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Review Article

From crop seeds to Brazilian forest seeds: history of validation methods for germination tests[1]

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ABSTRACT

For the commercialization of forest seeds, it is necessary to validate methods for germination tests. This review aimed to highlight the history of validation methods for germination tests of forest seeds based on reference research, classic and recent, as well as on researchers reports. These tests began in 1928, but only in 1954 methods were included in the official European rules of the International Seed Testing Association (ISTA). In Brazil, the Ministry of Agriculture, Livestock and Food Supply (MAPA) chose to adopt the methods by ISTA since the first edition of its rules, in 1967. Thus, the Brazilian official rules began to describe forest species with methods validated by international associations. The "Seeds Law" changed the scenario by demanding that analyzes be carried out using methods made official by the MAPA, and not just by mirroring the ISTA rules or bibliographic consultation. Based on this law, the first validation record for a species was made by the MAPA in 2013. The first records to make forest species methods official occurred in 2010 (10 species), 2011 (15 species) and 2013 (25 species). It is worth mentioning that the validation is a continuous and dynamic procedure, with no risk of stagnating the scientific research, since it does not exclude the possibility of incorporating new methods besides the validated ones.

KEYWORDS: Friedrich Nobbe, rules for seed germination, standardization of germination methodologies.

RESUMO

De sementes agrícolas a sementes florestais brasileiras: histórico da validação de métodos para testes de germinação

Para a comercialização de sementes florestais, é necessário validar métodos para testes de germinação. Esta revisão objetivou fazer um histórico de métodos de validação para testes de germinação de sementes florestais com base em bibliografias clássicas e recentes, bem como relatos de pesquisadores. Esses testes começaram em 1928, mas somente em 1954 métodos foram incluídos nas regras oficiais europeias da Associação Internacional para Teste de Sementes (ISTA). No Brasil, o Ministério da Agricultura, Pecuária e Abastecimento (MAPA) optou por adotar os métodos da ISTA desde a primeira edição de suas normas, em 1967. Assim, as normas oficiais brasileiras passaram a descrever métodos para espécies florestais validados por associações internacionais. A "Lei de Sementes" mudou o cenário ao exigir que as análises fossem feitas por meio de métodos oficializados pelo MAPA, e não apenas por espelhamento de regras da ISTA ou consulta bibliográfica. Com base nessa lei, o primeiro registro de validação de uma espécie foi feito pelo MAPA em 2013. Os primeiros registros de oficialização de métodos para espécies florestais ocorreram em 2010 (10 espécies), 2011 (15 espécies) e 2013 (25 espécies). Vale ressaltar que a validação é um procedimento contínuo e dinâmico, sem risco de estagnação da pesquisa científica, pois não exclui a possibilidade de incorporação de novos métodos além dos validados.

PALAVRAS-CHAVE: Friedrich Nobbe, regras para germinação de sementes, padronização de metodologias de germinação.

INTRODUCTION

In the context of seed technology, the term validation is relatively recent, because collaborative trials among laboratories for adjustments of methods for seed germination analysis did not have that designation. Detailed records of this process and, as a result, germination methods culminated in the inclusion of species in the official European International Seed Testing Association (ISTA)

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and North American Association of Official Seed Analysts (AOSA) rules (Rogers 1967, Steiner 2000, Steiner & Kruse 2006, Steiner & Kruse 2007, Steiner et al. 2008, Steiner et al. 2009, Muschick 2010, Steiner et al. 2011).

Studies by the ISTA aimed at standardizing methods back to the XIX century, specifically to 1877, and the principles adopted at that time for collaborative testing are still applied in the present days (Steiner 2000, Steiner & Kruse 2006, Steiner & Kruse 2007, ISTA 2007, Steiner et al. 2008). On the other hand, the AOSA prioritized researcher query for the standardization of germination methods, instead of collaborative testing (Rogers 1967).

In Brazil, from the publication of the first (1967) until the last (2009) official version of rules for seed analysis (Brasil 1967, Brasil 1992, Brasil 2009), advances in standardization of methods for Brazilian species were modest. The Brazilian option of officializing methods developed by international associations, especially those proposed by the ISTA, proved suitable for the seeds of agricultural species. However, the uniqueness and the particularities of the tropical environments require the formalization of methods for germination tests of forest species through validation processes carried out in Brazil.

A historical report by Friedrich Nobbe (Steiner & Kruse 2006, Steiner & Kruse 2007, Steiner et al. 2008, Muschick 2010) on the validation of methods for testing the germination of seeds of 50 Brazilian forest species revives the path traveled by national and international official organizations, in order to improve the quality of produced and marketed agricultural and forest seeds. This long trajectory, from 1869 to 2013, is marked by mishaps such as wars and technical difficulties to evaluate and classify seeds and seedlings, besides random and systematic errors in determinations.

In this way, this study aimed to demonstrate the history of the standardization of germination methodologies. The questions that led the study were: where, when and who participated in the advances of standardization of methods? What are the difficulties that seem to have led to the validation delay of native species? What are the foundations that supported the validation? What is the impact of the standardization of methods for germination tests of seeds of Brazilian forest species and what are the future prospects? Finally, what procedures were adopted, once by Nobbe, and which recently reverberate in the validation of methods for seed germination tests for Brazilian forest species?

For this, a review was delimited by reference research, classic and recent, as well as researchers' reports. Among the bibliographic sources and suitable sites used were Scielo, Google Scholar and the virtual library Capes periodicals, as well as the following keywords and expressions: AOSA; Association of Official Seed Analysts; Forest Seed Committee; ISTA; International Seed Testing Association; Rules for seed germination; Regras para análises de sementes; standardization of seed germination testing; padronização de teste de germinação de sementes; collaborative studies for seeds; ensaios colaborativos para sementes; seed histories; histórico de sementes; Friedrich Nobbe. After the exploratory procedure, the references used in those studies found in the bibliographic bases were also analytically consulted.

THE BEGINNINGS OF THE VALIDATION PROCESS

Nobbe is the author of the first official document on seed quality. Published in 1869, the "Statute Concerning the Testing of Agricultural Seeds" was a milestone for advances in seed quality and for the trade regulation (Steiner & Kruse 2006, Steiner & Kruse 2007) (Figure 1). The motivation for its publication took place when the Experimental Station of Tharandt, Germany, received commercial seeds of "Tall Fescue", a species of the Poaceae family used for lawns and pastures. In the botanical identification made by Nobbe, only 30 % of the seeds belonged to the species (Steiner & Kruse 2006, Steiner & Kruse 2007, Muschick 2010). Based on this experience, Nobbe published, in May 1869, the article "On the Necessity for Control of the Agricultural Seed Market". Considered a precursor of the Statute, the manuscript contained information about sampling, sample representativeness, capacity of the germination test to determine the quality of seed lots and reveal differences in the potential of seed germination (Steiner & Kruse 2007, Steiner et al. 2008, Muschick 2010).

In 1875, during the 1st Meeting of Directors of Seed Analysis Experimental Stations (Graz, Austria), 31 participants received from Nobbe a proposal of methods for laboratory analysis (Figure 1). The directors discussed these methods and unanimously

Figure 1. Chronology of advances since the first official document on seed analysis, in 1869, until the foundation of the International Seed Testing Association (ISTA), in 1924.

recommended their application. The ideas of uniformity and standardization consolidated in the Graz meeting culminated, in 1876, with the publication of the "Handbook of Seed Analysis" authored by Nobbe. The theme of the 2nd Meeting in 1876 (Hamburg, Germany), "uniformity in seed analysis", later became the motto of the International Seed Testing Association (ISTA) (Steiner & Kruse 2006, Steiner & Kruse 2007, Steiner et al. 2008, Muschick 2010).

In 1877, at the 3rd Meeting (Munich, Germany), the report of the collaborative testing between laboratories for the analysis of seeds of *Poa pratensis* L. was considered to be the first validation record (Figure 1). The results were discussed and the method for the species germination test was approved at the 4th Meeting, in 1878 (Kassel, Germany) (Steiner & Kruse 2006, Steiner & Kruse 2007, Steiner et al. 2008). It is important to emphasize that during the publication of the Statute, from 1869 to 1896, 119 laboratories and experimental stations were founded in 19 countries (Steiner & Kruse 2006, Muschick 2010). At the 2nd International Botanical Congress, in 1905 (Vienna, Austria), agricultural botanists realized the scope of the theme "seeds" and proposed a specific event on the subject with the following objectives: incorporating scientific bases to the laboratory testing, standardizing results and establishing standards and methods (Figure 1). This event occurred in 1906, in Hamburg (Germany), and was called 1st International Conference for Seed Analysis, in conjunction with the conference of the Association for Applied Botany.

With the difficulties generated by the World War I (1914-1918), the 3rd edition of the Conference occurred only in 1921 (Copenhagen, Denmark), when the European Seed Testing Association was founded. At the 4th edition of the Conference, in 1924 (Cambridge, England), the Association was renamed as International Seed Testing Association (ISTA), with the aim of broadening its scope and covering other countries. One of the first activities of the Association was the creation of the "Research Committee for Countries with Temperate Climate", which was responsible for drawing up a draft for international rules for seed analysis (Steiner & Kruse 2006, Steiner et al. 2008, Muschick 2010). As a result of the expansion of the scope, the Conference became known as International Congress of Seed Testing, with this amendment being consolidated in 1928, in Rome, Italy.

THE TRAJECTORY OF EUROPEAN AND AMERICAN FOREST SEEDS

Until the founding of the ISTA, in 1924, analyses and discussions were focused on agricultural seeds. Only at the 5th International Congress of Seed Testing, in 1928 (Rome, Italy), the Forest Seed Committee was formed (Steiner et al. 2008, Muschick 2010). In 1930, the Committee began the first study with four seed lots of the forest species *Larix europaea*, *Picea abies*, *Pinus sylvestris* and *Pinus strobus*, to determine the 1,000-seed weight, purity and germination, including the days for the final counting and utility value (Steiner et al. 2009). The results of the Committee, including tolerances, were statistically analyzed and presented in 1931, at the 6th edition of the Congress (Wageningen, Netherlands) (Steiner et al. 2009, Muschick 2010), revealing the presence of random errors in the weight, germination and utility value of 1,000 seeds, and systematic errors in the determination of purity (determination of mixtures of seeds and/or inert material in the sample) (Steiner et al. 2009). Most of the results were within tolerance (maximum variability expected between replications and between laboratories for the same test), except for purity. The difficulties of the germination test were concentrated on classifying abnormal seedlings and remaining seeds (Steiner et al. 2009, Muschick 2010). At this Congress, a draft of the first version of the ISTA international rules was presented (Steiner et al. 2008).

Between 1931 and 1934, six lots of *Picea excelsa* seeds were sent to laboratories and the methods were discussed at the 7th Congress, in 1934 (Stockholm, Sweden) (Figure 2). The Forest Seed Committee itself considered the methods unsatisfactory and proposed sending another six lots of seeds of the species to the laboratories. In 1937, at the 8th Congress (Zurich, Switzerland), discussions about the variability in germination analysis were intensify (Steiner et al. 2009). At this Congress, the Association of Official Seed Analysts (AOSA), founded in 1908, was invited to host the 9th Congress, but the outbreak of the World War II (1939-1945) delayed the event (Steiner et al. 2009, Muschick 2010). In 1938, the second version of the international rules for seed analysis was presented.

Figure 2. Timeline from the formation of the Committee for Forest Seed until the inclusion of the germination methods for 28 forest species in the third edition of the ISTA rules.

Even with the interruption of 13 years between the 8th and 9th Congresses, the activities of the Forest Seed Committee continued, and, in 1950, at the 9th Congress (Washington, USA), methods for 17 tree species, including forest and ornamental, were presented with recommendation of the tetrazolium test for 14 species, a test used to determine the viability of the seed embryo. Only in 1954, with the third edition of the ISTA rules, the inclusion of tree species occured, being 28 European species and five North American species (Steiner et al. 2009). From 1954 to the present days, continuous processes of method validations have been conducted by the ISTA, with the main objective of including new methods and species to its official rules. Twenty-three years had passed since the publication of the first official version of the ISTA rules, in 1931, until the inclusion of the first forest species, in 1954. This gap was even greater for the AOSA, and 49 years separated the first edition of their official rules (1916) and the officialization of germination testing methods of forest species (1965).

THE TRAJECTORY OF THE BRAZILIAN FOREST-TREE SEEDS

Both the first edition published in 1967 and the second edition (1992) of the rules for Brazilian Seed Analysis have contents analogous to the ISTA rules (November 2001). In its latest edition of 2009 (Brasil 2009), even with a broad participation of the Brazilian scientific community and more specific content to meet national demands, it still did not include new species from collaborative trials conducted in the country, and the mirroring of the ISTA international rules remained.

Since the first edition of the rules in 1967 until the latest publication in 2009, 42 years passed without the inclusion of methods calibrated in Brazil for germination tests with Brazilian forest species. Of the approximately 1,365 records of proposed methods for testing seed germination, a little more than 276 are forest and shrub species (Ferraz & Calvi 2010). Part of these forest species also occurs in Brazil, but the method was not validated by Brazilian official laboratories. For agricultural species, the first official record of validation in Brazil was also late (2013), and occurred by the Normative Instruction nº 41, of September 11, 2013 (Brasil 2013), officializing methods for the germination test of forage radish seeds (*Raphanus sativus* L.).

The fact that up to 2010 there were no official germination methods for forest species did not result in lack of information or efforts to organize the system of seed production. One has to consider the various contributions to the standardization of laboratory procedures for analysis of forest seeds (Piña-Rodrigues 1988, Oliveira et al. 1989, Figliolia & Piña-Rodrigues 1995, Piña-Rodrigues et al. 2007). In this line, the "Handbook for Forest Seed Analysis" was published in 1988, bringing laboratory recommendations for the species (Piña-Rodrigues 1988). This manual contained a collection of procedures adopted in seed technology, such as aspects of production, sampling, purity and determination of humidity up to the germination analysis itself. Only 15 years later, in 2003, the bibliography "Seed germination and seedling production of plants from the Cerrado" was released (Salomão et al. 2003). The contents of this publication cover the procedures for review of the Cerrado species based on the experience and procedures tested by the Brazilian Agricultural Research Corporation (Embrapa) and bibliographical consultation for more than 200 species. As in the 1988 manual, this information is an important record for the forest species distributed in Brazilian biomes; however, lacks validation from the Ministry of Agriculture, Livestock and Food Supply (MAPA) for official purposes. The "Handbook of Procedures of Forest Seed Analysis", published in 2011 (Lima Júnior 2010), has similarities with the "Handbook for Forest Seed Analysis" published in 1988 (Piña-Rodrigues 1988). Although both have on their composition a theme structure similar to the rules for analysis of seeds in the different editions published, the recommendation of methods is still based essentially on literature.

The scenario began to change with the law nº 10,711 of August 05, 2003, which, in its article 28 of the chapter VI, describes that "the analysis of seeds and seedlings should be performed in accordance with methodologies formalized by the MAPA" (Brasil 2003). This law mobilized the scientific community in the sense of contributing for the inclusion of methods in Brazilian official rules to happen through validation and no longer by mirroring the ISTA rules. Thus, in 2005, during a round table which took place in the XIV Brazilian Congress of Seeds (Foz do Iguaçu, Brazil), the results of a collaborative study with seeds of 10 forest species considered priorities, two of each biome of Brazil, were first presented by the researchers. For the first time, collaborative tests served as the underpinning of the discussions about the efficiency of germination methods of forest species.

Stimulated by the researchers who participated in the round table in 2005, the MAPA officialized the Group IV by the Ordinance nº 62 of March 10, 2006 (Brasil 2006), created with the purpose of including Brazilian forest seeds in the national rules by means of validation procedures. Supported by the ISTA document "Method Validation for Seed Testing" (ISTA 2007), the Group IV started, in 2007, a validation process (pilot project) with research and official MAPA laboratories for the inclusion of 10 forest species in the Brazilian rules. The analysis and discussion of these results by researchers and federal agricultural supervisors occurred in 2009, in the National Agricultural Laboratory (Lanagro), in Porto Alegre, and the problems of repeatability and reproducibility, essential to the process, rendered unsatisfactory results. However, this pilot project left a significant legacy by indicating the need for better detailing the protocols of each species, especially in relation to the description of normal and abnormal seedlings.

In 2008, the National Council for Scientific and Technological Development (CNPq) and the Secretariat of Agricultural Defense of the MAPA (SDA/MAPA) released the public notice nº 64/CNPq/SDA/MAPA (Brasil 2008), detailing the possibility of financing projects involving the validation of methods for germination tests. The Universidade Federal de Uberlândia (UFU) was one of the institutions awarded with the project "validation of methodologies for analysis of seed germination of native forest species", with the proposal of validating 100 native forest species (Santana et al. 2012b, Ribeiro-Oliveira & Ranal 2014). Derived from that project, others emerged and were developed by the partnership between UFU, MAPA, CNPq and Foundation of Support to Research of the State of Minas Gerais (Fapemig).

Between the beginning of the activities of the team of researchers and federal agricultural supervisors involved in 2008 and the publication of the normative instruction nº 44 of December 23, 2010 (Brasil 2010), which formalized methods for germination tests of 10 Brazilian forest species, the third issue of rules for seed analysis was published (Brasil 2009). The year 2010 is considered to be the first record of method validation for forest species in Brazil tested through collaborative trials involving national laboratories and anchored in statistical principles. The species whose methods were validated on this occasion were *Astronium fraxinifolium*, *Ceiba speciosa*, *Cybistax antisyphilitica*, *Enterolobium contortisiliquum*, *Guazuma ulmifolia*, *Lafoensi pacari*, *Mimosa caesalpiniaefolia*, *Peltophorum dubium*, *Pseudobombax tomentosum* and *Pterogyne nitens.*

Methods for another 15 forest species were officialized by the normative statement nº 35 of July 14, 2011, being them: *Acacia polyphylla*, *Cariniana estrellensis*, *Cedrela fissilis*, *Cedrela odorata*, *Cytharexylum myrianthum*, *Jacaranda cuspidifolia*, *Jacaranda micrantha*, *Ormosia arborea*, *Parapiptadenia rigida*, *Parkia pendula*, *Platymenia reticulata*, *Schizolobium parahyba* var. *amazonicum*, *Senna macranthera*, *Handroanthus chrysotrichus* and *Tabebuia roseo-alba* (Brasil 2011). In this same year, at the XVII Brazilian Congress of Seeds, in Natal, the project team met with the official MAPA and research laboratories for the presentation of results. In the subsequent year, 2012, the normative instruction nº 26 of September 10, 2012, was published with the officialization of over 25 species (Brasil 2012): *Albizia hassleri*, *Anadenanthera colubrina*, *Anadenanthera macrocarpa*, *Apuleia leiocarpa*, *Cariniana legalis*, *Cassia leptophylla*, *Copaifera langsdorffii*, *Cordia americana*, *Dalbergia miscolobium*, *Dalbergia nigra*, *Enterolobium maximum*, *Erythrina speciosa*, *Gallesia integrifolia*, *Hymenaea courbaril*, *Hymenaea stigonocarpa*, *Mimosa scabrella*, *Peltogyne confertiflora*, *Qualea grandiflora*, *Schinus terebinthifolius*, *Senna multijuga*, *Stryphnodendron barbadetiman*, *Stryphnodendron polyphyllum*, *Tabebuia aurea*, *Handroanthus impetiginosus* and *Zeyheria tuberculosa.* To verify the details by species and the statistical tests, it is recommended to consult Brasil (2010), Brasil (2011), Brasil (2012), Santana et al. (2012a), Pereira & Santana (2013) and Santana et al. (2013).

As for the seedlings, the normal ones with phanero-epigeal germination must have free cotyledons and a developed hypocotyl. For *Anadenanthera colubrina* and Erythrina *speciosa*, in addition to these characteristics, the epicotyl must be developed. For normal seedlings with phanerohypogynous germination, the cotyledons must be free and the epicotyl developed. *Pseudobombax tomentosum* seedlings do not have a developed epicotyl, and *Astronium fraxinifolium* has seedlings with pericarp attached to the cotyledons (cryptocotyledons), and both are still classified as normal seedlings of the phanero-hypogia type. For the 50 species, taproot formation is essential (Lobo et al. 2014).

The activities of the Group IV, formed in 2006, also generated, in 2013, a document entitled "Guidelines for Analysis of Forest Species" (Brasil 2013a), which established methods from extensive literature review and experience from technicians and researchers working in the area. This document defines procedures for sampling, analysis of purity and germination testing with validated methods of the 50 species (Brasil 2010, Brasil 2011, Brasil 2012) and others recommended from the literature for forest species seeds.

FINAL REMARKS

In view of the diversity of species present in the Brazilian biomes, the methods for testing seed germination for the 50 species may seem little expressive, but they are not. They represent a starting point for other studies to appear based on the legacy of this pioneering study. Such researches will no longer follow the character of the review when they indicate a method, as in the initial studies made in Brazil, and will adopt procedures that enable a robust analysis of their efficiency.

The validation of these 50 species will support the professionalization of the Brazilian forestry seed sector in all its farms, whether from the production, collection or formation of clonal forests to the sale and export of seeds. With this, other benefits may arise, such as genetic breeding, which aims at the production of wood and development of phytochemical molecules for different purposes, among others. In addition, it may diversify the species in reforestation, to the detriment of the planting of exotic species, and thus reduce deforestation and, as a result, there is a decrease in genetic erosion. All this is due to the recognition of forest essences that were previously less valued than cultivated ones. It should also be noted that, when carrying out collaborative tests, the validation of methods for testing the germination of forest seeds puts an end in the two biggest bottlenecks in the sector: lack of a method to determine the germination potential of a seed lot and the existence of laboratories that qualifies it.

The proposed methods for seed germination of the 50 native forest species are not the only efficient methods capable of ensuring the reliable record of the quality of a seed lot (Santana et al. 2012b). Validation is an ongoing process and must propose methods for both species that still do not have them as for those that have official methods. Because of this, there is no fear of stagnating seed science through laboratory standardization (Ribeiro-Oliveira & Ranal 2014).

Another important aspect of validation is that the best method is not always capable of reproduction, and the Nobbe's phrase from 1869, "The best is not too good", is a reflection of that. With this little phrase the author explains why the emergence in the field, although it is the method that most closely matches the actual driving conditions of a crop, cannot be the official reference for the germination of a seed. The reason is that the seedling emergence test does not present sufficient technical conditions to be standardized. The phrase will also have reflexes when new methods, besides the validated ones for forest seed germination, do not show repeatability and/or reproducibility. The non-validation does not necessarily imply that the method is not suitable, but that technically it cannot be reproduced under conditions of laboratory routines.

At first, the validation of a germination test method involving temperature, substrate, dormancy overcoming (when present), asepsis and description of abnormalities may be reckless, given the particularities of seeds from tropical forest species. However, when the method is validated, it is possible to know the inherent variability of the material under analysis, especially its ability to be reproduced in laboratories. This method capability is a necessary condition for official documents to be issued, such as Seed Analysis Bulletins required by law. The law does not improve the quality of the seed, nor the validated method, but both indicate to the forestry sector the quality of the material sold in the country.

The methods for germination tests validated for the 50 species are still insufficient in view of the diversity of the Brazilian flora. Even with investments and technical efforts, the inclusion of a large number of species in the official rules for seed analysis (Brasil 2009) would take decades. To reduce this time, forest seed research needs to test more robust methods. The robustness ensures that small variations in the method do not affect the final result of the germination percentage, in addition to keeping the repeatability and reproducibility of the process within acceptable limits, essential for the process.

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