Use of the tetrazolium test for estimating seed viability and vigour

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isss international society for seed science

Embrapa

ISTA Seed Quality Assurance

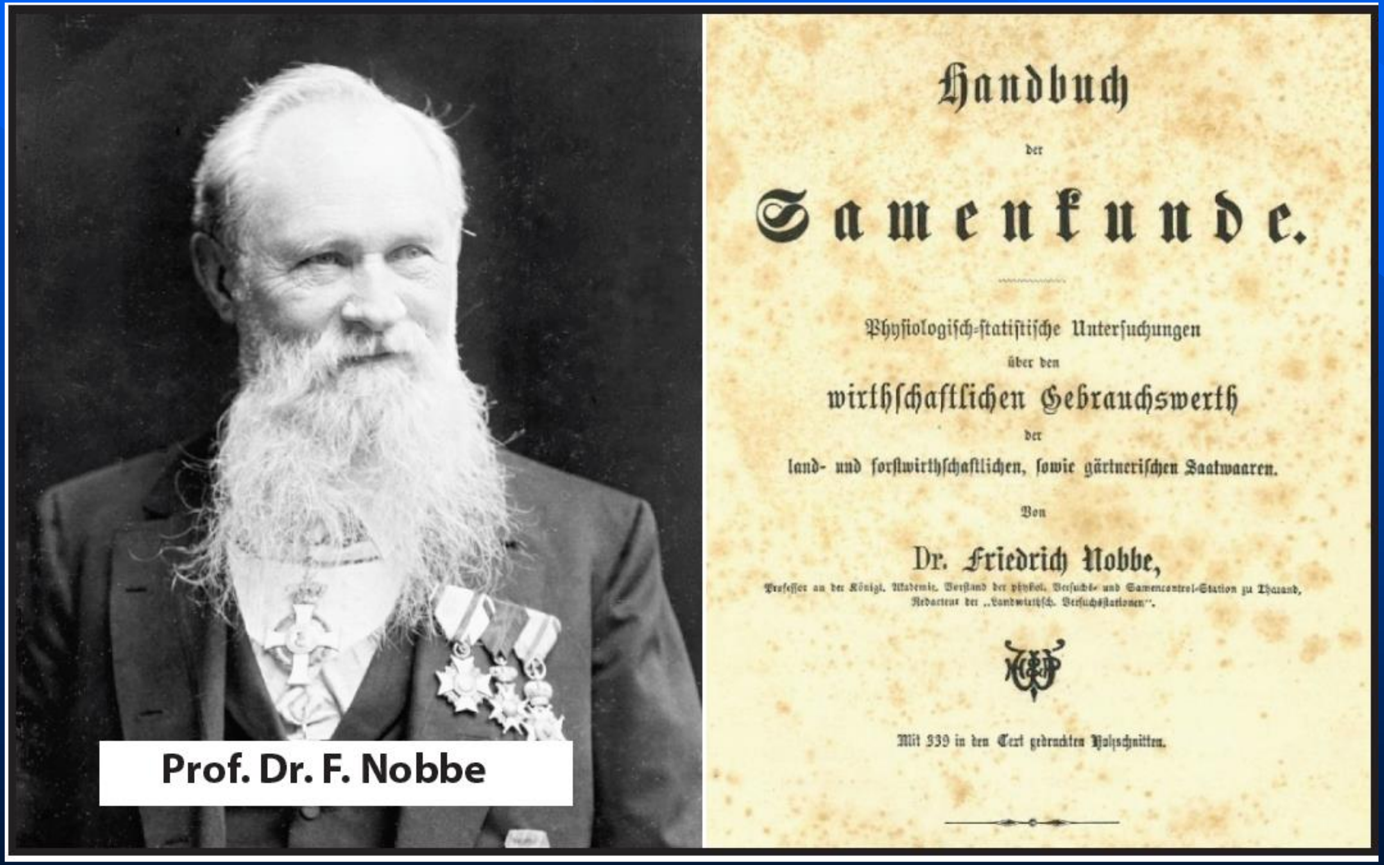
Introduction

The tetrazolium test (TZT) has been developed and improved since the early years of the 20th century; initially for determining seed viability. With the development of its methodology it has also been used to evaluate seed vigour of different seed species:

- Grain crops
- Forage crops
- Horticultural crops
- Forrest species: native, exotic and commercial
- Ornamental and medicinal species

Brief history

1869: **Johann Friedrich Nobbe established the first seed testing station in Tharandt, Saxony in 1869; he also published the first Rules for Seed Sampling and Testing.**



First successful attempts

1922: Turina, Yugoslavia:

- Reduction of tellurium and selenium salts in seed cells.

1925: Neljubow, Russia:

- Use of vital stains: indigo carmine for the identification of nonviable seed tissues: viable tissues did not stain.

1935: Hasegawa, Japan:

- Working with tree seeds: application of tellurium and selenium salts in embryo staining: tellurium (dark blue to black); selenium (bright red).

1935: Eidmann & Schmidt, Germany:

- Improved the selenium method;
- Introduced the concepts of seed viability and vigour: “total germinated”, “weak germinated” and “dead seeds”.

1937: ISTA Congress in Zurich:

- **Eidmann reports the "*Eidmann Method*" *for determining seed viability; later on it was recognized as the* "*Eidmann-Hasegawa Method*".**

1938: Eidmann is transferred and quit working with the TZT; Hasegawa´s lab is bombed during the war.

1939/1940: Georg Lakon, born in Greece, working in Hohenheim, Germany:

- **Perfected the selenium method developed by Hasegawa, Eidmann and Schmidt**
- **Developed the "topographical" selenium method**
- **After recognising the poisonous effects of selenium, he searched for a similar non-toxic salt.**

DR. G. LAKON

1941: **Based on information provided by Kühn and Jerchel:**

- **Lakon selected 2,3,5-triphenyl tetrazolium chloride (TTC) and perfected the test for several cereal and corn seeds.**

1945: **after the II World War**

- **Lakon´s work was first published in America**

1949: the TZT test was incorporated into the Official German Seed Testing Rules.

1950: Lakon presents his results during the ISTA Congress in Washington, DC.

1947-50: pioneer work with the TZT in the USA:

- Boyce Thompson Institute of Yonkers, New York
- Pioneer Hi-bred Corn Company, Johnston, Iowa
- Iowa State University (ISU), Ames, IA

1951-60: substantial achievements:

- **ISU: Isely, Bass, Smith and Throneberry**
- **University of Idaho: Parker**
- **University of Hohenhein: Bulat and Steiner**
- **North Carolina State University (NCSU): Robert Moore**

by Dr. R. P. Moore
Professor, Research, Crop Stands
North Carolina AES, Raleigh

1956: ISTA Tetrazolium Committee was founded.

1961-70: important developments:

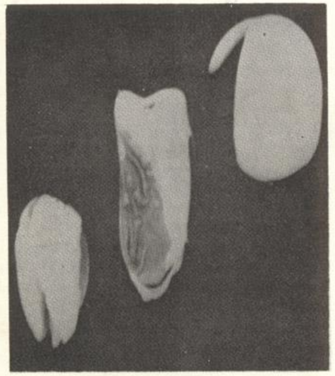
- **NCSU: Moore**
- **Mississippi State University (MSU): Delouche**
- **Oregon State Seed Laboratory: Grabe**

1962: “The TZ Test for Seed Viability” - MSU

- **Delouche et al. (1962)**

TECHNICAL BULLETIN 51 NOVEMBER 1962

The Tetrazolium Test for Seed Viability



MISSISSIPPI STATE UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
HENRY H. LEVECK, Director
STATE COLLEGE MISSISSIPPI

o teste de tetrazólio para viabilidade da semente

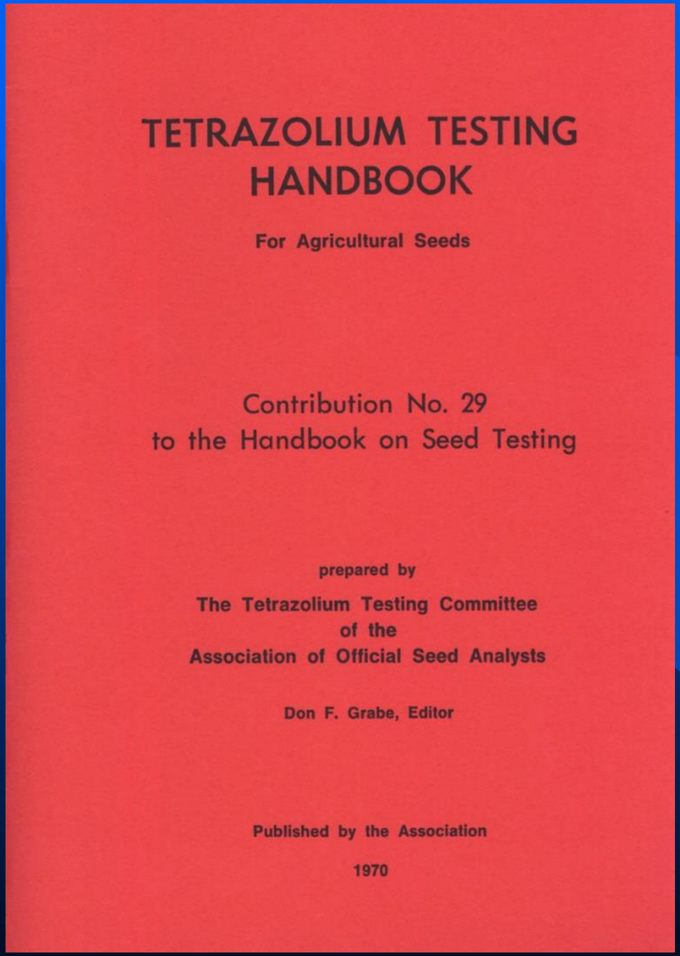
JAMES C. DELOUCHE
T. WAYNE STILL
MABEL RASPET
MYRTA LIENHARD

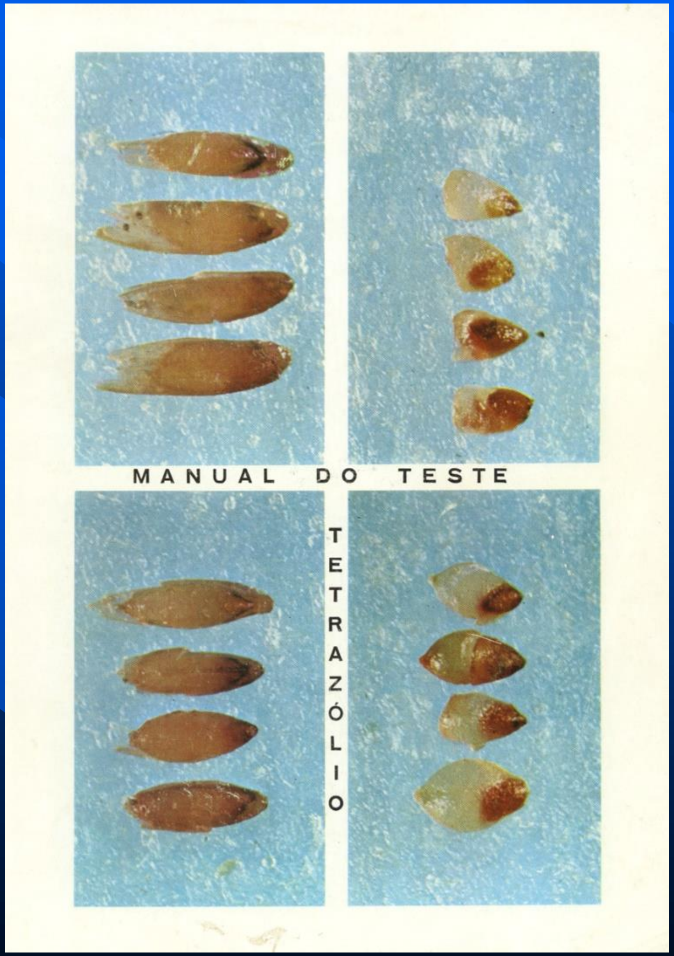
AGIPLAN
1976

1966: "Chapter 6: Biochemical Test for Viability – The Topographical Tetrazolium Test": incorporated into the ISTA Rules: Lakon and Bullat.

Viability procedures for over 90 species

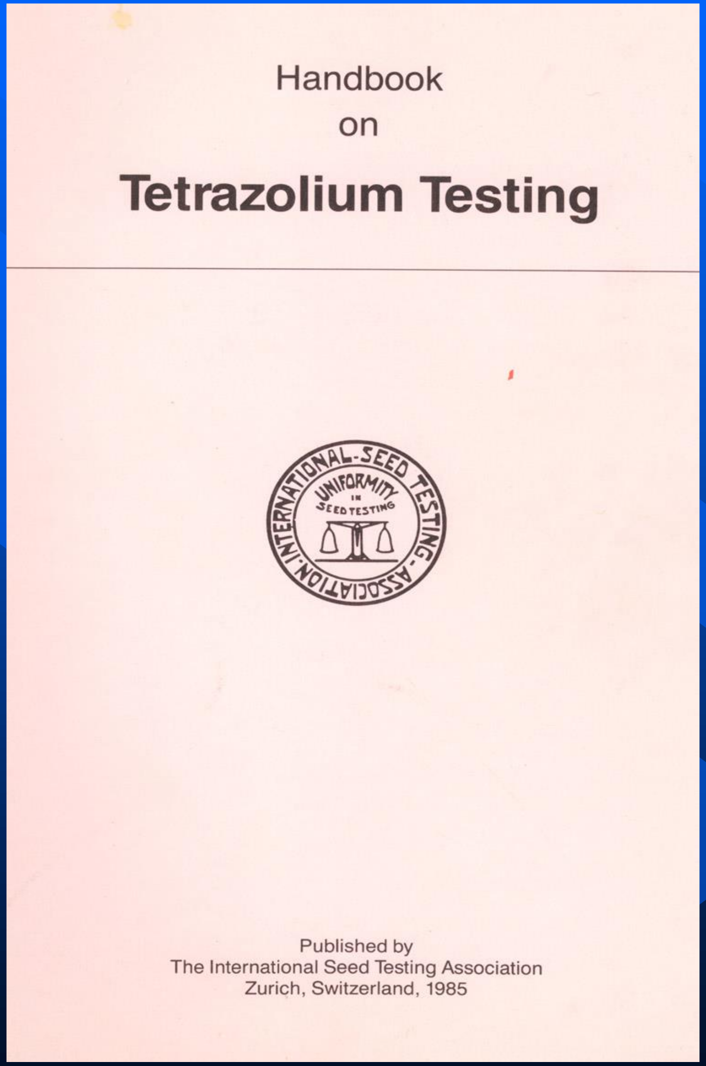
1970: The use of the TZT was accepted by the Association of Official Seed Analysts (AOSA) with the release of the "Tetrazolium Testing Handbook": Grabe - Oregon





1983: **AOSA published the *"Seed Vigor Testing Handbook"* which compiled important information about the TZT for soybean, cotton, corn and wheat.**

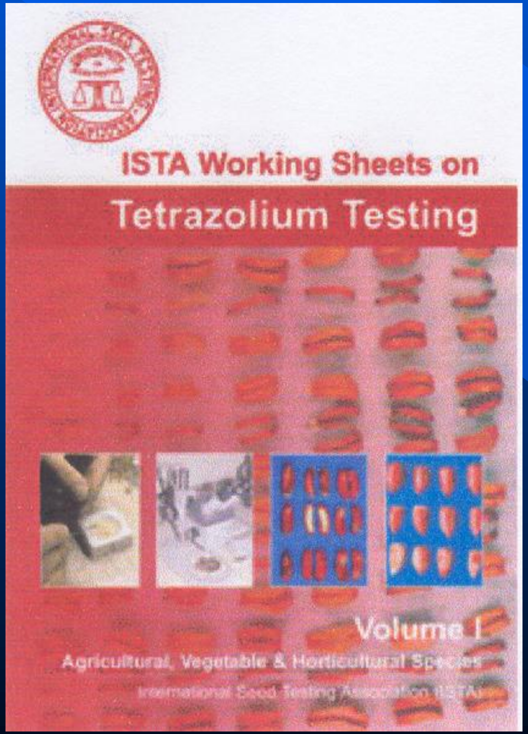
1985: *"ISTA Handbook on TZ Testing"* (Moore, 1985), containing details on the testing methodology for over 650 species.

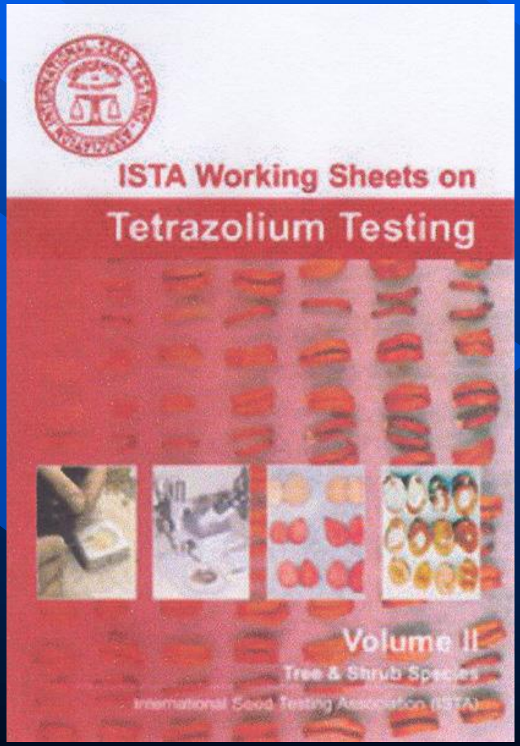


2000: **AOSA: “*Tetrazolium Testing Handbook*” (viability only). Further updates can be accessed on the AOSA website (www.analyzeseeds.com).**

2003: *"ISTA Working Sheets on Tetrazolium Testing"*

- Norbert Leist, Stefanie Kraemer and Andrea Jonitz
- LUFA Augustenberg, de Karlsruhe, Germany
- Volume I: Agricultural, Vegetable & Horticultural Species
- Volume II: Tree & Shrub Species
- Constantly updated





TZT for vigour determination

- 1960-1970: Moore refined a classification for vigour determination for corn and soybean seeds: 1 to 5 if viable, and of 6 to 8 if non-viable. The presence, location and nature of staining and the physical condition of embryo structures were used as criteria in this classification scheme.
- 1981-1995: TZT for vigour included "Handbook of Vigour Test Methods" published by ISTA (Perry, 1981) and later updated by Fiala (1987) and Hampton and TeKrony (1995) and in the "Seed Vigor Testing Handbook" published by AOSA (1983).

Soybean seeds

Embrapa Soybean: TZT was perfected for seed vigour determination; several handbooks with specific procedures of the tests.

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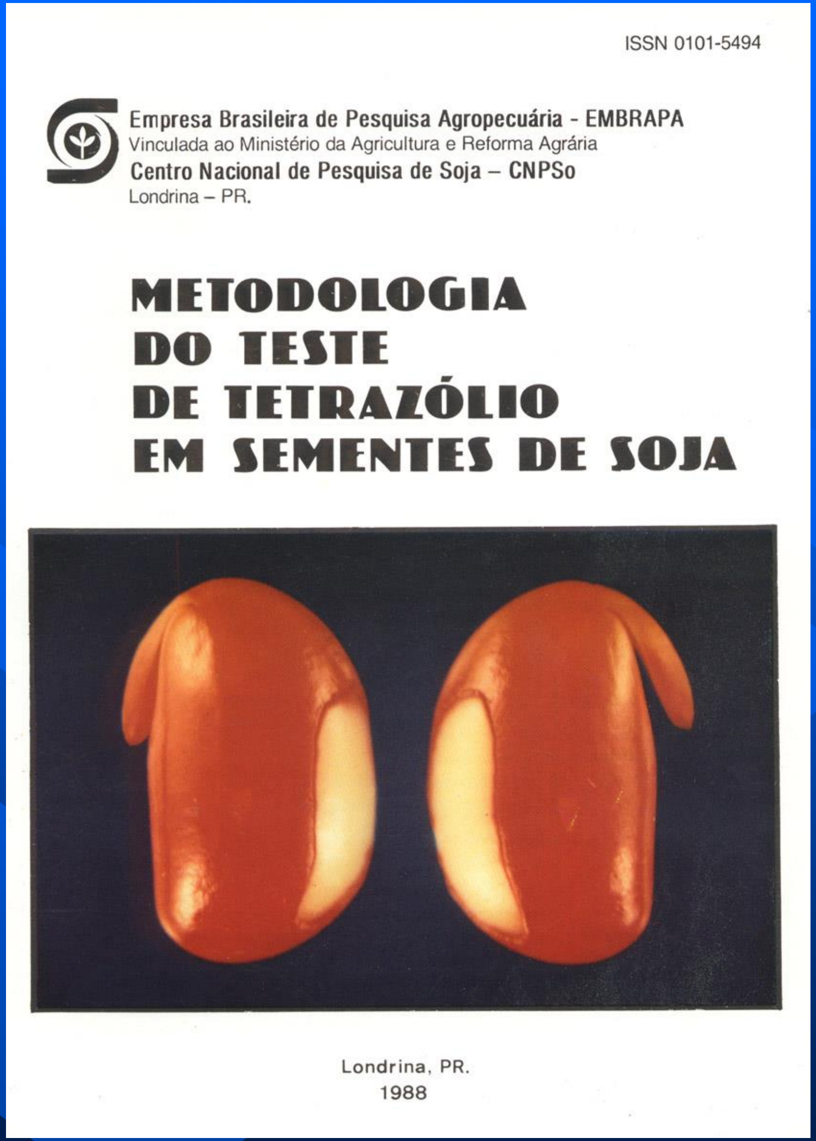
STS

1.981 Vol.3 Nº 1

1º Ciclo de Treinamento em Tetrazólio
(teste para sementes de soja)

CURITIBA PARANÁ

1981



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Centro Nacional de Pesquisa de Soja – CNPSo
Londrina – PR.

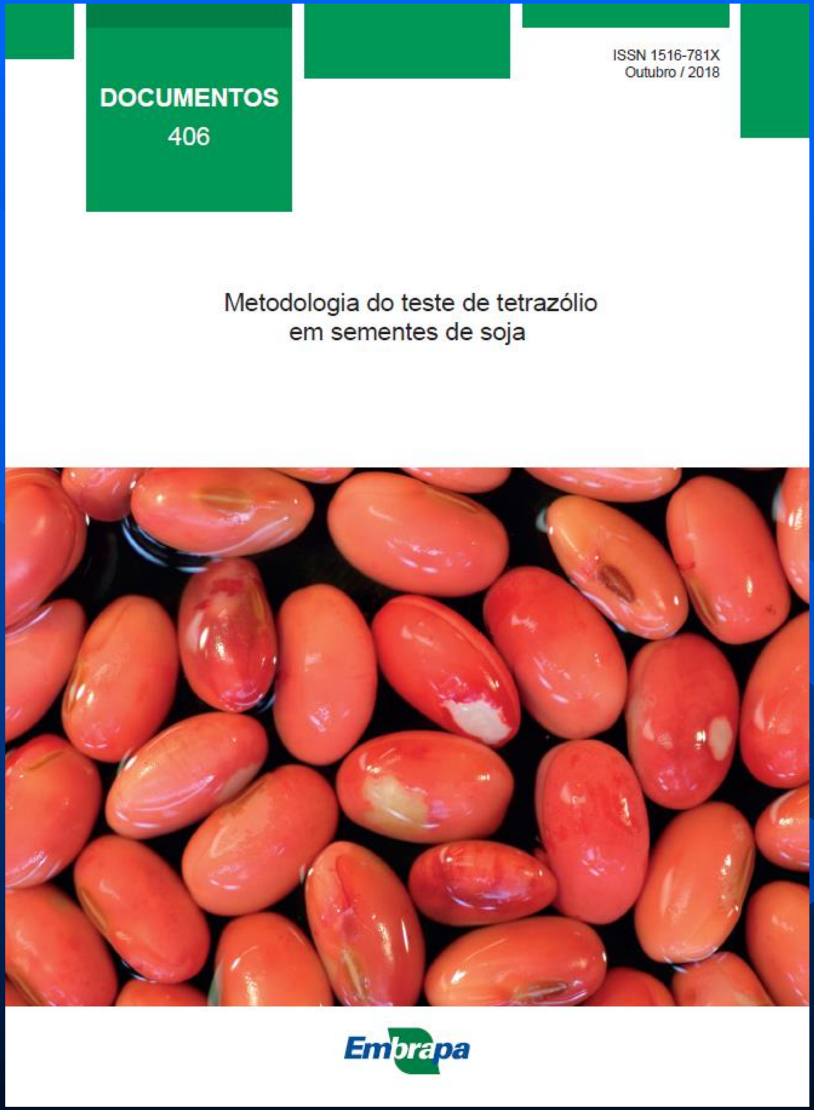
METODOLOGIA DO TESTE DE TETRAZÓLIO EM SEMENTES DE SOJA

Londrina, PR.
1988

1985

1998: 2nd edition of the handbook:
In English, Spanish and Portuguese

2018: 3rd edition of the handbook



Intensive Trainings

72 Courses in TZT and seed pathology since 1984:

- 1.634 participants.

Additionally: 24 trainings in the TZT: total of 96 courses; 2.345 trained personnel.

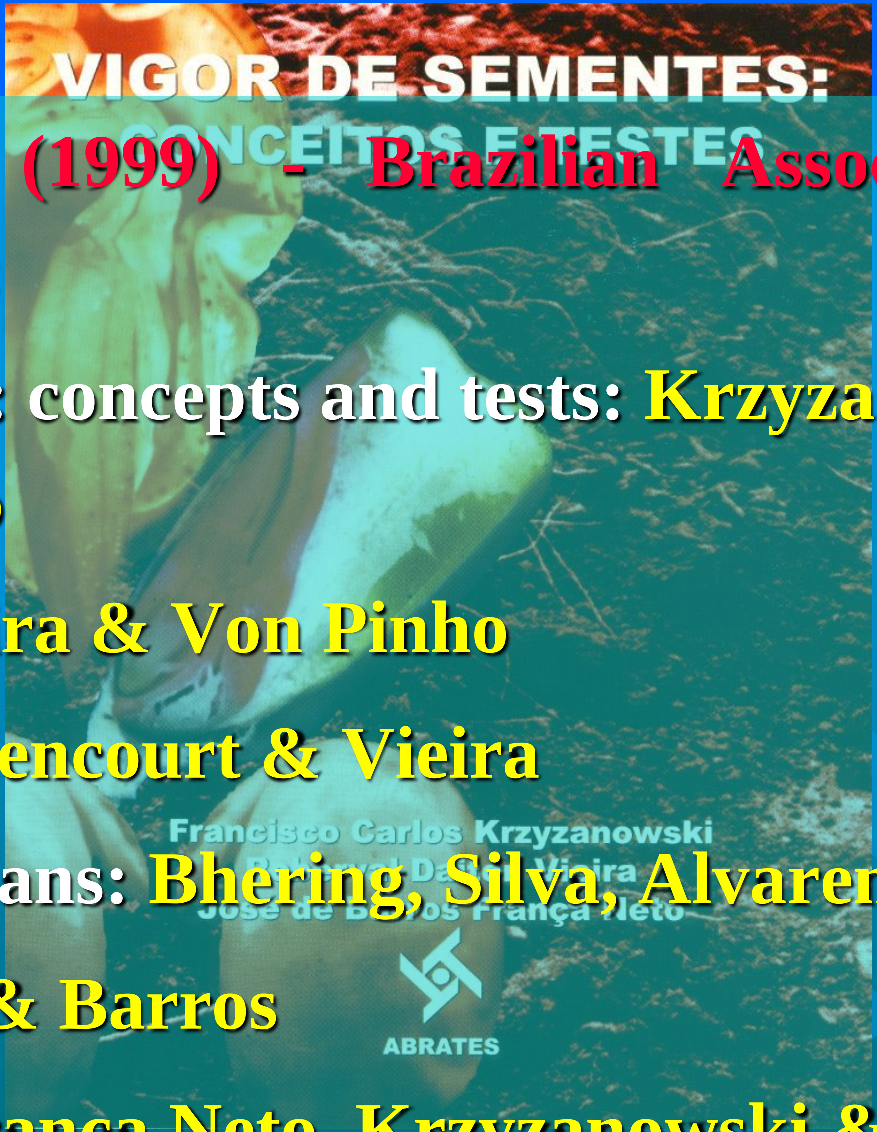
Soybean seeds

In Brazil: the TZT for estimation vigour and viability in soybean seeds is routinely used by all seed analysis laboratories.

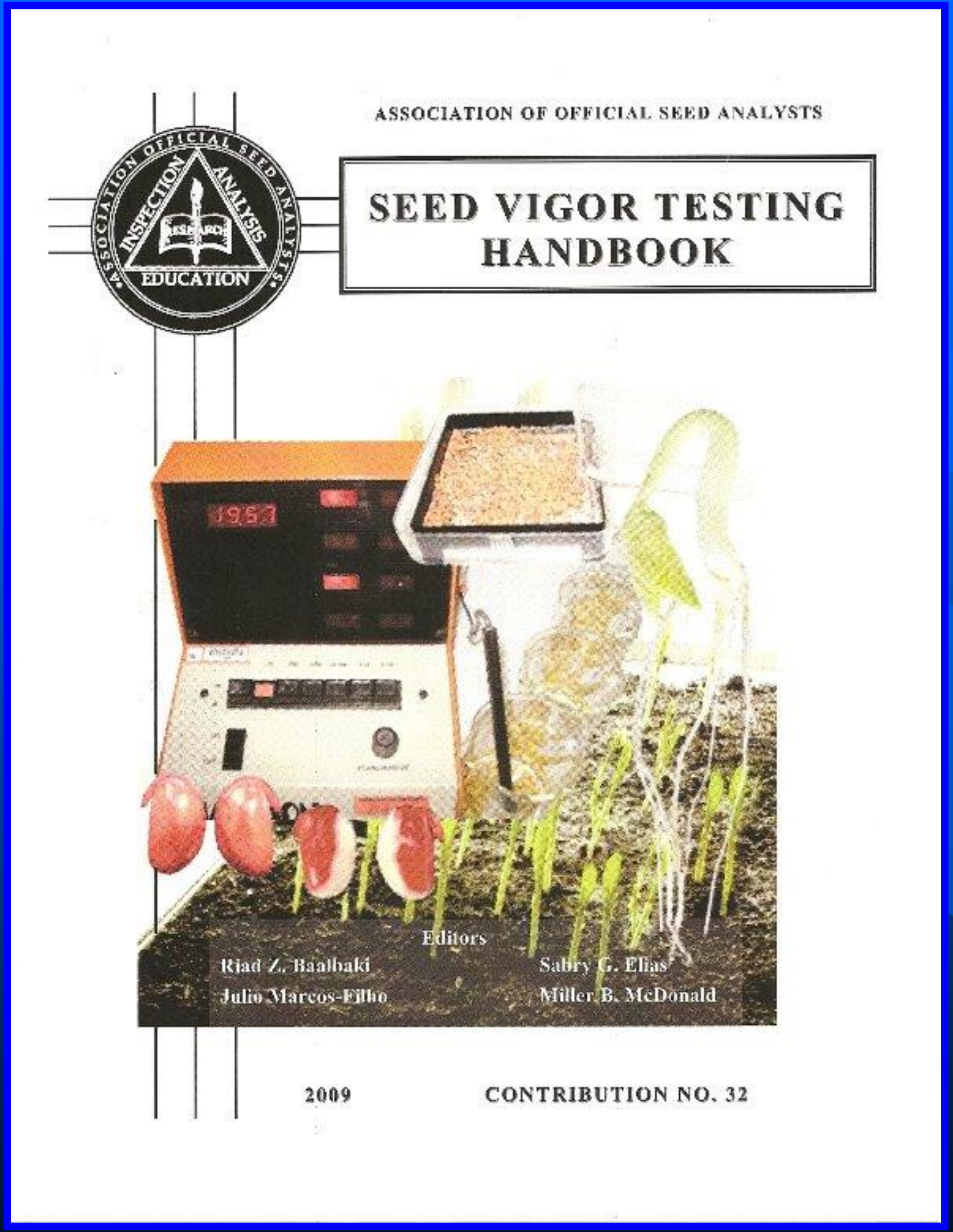
- Over 300,000 analyses per year;
- Additional work: coffee, tropical forage crops, sunflower, common beans, corn, wheat and peanuts.

Soybean seeds

In Argentina: the TZT procedure for vigour determination was improved at INTA by Craviotto et al. (1995; 2008a), who also published the methodology in English (Craviotto et al., 2008b).



- **ABRATES (1999) - Brazilian Association of Seed Technology:**
  - ✓ Seed vigour: concepts and tests: **Krzyzanowski, Vieira & França Neto**
  - ✓ Cotton: **Vieira & Von Pinho**
  - ✓ Peanut: **Bittencourt & Vieira**
  - ✓ Common beans: **Bhering, Silva, Alvarenga & Dias**
  - ✓ Corn: **Dias & Barros**
  - ✓ Soybean: **França Neto, Krzyzanowski & Costa**



Baalbaki et al., 2009:
AOSA
Seed Vigor Handbook

TZT: viability and vigour for seeds of soybean, corn, common beans, cotton and peanut

ISTA (2017): the TZT methodology for determining vigour in soybean seeds was included in the International Rules for Seed Testing, which are revised annually

Chapter 15 – Seed vigour testing

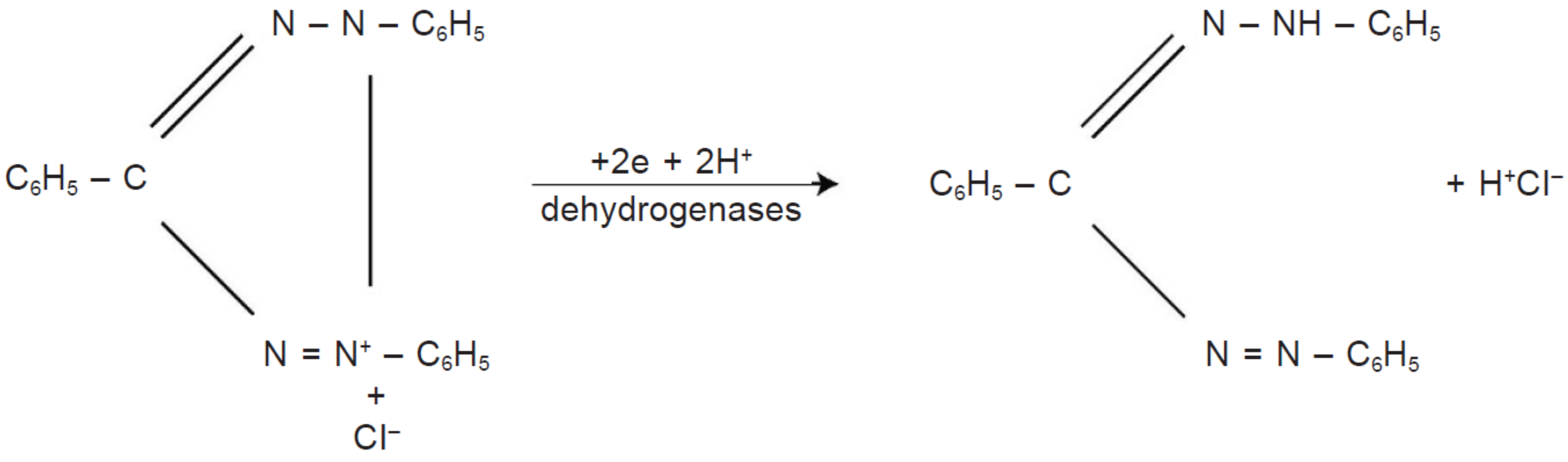
Principles of the test

- **TZT indirectly determines the respiratory activity in the cells that make up the seed tissues.**
- **The test relies on the activity of dehydrogenase enzymes, which catalyse respiratory reactions in the mitochondria, during glycolysis and the citric acid cycle (Krebs cycle).**

Principles of the test

- **These dehydrogenase enzymes, particularly malate dehydrogenase, reduce the tetrazolium salt in living tissues.**
- **When a seed is immersed in the colourless TTC-solution, the TTC penetrates into the seed tissues where it interferes with the reduction processes of the living cells by accepting a hydrogen ion.**
- **In the reduced form, the TTC-salt is a red-coloured, stable, non-diffusible substance called triphenylformazan or formazan.**

Reduction Reaction:



2,3,5-triphenyl tetrazolium chloride (colourless, diffusible)

Triphenylformazan (red, nondiffusible)

- **Normal faint red ⟶ vigorous tissue**
- **Intense red colour ⟶ deteriorating tissue**
- **Not-stained (white) ⟶ dead tissue**

Principles of the test

- **When TTC is reduced, forming triphenylformazan in the tissue, it indicates that respiratory activity is occurring in the mitochondria of seed tissue cells, which are considered alive.**
- **Respiring tissue can be found within the embryo of a seed, in cotyledons, radicle and scutellar tissue, in some nutritive endosperm tissues, in female gametophyte tissue in gymnosperms, and in the aleurone cell layer inside the pericarp of grasses.**

Use of the TTZ for vigour determination

- **In the recently published ABRATES (Brazilian Association of Seed Technologists) vigour handbook "Seed Vigour: Concepts and Tests", specific procedures are described, which include in detail the methodology for carrying out the TZT for the assessment of viability and vigour in seeds of 17 species.**
- **Some test information for some of these species will be illustrated.**

Abrates: 2020: Seed vigour: concepts and tests (2nd edition):

Editors: Krzyzanowski, Vieira, França-Neto, Marcos-Filho

- **Tetrazolium test: vigour and viability:** 17 species
  - ✓ Cotton: Von Pinho, Oliveira, Krzyzanowski, França-Neto
  - ✓ Peanut: Vieira, França-Neto, Krzyzanowski
  - ✓ Common beans: Krzyzanowski, França-Neto, Dias
  - ✓ Sunflower: Silva, França-Neto, Panobianco
  - ✓ Tropical forage grasses: 8 species: Custódio, Aguiar
  - ✓ Corn: França-Neto, Krzyzanowski, Dias, Barros
  - ✓ Soybean: França-Neto, Krzyzanowski
  - ✓ Solanaceae: tomato and bell pepper: Marcos-Filho
  - ✓ Wheat: Carvalho, Krzyzanowski, Ohlson, Panobianco

General procedures of the test

- Initially, the seeds are preconditioned in moist germination paper, so that its moisture content reaches values in which the respiration processes are activated in the mitochondria; for corn and soybeans – above 25% MC (Vertucci & Leopold, 1987);
- Seeds stained by placing them into the TTC solution in darkness; concentration varies from 0,075 to 0,5%, depending on the species; to speed up the processes, temperature can be raised to 30 - 40 ^{o}C;
- After the staining process, the seeds are rinsed with water to remove the excess of TTC solution;
- Reading is performed, following specific procedure for that particular species.

Soybean seeds [*Glycine max* (L.) Merrill]

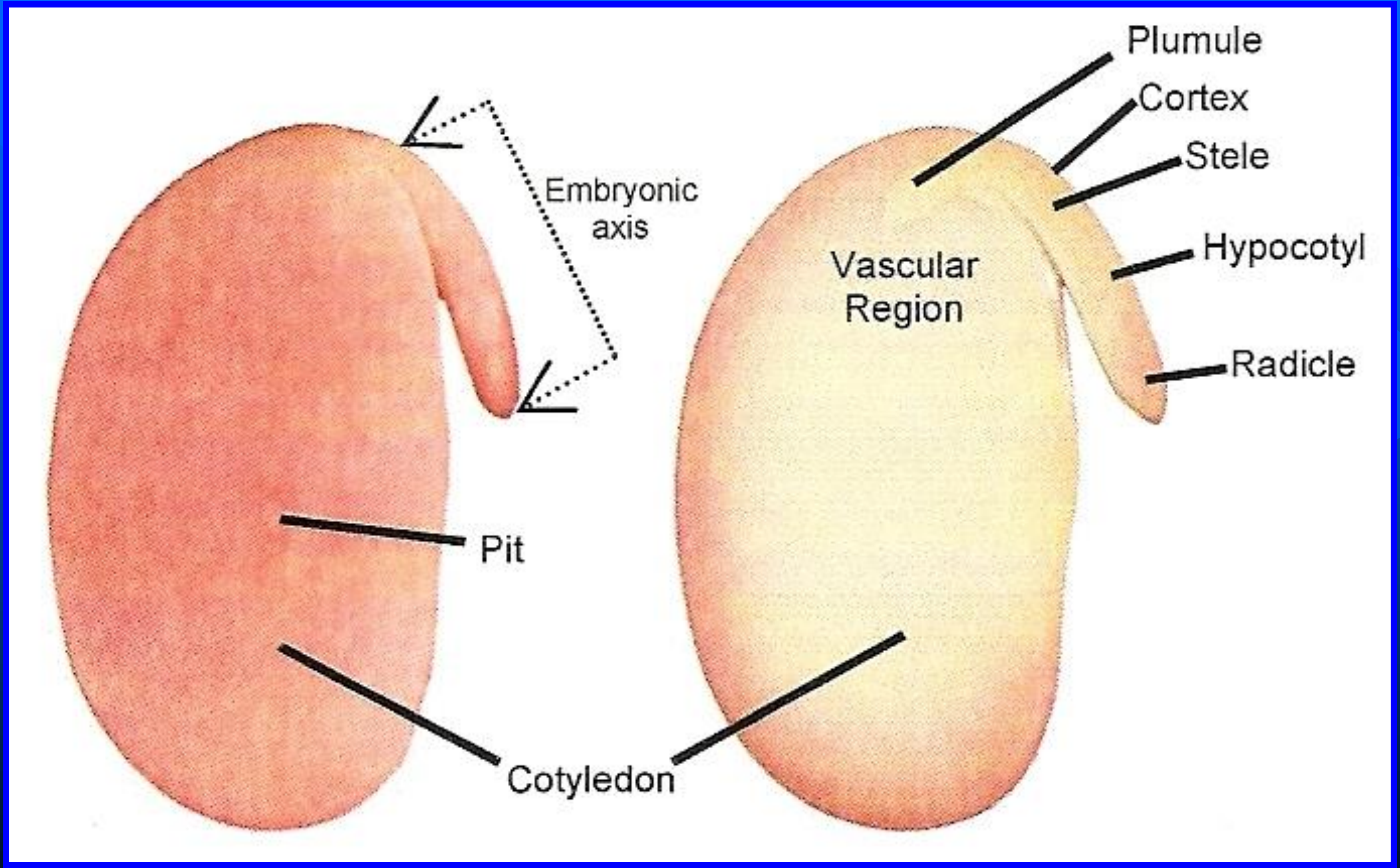
- França-Neto and Krzyzanowski (2020) cover in detail the methodology for testing soybean seeds;
- Preconditioning: rolled germination towel for 16 h at 25 ºC
- Staining in darkness: 0,075 – 0,1% TTC solution for 2,5-3,0 h at 35-40 ºC
- Seeds are rinsed and kept submersed in water;
- Reading is performed, according to specific procedures.




SANREMO
TOP
STOCK


SANREMO
TOP
STOCK

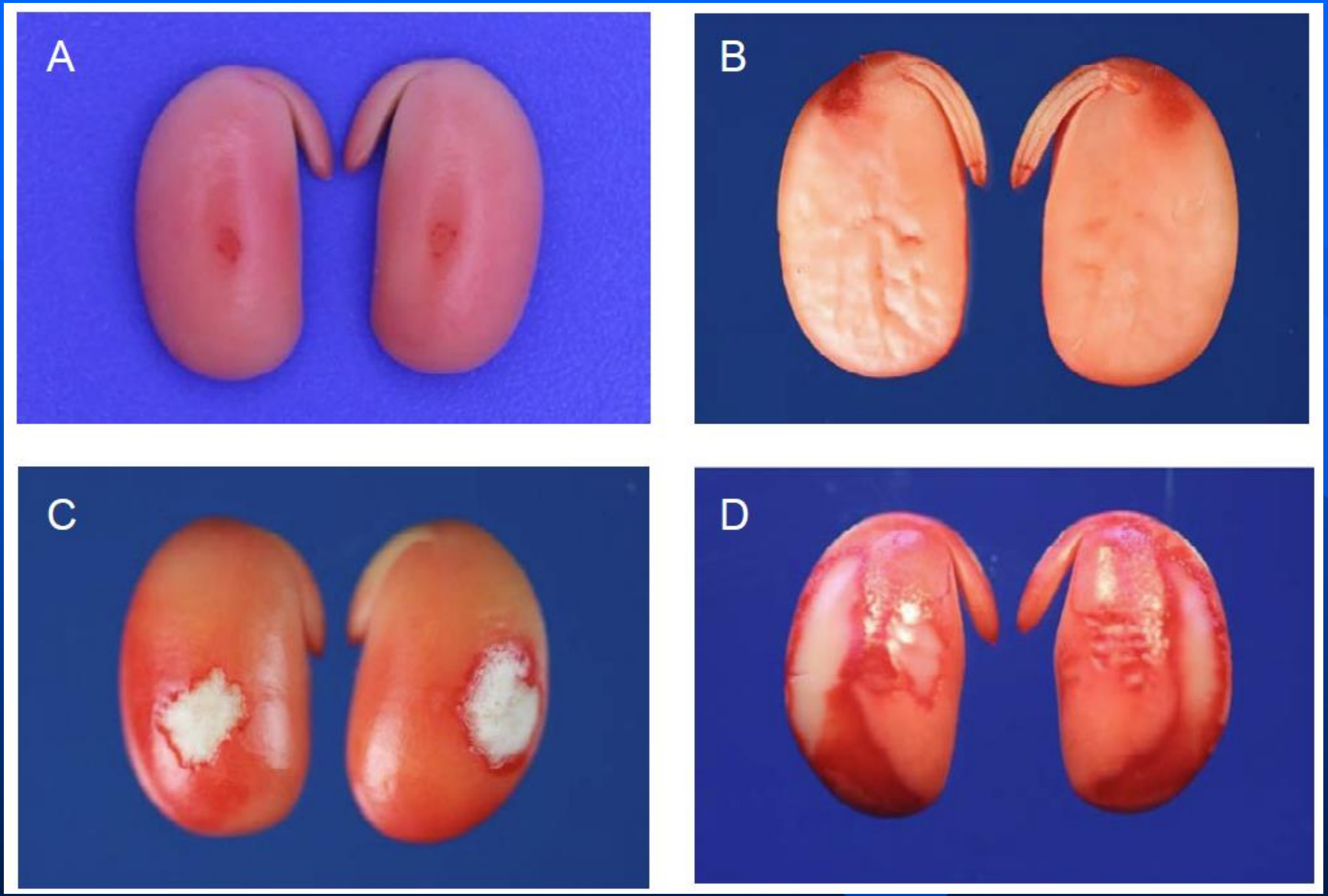
Soybean seeds [*Glycine max* (L.) Merrill]



Soybean seeds [*Glycine max* (L.) Merrill]

- **Seeds are classified into eight categories:**
- **Seeds from Classes 1 to 3 are viable and vigorous;**
- **Classes 4 and 5 are viable but non-vigorous;**
- **Classes 6 and 7 are non-viable; and**
- **Class 8 are dead seeds.**
- **The characterization of the main damages that affect the quality of soybean seeds is presented, such as mechanical damage, weathering damage, and that caused by stinkbugs.**

Soybean seeds [*Glycine max* (L.) Merrill]

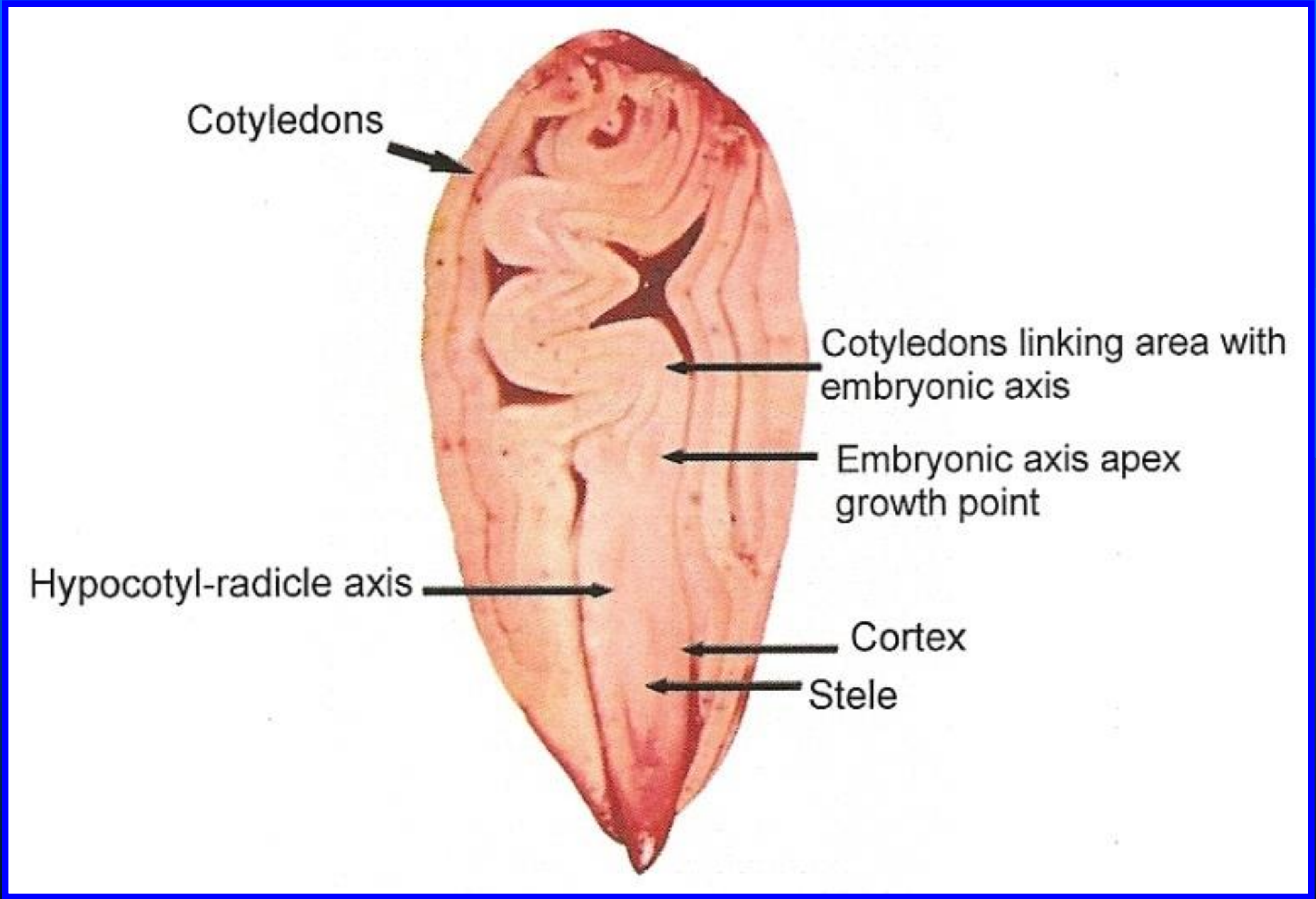


Soybean seeds, stained by tetrazolium solution. A, high vigour seeds; B, seeds with latent mechanical damage; C, seeds with stinkbug damage; D, seed with weathering damage.

Cotton seeds (*Gossypium hirsutum* L.)

- Von Pinho et al. (2020) classify the seeds into eight vigour classes.
- Seeds of Classes 1 to 3 are viable and vigorous;
- Classes 4 and 5 are viable but non-vigorous;
- Classes 6 and 7 are non-viable; and
- Class 8 are dead seeds.
- Details are presented to characterise the main damage that affects cotton seeds, such as mechanical damage, weathering damage and those caused by stinkbugs.

Cotton seeds (*Gossypium hirsutum* L.)



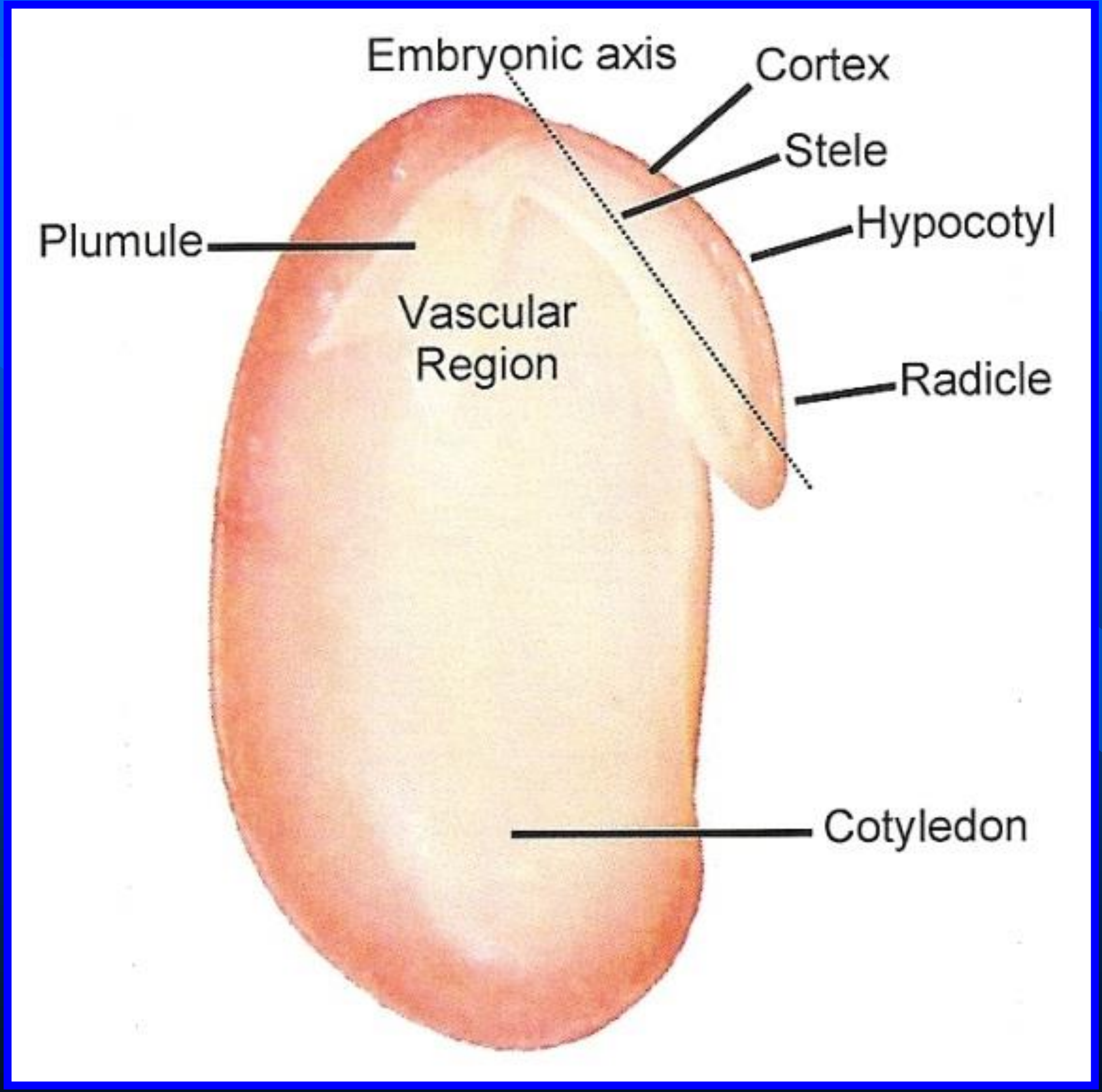
Cotton seeds (*Gossypium hirsutum* L.)

Cotton seeds stained by tetrazolium solution. A, high vigour seeds; B, seed with latent mechanical damage; C, seed with stinkbug damage; D, seed with weathering damage.

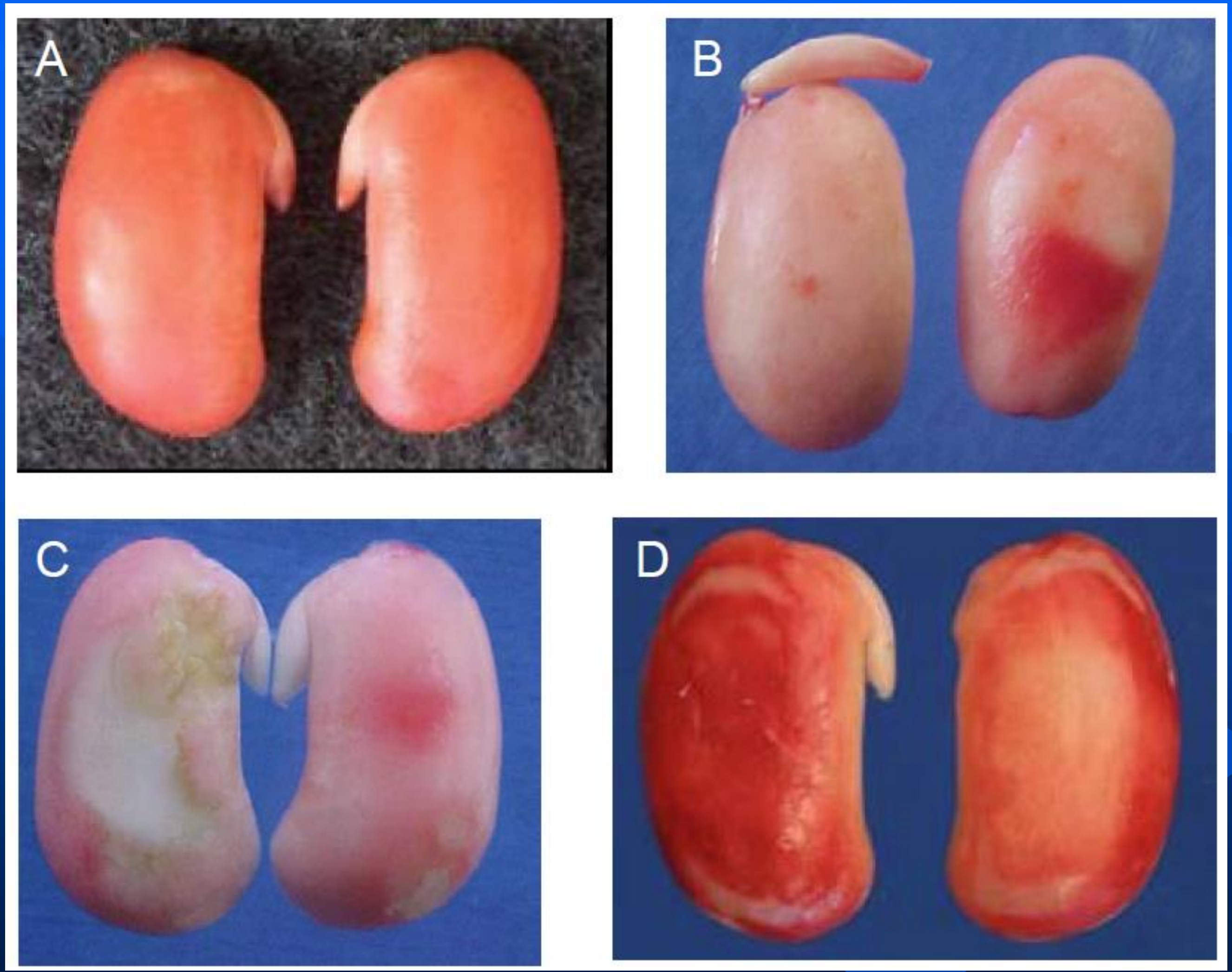
Common bean seeds (*Phaseolus vulgaris* L.)

- Krzyzanowski et al. (2020) give the detailed test methodology for classifying common bean seeds into eight vigour classes, with seeds from
- Classes 1 to 3 viable and vigorous;
- Classes 4 and 5 viable but non-vigorous;
- Classes 6 and 7 non-viable; and
- Class 8 dead seeds.
- The characterization of the main types of damage that affect the quality of common bean seeds is presented: mechanical damage; those of weathering and those caused by stinkbugs.

Common bean seeds (*Phaseolus vulgaris* L.)



Common bean seeds (*Phaseolus vulgaris* L.)

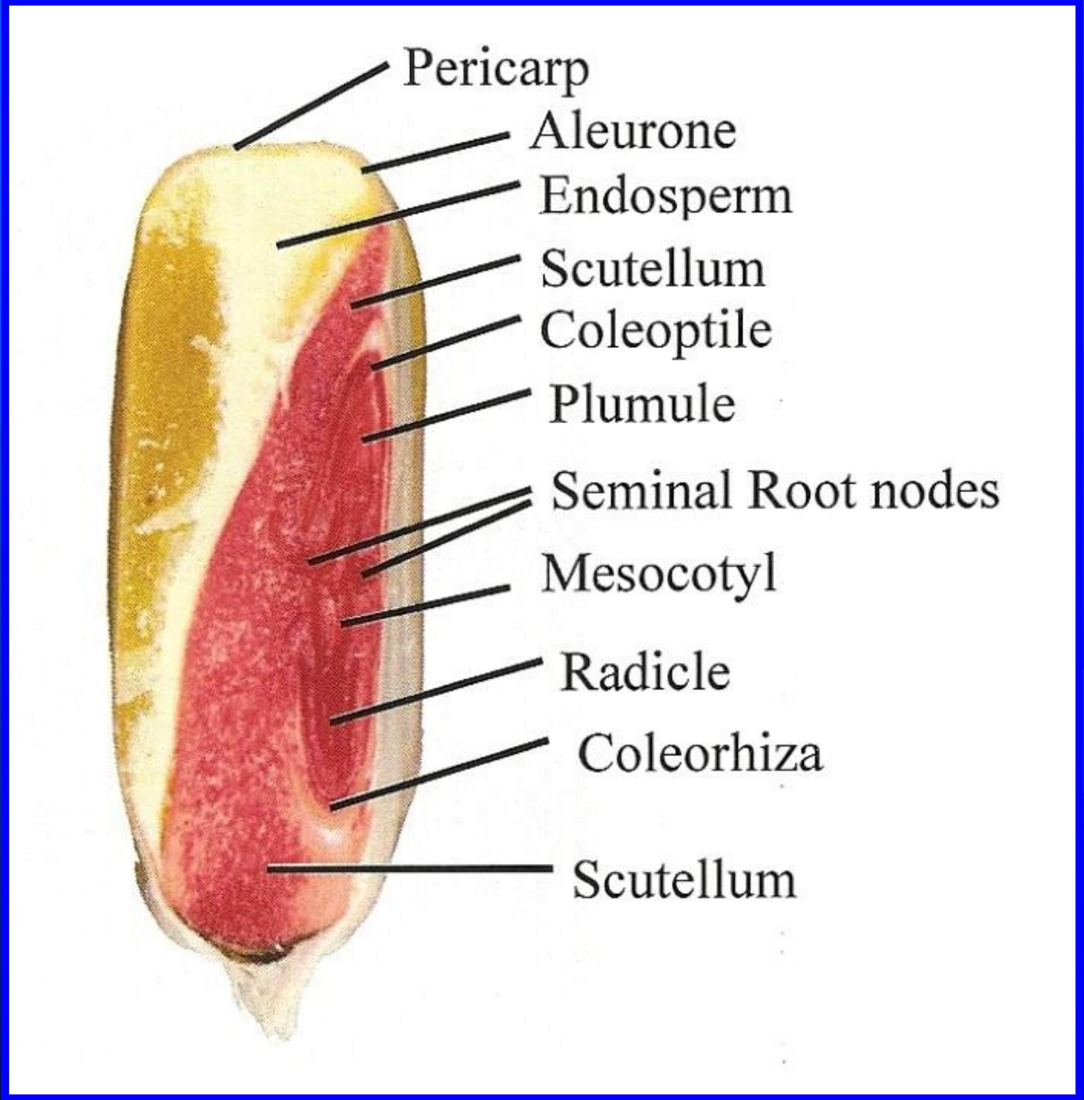


Common bean seeds, stained by tetrazolium solution. A, high vigour seeds; B, seeds with mechanical damage; C, seeds with stinkbug damage; D, seed with weathering damage.

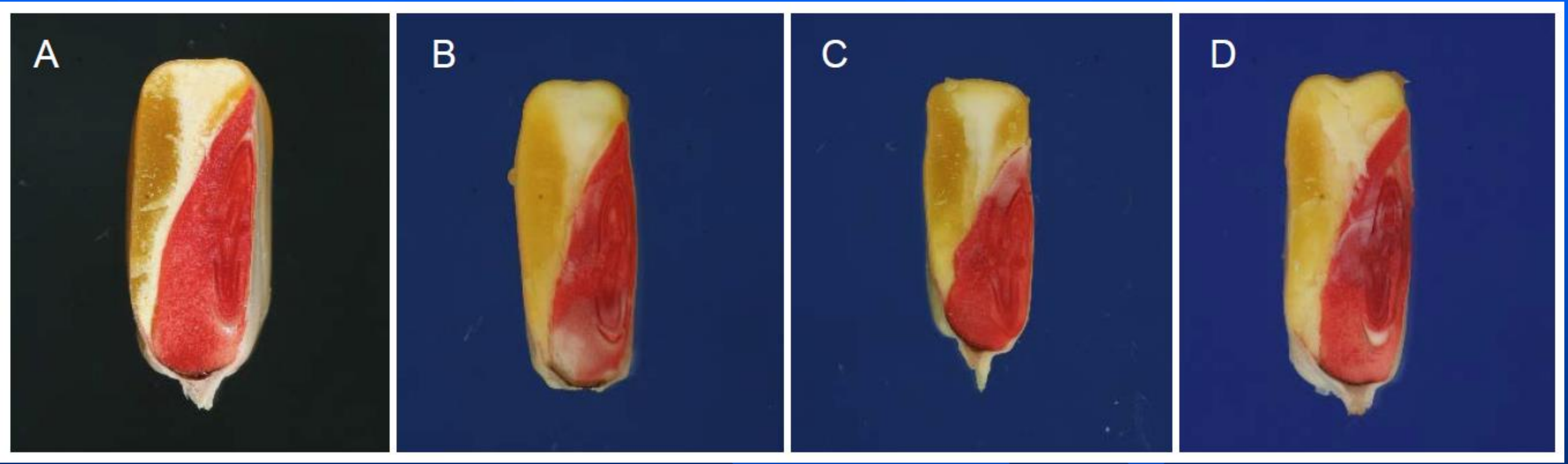
Maize seeds (*Zea mays* L.)

- França-Neto et al. (2020) cover in detail the test methodology for maize seeds, defining three vigour classes:
- Class 1 viable and vigorous;
- Class 2 viable and non-vigorous;
- Class 3 non-viable.

Maize seeds (*Zea mays* L.)



Maize seeds (*Zea mays* L.)

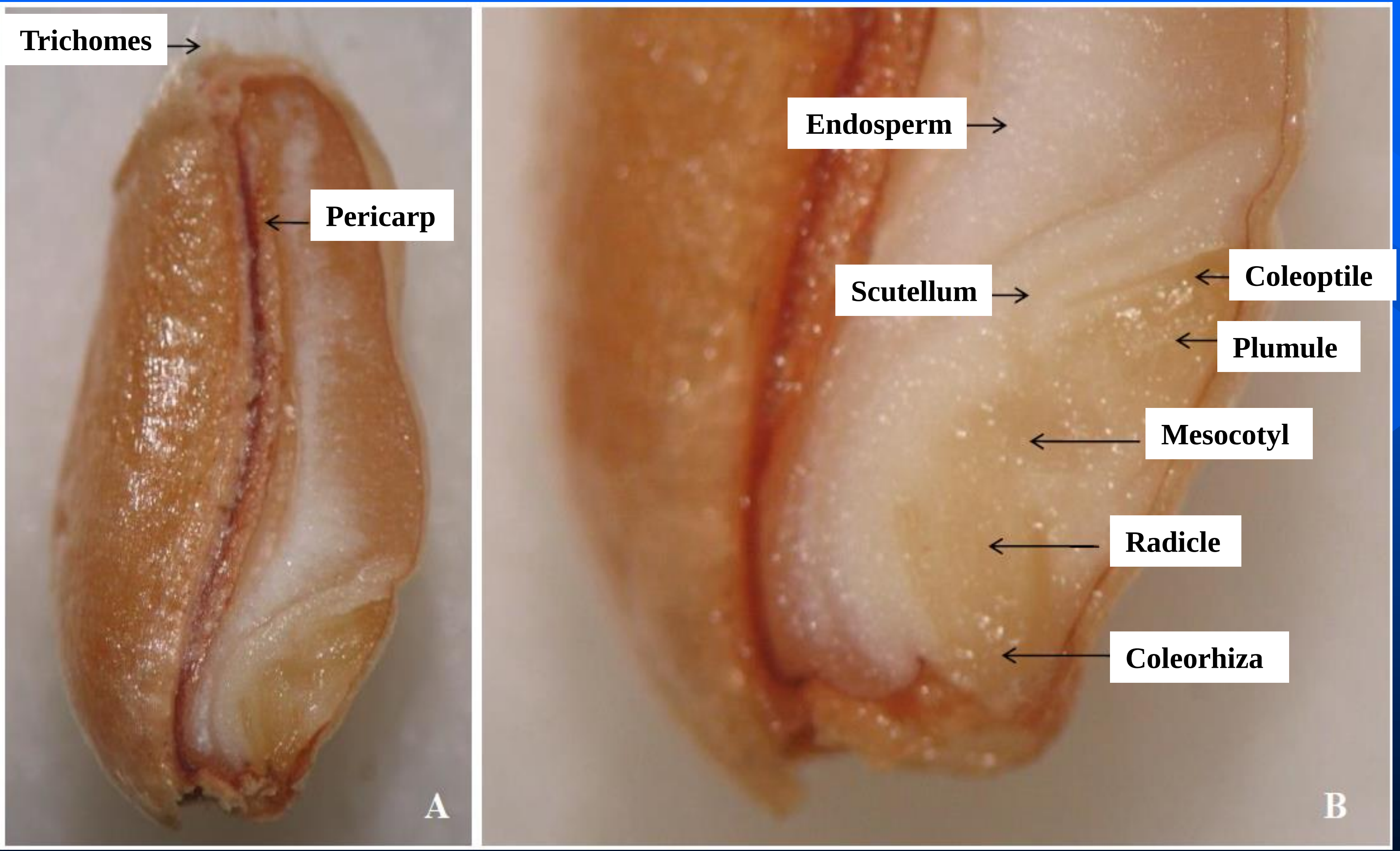


Maize seeds stained by tetrazolium solution. A, viable and vigorous seed; B, viable non-vigorous seed, with mechanical damage in the lower region of the scutellum, coleorriza and radicle; C, viable non-vigorous seed, with mechanical damage in the upper region of the scutellum; D, non-viable seed, with mechanical damage affecting part of the scutellum, damaging the coleoptile and plume.

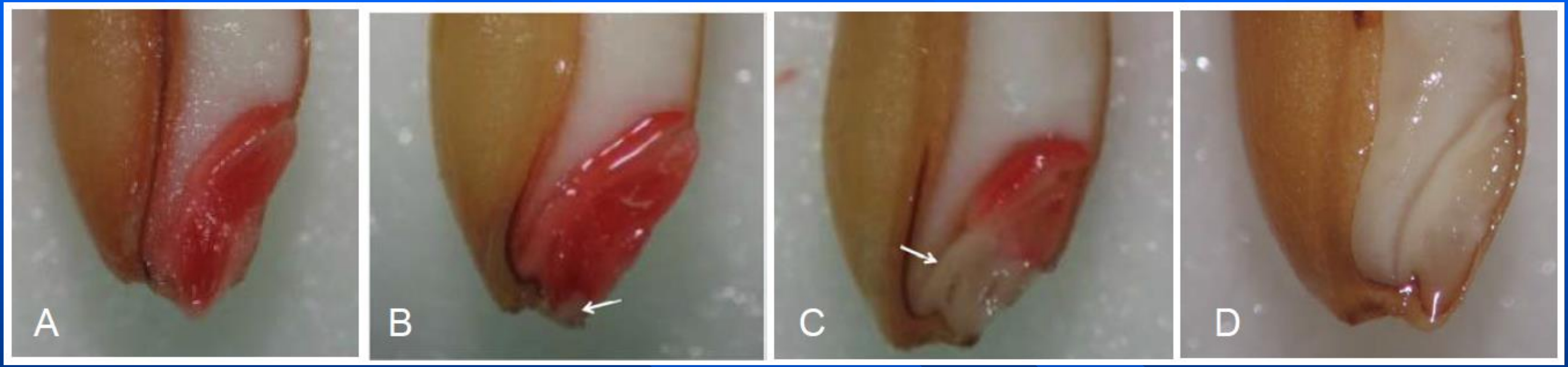
Wheat seeds (*Triticum aestivum* L.)

- Carvalho et al. (2020) describe the detailed methodology of the test for wheat seeds, which is classified into four classes:
- Class 1 are viable and vigorous seeds;
- Class 2 are viable and non-vigorous;
- Class 3 are non-viable; and
- Class 4 are dead seeds.

Wheat seeds (*Triticum aestivum* L.)



Wheat seeds (*Triticum aestivum* L.)



Wheat seeds, stained by tetrazolium solution. A, viable and vigorous seed; B, viable seed non-vigorous; C, non-viable seeds; D, non-viable (dead) seed.

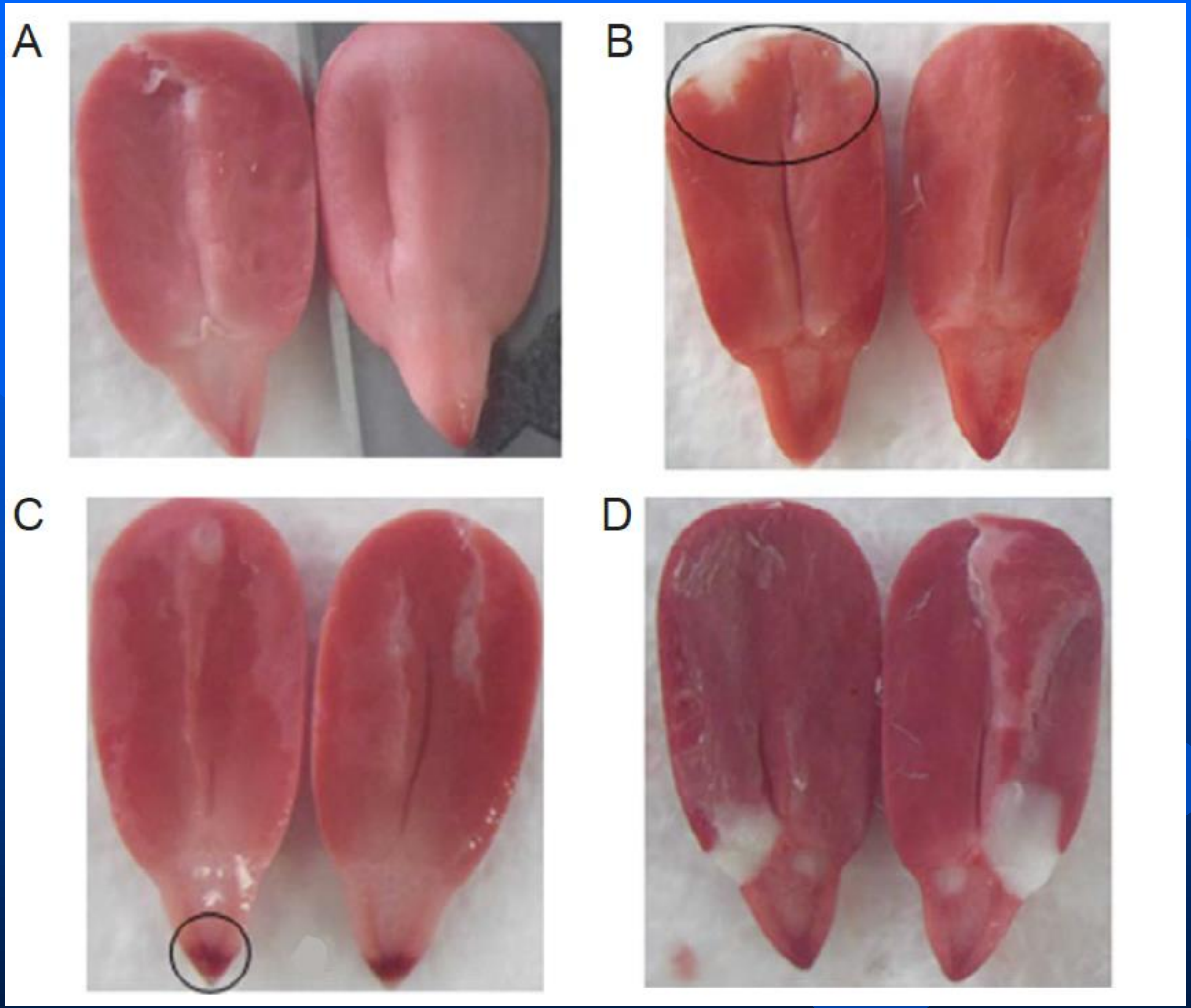
Sunflower seeds (*Helianthus annuus* L.)

- Silva et al. (2020) cover the test methodology for the determination of vigour in sunflower seeds, classifying the seeds into five classes:
- Class 1 are high vigour seeds;
- Class 2 are medium vigour seeds;
- Class 3 are low vigour seeds;
- Class 4 are non-viable seeds; and
- Class 5 are dead seeds.

Sunflower seeds (*Helianthus annuus* L.)

Evaluation of sunflower embryos: A. longitudinal cut between the cotyledons; B. internal face of the cotyledons; C. cross sectioned embryo, showing the vital its components: (v) vascular region; (p) plumule; © central cylinder; (e) embryonic axis: radicle-hypocotyl

Sunflower seeds (*Helianthus annuus* L.)

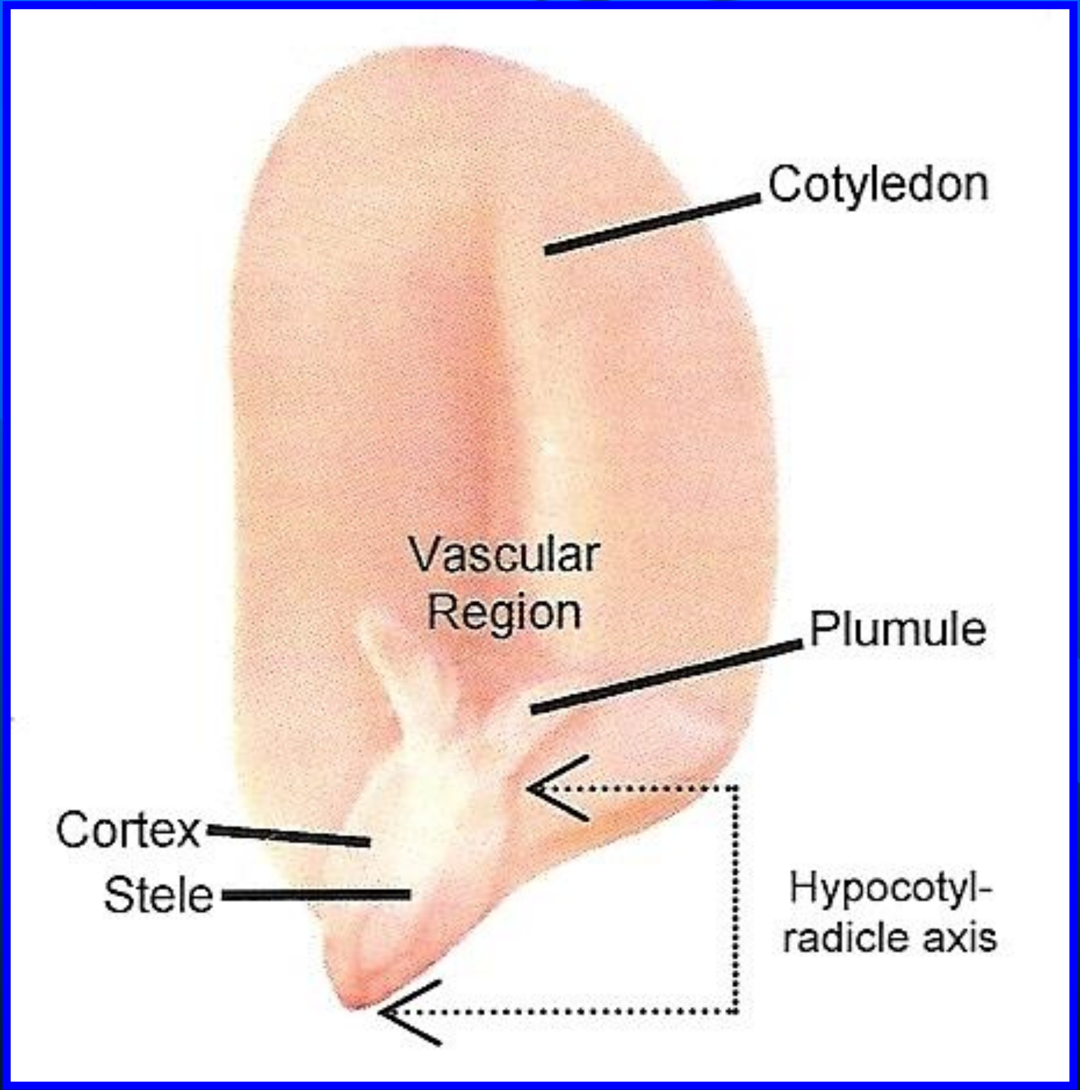


Sunflower seeds stained by tetrazolium solution. A, high vigour seed; B, medium vigour seed, with damage (circle) observed in one of the cotyledons; C, low vigour seed, with mechanical damage (circle) on the radicle; D, non-viable seed.

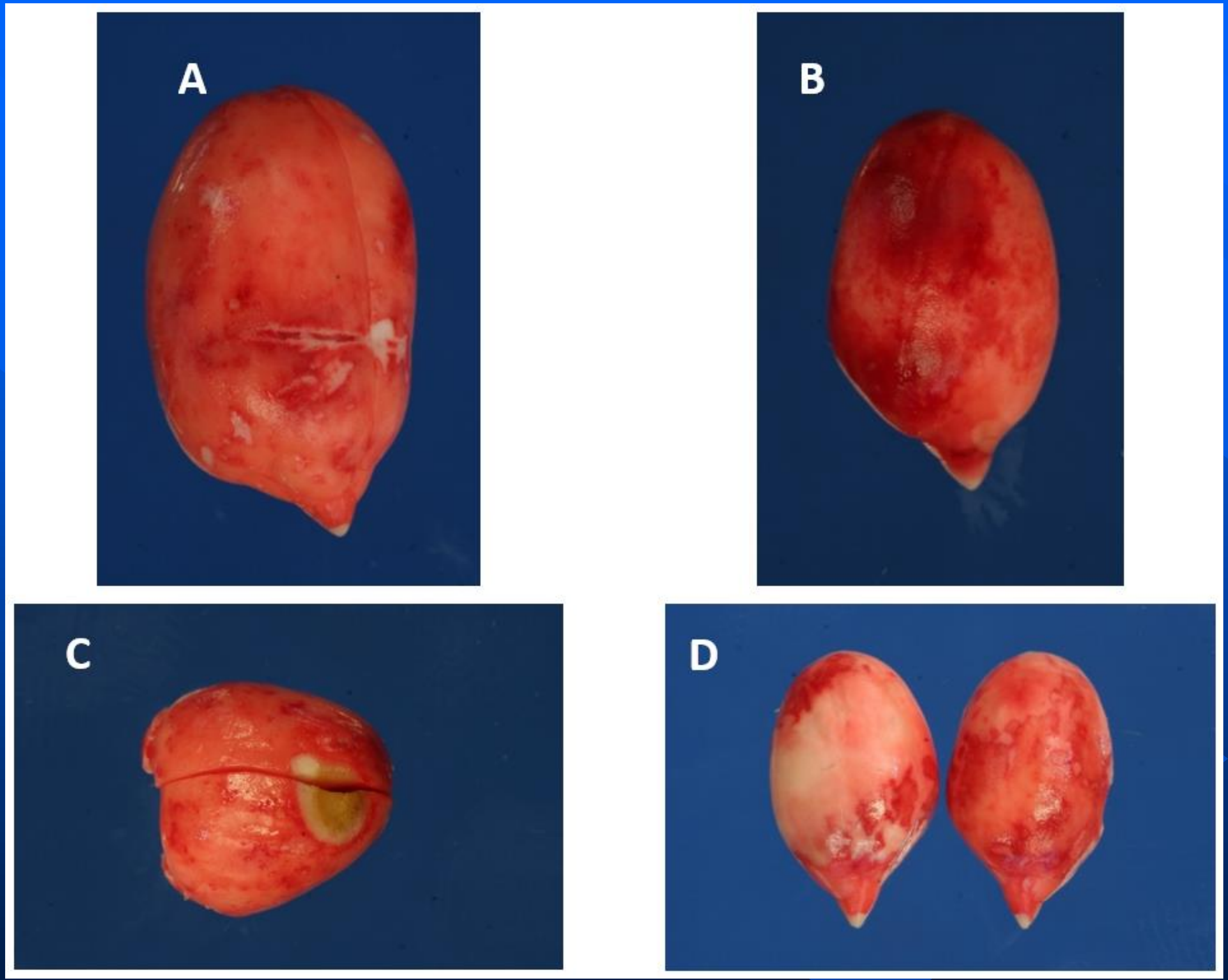
Peanut seeds (*Arachis hypogaea* L.)

- Vieira et al. (2020) illustrate the details the tetrazolium test, classifying peanut seeds into three vigour classes:
- Class 1 are viable and vigorous seeds;
- Class 2 are viable non-vigorous seeds; and
- Class 3 non-viable seeds.

Peanut seeds (*Arachis hypogaea* L.)



Peanut seeds (*Arachis hypogaea* L.)

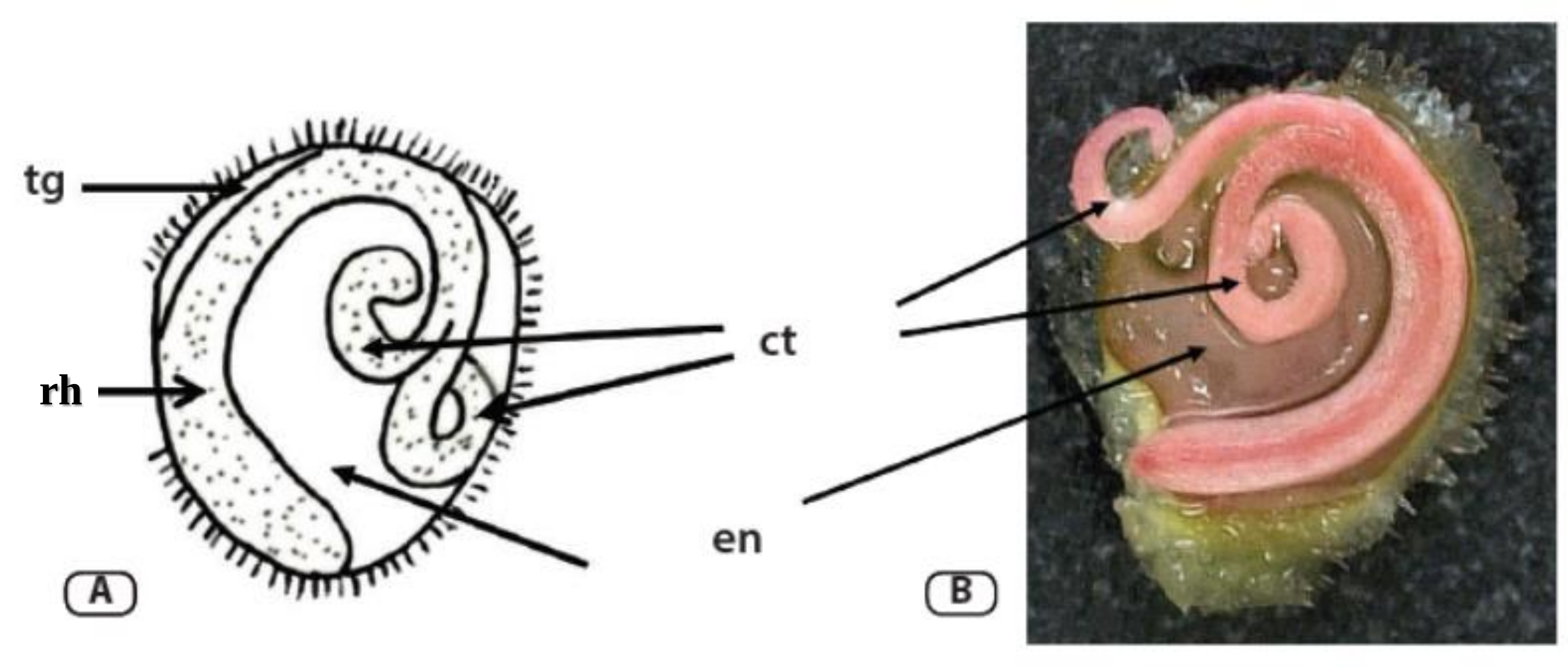


Peanut seeds stained by tetrazolium solution. A, seeds with immediate mechanical damage; B. seed with latent mechanical damage; C, seed with damage caused by stinkbug; D, seed with weathering damage.

Solanaceae: tomato (*Solanum lycopersicum* L.) and bell pepper (*Capsicum annuum* L.)

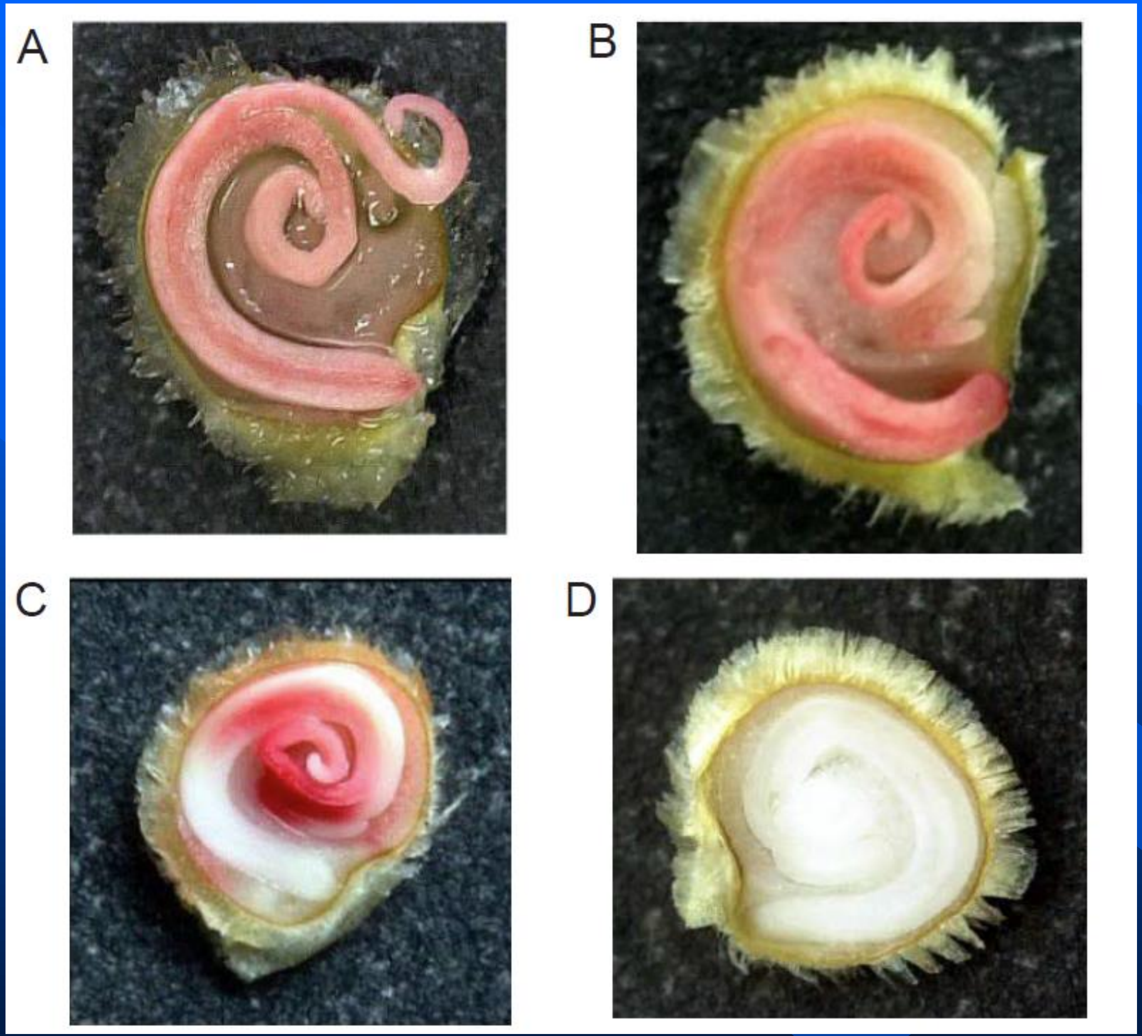
- Marcos-Filho (2020) addresses the test methodology for determining seed vigour in these two species.
- Seeds are classified into:
- Class 1 viable and vigorous;
- Class 2 viable non-vigorous; and
- Class 3 non-viable.

Solanaceae: tomato (*Solanum lycopersicum* L.) and bell pepper (*Capsicum annuum* L.)



A. Embryo structure of tomato/bell pepper seed; B. embryo stained by the tetrazolium solution. (tg) tegument/seed coat; (ct) cotyledons; (en) endosperm; (rh) radicle-hypocotyl axis.

Solanaceae: tomato (*Solanum lycopersicum* L.)

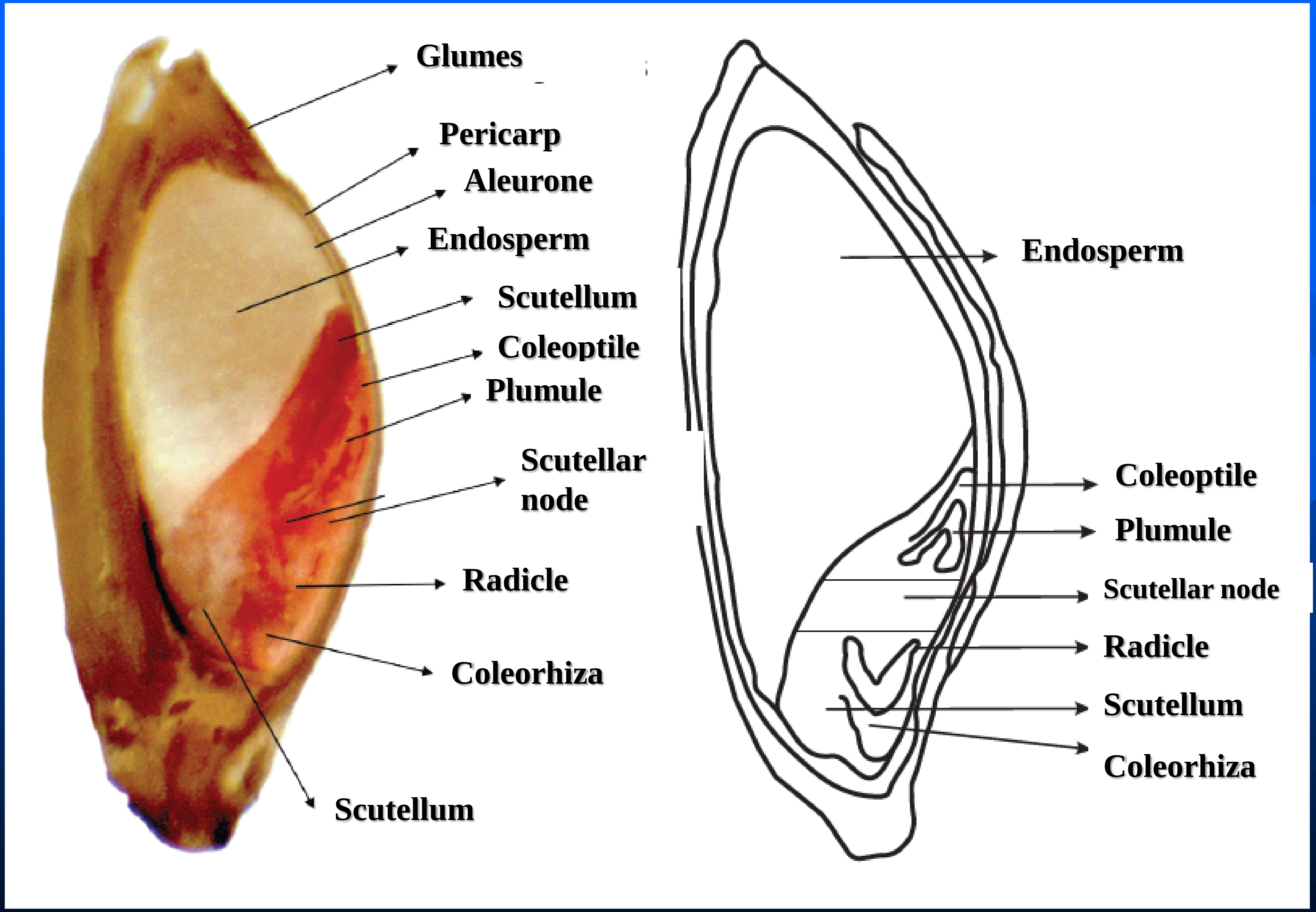


Tomato seeds stained by tetrazolium solution. A, viable and vigorous seed; B, viable non-vigorous; C, non-viable seed; D, non-viable (dead) seed.

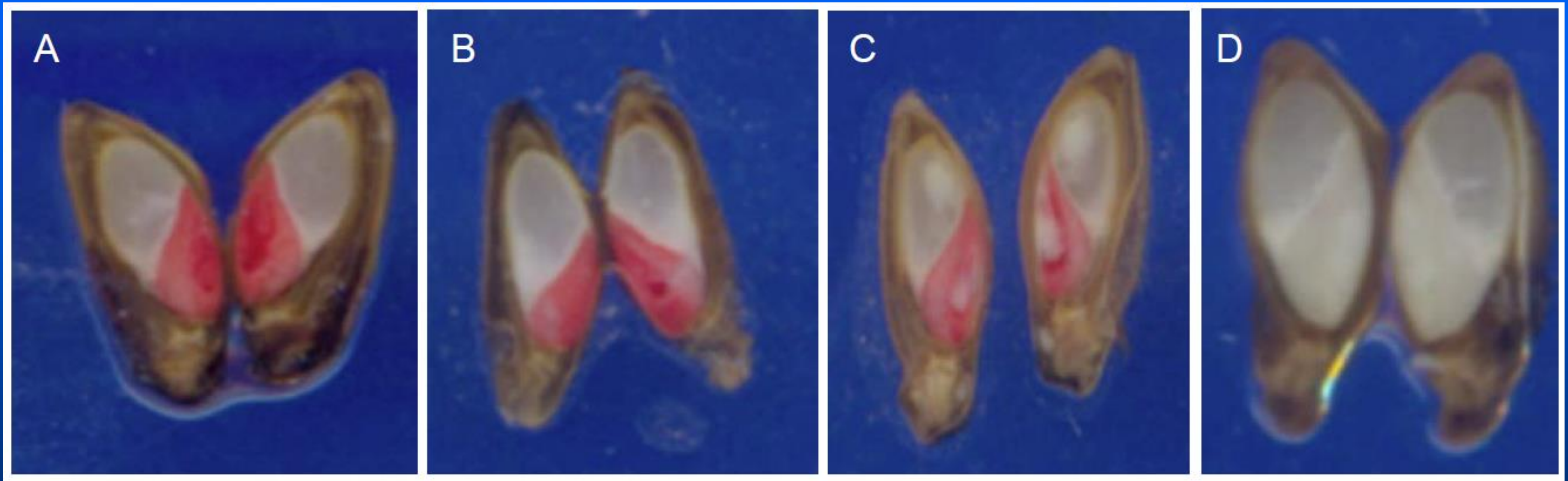
Tropical forage grass seeds

- **Custodio and Aguiar (2020) present the test methodology for vigour determination in seeds of eight species, with illustrations of *Urochloa brizantha* seeds, representing the other species, such as *Andropogon* spp., *Cynodon dactylon* (L.) Pers., *Malinis* spp., *Panicum* spp., *Paspalum* spp., *Pennisetum* spp. and *Setaria* spp.**
- **Seeds are classified into three classes:**
- **Class 1 viable and vigorous;**
- **Class 2 viable and nonvigorous; and**
- **Class 3 non-viable.**

Tropical forage grass seeds



Tropical forage grass seeds



Seeds of *Urochloa brizantha* cv. Marandu stained by tetrazolium solution. A. viable and vigorous seed; B. viable non-vigorous seed; C. non-viable seed; D. non-viable (dead) seed.

Final comments

- The use of tetrazolium results, as with any vigour test, can assist the categorisation of vigour levels for different seed lots and for estimating the performance of these lots in the field under optimum and stressful conditions.
- The method of the test has been perfected for determining viability and vigour of several seed species. However, due to the dynamism of the test, its methods will keep on being developed and perfected for different species.
- Referee tests for determining the accuracy and precision of the method should also be conducted to verify the accuracy of tetrazolium interpretation.
- Additionally, with the improvement of techniques of computerised image analysis, efforts should be dedicated to develop softwares for reading and estimating seed viability and vigour by digital means.

Thank you!

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