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Bibliographic review

Review on use of tetrazolium in seed viability and bioregulator in seedling emergence of araçá-boi (*Eugenia stipitata* McVaugh)

Uso de tetrazolio en la viabilidad de semillas y biorreguladores en la emergencia de plántulas de araçá-boi (*Eugenia stipitata* McVaugh)

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ABSTRACT : The objective of this study is to perform a bibliographic review of the published scientific material on the use of tetrazolium test and plant bioregulator in seeds of *Eugenia stipitata*, as well as in the emergence of its seedlings. Research on this species could reveal and record its production potential. *E. stipitata* is a fruit species native to the Amazon belonging to the Myrtaceae family. Undoubtedly, a prominent species potentially provides fruits with great benefits for human health and new alternatives for production systems. According to the bibliographic survey, the emergence of *E. stipitata* seedlings can exceed 180 days after sowing, while in the tetrazolium test the results can be obtained between 6 and 30 hours. In relation to seed physical and physiological dormancy, the plant bioregulator has potential to promote emergence of vigorous seeds and seedlings. Thus, the tetrazolium test can be an alternative to determine viability of *Eugenia stipitata* seeds. Different priming periods and concentrations of the tetrazolium salt solution are required for seeds of the genus *Eugenia*. Using the plant bioregulator can accelerate the emergence of seedlings of the genus *Eugenia*.

Key words: Eugenia, native species, seed quality, biostimulants.

RESUMEN: El objetivo de este estudio es realizar una revisión bibliográfica del material científico publicado sobre el uso de la prueba de tetrazolio en la determinación de la viabilidade de las semillas de *Eugenia stipitata*, así como de biorreguladores vegetales para la emergencia de sus plántulas. La investigación sobre esta especie podría revelar y registrar su potencial de producción. *E. stipitata* es una especie frutal originaria de la Amazonía, perteneciente a la familia Myrtaceae. Sin duda, es una especie destacada que potencialmente brinda frutos con grandes beneficios para la salud humana y nuevas alternativas para los sistemas productivos. Según el levantamiento bibliográfico, la emergencia de plántulas de *E. stipitata* puede superar los 180 días después de la siembra, mientras que en la prueba de tetrazolio los resultados se pueden obtener entre 6 y 30 horas. En relación con la latencia física y fisiológica de las semillas, el biorregulador de plantas tiene potencial para promover la emergencia de semillas y plántulas vigorosas. Así, la prueba de tetrazolio puede ser una alternativa para determinar la viabilidad de las semillas de *Eugenia stipitata*. Se requieren diferentes períodos de imbibición y concentraciones del cloruro de 2-3-5-trifenil tetrazolio para las semillas del género *Eugenia*. El uso de los biorreguladores vegetales puede acelerar la emergencia de plántulas del género *Eugenia*.

Palabras clave: especies silvestres, calidad de las semillas, bioestimulantes.

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INTRODUCTION

The genus *Eugenia* representing more than 100 species within the family Myrtaceae, whose importance is due to its nutraceutical potential (1) and numerous medicinal properties, such as leaves with secondary metabolites of interest and other benefits, which include from the reduction of chronic, cardiovascular diseases, antimicrobial effects to the control of type II diabetes and chronic inflammatory (2, 3). Thus, this species is an income alternative, through either the commercialization of its fruits or the production of seeds and seedlings.

However, the seed of this species has physical dormancy determined by the presence of the seed coat, requiring a period of 180 days, after sowing, to complete the evaluation of the traditional germination test (4).

In this sense, it is necessary to adhere to techniques that facilitate the rapid and effective characterization of seed lots with high level of sensitivity to desiccation (5). Thus, the tetrazolium test is an alternative to assist decision-making on the viability of the seed lot, and estimate vigor levels. This test based on the activity of dehydrogenase enzymes that are associated with the respiration process. Thus, the hydrogenation of 2-3-5-triphenyl tetrazolium chloride favors the production of a red-colored substance in living cells, which makes it possible to differentiate the living parts (stained red) from the dead parts (milky-white) (6).

Several studies prove the efficacy of the tetrazolium test in seeds of annual crops. On the other hand, the methodology of this test is incipient for native fruit species, such as *Eugenia stipitata*, so studies are required to obtain an effective technique that allows the differentiation of living parts from dead parts.

Thus, research has been conducted to adjust and improve the tetrazolium test methodology for seeds of some species of the genus *Eugenia*, such as *Eugenia pleurantha* (7), *Eugenia involucrata* DC., *Eugenia brasiliensis* Lam., *Eugenia uniflora* L., *Eugenia pyriformis* Cambess. (8, 9) and even the *Eugenia stipitata* (10).

Within these perspectives, scarification methods and biostimulants function as activators of metabolism reactivate physiological processes in different phases of germination and have been used as pre-germination treatments to optimize the germination process of dormant seeds.

The plant bioregulator has the function of establishing hormonal balance and enhancing the maintenance of physiological processes that culminate with cytokine activity (increase in cell division), with influence on cell elasticity and plasticity (growth), promoting different responses in seedling vigor (11, 12).

In view of the above, the objective of this study is to perform a bibliographic review of the published scientific material on the use of tetrazolium test and plant bioregulator in araçá-boi (*Eugenia stipitata*) seeds and in the emergence of its seedlings.

Seed characteristics of araçá-boi (*Eugenia stipitata* McVaugh)

Araçá-boi seed has slender, coriaceous and thick seed coat. Its outer surface is dark brown, with a suede-leather appearance. Internally, the seed coat is smooth and light brown. In relation to shape, it can be reniform, flattened, discoidal, with length greater than width or vice versa, almost spherical, but the predominant morphology is reniform with lateral flattening (13).

The seed is exalbuminous, starchy and pachychalazal, with a coat of complex structure impregnated with phenolic substances. Pachychalazal is an important structure for recalcitrant seeds, as it is related to micromorphological strategies to neutralize water loss, thus ensuring the water content in the embryo, which is eugenioid and has an undifferentiated embryonic axis, hence being pseudomonocotyledonous (14).

In general, many advances have been achieved with araçá-boi seeds, such as: the potential for regeneration of embryos when part of their tissues are suppressed, which can provide new complete seedlings (13), and differences in the limits of tolerance to desiccation and storage (15).

Germination aspect of araçá-boi

Seeds of the genus *Eugenia* are known for their intolerance to desiccation (recalcitrant) and resistant seed coat, which after drying becomes resistant to rupture by the embryo, resulting in delayed germination (13).

The dormancy of araçá-boi seeds may be due to the presence of germination-inhibiting chemicals such as abscisic acid and phenolic compounds. Being suggest leaching as a method for overcoming dormancy caused by these inhibitors. On the other hand, seed fractionation, can trigger the balance between production and elimination of reactive oxygen species (hydrogen peroxide and singlet oxygen), which promote biological processes that favor the emergence of roots, which acts as a positive sign in dormancy overcoming (16).

Although the germination test is the standard performed in the laboratory, it may be time consuming, depending on the species under analysis, and the delay in obtaining the germination leads to a serious limitation to the decision-making process in the seed industry; for *E. stipitate*, the emergence test lasts around 180 days after sowing (4).

These facts require scientific research to search for promising methodologies for analysis of seeds of native species, which will enable large-scale production of araçá-boi seedlings via seeds (17). Therefore, using the tetrazolium test can optimize the prediction of the physiological viability of seeds of the species under study.

Tetrazolium Test Importance of the tetrazolium test

Rapid evaluation of the physiological potential of seeds is important so that farmers, researchers and companies use their resources rationally. In that regard, different

procedures for evaluating seed viability have been used in the identification of lots with high or low vigor (18, 19). Among them, the tetrazolium test stands out for generating promising results to determine the viability of seeds of native fruit crops. This test is relatively simple, but the price of the salt is high, so adjusted and promising methodologies are recommended to perform the test. Consequently, the tetrazolium test has been shown to be an interesting alternative due to the quality and speed in determining seed viability and vigor, making it possible to obtain results usually in less than 24 hours (20).

Physiology of the tetrazolium test

The tetrazolium test is based on the immersion of seeds in a colorless solution of 2,3,5-triphenyl tetrazolium chloride salt, used as an indicator of the reduction process that occurs within living cells. In this process, H+ ions released during the respiration of living tissues are transferred by a group of enzymes, particularly malic acid dehydrogenase, and interact with the tetrazolium salt, which is reduced to a red, stable and non-diffusible compound called triphenylformazan, indicating that there is respiratory activity and cellular viability of the tissue (6).

The formation of light carmine red indicates viable tissue, while intense carmine red reveals deteriorating tissue, since decayed tissues release very small amounts of hydrogen ions, insufficient for the reaction with the tetrazolium salt (21), so the reduction of the salt will not occur and the dead tissue will contrast as white (6).

These staining differences, observed in the seeds after they are subjected to the tetrazolium solution, are the main characteristics that must be considered in the interpretation of the test results, in addition to whether the intensity of seed staining varies between species (22).

Adjust of the tetrazolium test

The effectiveness of the test in assessing seed viability and vigor is related to the application of methodologies adjusted for each species, in order to define the most appropriate conditions for the preparation (23). However, some species need to go through preparatory steps prior to immersion in the tetrazolium solution so that it is adequately absorbed by the seeds (24). Thus, the imbibition process triggers the physiological activity of the seed and facilitates seed coat removal and exposure of the embryo to the contact with the solution used (25). After priming, many species still require preparatory techniques, which involve puncture, cutting and/or removal of the seed coat (24).

The longitudinal sectioning, followed by seed coat removal, prior to immersion in tetrazolium solution, despite being a delicate and laborious operation, makes it possible to reduce the time necessary for seeds to acquire the staining (26).

Therefore, the conduction of the tetrazolium test it is indispensable to perform procedures to determine the priming and cutting of the seeds before they are subjected to the tetrazolium salt, as well as the staining, varying the concentration of the tetrazolium salt solution, time and priming temperature, which are determined for each species (9, 21, 23).

In some species to obtain satisfactory results of staining by the tetrazolium salt, priming needs to be carried out to favor the penetration of the solution into the tissues of interest to be evaluated, as well as adjustments in the tetrazolium solution concentration, time and priming temperature (27). Air temperature contributes to increasing water viscosity, which accelerates water absorption by the seed in phase I of the three-phase germination pattern (28).

The choice of the appropriate method should be based on the ease of identification of viable and unviable tissues and on the ability to differentiate lots with different physiological qualities (29). For temperature, the staining color should be developed between 35 and 40 ºC, because it is faster, but the test can be performed normally at temperatures from 20 to 40 ºC (30).

Potential of the tetrazolium test in the viability of *Eugenia* spp. seeds

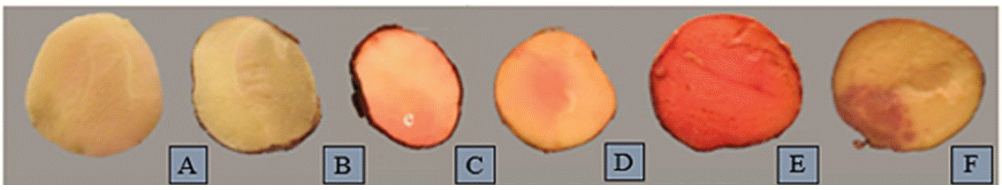
Table 1 lists the best results of the tetrazolium test, and Figure 1 shows the results of staining of tetrazolium test in different species of the genus *Eugenia*.

The Figure 1 (9) shows the results of staining of the tetrazolium m test in different species of the genus *Eugenia*.

As shown in Table 1 and Figure 1, despite belonging to the same genus, the species have differences between the ideal combinations for the use of the tetrazolium test. Such variations emphasize the need to adapt the tetrazolium test methodology for each species, as these results may be related to specificity and genetic variation, reduction of moisture content during the storage period or maturity degree at the time of fruit harvest, which may alter the hormonal balance of seeds (26).

Table 1. Priming, concentration, immersion time/temperature and seed viability obtained by the tetrazolium test in different species of *Eugenia*

Species	Priming	Concentration	Time/ Temperature	Viability	Reference
E. involucrate	Immersion in distilled water (24h/30 °C)	0.5%	2h/30 °C	87 %	(8)
E. pyriformis	Immersion in distilled water (24h/30 °C)	0.5%	6h/30 °C	83 %	(8)
E. pleurantha	Immersion in distilled water (12h/30 °C)	0.1%	4h/30 °C	88 %	(7)
E. brasiliensis	Immersion in distilled water (3h/25 °C)	0.250%	3h/35 °C	85 %	(9)
E. uniflora	Immersion in distilled water (3h/25 °C)	0.125%	3h/35 °C	83 %	(9)
E. stipitata	-	1%	24h/30 °C	-	(10)



A to F= Staining of *E. brasiliensis*, *E. uniflora* and *E. pyriformis* seeds in the tetrazolium test; A and B = intact seeds, demonstrating that they do not acquire sufficient staining for analysis; C and D = viable seeds; E and F = unviable seeds; and surface of the embryo after longitudinal cutting. Scale of 1 cm

Figure 1. Viability staining in seeds of different species of *Eugenia* by the tetrazolium test

Influence of bioregulators on seed germination process

Growth bioregulators, also called biostimulants or phytohormones, contain in their composition: amino acids, humic substances (humic acids and fulvic acids), plant growth hormones, vitamins and various other elements, and may contain organic substances from algae extract (11).

Bioregulators function as activators of seed and plant cell metabolism, reactivate physiological processes in different stages of development and stimulate root growth (12, 31). Stimulate® is a commercial product known to contain three plant hormones in its composition, 0.009 % kinetin, 0.005 % gibberellic acid (GA3) and 0.005 % indolyl butyric acid (IBA), phytoregulators such as auxins, gibberellins and cytokines (32).

Auxins act mainly in the regulation of growth and promotion of rooting of root primordia, whereas gibberellins have as one of their main functions the stimulation of cell division and elongation, besides having greater influence on the germination process. They have a stimulating effect, in both the presence and the absence of dormancy in the seed, acting in the activation of vegetative growth of the embryo, mobilization of endosperm reserves and weakening of the endosperm layer surrounding the embryo (12). In turn, cytokinins mainly stimulate cell division processes (cytokinesis) (33).

The ideal concentration of the commercial product composed of kinetin, gibberellic acid and indole butyric acid optimized the percentage of emergence in *Acacia mangium* (34). In *Hymenaea courbaril* the 2.0 ml L^{-1} dose of plant regulator resulted in an 18 % increase in shoot height, compared to the control at 90 DAT. however, did not promote an increase in chlorophyll a and b (32). Inadequate concentrations of plant bioregulators can lead to deterioration by phytosanitary, physiological, biochemical, and cytological alterations in the seeds, culminating in low emergence or even embryo death (35).

However, there are few studies with bioregulators in native seeds of the Amazon (31), which can be a field of study to enhance the vigor of plantlets, consequently leading to quality seedlings.

In view of the above, species of the genus *Eugenia* have potential as fruit crops, and the seed is the main pathway of propagation. In addition, the fractionation of araçá-boi seeds has the advantage of homogenizing emergence and improving plant stand in the nursery, resulting in better yield in the use of propagative material and in the quality of the seedlings produced.

CONCLUSIONS

The studies conducted allowed exploring; relating and understanding the physical and physiological responses of seeds of the genus *Eugenia* subjected to different techniques in seed analysis (fractionation, tetrazolium test and plant bioregulator), should be judicious and based on technical and scientific information, avoiding inferences and contradictory results. It is worth mentioning that these and other studies that provide information for improving the traditional system of *Eugenia stipitata* seedlings require investments and conceptual, managerial and technical changes in seed analysis for its real adoption. For instance, the tetrazolium test may be an alternative to determine the viability of *Eugenia stipitata* seeds faster; in turn, different priming periods and concentrations of the tetrazolium salt solution are required in the imbibition of seeds of the genus *Eugenia*. Using the bioregulator at the appropriate concentration can optimize the emergence rate for large-scale production of seedlings of the genus *Eugenia*.

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