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REVIEW ARTICLE

Tetrazolium: an important test for physiological seed quality evaluation[1]

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ABSTRACT – The production of high quality seeds is linked to a good quality control system. In this system, the tetrazolium test plays an important role in the evaluation of the seed physiological quality, not only due to its relative quickness, but also because of the amount of information that the test presents, such as the indexes of viability and vigor, in addition to providing the diagnosis of possible seed quality problems, such as mechanical damage, insect damage, pre-harvest weathering and deterioration during storage. The development of the test had its beginnings at the end of the 19th century, with great evolution in its concepts and methods in the 20th century. For that, there was the dedication of dozens of professionals in several countries, such as Germany, Japan, Russia, Serbia (former Yugoslavia), United States, Argentina and Brazil. The test indirectly measures the respiration processes that occur in the mitochondria of the cells that make up the tissues of the seeds. The reducing reaction of the solution of the tetrazolium salt under the action of dehydrogenase enzymes results in triphenylformazan, which presents a red carmin coloration. By the interpretation of the resulting staining patterns, seed viability, vigor and the main problems affecting seed quality are determined.

Index terms: viability, vigor, quality diagnosis, history of the test.

Tetrazólio: um teste de importância para a avaliação da qualidade fisiológica das sementes

RESUMO – A produção de sementes de alta qualidade está atrelada a um bom sistema de controle de qualidade. Nesse sistema, o teste de tetrazólio é um dos testes que se destaca para a avaliação da qualidade fisiológica das sementes, não apenas por sua relativa rapidez, mas também pela quantidade de informações que o teste pode apresentar, por meio dos índices de viabilidade e de vigor, além de propiciar o diagnóstico dos possíveis problemas de qualidade das sementes, como os danos mecânicos, danos causados por insetos e os de intempéries em pré-colheita e de deterioração durante a armazenagem. O desenvolvimento do teste teve seus primórdios no final do século 19, com grande evolução em seus conceitos e métodos no século 20. Para tanto, houve a dedicação de dezenas de profissionais em diversos países, como Alemanha, Japão, Rússia, Sérvia (antiga Iugoslávia), Estados Unidos, Argentina e Brasil. O teste mede, indiretamente, os processos de respiração que ocorrem nas mitocôndrias das células componentes dos tecidos das sementes. A reação de redução da solução do sal de tetrazólio sob a ação das enzimas desidrogenases resulta no trifenilformazan, que apresenta a coloração vermelho carmin e, por meio da interpretação dos padrões de coloração, são identificados os índices de viabilidade, de vigor e dos principais problemas que podem afetar a qualidade das sementes.

Termos para indexação: viabilidade, vigor, diagnóstico da qualidade, histórico do teste.

Introduction

The production and utilization of high quality seeds are important and basic keys for the success of crop production. To achieve these requisites, seed industry quality control programs must be versatile and dynamic, promptly providing accurate results. In this sense, the tetrazolium test has assumed a prominent position for some cultures, mainly due

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to the large number of information provided by the test. In addition to viability, it provides valuable information about vigor, as well as making it possible to diagnose the main problems that may affect seed quality, such as the indexes of mechanical damage, field and storage deterioration and insect damage, such as those caused by stinkbugs.

In Brazil, the tetrazolium test is widely used in seed quality control, assuming proportions never recorded even in the countries where the test was developed. Its methodology has been improved, especially with regard to the determination of the vigor index, making the test much more attractive, especially for seeds of common beans, corn, cotton, peanuts, soybean, sunflower, wheat and seeds of forage and horticultural species. For such crops, there are today specific methodologies, which contemplate in detail the methodology of the test.

Test History

The development of rapid and accurate methods for estimating the physiological quality of seeds has been studied by seed physiologists and technologists for many years, primarily at the beginning of the 20th century when the early stages of organized seed production were established, initially in European countries. One of the pioneering milestones that provided the impetus for seed analysis, including the development of quick viability methods, was the establishment of the first seed testing station in Tharandt, Saxony in 1869 by Frederich Nobbe, who also published the first rules for seed sampling and testing (Steiner, 1997a).

Many early testing methods relied on specific seed characteristics such as color, appearance, volumetric weight, density, rate of imbibition, electrical conductivity and respiratory intensity of the seeds to estimate seed viability (Moore, 1969). Unfortunately, results obtained by these methods often were not accurate. During the early 1920s, the activity of certain enzymes, such as peroxidase, catalase, oxidase, reductase and phenolase, was studied, but these too were unsuccessful probably because individual seeds were not evaluated. Several stains, such as indigo carmine, sulfuric acid, methylene blue, neutral red, and malachite green, were also evaluated. Again, the lack of precision of these tests was a major problem.

As reported by Moore (1969) and Steiner and Kruse (2003), the first successful attempts to evaluate seed viability by vital stains were accomplished by Turina of Yugoslavia in 1922 and by Neljubow of Russia in 1925. Turina worked with the reduction of tellurium and selenium salts in seed cells and Neljubow reported success with indigo carmine.

Hasegawa (1935) of Japan, working with tree seeds in the early 1930s, improved the application of selenium and tellurium salts in seed embryo staining. Most of his work was published in Japanese, thus making his achievements unknown to the scientific community for many years. Many of these studies, however, became widely publicized after he released his findings in English (Hasegawa, 1935) and in German during a meeting of the International Seed Testing Association (ISTA) in Europe. During that trip, Hasegawa revealed details of his testing procedures to the German scientists, F.E. Eidmann and W. Schmidt, who later improved the selenium method (Moore, 1969; Steiner, 1997a; Steiner and Kruse, 2003).

Dr. Georg Lakon, born in Athens, Greece and working in Hohenheim, Germany, had shown a great interest in seed physiology in the early 1920s. He perfected the selenium method developed by Hasegawa, Eidmann and Schmidt, culminating in the development of the "topographical" selenium method (Lakon, 1940). After he recognized the poisonous characteristics of selenium during laboratory use, however, Lakon searched for a similar but non-toxic salt that could be used for the same purpose. After Kühn and Jerchel (1941) first called attention to the reduction of tetrazolium compounds in living tissues, Lakon tested several of these salts and concluded that 2,3,5-triphenyl tetrazolium chloride (TTC) was the most appropriate for the topographical test. Lakon developed his test on several cereal crops and corn.

Among several basic concepts developed by Lakon, the definition and characterization of "seed viability" had the greatest impact on the establishment of the tetrazolium test for seeds. He envisaged viability as an "in-borne" germination or potential germination and, hence, viewed a resting seed as a type of potential seedling (Steiner and Kruse, 2003).

Moore (1976), Steiner (1997a), and Steiner and Kruse (2003) reported that an understanding of the existence and merits of tetrazolium (TZ) testing first occurred in the United States in 1945 by U.S. Military personnel who investigated research in Germany after World War II. The first TZ research conducted in the U.S. was published by Porter et al. (1947) at Iowa State University. Other pioneering TZ studies in America were published in 1948 by Flemion and Poole from the Boyce Thompson Institute of Yonkers, New York, by Goodsell from Pioneer Hi-Bred Corn Company in Johnston, Iowa, and by Bennett from Iowa State University (Moore, 1976). In 1949, tetrazolium testing was incorporated into the Official German Seed Testing Rules (Steiner, 1997a).

Substantial achievements and improvements in the TZ test were made by different U.S. and German universities during the 1950s. These included Isely, Bass, Smith and Throneberry from Iowa State University, Parker from the University of Idaho, and Bulat and Steiner from the University of Hohenhein. In 1956, the first ISTA Tetrazolium Committee

was founded and contributed excellent TZ research and training activities (Steiner and Kruse, 2003).

In the 1960s, important developments concerning the practical application of the TZ test were reported by Delouche, Still, Raspet, and Leinhard from Mississippi State University who published the first TZ test handbook for a large number of seed species (Delouche et al., 1962). Jensen, Pierpoint, Hayes and Grabe from Oregon State Seed Laboratory, and Copeland, Bruce, and Midyette from Virginia also provided important improvements to the test. In 1966, "Chapter 6: Biochemical Test for Viability - The Topographical Tetrazolium Test" was incorporated into the ISTA Rules.

In 1970, another important milestone in TZ testing occurred. The use of the TZ test was accepted by the Association of Official Seed Analysts (AOSA) with the release of the "Tetrazolium Testing Handbook" (Grabe, 1970). In 1983, AOSA published the "Seed Vigor Testing Handbook" (AOSA, 1983) which compiled important information about the methodology of the TZ test for soybean, cotton, corn, and wheat.

Special recognition and tribute are given to Dr. Robert P. Moore from the North Carolina State Seed Laboratory. Between 1955 and 1985, he published more than 230 articles concerning the TZ test and edited the outstanding "ISTA Handbook on Tetrazolium Testing" (Moore, 1985) published by the International Seed Testing Association. This publication contains details and procedures for the application of the test to more than 650 species.

In 2000, AOSA completed the first revision of the "Tetrazolium Testing Handbook", which was subsequently updated in 2001, 2002, 2004, 2005, 2006 and 2007 (Peters, 2007) and in 2010 (Miller, 2010). Further updates can now be accessed on the AOSA website (www.aosaseed.com).

In 2003, ISTA published the "ISTA Working Sheets on Tetrazolium Testing" edited by Norbert Leist, Stefanie Kramer and Andrea Jonitz (Leist et al., 2003) in two volumes: Volume I for agricultural, vegetable, and horticultural species; and Volume II for tree and shrub species. Both AOSA and ISTA publications determine seed viability using the tetrazolium test.

The first attempts in developing staining methods for the determination of seed vigor were conducted in Russia by Neljubow in the 1920s who formulated five classes of staining patterns according to the intensity of color in the seed tissues (Moore, 1969). In the 1930s, Eidmann, improving on the work of Hasegawa, proposed three evaluation classes (full germinative seeds; weak germinative seeds; and dead seeds) depending on the intensity and extent of staining (Steiner and Kruse, 2003). In 1950, Lakon discriminated between high and low vigor seeds on the basis of the location and extent of color as well as appearance of staining and tissue texture (Steiner, 1997b). These same principles were used and improved by Moore and Smith (1956) who classified seeds in Classes "A" (vigorous), "B" (viable but non-vigorous) and "C" (non-viable).

In the 1960s, Moore (1961, 1962a, 1962b, 1967a, 1967b) refined a relative classification vigor scheme for corn and soybean seed. Each seed was assigned a soundness rating of 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.

One of the first proposals to use the tetrazolium test for determining seed vigor and to diagnose the possible causes that affect soybean seed quality was carried out by the former Embrapa Soybean researcher, Luiz Antonio Geraldo Pereira, in his Master's thesis, held at Mississippi State University (Pereira, 1974). He evaluated the quality of 39 soybean seed samples, classifying them by two vigor methods: TZ Energy 1-2 and TZ Energy 1-3. In addition, he described in detail the characteristic symptoms of the main types of damages that may affect the quality of soybean seeds: field weathering; mechanical damage; and those caused by stinkbugs. He concluded that the vigor index determined by the sum of the seeds in classes 1-3 was the one that best correlated with seedling emergence in the field.

The inclusion of the tetrazolium test for vigor determination in the "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) greatly contributed to the dissemination and development of the notion that the tetrazolium test is a reliable method for determining seed vigor for several species.

In the case of soybeans, the tetrazolium test was perfected for seed vigor determination by seed technology specialists at Embrapa Soybean, Brazil, who published six manuals containing specific procedures for the test with seeds of this species (França-Neto, 1981, 1989; França-Neto et al., 1985; 1988; França-Neto and Krzyzanowski, 2018), one of them being published in three languages: English (França-Neto et al., 1998a); Spanish (França-Neto et al., 1998b); and Portuguese (França-Neto et al., 1998c). In Argentina, the test procedure for vigor determination was improved by Craviotto et al. (1995; 2008a), who also published the methodology in English (Craviotto et al., 2008b).

Detailed procedures for the tetrazolium test for seed vigor determination have been published by ABRATES (Brazilian Association of Seed Technology) in the handbook "Seed Vigor: Concepts and Tests ("Vigor de Sementes: Conceitos e Testes"). In this manual, the test methodologies for cotton seeds (Vieira and Von Pinho, 1999), peanut (Bittencourt and Vieira, 1999), common beans (Bhering et al., 1999), corn (Dias and Barros, 1999) and soybean (França-Neto et al., 1999)

were published. AOSA also published the methodologies of tetrazolium tests for the determination of seed vigor for these same species in the Seed Vigor Testing Handbook, edited by Baalbaki et al. (2009).

In 2017, the test methodology for determining vigor in soybean seeds was included in the International Rules for Seed Testing, which are annually edited by ISTA (2017). The methodology described in this publication is a hybrid of the procedures indicated by Embrapa (França-Neto et al., 1998c) and the one published in Argentina by "INTA - Instituto Nacional de Tecnologia Agropecuária" (Craviotto et al., 2008a).

In Brazil, Embrapa Soybean had and has a very important role in the adoption of the tetrazolium test in soybean seeds, due to the different training offered: since 1984, this institution offered more than 90 courses on the test, which counted on the participation of more than 2,200 professionals involved in Seed Science and Technology.

"The successful development of the TZ test represents the accomplishment of many milestones in the history of seed research and in the attainment of new insights into seed life" (Moore, 1985). Other detailed reviews about the history of the TZ test can be found by Cottrell (1948), Delouche et al. (1962), Gadd (1950), Isely (1952), Lakon (1953), Lindenbein (1965), Moore (1962a, 1966, 1969, 1976), Steiner (1997a) and Steiner and Kruse (2003).

Principles of the Test

The tetrazolium test indirectly determines the respiratory activity in the cells that make up the seed tissues. The test relies on the activity of dehydrogenase enzymes (AOSA, 1983; Bulat, 1961; Copeland et al., 1959; Moore, 1973; Smith, 1952; Smith and Throneberry, 1951), which catalyze respiratory reactions in the mitochondria, during glycolysis (Figure 1) and citric acid cycle, or Krebs cycle (Figure 2). In glycolysis, there is the activity of one of these enzymes, glyceraldehyde-3-phosphate dehydrogenase, and in the citric acid cycle another five enzymes: pyruvate dehydrogenase; isocitrate dehydrogenase; α-ketoglutarate dehydrogenase; succinate dehydrogenase; and malate dehydrogenase. These enzymes, particularly malate dehydrogenase, carry out the reduction of the tetrazolium salt (2,3,5-triphenyl tetrazolium chloride - TTC) in living tissues. When a seed is immersed in the colorless 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-colored, stable, nondiffusible substance called triphenylformazan or formazan (Figure 3).

When TTC is reduced, forming triphenylformazan in the tissue, it indicates that respiratory activity is occurring in the mitochondria of seed tissue cells, which is are considered alive (Peters, 2007). Therefore, the resulting red color in the seed tissue is a positive indication of viability by indirectly detecting respiratory activity at the cellular level. Non-viable seed tissues do not react with TTC and consequently do not stain.

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 (Peters, 2007).

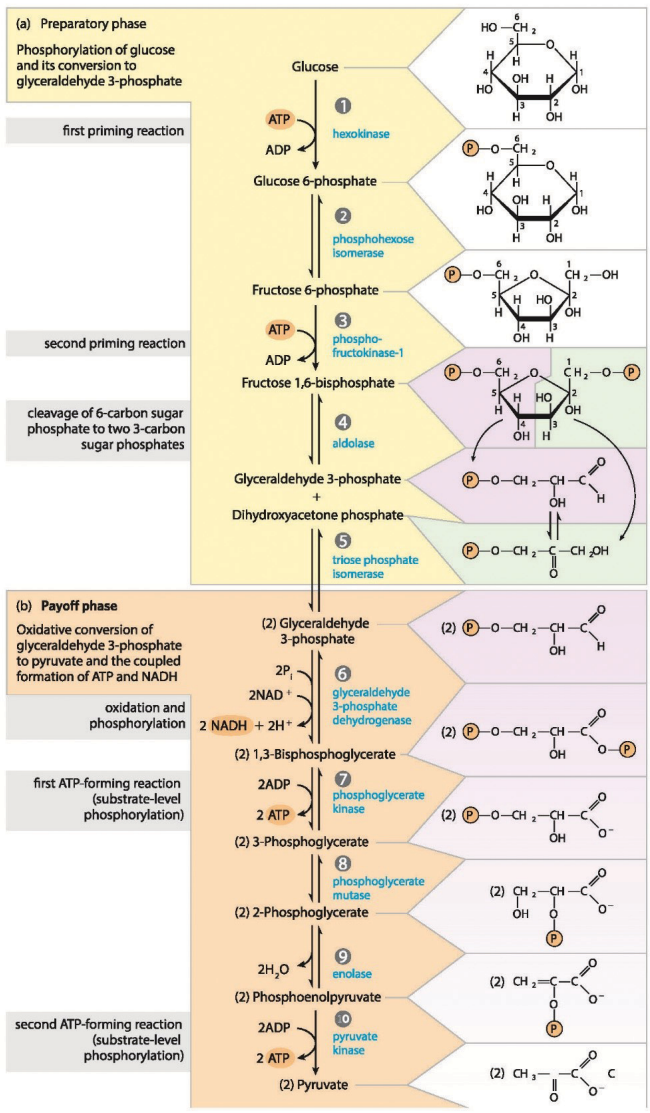

Figure 1. Glycolysis scheme, whereby glucose will be metabolized in two ATP per molecule of glucose and in pyruvate, initiating the oxidative processes of the citric acid cycle, or Krebs cycle. Source: Lehninger et al. (2013).

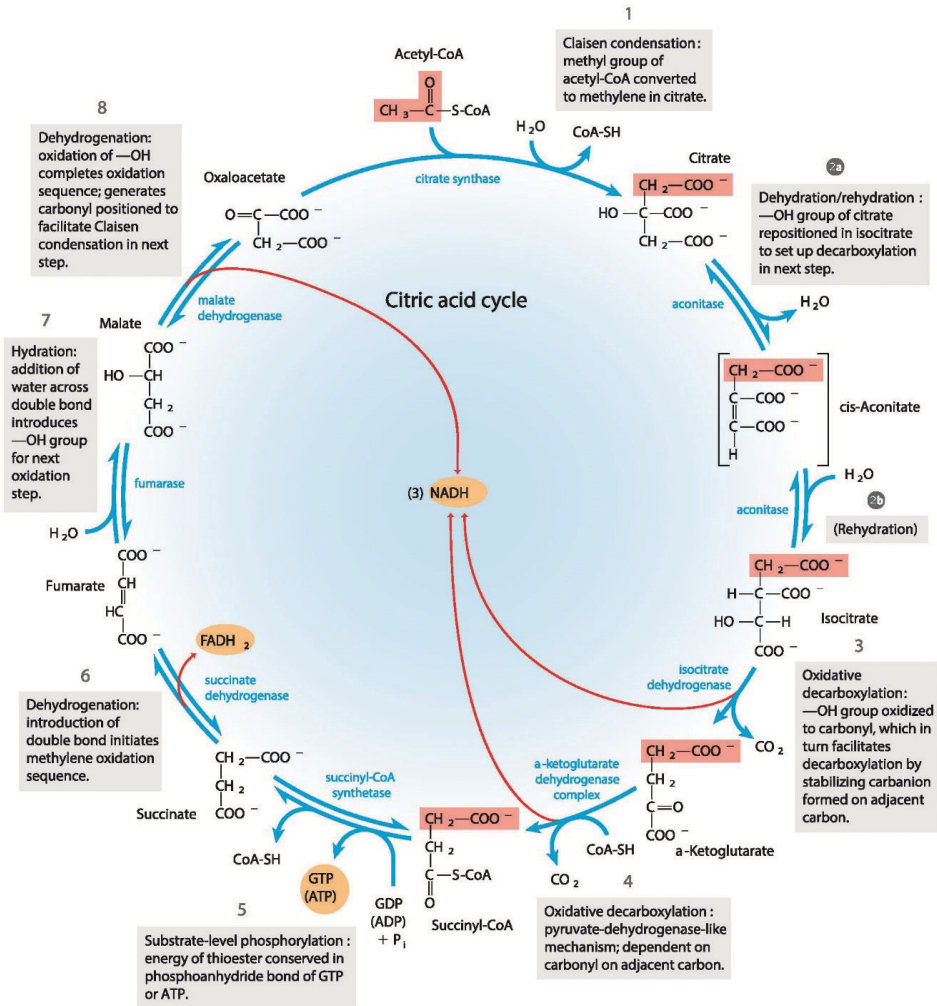

Figure 2. Illustration of the citric acid cycle, or Krebs cycle, through which pyruvate, from glycolysis, is metabolized under the action of several enzymes, including five dehydrogenases, in energy (ATP), carbon dioxide and water. Source: Lehninger et al. (2013).

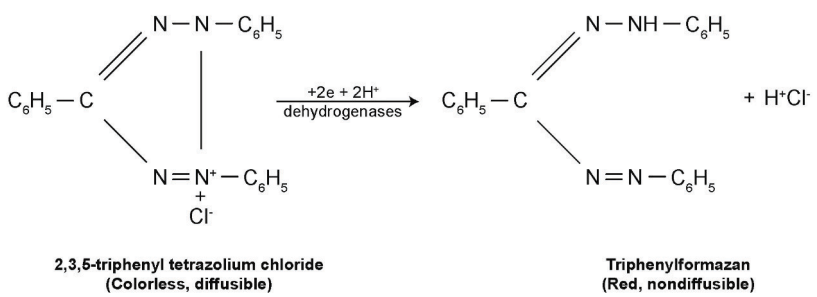

Figure 3. Reduction reaction that results in tetrazolium salt staining within living seed cells. Adapted from Peters (2007). Art: Thais Sofia Ribeiro Santos.

If the tissue is vigorous, a normal faint red color will result; if it is weak, an intense red color will develop due to the rapid diffusion of the TTC-solution through the damaged cell membranes of the seed tissue; if it is dead, no reduction will occur and the dead tissue will be observed as a contrasting white color compared to the stained living tissue. These color differences, together with the knowledge of seed features and function, permit an assessment of the

presence, location, and nature of weaknesses within embryo tissues (Moore, 1973).

Advantages and Limitations of the Test

Listed below are the major advantages and disadvantages of the tetrazolium test:

Advantages

The major advantages of the tetrazolium test are:

a) It excludes major environmental disturbances that could affect seedling growth and evaluation tests, such as the germination test;

b) Focuses evaluation on the physical and physiological conditions of each seed embryo structure;

c) Provides rapid evaluation: less than 20 h for most crops;

d) Allows identification of seed vigor level;

e) Diagnosis the cause(s) of seed deterioration;

f) Requires simple and inexpensive equipment;

g) It is not influenced by factors such as dormancy;

h) An experienced analyst may analyze between four to five seed samples (2 x 50 seeds) per hour.

Limitations

The major disadvantages of the tetrazolium test are:

a) Requires training and knowledge of seed structures and proper tetrazolium interpretation;

b) Is tedious due to the examination of individual seeds that requires patience and experience;

c) Consumes more time per sample than the standard germination test in spite of being a quick test; however, the tetrazolium test provides more seed quality information than the standard germination test;

d) Shows neither the efficacy of chemical seed treatments nor their potential phytotoxicity;

e) Requires a decision-making capability of the seed analyst.

When the Test Should Be Applied

Seed quality control is improved with the use of the tetrazolium test in all phases of seed production such as harvesting, receiving, before and after seed processing and drying, during storage and before sowing.

The test has been applied with success even before harvest: mature plants or plant parts are daily sampled from the seed production field about six to seven days before harvest; pods or other fruit structures are hand threshed, and seeds are then taken for analysis. The tetrazolium test will provide information on viability, vigor, weathering and stinkbug damage. Depending on these results, the seed producer can make decisions regarding whether seeds from the field possess sufficient quality to warrant being harvested as seed or grain. The adoption of this procedure may result in significant savings for the seed producer, avoiding unnecessary expenses related to transportation, drying, processing, bagging and storage of low quality seed lots.

Areas for Improvement

The utilization of tetrazolium results, as with any vigor test, can assist the categorization of vigor level for different seed lots and for estimating the performance of these lots in the field under optimum and stressful conditions. Referee tests for determining the accuracy and precision of the method should also be conducted to verify the accuracy of tetrazolium interpretations.

References

AOSA. Association of Official Seed Analysts. *Seed vigor testing handbook*. Stillwater: Seed Vigor Test Committee of the Association of Official Seed Analysts, 1983. 93p. (AOSA. Contribution to the Handbook on Seed Testing, 32).

BAALBAKI, R.; ELIAS, S.; MARCOS-FILHO, J.; MCDONALD, M.B. (Ed.). *Seed vigor testing handbook*. Ithaca: Association of Official Seed Analysts, 2009. 341p. (AOSA. Contribution to the Handbook on Seed Testing, 32).

BHERING, M.C.; SILVA, R.F; ALVARENGA, E.M.; DIAS, D.C.F.S. Metodologia do teste de tetrazólio em sementes de feijão. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B. (Ed.). *Vigor de sementes*: conceitos e testes. Londrina: ABRATES, 1999. p.8.3.1–8.3.10.

BITTENCOURT, S.R.M.; VIEIRA, R.D. Metodologia do teste de tetrazólio em sementes de amendoim. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B. (Ed.). *Vigor de sementes*: conceitos e testes. Londrina: ABRATES, 1999. p.8.2.1–8.2.8.

BULAT, H. Reduction processes in living tissue, formazan, tetrazolium salts and their importance as reduction-oxidation indicators in resting seed**.** *Proceedings of the International Seed Testing Association,* v.26, p.686-696, 1961.

COPELAND, T.G.; BRUCE, C.F.; MIDYETTE, J.W. The unofficial application of tetrazolium tests as an aid in checking germination claims. *Proceedings of the Association of Official Seed Analysts*, v.49, p.134-141, 1959.

COTTRELL, H.J. Tetrazolium salt as a seed germination indicator. *Annals of Applied Biology*, v.35, p.123-131, 1948.

CRAVIOTTO, R.M.; FARED, M.; MONTERO, M. *Prueba topografica por tetrazolio*: patrones para la especie soja. Santa Fé: INTA, Centro Regional Santa Fé, E.E.A. Oliveros, 1995. 20p.

CRAVIOTTO, R.M.; PEREARNAU, M.A.; GALLO, C. *Prueba topográfica por tetrazólio en soja*. Santa Fé: INTA, Centro Regional Santa Fé, E.E.A. Oliveros, 2008a. 96p.

CRAVIOTTO, R.M.; PEREARNAU, M.A.; GALLO, C. *Topographic tetrazolium test for soybean*. Santa Fé: INTA, Centro Regional Santa Fé, E.E.A. Oliveros, 2008b. 96p.

DELOUCHE, J.C.; STILL, T.W.; RASPET, M.; LIENHARD, M. *The tetrazolium test for seed viability*. Mississippi State: Mississippi State University, 1962. 64p. (Agricultural Experiment Station Technical Bulletin, 51).

DIAS, M.C.L.L.; BARROS, A.S.R. Metodologia do teste de tetrazólio em sementes de milho. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B. (Ed.). *Vigor de sementes*: conceitos e testes. Londrina: ABRATES, 1999. p.8.4.1–8.4.10.

FIALA, F. *Handbook of vigour test methods*. 2nd. ed. Zurich: International Seed Testing Association, 1987. 71p.

FRANÇA-NETO, J.B. Methodology of the tetrazolium test for soybean seeds. In: *Complete diagnosis of soybean quality problems*. Gainesville: University of Florida, Institute of Food and Agricultural Services, USDA-ARS, 1989. p.6-51.

FRANÇA-NETO, J.B. Princípios do teste de tetrazólio para a semente de soja. *Boletim LASP*, v.3, n.1, p.1-8, 1981.

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C. *Metodologia do teste de tetrazólio em sementes de soja*. Londrina: Embrapa Soja, 2018. 108p. (Embrapa Soja. Documentos, 406).

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; COSTA, N.P. *El test de tetrazolio en semillas de soja*. Londrina: EMBRAPA-CNPSo, 1998b. 72p. (EMBRAPA-CNPSo. Documentos, 117).

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; COSTA, N.P. Metodologia do teste de tetrazólio em sementes de soja. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B. (Ed.). *Vigor de sementes*: conceitos e testes. Londrina: ABRATES, 1999. p.8.5.1–8.5.28.

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; COSTA, N.P. *O teste de tetrazólio em sementes de soja*. Londrina: EMBRAPA-CNPSo, 1998c. 72p. (EMBRAPA-CNPSo. Documentos, 116).

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; COSTA, N.P. *The tetrazolium test for soybean seeds*. Londrina: EMBRAPA-CNPSo, 1998a. 71p. (EMBRAPA-CNPSo. Documentos, 115).

FRANÇA-NETO, J.B.; PEREIRA, L.A.G.; COSTA, N.P. Metodologia do teste de tetrazólio em sementes de soja. In: FRANÇA-NETO, J.B.; HENNING, A.A. (Ed.). *Diagnóstico completo da qualidade da semente de soja - versão preliminar*. Londrina: EMBRAPA - CNPSo, 1985. p.9-43.

FRANÇA-NETO, J.B.; PEREIRA, L.A.G.; COSTA, N.P.; KRZYZANOWSKI, F.C.; HENNING, A.A. *Metodologia do teste de tetrazólio em semente de soja*. Londrina: EMBRAPA-CNPSo, 1988. 58p. (EMBRAPA-CNPSo. Documentos, 32).

GADD, I. Biochemical tests for seed germination. *Proceedings of the International Seed Testing Association*, v.16, p.235-253, 1950.

GRABE, D.F. *Tetrazolium testing handbook for agricultural seed*. Stillwater: Association of Official Seed Analysts, 1970. 62p.

HAMPTON, J.G.; TEKRONY, D.M. *Handbook of vigour test methods*. 3rd. ed. Zurich: International Seed Testing Association, 1995. 117p.

HASEGAWA, K. On the determination of viability in seed by reagents. *Proceedings of the International Seed Testing Association*, v.7, p.148-153, 1935.

ISELEY, D. Employment of tetrazolium chloride for determining viability of small grain seeds. *Proceedings of the International Seed Testing Association*, v.42, p.143-153, 1952.

ISTA. *International Rules for Seed Testing*. Bassersdorf: International Seed Testing Association, 2017. 296p.

KÜHN, R.; JERCHEL, D. Uber invertseifen, VIII. Mitteil.: reduktion von tetrazoliumsalzen durch bakterien, gärende hefe und keimende samen. *Berichte der Deutschen Chemischen Gesellschaft*, v.74, p.949-952, 1941.

LAKON, G. Germination potential and germination tendency according to the present state of research. *Saalgutwirtsch*, v.5, p.180-183–205-207, 1953.

LAKON, G. The topographical selenium method, a new method for the establishment of viability of cereal seed without a growth test. *Proceedings of the International Seed Testing Association*, v.12, p.1-18, 1940.

LEHNINGER, A.L.; NELSON, D.L.; COX, M.M. *Principles of biochemistry*. 6. ed. New York: W.H. Freeman and Company, 2013. 1198p.

LEIST, N.; KRAMER, S.; JONITZ, A. *ISTA working sheets on tetrazolium testing*. Volumes I and II. Zurich: International Seed Testing Association, ISTA Tetrazolium Committee, 2003.

LINDENBEIN, W. Tetrazolium testing. *Proceedings of the International Seed Testing Association*, v.30, p.89-97, 1965.

MILLER, A.L. (Ed.). *AOSA/SCST Tetrazolium Testing Handbook*, 2010 edition. Moline, IL: Association of Official Seed Analysts and Society of Commercial Seed Technologists, 2010. 402p.

MOORE, R.P. Freeze injury to seed corn as evaluated in tetrazolium and growth tests. *Proceedings of the International Seed Testing Association*, v.57, p.138-140, 1967a.

MOORE, R.P. *Handbook on tetrazolium testing*. Zurich: International Seed Testing Association, 1985. 99p.

MOORE, R.P. History supporting tetrazolium seed testing. *Proceedings of the International Seed Testing Association*, v.34, p.233-242, 1969.

MOORE, R.P. Seed facts from tetrazolium tests: freeze injury to seed corn as evaluated in tetrazolium and growth tests. In: 1967 Short course for seedsmen and seed technology laboratory. *Proceedings*… Mississippi State: Mississippi State University, 1967b. p.73-78.

MOORE, R.P. Tetrazolium as a universally acceptable quality test of viable seed. *Proceedings of the International Seed Testing Association*, v.27, p.795-805, 1962a.

MOORE, R.P. Tetrazolium best method for evaluating seed life. *Seedsmen's Digest*, v.17, n.11, p.38-40, 1966.

MOORE, R.P. Tetrazolium evaluation of the relationship between total germination and seed quality. *Proceedings of the International Seed Testing Association*, v.51, p.127-130, 1961.

MOORE, R.P. Tetrazolium seed testing developments in North America. *Journal of Seed Technology*, v.1, p.17-30, 1976.

MOORE, R.P. TZ checks your seed for quality. *Crops and Soils*, v.15, n.1, p.1012, 1962b.

MOORE, R.P. Tetrazolium staining for assessing seed quality. In: HEYDECKER, W. (Ed.). *Seed Ecology*. London: Butterworth, 1973. p.347-366.

MOORE, R.P.; SMITH, E. Tetrazolium: a useful research tool in studying causes for seed germination difficulties. In: *Annual meeting of the association of seed control officials of the southern states*. Nashville, 1956. p.15-22.

PEREIRA, L.A.G. Comparisons of selected vigor tests for evaluating soybean seed quality. 1974. 74f. Master Thesis – Mississippi State University, Mississippi State.

PERRY, D.A. *Handbook of vigour test methods*. Zurich: International Seed Testing Association, 1981. 72p.

PETERS, J. (Ed.). *Tetrazolium testing handbook*. Ithaca: Association of Official Seed Analysts, 2007. 88p. (AOSA. Contribution to the Handbook on Seed Testing, 29).

PORTER, R.H.; DURREL, M.; ROMM, H.J. The use of 2, 3, 5-triphenyl-tetrazolium chloride as a measure of seed germinability. *Plant Physiology*, v.22, p.149-159, 1947.

SMITH, F.G. The mechanism of the tetrazolium reaction in corn embryos. *Plant Physiology*, v.27, p.445-456, 1952.

SMITH, F.G.; THRONEBERRY, G.O. The tetrazolium test and seed viability. *Proceedings of the Association of Official Seed Analysts*, v.40, p.105-109, 1951.

STEINER, A.M. History of the development of biochemical viability determination in seeds. In: DON, R.; LEIST, N.; STEINER, A.M. (Ed.). ISTA Tetrazolium Workshop 1997. Edinburgh: *Proceedings*… ISTA, 1997a. p.7-16.

STEINER, A.M. Seed vigour determination by means of the topographical tetrazolium test. In: DON, R.; LEIST, N.; STEINER, A.M. (Ed.). ISTA Tetrazolium Workshop 1997. Edinburgh: *Proceedings*… ISTA, 1997b. p.27-37.

STEINER, A.M.; KRUSE, M. On the origin and rise of topographical tetrazolium testing – a brief historical retrospect. In: LEIST, N.; KRAMER, S.; JONITZ, A. (Ed.). Bassersdorf: ISTA Tetrazolium Committee. *Working Sheets on Tetrazolium Testing*, v.1, p.1-5, 2003.

VIEIRA, M.G.G.C.; VON-PINHO, E.V.R. Metodologia do teste de tetrazólio em sementes de algodão. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B. (Ed.). *Vigor de sementes*: conceitos e testes. Londrina: ABRATES, 1999. p. 8.1-1–8.1.13.

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