segura entre semillas latentes y semillas germinables. Esto se logra correlacionando; la forma, función, y cantidad de sustrato metabólicos presentes en las semillas y permite hacer estudios durante el almacenamiento u otras condiciones de las semillas. A través de TC se pueden crear imágenes de cortes delgados, continuos y no destructivos, útiles para identificar y cuantificar el área y volumen de cualquier tejido que este en un rango de 5000 Hounsfield de biodensidad. Los datos son expresados ya sea en histogramas de densidades o en mapas numéricos de la misma unidad de densidad. La RM evidencia la presencia y distribución de los iones hidrógeno en las moléculas de agua y/o ácidos grasos. La móvil distribución de estos protones pueden ser representada tanto en área como en volumen (determinación de píxeles y voxels). La técnica de biopsia consiste en la extracción de una muestra de tejido a través de una cavidad que puede medir desde 1.0 hasta 3.0 mm. Análisis químicos (nitric oxide synthase) permiten evaluar cuantitativamente los cambios orgánicos y elementales de tejidos activos.

Nuevas modalidades de obtención de imágenes y nuevas técnicas desarrolladas inicialmente para ser aplicadas en medicina, están siendo cada día más comúnmente usadas en semillas (Vozzo, Patel, and Terrell, 1999)

La tomografía computarizada (TC) es un procedimiento radiográfico que permite fotografiar las densidades de las semillas y determinar su morfología. La resonancia magnética (RM) define la concentración de las moléculas de hidrógeno presentes en las masas de agua y sustancias grasas, obteniéndose datos fisiológicos. La presencia de la enzima óxido nítrico sintetasa es determinada a través de procedimientos bioquímicos. La extracción de las muestras de tejidos desde las semillas, para ser usados en los análisis de NOS, pueden hacerse a través de la fitobiopsia. En este último procedimiento las técnicas de TC y RM pueden ser herramientas útiles para localizar con precisión los tejidos deseados y obtener muestras entre planos de hasta 0.5 mm.

TC y RM son técnicas no invasivas, y no destructivas por lo cual no traumatizan el tejido vegetal. En cambio ONS, requiere un procedimiento destructivo del tejido que pudo haber sido

obtenido desde la semilla por medio del procedimiento medianamente destructivo phytobiopsia. (Figuras 1-3).

Semillas de *Juglans nigra* L (walnut negro) and *Vigna unguiculata* L. (frijol) fueron seleccionadas para este estudio por su respectiva importancia en arboles forestales y cultivos agronomicos. Las semillas de walnut negro fueron colectadas de arboles silvestres en el este de Mississippi, en tanto que las semillas de frijoles fueron seleccionadas entre varias variedades comerciales. Walnut negro fueron ademas consideradas por su mayor tamaño (25-mm de diametro) lo cual permite mejor interpretacion de los estudios de TC y RM. Las semillas de frijol por su menor tamaño no permite una buena interpretacion de esas tecnicas. Semillas de ambos cultivos fueron usadas para los bionalisis de enzimas.

Los procedimientos aqui descritos han sido usados bien sea individualmente o en conjunto. Por ejemplo, analisis de ONS para la obtencion de la muestra a ser analizada para la actividad enzimatica ONS, fue obtenidas a traves de la phytobiopsia y el punto de obtencion del tejido de dentro de la semilla fue preestablecido dentro del especimen usando imagenes temporales de TC o RM.

TC provee una absoluta replicacion de interpretacion de biodensidad usando Hounsfiels units HU, figura 4. La densidad de electrones de la muestra dentro cualquier preseleccionado plano es mostrado sobre una imagen en blanco y negro. Numericamente , la densidad es medida in HU con una escala que oscila from -3000 hasta +5000 aplicandole valores a la densidad del agua de HU= 1. HU puede ser ubicada sobre una region de interes (ROI) o sobre un simple pixel y luego ser mostrado como un grafico de barras o un mapa numerico, figura 5-6.

TC permite expresar la biodensidad (HU) en valores numericos absolutos y relacionarlos con la hidratacion. La hidratacion puede ser vista como inicial, progresiva, or final dentro de la semilla, puesto que ella es medida a diferentes intervalos de tiempo. Usado como una herramienta en las investigaciones de anatomia, HU reflejara la interaccion del agua. Imagenes reconstruidas a traves de la computadora muestran la biodensidad (anatomia) en cualquier plano de una imagen de tres dimensiones. Las estructuras hasta de 0.5-mm, dentro de esas imagenes pueden ser facilmente medidas. Ademas el area es medida en mm² por calculaciones de pixeles y el volumen en mm³ por determinaciones de voxeles.

Imagenes obtenidas por TC son coupled con la tecnica de phytobiopsia paara fijar la extraccion de la muestra en un plano determinado dentro del sujeto en estudio. Como la fijacion de la imagen tomografica es estrictamente controlada por penetracion y atenuacion de rayos X, el muestreo del tejido es extremadamente seguro, cuando se aplica el estilete dentro del tejido.

RM depende de la distribucion quimica y enlaces de los nucleos de hidrogeno (proton). Imagenes con resonancia magnetica se basa en la medicion dentro de un campo magnetico de las frecuencias de radio transmitidas y recibidas, las cuales se producen al detectar y cuantificar los protones hidrogenos contenidos en las diferentes moleculas del organismo. Figura 7. Los signos son usados para reconstruir secciones de imagenes en cualquiera de los tres planos axiales.

Usando supresion quimica, las moleculas grasas son separadas de las moleculas de agua. Figuras

8 y 9. Las áreas y los volúmenes de lípidos y agua pueden ser representados por imágenes o por valores absolutos.

Durante el proceso de germinación y desarrollo redistribución de los protones de hidrógeno ocurre debido a transformaciones moleculares. MRM permite estudios no destructivos durante estas transformaciones. Actualmente los protones de hidrógeno son más fácilmente fotografiados, debido a que ellos son metabolizados en mayor cantidad. Sin embargo, técnicas para fotografiar otros protones, principalmente en fósforo están siendo desarrolladas.

Oxido nítrico es ampliamente reportado como un radical libre el cual funciona como molécula mensajera tanto intercelular como intracelularmente. La enzima oxido nítrico sintetasa actúa como catalizador para convertir arginina a citrulina y oxido nítrico, por lo cual determinando la citrulina disponible en la arginina tritiated en los tejidos vegetales se tiene un indicativo de las cantidades relativas de ON presente en la muestra (Bredt y Snyder, 1990). The arginine determinada en la muestra sirve como control (OJO). Mucho ha sido recientemente reportado concerniente a la presencia e importancia de la enzima ONS en las células de las plantas, relacionándolas con desarrollo y crecimiento incluyendo trabajos acerca de su importancia en promover germinación en las semillas de lechuga (*Lactuca sativa* L.), cuando ONS es aumentada con la adición de sodio nitropruside (Beligni and Lamattina, 2000), en la patología del virus del mosaico del tabaco (TMV) en tabaco (*Nicotiana tabacum* cv. Xanthi) (Durner, Wendehenne, y Klessig, 1998). Barroso et al (1999) reporta actividad de ONS (usando bionalisis y microscopía electrónica) en los peroxisomas de guisantes (*Pisum sativum* L.) Estudios in vivo han demostrado que ON es inhibido por el ácido sodico (Yamasaki y Sakihama, 2000).

Generalmente, ONS reacciona con el oxígeno en las células y con arginina para liberar citrulina y oxido nítrico. La cantidad de citrulina es un indicador de la actividad del oxido nítrico. Es de gran interés que ON de por sí tiene un electron spin resonancia y puede ser posible exponerlo a RM. Sin embargo su sensibilidad es medida en picomoles por unidad de enzima. Después que arginina se degrada a ON, este puede seguir la degradación hasta nitrito y nitrato. Los cuales son ambos componentes comunes de la célula y pueden complementar la importancia de las pruebas de ON.

Como lo reporta Barroso et al (1999), la actividad del ONS en los peroxisomas de las plantas está en el rango de los 5.6 nmoles de citrulina. Nuestros datos en ambos walnut negro y frijol muestran menor rango de actividad. Tabla 1.

Sin embargo, no ha sido demostrado que ON afecte el metabolismo celular en las plantas tan claramente como lo afecta en los tejidos de los mamíferos. El efecto de ON puede ser controlado por el uso de sodio nitropruside, un promotor de ON, o usando sodium azide; un inhibidor de ON. Los próximos pasos en estos estudios de ONS en semillas podría ser determinar los contenidos de ONS en las células de semillas recién cosechadas, durante germinación, o durante almacenamiento. Obtención de imagen de RM específicamente para el spin electron de ON puede ser útil para hacer análisis no destructivos durante ensayos de pregerminación y almacenamiento. RM tiene la ventaja de ver los píxeles individuales de cada célula y presentarlos en imágenes al momento de observarlos. TC permite la exacta localización y

permite el muestreo usando la tecnica de biopsia. Figura 10. Localizacion de anticuerpos puede determinar cual isomorfo de ONS esta siendo usado en el metabolismo de las plantas.

Barroso, Juan B., Francisco J. Corpas, Alfonso Carreras, Luisa M. Sandalio, R. Valderrama, Jose M. Palma, Jose A. Lupianez and Luis A. del Rio. 1999. Localization of Nitric-oxide synthase in plant peroxisomes. *Journal of Biological Chemistry*. Vol 274 (51):36729-36733.

Beligni, Maria Veronica, and Lorenzo Lamantina. 2000. Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyls elongation, three light-inducible responses in plants. *Planta* 210:215-221.

Bredt, Ds. and S. H. Snyder. 1990. Isolation of nitric oxide synthase, a calmoduli- requiring enzyme. *Proceedings National Academy of Sciences. USA.* 87:682-685.

Durner, Jorg, David Wendehenne, and Daniel F. Klessig. 1998. Defense gene induction in tobacco by nitric oxide, ciclic GMP, and cyclic ADP-Ribose. *Proceeding National Academy of Sciences, USA.* 95:10328-10333.

Vozzo, J. A., Ramesh Patel, MD, and A. Terrell. 1999. In vivo Digital Phyto Imaging(IDPI) in *Juglans nigra* seeds. In: Marzalina, M.; Khoo, K. C.; Jayanthi, N.; et al., eds. *Recalcitrant Seeds: Proceedings of the Conference; IUFRO seed Symposium 1998; October 12-15; Kuala Lumpur, Malaysia: Forest Research Institute of Malaysia: 74-78.*

Yamasaki, Hideo and Yasuko Sakihama. 2000. Simultaneous producoon of nitric oxide and peroxinitrite by plant nitrate reductase: in vivo evidence for the NR-dependent formation of active nitrogen species. *Federation of European Biochemical Societies.* 468:89-92.

Tabla 1. Actividad representativa de citrulina tritiated (picomoles tritiated citrulina?mg protein/min) obtenidos de los analisis en walnut negro y frijol.

	Walnut negro	frijol
Cotiledones	1.21	1.76
Radiculas	0.74	0.71
Hipocotilos	9.89	3.72

Figura 1. Un specimen de walnut negro disertado mostrando la localizacion del instrumento (aguja hipodermica + estilete) usados en la tecnica de biopsia.

Figura 2. Fotos de la aguja hipodermica el estilete y muestra de la semilla de walnut negro usados en este estudio.

Figura 3. Tejido representativos de dos muestras de walnut negro obtenidos mediante la tecnica de fitobiopsia. El peso de cada muestra se estima ser en mgm. Estas muestras fueron usadas en determinacion de ONS.

Figura 4. Imagenes obtenidas a ttraves del uso de TC de alta resolucio, de los planos Axiales (Ax, Bx y Cx) y perpendiculares (Ap, Bp y Cp) de la semillas de walnut negro. Cx y Cp representan una semilla intacta pero vacia de walnut, la cual no tiene potencial para germinar.

Figura 5. Seccion axial de una tomografia de la semilla de *Butia capitata* L. Los circulos representa las regiones de interes (RDI). Mapas numericos de las unidades en Hounsfield son tambien mostradas. Numeros negativos representan el area de acidos grasos.

Figura 6. Seccion axial de una TC en la semilla de *B. capitata* L. Los circulos representan las RDI dentro de las cuales las unidades Hounsfield son medidas. El grafico de barras representa la frecuencia de las UH medidas. El signo menos denota los pixels de las moleculas de acido grasos.

Figura 7. Imagenes en blanco y negro de secciones axiales obtenidas a traves de RM de las semillas de walnut negro. A=T-1 sping eco secuencia. B=T-1 spin eco secuencia con supresion del agua y C=T-1 sping eco frecuencia con supresion de las grasas.

Figura 8. Izquierda supresion de grasas. Derecha: supresion del agua. Modelo en tres dimensiones creado de cortes axiales T-1 y T-2 spin eco secuencias con supresion de las grasas o del agua.

Figura 9. Tridimensional imagenes de la semilla de walnut negro expuesta a RM. Las imagenes transversales fueron obtenidas con T-1 y T-2 spin eco secuencia. Tecnicas para la inhibicion de grasa y agua fueron aplicadas. Adicion de colores mediante la computadora fueron usados para la reconstruccion de las imagenes. A= supresion del agua. B= supresion de grasas.

C= combinacion de las imagenes A y B. el volumen de grasa fue calculado ser 2911.95 cu.mm.
El volumen de agua fue 2539.28 cu.mm.