

GERMINATION AND SEEDLING GROWTH OF *Coffea arabica* L. SEEDS

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Introduction

Coffee

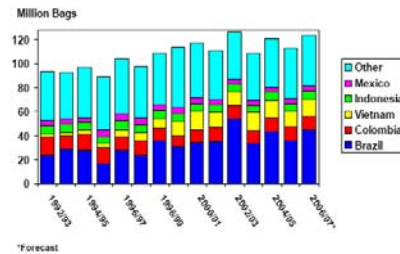
- the genus *Coffea*, a member of the Rubiaceae family, has more than 70 species, but only two species, *Coffea arabica* L. and *Coffea canephora* Pierre, have commercial value
- *Coffea arabica* L. produces a better quality of beverage and *Coffea canephora* Pierre is used to make blend with arabica



Brazilian production

- Brazil is the largest producer of coffee in the world, contributing approximately 30% of the global production
- *Coffea arabica* L. comprises 70% of the Brazilian annual production at 2.55 million tons in 2006/2007 (Conab, 2007)
- *Coffea arabica* L. being produced in the southeast and *Coffea canephora* Pierre in the north and northwest regions of the country

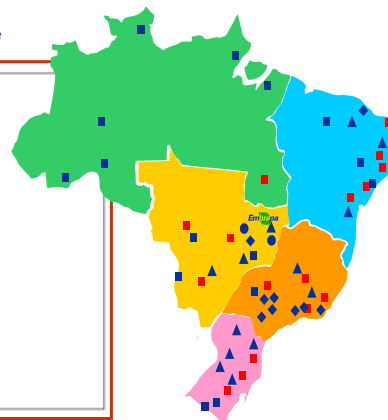
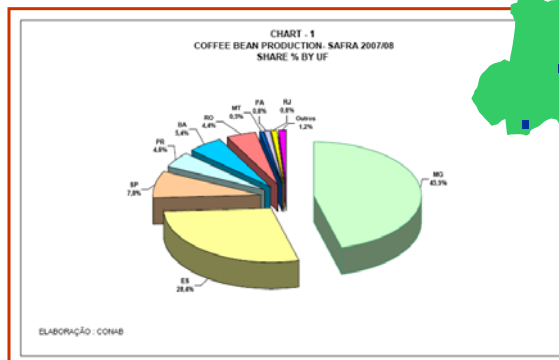
World Coffee Production in 2006/07
Forecast Up Nearly 10 Percent



USDA / Foreign Agricultural Service (June 2006)

Brazil

Distribution of coffee production per state



Coffea arabica L. is produced in the southeast and *Coffea canephora* Pierre in the north and northwest regions of the country.

Coffee propagation

- despite considerable effort at vegetative and micro-vegetative propagation of coffee plants, they still are primarily propagated by seedlings produced directly from seeds
- the fruits are collected at cherry fruit maturation stage and the parts of the fruit are removed by using a mechanical peeler and fermentation process



Coffee mature fruits



Fermentation process to remove the mesocarp

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Seedling production

- coffee seeds have slow and non-uniform germination and seedling growth, and they are sensitive to desiccation
- as a consequence they have poor storage potential
- mechanical restrictions and slow germination process

Problable causes

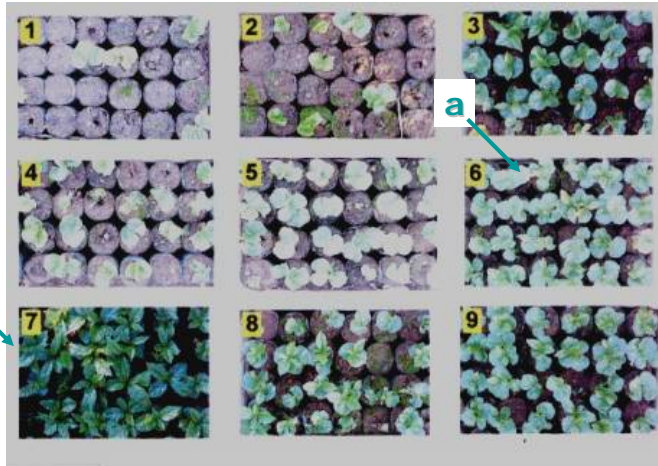
- the slow germination of coffee seeds has not been elucidate yet and several causes are pointed to, such as, the low water and O₂ uptake and presence of natural inhibitors, the hormonal balance and the presence of the endocarp



Coffee seeds with parchment



Coffee seeds without parchment



Seedlings from nine sowing treatments, 6 (a) and 7 (b) are seedlings from seeds with and without parchments. (Rosa et al., 2007)

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Seedling production

- seedling production requires 6 months and occurs from Jun/Jul to Dec/Jan, and seedling ready for planting usually takes place in the low rainfall season
- emergence can be 120 days long depending on soil temperature



“orelha de onca” (“Jaguar ear”)
Around 60 days



Six months seedling

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Slow and asynchronous seedling growth makes:

- it difficult to obtain high quality seedlings of the desired standard at the ideal time for planting in coffee production regions in Brazil
- rapid viability and/or vigor evaluations difficult because of the excessive time required to obtain the results



180 days



30 days (Brazilian rules for testing seeds (Brasil, 1992))

Staging coffee seedling growth
(based on morphological changes)

- can permit more accurate characterization of coffee seed/seedling development
- can enable improved physiological understandings of slow and non-uniform coffee seed germination and seedling growth
- can better standardize seed quality assessments in researches
- can produce a rationale that permits a justification to reduce the seed quality evaluation time in coffee that currently requires 30 days
- can shorten the coffee seedling production period allowing more rapid utilization of seedlings for plug production



Objective

- to describe coffee seedling growth developmental stages by documenting morphological changes during germination and post-germination growth

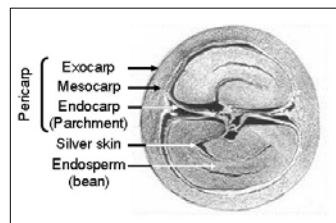


Material & Methods

- *Coffea arabica* L., cv. 'Rubi' - cherry fruit – peeled - fermented – dried
- germination test – according to the Rules for Seed Testing (Brasil, 1992)
- the germination and post-germination events were followed, scanned daily by an inverted UMAX 15000 scanner, to identify and characterize various growth stages
- each stage was characterized and described based on consistent and visible morphological changes

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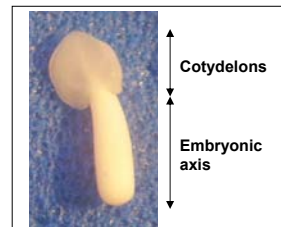
- a mature coffee fruit is a red/yellow drupe with two seeds 10 to 18 mm long and 6.5 to 9.5 mm wide (Dedeca, 1957) with an enclosing pericarp
- the pericarp is composed of an: *exocarp* (single layer of hardened and lignified cells); *mesocarp* (a mucilaginous tissue rich in sugar and water 0.5-2.0 mm in thickness; and *endocarp* (5-6 layers of a hard and lignified tissue 150 μ m that ultimately becomes the parchment layer surrounding the dry seed)



(De Castro and Marrancini, 2006)

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- seed – covered by the “silver skin” (thin pellicle remnant from the perisperm, referred to as the integument or spermoderm (Dedeca, 1957; Estanislau, 2002) and contains the endosperm and embryo
- the endosperm is live tissue (endosperm cap being formed by smaller and thinner cell walls compared to the rest of the endosperm where the radicle ultimately protrudes) (Dedeca, 1957; Dentan, 1985, Silva et al., 2004) and its composition consists of proteins, lipids and minerals with polysaccharides, cellulose and hemicelluloses deposited in the cell walls (Wolfrom et al., 1961; Wolfrom and Patin, 1964; Silva et al., 2004)
- the small (3 to 4 mm long) embryo contains a differentiated axis and two adherent cordiform cotyledons that make it dependent on endosperm reserves for successful seedling development (Dentan, 1985; Silva, 2002).



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- Eight embryo or seedling growth stages were characterized based on consistent, visible morphological changes:

from the seed imbibition to germination →

I1 - Seed Imbibition 1
I2 - Seed Imbibition 2

G - Germinated Seed

from the seed germination to cotyledonary leaves →

S1 - Seedling 1
S2 - Seedling 2
S3 - Seedling 3
S4 - Seedling 4
S5 - Seedling 5



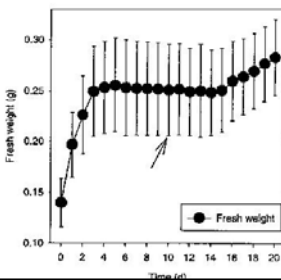
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I1 - Seed Imbibition 1

- a fully imbibed seed with no visible protuberance up to 3 days of imbibition. This stage is consistent with Silva (2002) and in disagreement with Camargo (1998) who observed a completed phase I after 6 days and a phase III that begins after approximately 9 days following the onset of imbibition



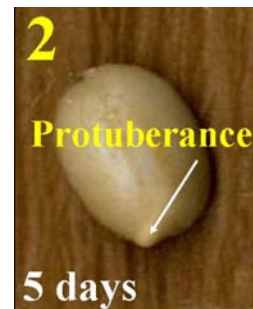
Imbibition curve (Silva, 2002)



Results

I1 - Seed Imbibition 2

- a visible protuberance can be noticed on the endosperm cap at five days which is a result of the embryo growing inside the endosperm, but the radicle does not penetrate through the outer layer of the endosperm at this stage
- there are regions of the endosperm with different appearance at six days where the endosperm cap region is more fully imbibed with compressed cells compared to the rest of endosperm that creates space for the embryo to expand during endosperm cell wall degradation
- this imbibition phase includes numerous physiological events that transform the dehydrated and quiescent embryo with little metabolism into an embryo with active metabolism culminating in growth.



G - Germinated Seed

the radicle penetrates through the outer endosperm layer completing *sensu stricto* germination at approximately 7 days

before germination, the major metabolic events take place in preparation for radicle emergence from non-dormant and viable seeds, such as protein hydration, sub-cellular structural changes, respiration, macromolecular syntheses and cell elongation (Bewley and Black, 1994)

this phase includes the mobilization of storage reserves necessary for transforming the seedling into an independent autotrophic organism capable of photosynthesis.



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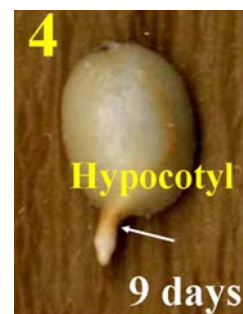
S1 - Seedling 1

- from the *Germinated Seed* stage, the embryo is considered a seedling that depends on the major storage reserves, and morphological activities are considered post-germination events

- after germination, and at approximately 9 days of imbibition, the hypocotyl grows and possesses a distinct pink color from the white radicle

- the radicle appears as an arrow shape which is a result of the enlarged junction between the hypocotyl and root caused by the growing root primordia

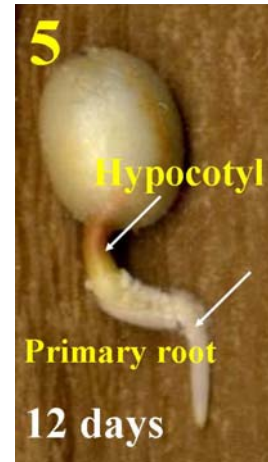
- root primordia are not noticeable, but lateral roots will later appear in this region; the enlarged junction between the hypocotyl and root contains an organized series of cells in their earliest stages of differentiation



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S2 - Seedling 2

- this stage is characterized by the appearance of root primordia at the junction between the hypocotyl and primary root that increase in size at approximately day 12
- the radicle, an embryonic root at the lower end of the hypocotyl, continues to grow and develops into the primary root (Sutton and Tinus, 1983)



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S3 - Seedling 3

- the first two or three lateral roots arise at the junction between the hypocotyl and primary root and more root primordia and root hairs form around the surface of the primary root
- the primary root is well developed and the root cap, zone of cell division and elongation can be differentiated with no root hairs, but root hairs can be detected in the zone of maturation
- root hairs develop from epidermal cells on the root surface in the zone of maturation and they are absent in others regions of the root, such as in the root apex (Raven and Johnson, 2002)



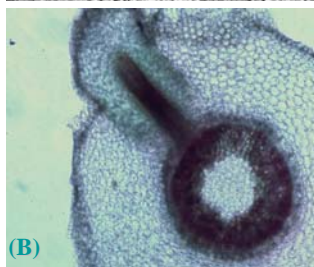
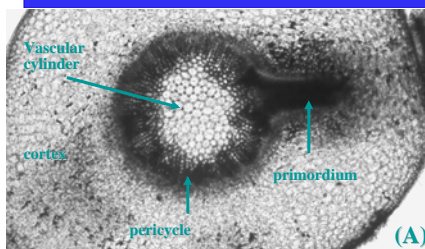
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S4 - Seedling 4

- from day 20 until day 30, lateral roots are initiated from the primary root, increase in size and number and root hairs are detected in every root in the zone of maturation, but they are not present on the root apices
- lateral roots that were initiated at the junction between the hypocotyl and primary root begin to form secondary roots
- a lateral root is defined as a root that arises by cell division in the pericycle of the parent root (Sutton and Tinus, 1983) and, depending on the rank of the root developing lateral roots, they are designated as secondary roots (laterals on a primary root), tertiary roots (laterals on a secondary root), etc. (Esau, 1977; Sutton and Tinus, 1983)



Two stages of lateral root development in coffee root system:
 (A) One lateral root primordium starting its growth from the pericycle
 (B) The lateral roots reach the exterior of the primary root



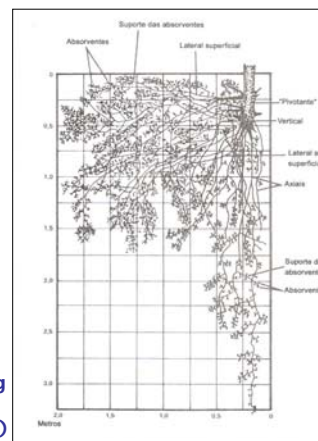
S5 - Seedling 5

- beginning at approximately 30 days, the seedling continues growth and forms a more substantial root system with a hypocotyl increasing in height, but the only significant morphological change occurring during this period is the consumption of the endosperm by the cotyledonary leaves
- at approximately 45 days, the seedlings have their cotyledons open and the remaining endosperm tissue is a thin layer covering the cotyledonary leaves, indicating that the endosperm has been substantially consumed and the apical meristem that will form the shoot still can not be detected
- the cotyledonary leaves are green, indicating that energy for seedling growth is coming from photosynthesis in addition to seed storage reserves, and between days 30 and 45, the plant can be considered a “young plant” instead of a “seedling”, since it is an independent autotrophic organism capable of photosynthesis



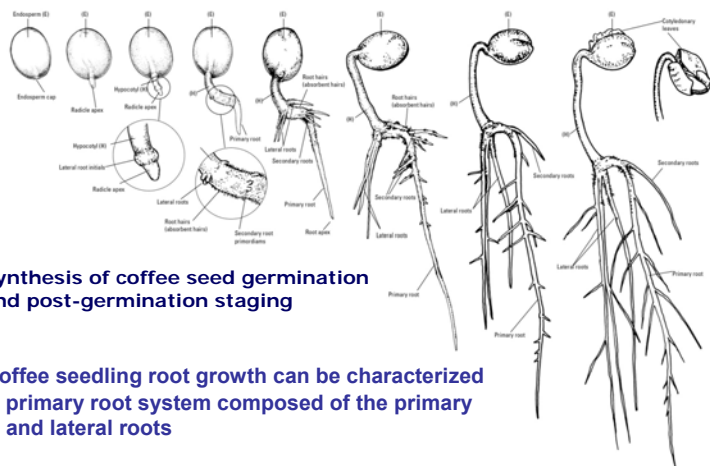
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- two distinct stages are defined in this study based on the appearance of lateral roots: one when the lateral roots arise from the root-stem junction region and the other when the lateral roots arise from the primary root that is temporally and spatially different from the first stage
- no descriptive information concerning root system terminology has been found for coffee seedlings, but adult coffee plants have been characterized as not having a typical primary root system (Dedeca, 1957; Rena and Guimaraes, 2000)
- this may be attributed to the physical and chemical properties of the soil and because the primary root is usually damaged during seedling transplanting before they are established in the field (Dedeca, 1957; Rena and Guimaraes, 2000)



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I2 G S1 S2 S3 S4 S5

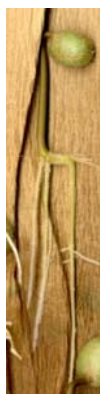


Synthesis of coffee seed germination and post-germination staging

- coffee seedling root growth can be characterized as a primary root system composed of the primary root and lateral roots



45 days



30 days

- normal seedlings are seedlings possessing the essential structures that are indicative of their ability to produce plants under favorable conditions (AOSA, 1998)
- the germination test for coffee seed requires 30 days (Brasil, 1992) before a seedling can be considered normal even though seedlings at this stage still do not possess all the essential structures, since it shows a general healthy condition, with a non-infected or non-abnormal endosperm, a normal hypocotyl and primary root system which possesses a primary root and lateral roots
- having normal and healthy cotyledons is not a requirement for considering a seedling as normal and this part of the seedling can only be typically observed after 30 or until 45 days as demonstrated in this study

- these results demonstrate that all essential seedling parts are present in coffee seedlings at stage S3 – Junction Lateral Roots, which occurs at day 15
- such data suggest that the standard germination test for coffee seeds can be substantially shortened to provide more rapid seed quality assessment

STAGING COFFEE SEEDLING GROWTH: A RATIONALE FOR SHORTENING THE COFFEE SEED GERMINATION TEST

15 days



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Shortening a germination test for any species has many technical advantages, such as:

- reducing the need for laboratory space, facilities and staff
- reducing the incidence of storage fungi development on low quality seed lots thereby making results more repeatable because coffee seeds germinate slowly
- obtaining a faster quality evaluation result and official quality certificate that requires a long time (30 days)
- extending the coffee seed marketing process that makes it difficult to obtain high quality seedlings for planting (normally the seedlings are ready for planting typically in the low rainfall season making seedling establishment difficult for this crop in the main coffee production areas in Brazil)

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Final considerations

Staging coffee seedling growth by defining and describing each morphological change permits:

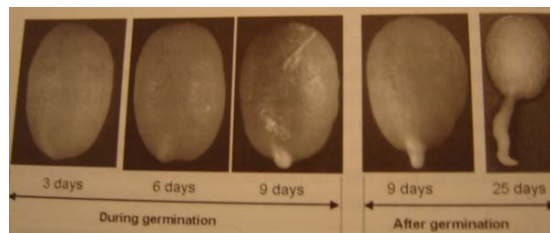
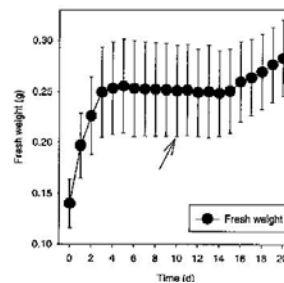
- to standardize the seed quality assessments in research enabling a better physiological understanding of slow and non-uniform coffee seed germination and seedling growth
- permit consistent and common terminology that allows successful communication in research studies
- permits a more accurate characterization of coffee seed/seedling development than days after planting since environmental factors have a major bearing on the speed and uniformity of seedling growth over this 45 day period

Final considerations

"Coffee seed germination occur in 50% of the seed population at 10 days following imbibition, but during imbibition, the fresh weight of intact seeds increased to a plateau at day 3, and remained constant until day 15, when phase III imbibition was achieved."

As a result, these findings suggest:

"The phases of coffee seed germination are not concurrent with the phases of water uptake as found in most seeds."



Final considerations

It may be that coffee seeds failed to have water uptake phases in conjunction with germination phases in this study because of abnormal growth since these seedlings did not develop into normal seedlings.

After the lag phase, only germinating seeds and normal seedlings enter into phase III imbibition as signaled by radicle elongation, and the increase in water uptake in phase III is initially related to changes in radicle cells requiring increased turgor for extension (Bewley and Black, 1994).



Thank you!

