ENSURING SEED QUALITY: A MULTIDISCIPLINARY PERSPECTIVE ON LABORATORY TESTING

Elena NICOLAE[1,2], Valentina VASILE[1,2], Costică CIONTU[1]

e-mail: petrescuelena2013@gmail.com

Abstract

Seeds are fundamental to plant production. The seed industry and its representatives, at global, regional, and national levels, require analytical methods to meet trade requirements and manage quality-related risks. Standardized interpretation and reporting of laboratory test results are essential. Laboratory seed quality testing provides a rapid assessment of a seed lot's biological potential, specifically its cultural and utilization value. This comprehensive evaluation encompasses multiple parameters including physical purity, germination capacity, vigor assessment, and thousand seed weight determination. The aim of this paper is to describe and provide a multidisciplinary perspective on the most important seed quality indicators and the standardized tests required for commercial sowing seeds.

Keywords: methods, seeds, quality, testing

Seed is the most important input for increasing plant production. Whether intended for consumption or sowing, seeds are a fundamental input with a special place in optimizing yields and investments (Păcurar, 2007). When evaluating seed quality, clarifying the term 'seed' is essential, because seed testing deals with the devisions of seed plants only. Botanically, a seed (or sperma) is a higher plant organ derived from the fertilized egg, exhibiting varied shapes and sizes, consistent at the species level.

A true seed comprises an embryonic plant, stored food (rarely absent), and a protective coat. However, Kozlowski (1972) notes that 'seed' in seed testing is often used functionally as a unit of dissemination or a disseminule, embracing dry, one-seeded (or few-seeded) fruits as well as true seeds.

Specialized legislation (European Commission, 2020) defines 'seed' more broadly as reproductive material, including seeds and vegetative propagating material, which must belong to a high-yielding variety adapted to specific environments, possessing biological purity, high productivity, superior quality traits, physiological properties, physical purity, germination, and health. This description encompasses a broad range of seed types, including agricultural species (forage grasses, cereals, small-seeded legumes, large-seeded legumes, and other agricultural species such as Beta, Brassica, Helianthus, etc.), vegetables, herbs, flowers, spices, and medicinal plants.

Given this diversity, the review focuses on laboratory testing methods for seed quality that are both reproducible under field conditions and provide a reliable indication of expected seed lot performance. The discussion will emphasize rapid and effective techniques relevant across these diverse plant categories.

THE SEED QUALITY SYSTEM IMPORTANCE

Seed certification relies heavily on seed testing. Seed certification is a system designed to maintain and make available to the public sources of high-quality seed of superior crop varieties, ensuring their genetic identity and purity. Seed testing is the scientific process used to assess seed quality against pre-defined standards. Without seed testing, it would be impossible to verify that seed meets the standards required for certification.

Certification programs set specific standards for various seed quality parameters, such as: genetic purity - ensuring the seed represents the claimed variety, physical purity - absence of weed seeds, other crop seeds, and inert matter,

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania, Email: post@info.usamv.ro
[2]Central Laboratory for Quality of Seeds and Planting Material, 10 Constantin Sandu Aldea Alley, District 1, Bucharest, Romania, Email: lccsms@lccsms.ro

germination - the percentage of seeds that are capable of producing normal seedlings etc.

Seed testing provides the data that confirms whether a seed lot meets these certification standards and enables informed decisions in the certification process. The results of seed tests can determine if a seed lot is eligible for certification, if it requires further processing or treatment, or if it should be rejected altogether.

A country's seed delivery system is best conceived as a value chain composed of interrelated components – from the development of well-adapted and nutritious crop varieties and their adoption by farmers, through the production and distribution, including sales, of quality seeds and planting materials, to on-farm utilisation of these inputs by farmers. The effective functioning of the value chain, enabled by the applicable national seed laws, policies, strategies, action plans and regulations, depends largely on the extent to which the stakeholders are able to put into practical use the relevant knowledge and skills required for producing quality seeds and planting materials.

Obtaining seeds with special qualitative properties is conditioned by the scientific substantiation of all activities which consist, on the one hand, in knowing the theoretical bases of genetics, breeding and seed production, and on the other hand, in the in-depth knowledge and application in this activity of modern methods and techniques standardized and regulated at national and international level (FAO, 2018a).

Benech – Ardold and Sánchez (2004) describe seeds as the beginning and the end of most agricultural practices and the way in which seeds function: their physiology, biochemistry, molecular biology, and genetics are critically important for agricultural success.

There are four basic parameters to classify the seed quality attributes: physical qualities - referring mostly to the seed lot, physiological qualities describing aspects of performance of the seed, genetic quality - relates to specific genetic characteristics of seed variety. When seed has good physical, physiological, seed health and genetic qualities, farmers have greater prospects of producing a healthy crop with improved yields. High quality seed is a major factor in obtaining a good crop stand and rapid plant development even under adverse conditions, although other factors such as rainfall, agronomic practices, soil fertility and pest control are also crucial (FAO, 2018c).

Seed is a living product and must be grown, harvested and processed correctly to maximize its viability and subsequent crop productivity (Rahman, 2016). Good seed quality can increase yield significantly.

Seed quality depends on the health, physiology, germinability and physical attributes of seeds, including the presence or absence of disease, chemical composition, insect infestation, and the presence or absence of weed seeds or other plant varieties. Quality of seeds and their products is directly or indirectly related to human health; nevertheless, the evaluation of seed quality parameters is a time-consuming process (Huang *et al.,* 2015, Rahman *et al.,* 2016).

According to FAO (2018b) the organization of seed certification requires the following steps: institute a seed certification agency – independent from the industry, establish a minimum seed certification standards and devise procedures for field inspection, processing, sampling, testing and labelling.

A seed law without some provisions for unbiased seed testing basis is meaningless and seed testing laboratories which are not properly integrated into a seed certification and seed control programme have questionable value (Seed Testing Manual, 2021). In industrialized countries, the existence of methods for verifying seed quality has always been an important support for the seed industry and for governments in seed production programs. In developing countries, where seed production is insufficient, the availability of internationally recognized methods represents a first step in initiating national seed production schemes: it facilitates the development of regulatory standards, such as seed certification (Léchappé, 2009).

THE SEED SAMPLING

Sampling is the first step and one of the most important when assessing the quality of a seed lot. In the second edition of the ISTA Handbook on Seed Sampling, Dr. Michael Kruse, Professor for Seed Science and Technology, quotes Arne Wold in the foreword: 'Sampling of seed is considered an important part of seed quality control. Correct sampling is a prerequisite for the reliable estimation of the quality of the seed lot. Accurate description and detailed information of the sampling procedures are therefore necessary. Uniformity in sampling seed lots as well as in drawing working samples is as important as uniformity in test methods in order to obtain accurate and reproducible results. Incorrect sampling may lead to misleading test results, discarding seed lots of high quality or to approval of seed lots of low quality which may reduce crop yield or even result in complete failure'.

According to International Seed Testing Association Rules (2024a) the object of sampling

is to obtain a sample of a size suitable for tests, in which the probability of a constituent being present is determined only by its level of occurrence in the seed lot which is a specified quantity of seed that is physically and uniquely identifiable. A good sample for seed testing must be representative of the lot (Bányai & Barabás, 2002).
The qualification of seed lots should be evaluated on the basis of their characteristics which can be examined on small representative samples drawn from them. Data collected on such small samples can then be useful for the entire lot following the application of appropriate mathematical statistical methods (Bányai & Barabás, 2002).

The reliability of the inference made about the quality of the seed lot depends primarily on two components: the accuracy with which the sample represents the lot and the accuracy and precision of laboratory tests (Kruse, 2004). For instance, the size of the purity tolerance depends on the size of the seed lot and on the number of bags from which sample cores are taken. Estimates of the quality of various attributes of seed in a lot are made from samples and these are subjected to error because of variation in the seed, in methods and in apparatus (Milles, 1963).

After sampling has been carried out according to standard requirements and the seed samples have been accepted and received in the seed testing laboratories, they enter the specific seed testing workflow, with the first test performed being usually the purity analysis.

THE PURITY ANALYSIS

Purity is an expression of how 'clean' the seed lot is. Physical purity is a critical factor affecting the value of seed. It indicates the proportion of 'Pure Seed', 'Inert Matter' and 'Other Seeds' within a seed sample. Testing for seed purity it's important for ensuring that seed lot doesn't devalue due to the presence of unwanted seeds or specific amounts of inert matter. According to International Rules for Seed Testing (2024b) the purity is referring to the percentage composition by weight of the sample being tested and by inference the composition of the seed lot and to identification of the various species of seeds and inert particles constituting the sample.

The pure seed fraction must refer to the species stated by the applicant or found to predominate in the test and must include all botanical varieties and cultivars of that species. Will be included structures like intact seed units and commonly found dispersal units i. e. achenes and similar fruits, schizocarps, florets with an obvious caryopsis containing endosperm, free caryopses and pieces of seed units larger than one-half their original size. In this fraction will be also included the structures which are immature, undersized, shrivelled, diseased or germinated, providing they can be identified as of that species, unless transformed into partially or fully ergotised visible fungal sclerotia, smut balls or nematode galls.

Mureşan, Pană and Cseresnyes (1986) consider the determination of physical purity the first of the two essential objectives pursued in seed quality control. The second essential objective is to determine the germination from the pure seed fraction separated at the purity analysis. The purity analysis provides information about the gravimetric composition of the working sample and by this about the whole seed lot, about the determination of the identity of different seeds and inert particles found in the working sample. The purity analysis is done on a working sample with a specific weight depending on species performing the following successive operations: dividing the sample in pure seed fraction, other seeds and inert matter, identifying the other seed species and type of inert matter and then weight them with specific decimal places.

Short history of Purity Test. Before the International Seed Testing Association was founded in 1924, according to Jensen (2008), two methods were used for Purity analysis in Europe: the Continental method or the Stronger method, and the Irish method also called the English method. By the Continental method, all seeds were examined first by the naked eye or using magnification, and then under reflected light. It required that the seeds, classified as pure seed, did not have any damage on vital parts of the seed. Undamaged seeds were, accordingly, considered to be able to germinate. The Quicker method, which was a modified form of the Irish Method, was based on the assumption that all pieces of seeds of the species tested, with a size of more than half the original size, should be classified as pure seed. The evaluation of whether the seeds were able to germinate was supposed to be determined in the germination test. The same author mentioned that the subject of what method to be used was intensively discussed during the ISTA Congresses at Copenhagen - 1921, Cambridge - 1924 and Rome - 1928. During the Congress at Wageningen - 1931 it was not possible to obtain an agreement about a preferred method, and both the Stronger method and the Quicker method were included in the first ISTA Rules of 1931. In time, those two methods for purity testing became an increasing inconvenience for the seed trade, and the Russian delegation in the International Organisation for

Standardisation - ISO raised the question whether ISO should go into standardisation of seed testing and that they should begin with the purity. This question was submitted via ISTA member countries standardisation organisations to the ISTA laboratories and to the ISTA Secretariat. It was taken as a serious warning that decisions on formulation of the Rules could move to other organisations, and the ISTA Congress in 1950 finally decided that the stronger method should be deleted and the quicker method should be the only method for purity analysis in the ISTA Rules.

THE THOUSAND SEED WEIGHT (TSW)

The object of the test is to determinte the weight of 1000 pure seeds from the submitted sample, either by counting the entire fraction or by counting in replicates (International Seed Testing Association Rules, 2024d). In general, the pure seed fraction must refer to the species stated by the applicant, or found to predominate in the test, and must include all botanical varieties and cultivars of that species including structures like intact seed units and pieces of seeds units larges than one-half their original size International Seed Testing Association Rules, 2024b). It varies from species to species, and within the same species from one cultivar to another. TSW provides an indirect measure of seed size and uniformity. A higher TSW generally indicates larger seeds, while a consistent TSW across samples from the same lot suggests greater uniformity. In contrast a low TSW can indicate that seeds were not fully mature at harvest or that they experienced stress during development, leading to poor "fill" (i.e., not being completely full of reserves). Immature or poorly filled seeds often have reduced germination and vigor.

Thousand seed weight is an important parameter for the evaluation of any variety being not only directly related to the grain yield and milling quality of grain, but also has an impact on the seedling vigour and growth indirectly affecting the wheat (*Triticum aestivum* L. subsp. *aestivum*) yield (Botwright *et al.,* 2002). TSW was also found to be closely associated with kernel size traits as well, such as kernel length, kernel width, kernel thickness, and the kernel length/width ratio (Dholakia *et al.,* 2003). For rice (*Oryza sativa* L.), thousand seed weight is frequently used in crop research as a measurement indicator (Li *et. al.,* 2004). TSW along with spike number per m^2 and kernel number per spike are the three main components determining wheat (*Triticum aestivum* L. subsp. *aestivum*) yield. Zhang *et al,* (2021) describe this trait is controlled by multiple genes being affected by environment as well as genetic background and positively correlated with kernel length and kernel width.

In hybrid broccoli, plants from large seeds had higher vegetative yields than plants from medium or small seeds (Heather & Sieczka 1991). In some *Brassica rapa* L. cultivars, plants from large seeds had more pods, larger pods, heavier seeds and higher seed yields than plants from small seeds (Ahmed & Zuberi, 1973).

Seed size is correlated with factors like seedling vigor and establishment. TSW helps characterize and differentiate seed lots. It allows for the comparison of seed quality between different varieties, production years, or geographical origins. This information is valuable for seed producers, breeders, and farmers in making informed decisions about seed selection.

In the same time TSW is not a standalone indicator: it should be used in conjunction with other seed quality parameters, such as germination percentage, purity, and vigor tests, to get a complete picture of seed quality.

THE GERMINATION

Theoretical Considerations. Seed germination is a critical process in the life cycle of higher plants. As stated by Mayer and Poljakoff-Mayber (2014), the germination of the seed of the higher plant may be regarded as that consecutive number of steps which causes a quiescent seed, with a low water content, to show a rise in its general metabolic activity and to initiate the formation of a seedling from the embryo.

Physiologically, seed germination is a multi-stage process where an embryo transforms into a seedling. It starts with water imbibition, which softens the seed coat and activates enzymes. A lag phase follows, during which food reserves (carbohydrates, fats/oils, and proteins) are mobilized by enzymes. Germination then occurs with the rupture of the seed coat and radicle emergence. Early growth relies on water uptake for cell elongation, consuming the storage tissues. Seedling growth patterns are classified as either hypogeal (cotyledons remain underground) or epigeal (cotyledons emerge above ground).

Germination is an energy-demanding process that requires functioning mitochondria. One of the earliest events of seed germination is progressive development of structurally simple and metabolically quiescent mitochondria into fully active ones, known as mitochondrial biogenesis. This is a complex and tightly regulated process, which is accompanied by sequential and dynamic gene expression, protein synthesis, and post-

translational modifications (Czarna *et al.,* 2016). The data highlight diverse regulatory and metabolic mechanisms upon seed germination, including induction of environmental factor-responsive signaling pathways, seed storage reserve mobilization and utilization, enhancement of DNA repair and modification, regulation of gene expression and protein synthesis, modulation of cell structure, and cell defense (Tan *et al.,* 2013).

While the summarized process outlines the general physiological stages of germination, it's important to note that these stages are not universal. Each species has unique germination characteristics, influenced by various factors.

These factors influencing the germination are: water availability, temperature, oxygen, dormancy mechanisms, light requirements and even storage based on the seed tolerance to desiccation. These factors can modify or even dictate the specific steps of germination for a given species.

Some plant species may require specific light conditions; usually, plants need the presence of light to germinate, but there are species, e.g., *Phacelia tanacetifolia* (ISTA Rules, 2024c) that germinate better in darkness. Other factors influencing the germination are dormancy, chemical deficiency, weather conditions during seed development, immaturity, mechanical damage, phytotoxicity caused by some chemicals, insects and mites, plant pathogens and longevity. The germination will not occur above or below the critical temperatures. Each species has a minimum, an optimum, and a maximum temperature for seed germination. Critical moisture levels vary among crop seeds. Most starchy seeds (monocots) will begin germination when they have a moisture content of approximately 30 percent. Most oily seeds (dicots), however, will not begin germination until they have a moisture content of at least 50 percent. Lack of oxygen is not usually a limiting factor for germination. However, wet or soggy substrate may not contain enough oxygen for germination to begin. Seeds planted in such conditions will absorb water quickly and will have the tendency to decay.

Very strongly connected to germination potential is the seed vigour which is a concept describing several seed performance associated characteristics, and not a single property (Perry, 1981). Seeds which are able to produce a seedling, respectively to achieve germination from a botanical point of view, will not necessarily have the vigour to produce a plant in field conditions.

Practical Considerations. The purpose of the germination test is to determine the germinative capacity of a seed lot, a determination that can subsequently be used both to compare different lots and to estimate the sowing value for cultivation in the field. The germination test reports the percentage of normal seedlings, abnormal seedlings and dead or ungerminable seeds in a seed lot.

Standard germination determined in the laboratory and expressed as a percentage involves an estimate of the potential of a seed lot for germination and emergence under favorable environmental conditions. When maximum viability is reached, a seed lot should theoretically have a germination of nearly 100%, providing dormancy is not a factor. Loss on viability from this point on results from the deterioration processes involved with seed ageing. However, performing the test under field conditions is normally unsatisfactory because the results cannot be reliably repeated. Therefore, laboratory methods have been developed in which external conditions are controlled to ensure the most homogeneous, rapid, and complete germination for most samples of a given species (International Seed Testing Association Rules, 2024c).

Germination tests are successful in two aspects (Matthews, 1981): they are repeatable, and they provide information about the potential of a seed lot to germinate under optimum conditions.

Other species have dormant seeds, meaning that seeds are viable but in a non-germination state. When testing, this situation might be solved by exposing the seeds to specific actions like removing or breaking seed coverings, cold, light, time, or hormones. The major limitation of the germination test as an assessment of seed lot potential performance is its inability to detect quality differences among high germinating seed lots (Roberts, 1984).

To improve germination, modern techniques are taken into consideration: seed priming and exposure to specific magnetic fields. According to Vashisth and Nagarajan (2010), treatment of sunflower seeds in static magnetic fields of strength from 0 to 250 mT in steps of 50 mT for 1–4 h in steps of 1 h increased the speed of germination, seedling length and seedling dry weight under laboratory germination tests. The treated seeds planted in soil resulted in statistically higher seedling dry weight, root length, root surface area and root volume in 1-month-old seedlings and in germinating seeds, enzyme activities of a-amylase, dehydrogenase and protease were significantly higher in contrast to controls. In a research experiment, Afzal et al. (2012) showed the positive impact of magnetism at 100 mT on improving early growth, germination

rate and biochemical parameters on seeds of French marigolds (*Tagetes patula* L.). This was because the seeds after exposure to magnetic field lines showed higher levels of α-amylase activity which improved germination. According to Dotto and Silva (2017), the physiological priming of beet seeds with water or salicylic or gibberellic acids, respectively, alters seed germination and vigour potential and the response varies according to the cultivar used and the type of conditioning adopted. Germination of beet seeds is promoted at 1−2 mM ascorbic, gibberellic or salicylic acids, respectively, while at 1−3 mM, the growth of roots and shoot of beet seedlings are promoted.

While laboratory germination tests provide valuable information about a seed lot's potential, they are limited by their artificial environment and inability to fully capture complex factors like seed vigor and dormancy.

THE SEED VIGOUR

The subject of seed vigour is complex (Hampton & TeKrony, 1995) because it is not a single measurable property, like germination, but a concept describing several characteristics associated with various aspects of the seed, both in the field (Perry, 1978, 1981) and in storage (Hampton & Colbear, 1990). Seed vigour consists of those attributes that determine the potential for rapid, uniform development of seedlings under variable field conditions (Al-Amery *et al,* 2018, Marcos-Filho, 2015). Therefore, seed vigour is often a better indicator of stand establishment in the field (Saux *et al.,* 2020, Al-Amery *et al.,* 2018, Torres & Marcos-Filho, 2005, TeKrony & Egli, 1993).

In the acceptance of the International Seed Testing Association (2024e), 'Seed vigour is the sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environmental conditions. Seed vigour is not a single measurable property, but a concept describing several characteristics associated with the following aspects of seed lot performance: rate and uniformity of seed germination and seedling growth, emergence ability of seeds under unfavourable environmental conditions and performance after storage, particularly the retention of the ability to germinate'. A vigorous seed lot is one that is potentially able to perform well even under environmental conditions which are not optimal for the species (Hampton & TeKrony, 1995). Results of vigour tests can be used in deciding whether the seed lots can be sown earlier in the season, when the occurrence of stressful conditions is possible, or it should be sown later, when the soil is warmer, and the conditions become more favourable for germination and seedling growth (Milošević & Zlokolica, 1996).

Seed vigour tests fall roughly into three general categories (Al-Amery et al, 2018):

1. Stress-Imposition Tests: Measure standard germination percentage after a pre-germination stress imposition. Examples include accelerated ageing, controlled deterioration, and cold test.
2. Indirect Measurement Tests: Indirectly measure germination potential utilizing an accepted physiological or biochemical aspect of germination. The most utilized indirect method uses electrolyte leakage (Marcos-Filho, 2015). Measurement of the electrical conductivity of leachates provides an assessment of the extent of electrolyte leakage from plant tissues. Conductivity measurement of the soak water in which a bulk sample of seeds has been steeped gives an estimate of seed vigour. Seed lots with high electrolyte leakage, i. e. high leachate conductivity, are considered to have low vigour, whilst those with low leakage (low conductivity) are considered to have high vigour (International Rules for Seed Testing, 2021e). The conductivity test was first recognized for seeds of several crop species by Hibbard & Miller in 1928 and later developed into a routine vigour test to predict field emergence of garden pea (*Pisum sativum* L.) (Matthews & Bradnock, 1967).
3. Visual Post-Germination Analysis: Uses visual assessment of seedlings after germination.

Accelerated Ageing Test: The accelerated ageing stress test exposes seeds for short periods to high temperature and high relative humidity (≈ 95 %). During the test, the seeds absorb moisture from the humid environment and the raised seed moisture content, along with the high temperature, causes rapid seed ageing. High vigour seed lots will withstand these extreme stress conditions and age more slowly than low vigour seed lots. Thus, after testing, high vigour lots retain a high germination, whilst that of low vigour lots is reduced (International Rules for Seed Testing, 2021e).

This test was initially developed as a test to estimate the longevity of seed in commercial storage (Delouche & Baskin, 1973) and has been used to predict the lifespan of a number of different species. It was correlated with the field emergence for the following species: *Arachis hypogaea* L., *Brassica oleracea* L. var. *capitata*, *Brassica rapa* L., *Capsicum* spp., *Glycine max* L., *Gossypium hirsutum* L., *Helianthus annuus* L., *Lolium perenne* L., *Phaseolus mungo* L., *Phaseolus vulgaris* L., *Sorghum bicolor* (L.)

Moench subsp. *bicolor*, *Trifolium incarnatum* L., *Trifolium pratense* L., *Triticum aestivum* L. subsp. *aestivum* and *Zea mays* L. and with storage conditions for: *Allium cepa* L., *Bromus willdenowii* Kunth., *Citrullus lanatus* Thunb. var. *caffer*, *Cynosurus cristatus* L., *Festuca arundinacea* L., *Glycine max* L., *Raphanus sativus* L., *Sorghum bicolor* (L.) Moench subsp. *bicolor*, *Trifolium incarnatum* L., *Trifolium pratense* L., *Triticum aestivum* L. subsp. *aestivum* and *Zea mays* L.

According to Zhang *et al.* (2021), high relative humidity and high temperature are two factors that accelerate seed deterioration. As seeds age, frequently observed changes include membrane damage and the destruction of organelle structure, an increase in the loss of seed leachate, decreases of respiratory rates and ATP production, and a loss of enzymatic activity. These phenomena could be interrelated and reflect the general breakdown in cellular organization. Many processes can result in seed ageing; it is likely that oxidative damage caused by free radicals and reactive oxygen species which can have vital interactions with any macromolecule of biological interest that result in damage to various cellular components caused by protein damage, lipid peroxidation, chromosomal abnormalities, and DNA lesions.

Controlled Deterioration Test: This test was developed for detecting seed lots of small-seeded vegetable species (carrot, onion, lettuce, brassicas) with poor field performance potential (Hampton & TeKrony, 1995, Powell & Matthews, 1981), and storage potential (Hampton & TeKrony, 1995, Powell & Matthews, 1984a). The test can consistently identify low and high vigour seed lots (Powell *et. al,* 1984b). The basis of the test is an ageing technique similar in principle to the accelerated ageing test i.e., seeds are exposed to the two most important environmental variables which influence seed deterioration: high temperature (40-45°C) and high seed moisture for a short period of time (24 - 48 hours depending on the species). The initial seed moisture content is raised to the same level for all lots prior to the period of deterioration at high temperature. Thus, the test provides a constant seed moisture content during the deterioration period, in contrast to accelerated ageing test where seed moisture is variable (TeKrony, 2003). The test can be run in a standardized manner for the following species: *Brassica* spp., *Daucus carota* L., *Lactuca sativa* L., *Beta vulgaris* var. *altissima* Doll., *Allium cepa* L., *Pisum sativum* L., *Trifolium pratense* L., *Medicago sativa* L., *Lolium perenne* L. and *Festuca arundinacea* Schreb. (Hampton & TeKrony, 1995).

Cold Test: This vigour test developed to simulate adverse field conditions like high soil moisture, low temperatures and soilborne fungi usually occurring in early spring. The test was first developed for maize, soybean and sorghum. Beyond the assessment of field performance, The Association of Official Seed Analysts (AOSA, 1983) lists several uses for the results of this test: evaluating fungicide efficacy, selecting genetic material demonstrating an ability to germinate in cold wet soil, evaluating physiological deterioration resulting from prolonged or adverse storage, freezing injury, immaturity, injury from drying and other causes, measuring the effect of mechanical damage on germination in cold, wet soil, selecting seed lots for early spring planting and providing a basis for adjusting planting rates for individual seed lots.

The cold test exposes seeds to cold temperatures (10°C, 7 days) in non-sterile field soil at approximately 60-70% of water holding capacity prior to a 4-7day growth period in ideal conditions (25°C). The ability of seeds to germinate and emerge in cold wet soil is affected by genotype, seed quality (both physical and physiological), pathogens and seed treatment. Because the test requires the use of soil, which is a variable material both physically and biologically, it cannot be readily standardized between laboratories.

Seed vigour testing provides valuable insights into a seed lot's ability to perform under a wide range of conditions, supplementing the information obtained from standard germination tests. However, it's essential to recognize that vigour tests, due to their inherent complexity and sensitivity to environmental factors, necessitate highly skilled and experienced analysts.

The subjectivity involved in some vigour assessments, as well as the need for precise execution of the test procedures, emphasizes the importance of well-trained professionals in order to obtain reliable and reproducible results. In other words, vigour tests require highly skilled professionals due to their inherent complexity and sensitivity to environmental factors.

CONCLUSIONS

The laboratory seed quality testing serves as a cornerstone for a thriving and sustainable seed system, moving far beyond mere academic exercise. Standardized methods, rigorously applied, provide the data-driven insights essential for mitigating risk, fostering trust, and driving economic prosperity across the seed value chain. This review has highlighted the diverse range of methods employed to assess seed purity,

germination, vigor, and overall health, demonstrating how each contributes to a comprehensive understanding of seed lot potential.

The economic impact of accurate seed testing cannot be overstated. From enabling farmers to make informed decisions about sowing rates and pre-planting treatments, to facilitating international trade and protecting the value of plant breeding innovation, reliable seed quality data underpins the stability and competitiveness of the seed market.

By reducing the risk of crop failure and promoting efficient resource utilization, high-quality seed, assured by rigorous testing, contributes directly to increased agricultural productivity and food security.

While the existing arsenal of seed testing methods is robust, there remains scope for further refinement and innovation.

Future research should focus on developing rapid, non-destructive techniques for assessing seed vigor and health, as well as exploring the potential of molecular markers and other advanced technologies to enhance the accuracy and precision of seed quality assessment. Furthermore, continued harmonization and standardization of seed testing methods at the international level are crucial for facilitating global seed trade and ensuring that farmers worldwide have access to high-quality seed.

Investing in robust seed testing infrastructure, adhering to internationally recognized standards, and fostering collaboration among seed scientists, regulators, and industry stakeholders are essential for maintaining a vibrant and resilient seed system that supports sustainable agriculture and meets the growing demand for food in a changing world.

REFERENCES

Afzal, I., Mukhtar, K., Qasim, M., Basra, S., Shahid, M., Haq, Z. (2012). *Magnetic stimulation of marigold seed.* International Agrophysics, 26(4), 335–339. https://doi.org/10.2478/v10247-012-0047-1.

Ahmed, S.U., Zuberi, M.I. (1973). Effects of seed size on yield and some of its components in rapeseed, Brassica campestris L. var. Toria. Crop Science, 13, 119–120.

Al-Amery, M., Geneve, R., Sanches, M., Armstrong, P., Maghirang, E., Lee, C., Hildebrand, D. (2018). *Near-infrared spectroscopy used to predict soybean seed germination and vigour.* Seed Science Research, 28(3), 245-252. doi:10.1017/S0962508518000119.

Bányai, J., Barabás, J. (2002). *Handbook on Statistics in Seed Testing.* International Seed Testing Association, Zurich, Switzerland.

Benech-Arnold, R.L., Sánchez, R.A. (2004). *Handbook of seed physiology: applications to agriculture,* The Haworth Press, NY, USA, ISBN: 1-56022-928-4.

Botwright, T.L., Condon, A.G., Rebetzke, G.J., Richards. (2002). *Field evaluation of early vigour for genetic improvement of grain yield in wheat*. Australian Journal of Agricultural Research, 53, 1137–1145.

Czarna, M., Kolodziejczak, M., Janska, H. (2016). *Mitochondrial Proteome Studies in Seeds during Germination.* Proteomes, Volume 4, Number 2, 19-19(1). Directory of Open Access Journals. DOI: https://doi.org/10.3390/proteomes4020019.

Delouche, J.C., Baskin, C.C. (1973). *Accelerated ageing technique for predicting the relative storability of seed lots,* Seed Science and Technology, 1, 427-452. International Seed Testing Association. Zurich. Switzerland.

Dholakia, B.B., Ammiraju, J.S.S., Singh, H., Lagu, M.D., Röder, M.S., Rao, V.S., Dhaliwal, H.S., Ranjekar, P.K., Gupta, V.S., Weber, W.E. (2003). *Molecular marker analysis of kernel size and shape in bread wheat.* Plant Breeding, 122(5), 392–395. Retrieved from: https://doi.org/10.1046/j.1439-0523.2003.00896.x.

Dotto, L., Silva, V.N. (2017). *Beet seed priming with growth regulators.* Semina: Ciências Agrárias, 38(4), 1785. https://doi.org/10.5433/1679-0359.2017v38n4p1785.

European Commission. (2020). *Study on the Union's options to update the existing legislation on the production and marketing of plant reproductive material,* Brussels.

Food and Agriculture Organisation of the United Nations. (2018a). Seeds Toolkit. Module 1: Development of small-scale seed enterprises. The Food and Agriculture Organization of the United Nations and Africa Seeds. Rome. Italy.

Food and Agriculture Organisation of the United Nations. (2018b). Seeds Toolkit. Module 2: Seed processing: principles, equipment, and practice. The Food and Agriculture Organization of the United Nations and Africa Seeds. Rome. Italy.

Food and Agriculture Organisation of the United Nations. (2018c). *Seeds Toolkit. Module 3: Seed quality assurance.* The Food and Agriculture Organization of the United Nations and Africa Seeds. Rome. Italy.

Government of India, Ministry of Agriculture & Farmers Welfare. (2021, November 9). *Seed Testing Manual, Chapter 18: The role of seed testing laboratories under the seed law*, Retrieved from: https://seednet.gov.in/CMS/QualityControl/Seed_Testing_Manual/CHAPTER-18.pdf.

Government of India, Ministry of Agriculture & Farmers Welfare. (2021, November 9). *Seed Testing Manual,* Chapter 4: Seed testing procedures in brief, Retrieved from: https://seednet.gov.in/CMS/QualityControl/Seed_Testing_Manual/CHAPTER-4.pdf.

Hampton, J.G., Colbear, P. (1990). *Potential versus actual seed performance - can vigour testing provide an answer?* Seed Science and Technology, 18, 215-228.

Hampton, J.G., TeKrony, D.M. (1995). *Handbook of Vigor Test Methods,* 3rd Edition, International Seed Testing Association. Zurich. Switzerland.

Heather, D.W., Sieczka, J.B. (1991). *Effect of seed size and cultivar on emergence and stand*

establishment of broccoli in crusted soil. Journal of the American Society for Horticultural Science 116: 946–949.

Hibbard, R.P., Miller, E.V. (1928). *Biochemical studies on seed viability.* Measurements of conductance and reduction. Plant Physiology, 3, 335-352.

International Rules for Seed Testing. (2024a). *Chapter 2: Sampling*. International Seed Testing Association. Zurich. Switzerland.

International Rules for Seed Testing. (2024b). *Chapter 3: The Purity Analysis, Chapter 4: Other Seeds by number.* International Seed Testing Association. Zurich. Switzerland.

International Rules for Seed Testing. (2024c). *Chapter 5: The germination test.* International Seed Testing Association. Zurich. Switzerland.

International Rules for Seed Testing. (2024d). *Chapter 10: Thousand-seed weight (TSW) determination.* International Seed Testing Association. Zurich. Switzerland.

International Rules for Seed Testing. (2024e). *Chapter 15: The vigour test.* International Seed Testing Association. Zurich. Switzerland.

Jensen, H.A. (2008). *Development of ISTA Purity Analysis and Determination of Other Seeds by Number from 1924 to 2006, Historical Report from the Purity Committee,* International Seed Testing Association, Zurich, Switzerland.

Kozlowski, T.T. (1972). *Seed Biology: Importance, Development, and Germination,* Volume 1, Academic Press, New York, USA.

Kruse, M. (2004). *ISTA Handbook on Seed Sampling, 2nd Edition,* International Seed Testing Association. Zurich, Switzerland.

Léchappé, J. (2009). *Harmonization of seed testing for the facilitation of trade. In Responding to the challenges of a changing world: The role of new plant varieties and high quality seed in agriculture:* Proceedings of the Second World Seed Conference, Session 5 (UPOV Publication No. 354(E)). International Union for the Protection of New Varieties of Plants.

Li, J., Thomson, M., McCouch, S.R. (2004). *Fine Mapping of a Grain-Weight Quantitative Trait* Locus in the Pericentromeric Region of Rice Chromosome 3. Genetics, 168(4), 2187–2195. https://doi.org/10.1534/genetics.104.034165

Marcos-Filho, J. (2015). *Seed vigor testing: an overview of the past, present and future perspective.* Scientia Agricola 72, 363–374.

Matthews, S. (1981). *Evaluation of techniques for germination and vigour studies*. Seed Science and Technology, 9, 543-551.

Matthews, S., Bradnock, W.T. (1967*). The detection of seed samples of wrinkled-seeded peas (Pisum sativum L.) of potentially low planting value.* Proceedings of the International Seed Testing Association, 32, 553-563.

Mayer, A.M., Poljakoff-Mayber, A. (2014). *The Germination of Seeds,* Elsevier Science, Pergamon Press, England. Retrieved from: https://www.scribd.com/book/282590253/The-Germination-of-Seeds.

Milles, S.R. (1963). *Handbook of Tolerances and of Measures of Precision for Seed Testing.* Proceedings of the International Testing Association, vol. 28, no 3, International Seed Testing Association. Zurich. Switzerland.

Mureşan, T., Pană, N.P., Cseresnyes, Zoia. (1986). *Producerea şi controlul calităţii seminţelor agricole,* Editura Ceres, Bucureşti, România.

Păcurar, I. (2007). *Producerea seminţelor de cereale, leguminoase pentru boabe şi plante tehnice,* Editura Phoenix, Braşov, România.

Perry, D.A. (1978). *Report of vigour Test Committee 1974-1977.* Seed Science and Technology, 6, 159-181, Zurich, Switzerland.

Perry, D.A. (1981). *Introduction in: Handbook of vigour Test Methods.* International Seed Testing Association. Zurich, Switzerland.

Powell, A.A., Matthews, S. (1981). *Evaluation of controlled deterioration, a new vigour test for small seeded vegetables.* Seed Science and Technology, 9, 633-640.

Powell, A.A., Matthews, S. (1984a*). Prediction of the storage potential of onion seed under commercial storage conditions*. Seed Science and Technology, 12, 641-647.

Powell, A.A., Matthews, S. (1984b). *Application of the controlled deterioration vigour test to detect seed lots of Brussels sprouts with low potential for storage under commercial conditions.* Seed Science and Technology, 12, 647-657.

Roberts, E.H. (1984). *The control of seed quality and its relationship to crop productivity.* Proceedings of the Australian Seed Research Conference, 11-25.

Saux, M., Bleys, B., Thierry, A., Bailly, C., El-Maarouf-Bouteau, H. (2020). *A Correlative Study of Sunflower Seed Vigor Components as Related to Genetic Background.* Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7154842/, USA.

Tan, L., Chen, S., Wang, T., Dai, S. (2013). *Proteomic insights into seed germination in response to environmental factors.* PROTEOMICS, Volume 13, Numbers 12-13, 1 June 2013, pp. 1850-1870(21). Wiley-Blackwell. Retrieved from: https://doi.org/10.1002/pmic.201200394.

TeKrony, D.M. (2003). *Precision is an essential component in seed vigour testing*. Seed Science and Technology, 31, 435-447, International Seed Testing Association, Zurich, Switzerland.

TeKrony, Y.R., Egli, D.B. (1993). *Relationship of seed vigour to crop yield: A review.* Crop Science, 31, 819-822.

The Association of Official Seed Analysts (AOSA). (1983). *Seed vigour testing handbook.* Contribution No. 32 to the Handbook on Seed Testing, Association of Official Seed Analysts. Las Cruces, NM, USA.

Torres, S.B., Marcos-Filho, J. (2005). Physiological potential evaluation in melon seeds (*Cucumis melo* L.), Seed Science and Technology, Volume 33, 341–350, Switzerland.

Vashisth, A., Nagarajan, S. (2010). *Effect on germination and early growth characteristics in sunflower (Helianthus annuus) seeds exposed to static magnetic field,* Journal of Plant Physiology 167, 149–156.

Zhang, K., Zhang, Y., Sung, J., Meng, J., Tao, J. (2021). *Deterioration of orthodox seeds during ageing: Influencing factors, physiological alterations and the role of reactive oxygen species,* Plant Physiology and Biochemistry 158, 475–485.