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Tetrazolium test to assess the viability of kale seeds

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ABSTRACT: Kale (*Brassica oleracea* var. *acephala*) has great importance due to its easy propagation, acceptability and nutraceutical properties. The aim of the present work was to make methodological adjustments to conduct the tetrazolium test in kale seeds. Pre-tests were initially carried out to assess the priming time at 20 °C for 10 and 14 hours (times defined by the imbibition curve) and methods of removing the coat of kale seeds (total removal of the seed coat; cut in the distal region to the embryonic axis; longitudinal cut along the longest axis and whole seeds). Subsequently, the most efficient methodologies were evaluated using different concentrations of the tetrazolium salt solution (0.075%; 0.2%; 0.5% and 1.0%) and times (2, 4 and 6 h) of seed immersion in the solution, using four lots. The seeds were analyzed individually and classified as viable or non-viable. The tetrazolium test is efficient for evaluating the viability of kale seeds, providing results correlated with germination. Kale seeds should be primed between paper for 10 hours at 20 °C, and the seed coat should be removed for immersion in a 0.5% tetrazolium salt solution for 4 hours at 30 °C.

Index terms: *Brassica oleracea* var. *acephala*, priming, physiological quality.

RESUMO: A couve (*Brassica oleracea* var. *acephala*) apresenta grande importância devido a sua facilidade de propagação, aceitabilidade e às suas propriedades nutracêuticas. O objetivo no presente trabalho foi realizar ajustes metodológicos para a condução do teste de tetrazólio em sementes de couve. Inicialmente foram realizados pré-testes com relação ao tempo de pré-condicionamento a 20 °C por 10 e 14 horas (tempos definidos pela curva de embebição) e métodos de remoção do tegumento das sementes de couve (remoção total do tegumento; corte na região distal ao eixo embrionário; corte longitudinal no maior sentido e sementes inteiras). Posteriormente, as metodologias mais eficientes foram avaliadas usando diferentes concentrações da solução do sal de tetrazólio (0,075%; 0,2%; 0,5% e 1,0%) e tempos (2, 4 e 6 h) de imersão das sementes na solução, utilizando-se quatro lotes. As sementes foram analisadas individualmente e classificadas como viáveis ou não viáveis. O teste de tetrazólio é eficiente para a avaliação da viabilidade de sementes de couve fornecendo resultados correlacionados com a germinação. As sementes de couve devem ser pré-condicionadas entre papel por 10 horas a 20 °C, e ter o tegumento removido para imersão em solução do sal de tetrazólio a 0,5% por 4 horas a 30 °C.

Termos para indexação: *Brassica oleracea* var. *acephala,* pré-condicionamento, qualidade fisiológica.

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INTRODUCTION

Kale, Brassica oleracea var. acephala, belonging to the Brassicaceae family, is a traditional vegetable on the menu of Brazilians and one of the main leafy vegetables in Brazil (Vilela and Luengo, 2017). It stands out due to its easy propagation by seeds in the case of hybrid kale, the increase in its consumption due to the various forms of use in cooking and the recent scientific discoveries as to its nutraceutical properties (Trani et al., 2015). It is also a crop rich in carotenoids, which arouse great interest due to studies that prove its relation to the prevention of certain types of cancer and chronic ophthalmologic diseases such as cataracts (Šamec et al., 2019).

One of the main interests and challenges for producers is the production and obtaining of quality seeds, since the uniformity of seedlings and their rapid establishment in the field are one of the essential conditions to obtain a good stand with high yield and quality of the harvested product, which highlights the importance of seed vigor and the need to evaluate it (Paiva et al., 2016; Marcos-Filho, 2020).

The physiological quality of seeds is evaluated by tests such as the germination test, which is conducted under ideal and controlled conditions that allow the manifestation of maximum germination potential. According to the Rules for Seed Testing (Brasil, 2009), the evaluation of the physiological quality of kale seeds by the germination test has an established run period of 10 days (Brasil, 2009; ISTA, 2020).

In addition to the germination test, considered official by Brazilian regulations, another option for evaluating seed quality is the tetrazolium test, which is successfully used in quality control programs, because it is a fast method that estimates the viability of seed lots of various species (França-Neto and Krzyzanowski, 2019).

The efficiency of the tetrazolium test in the evaluation of seed viability depends on the development of appropriate methods for each species, determining adequate hydration conditions, preparation of seeds, concentration of the tetrazolium solution, as well as priming time and temperature (Fantazzini et al., 2020). Some authors working with several species of vegetables, such as crambe (Rezende et al., 2015), forage turnip (Nery et al., 2015), canola (Flores et al., 2015) and coriander (Silva et al., 2021), have found that it is necessary to define the criteria for interpreting the staining and for classifying seeds for viability and vigor.

For vegetable seeds, the tetrazolium test does not yet have widespread use, mainly due to the lack of information on the most appropriate methodology for the different species. It is noteworthy that the methodology of the tetrazolium test for kale seeds can still be improved. Thus, the aim with this study was to make methodological adjustments to conduct the tetrazolium test of this species.

MATERIAL AND METHODS

The experiments were conducted at the Seed Laboratory of the Agronomy Department of the *Universidade Federal do Vale do Jequitinhonha e Mucuri* (UFVJM) in Diamantina, MG, Brazil. The seeds were obtained from plants of half-sib progenies of kale in an experiment set up in the Olericulture Sector of UFVJM, which is located at 1,400 m altitude, with coordinates 18° 9’ S latitude and 43° 21’ W longitude. The original progenitor plants of this work are those of the kale germplasm bank of UFVJM.

Seed harvest was performed gradually, as the siliquas changed from a light green to a brown color, with the seeds inside showing a brown color. Then, they were extracted from the siliquas manually, and the seeds obtained from each plant and at each harvest time were joined according to the lots to which they belonged and stored in paper bags, under controlled conditions in cold chamber at 10 °C and 50% relative humidity, until the experiments were carried out.

Four lots were selected based on the largest number of seeds produced. These seeds were characterized for physical and physiological quality by the following tests:

Moisture content was determined by the oven method, at 105 °C for 24 hours (Brasil, 2009), with two replications of 0.3 g of seeds for each lot.

For the germination test, sowing was performed on germitest paper towel substrate, moistened with an amount of water equivalent to 2.5 times the dry weight of the paper, in gerbox boxes, placed in Biochemical Oxygen Demand (BOD) germination chamber, regulated at 20 °C, with constant light (Brasil, 2009). Four replications of 50 seeds were used, with results expressed as percentage of normal seedlings on the fifth day (first count), and the test was ended on the tenth day (final count) (Brasil, 2009). The counts were made daily to determine the germination speed index (GSI), calculated according to the formula proposed by Maguire (1962), by counting the number of seeds with protrusion, from the emergence of 1 mm of radicle.

The seedling emergence test was conducted with four replications of 50 seeds in trays with sand and earth substrate in the ratio of 2:1. The moisture content of the substrate was adjusted to 60% of its holding capacity. After sowing, the trays were kept at a temperature of 20 °C. Seedling emergence was computed on the fifth day (initial stand) and on the tenth day (final stand), after sowing, with calculation of the percentage of emerged seedlings. For the emergence speed index (ESI), the number of seedlings emerged from the beginning of the emergence was counted daily and the parameter was calculated according to Maguire (1962).

The imbibition curve was constructed from two replications of 50 seeds using two lots of kale seeds, a lot of seeds used in the experiment and a lot of commercial seeds of kale from Feltrin®. The seeds were placed to soak between germitest paper, moistened with an amount of distilled water equivalent to 2.5 times the weight of dry paper in gerbox boxes, and kept in BOD chamber at 20 °C. During the evaluation, the seeds were removed from the gerbox, dried with paper towels, and weighed on a digital scale with accuracy of 0.0001 g (Rodrigues et al., 2008).

The seeds were weighed before the beginning of the imbibition and after at time intervals of 15 minutes with duration of 7 hours, 30 minutes with duration of 3.5 hours, 1 hour with duration of 10.5 hours and 2 hours with duration of 17.5 hours until the stabilization of the curve and root protrusion in 50% of all seeds. Moisture content was calculated indirectly, based on the initial moisture content of the seeds and their wet weight at the different intervals (Armondes et al., 2016).

To define the procedures to be performed in the tetrazolium test, preliminary tests were initially carried out using different combinations of hydration time and forms of preparation of kale seeds, as described in Table 1. The seeds were primed between moistened paper for 10 and 14 hours at 20 °C, followed by total removal of the seed coat, cut in the distal region to the embryonic axis and longitudinal cut along the longest axis, in addition to seeds that were kept whole (Figure 1).

From the results obtained by the procedures described above for the tetrazolium test, staining and immersion times of 2, 4 and 6 hours in a tetrazolium solution at concentrations of 0.075%, 0.2%, 0.5% and 1.0% at 30 °C were evaluated. Four replications of 50 seeds were used for each lot.

After the development of the staining, the seeds were washed in running water and left submerged in water until the time of evaluation. Subsequently, they were examined under a stereoscopic microscope, observing mainly their vital area (embryonic axis) (Figure 1), as well as the extent and intensity of reddish tones, presence of milky white areas, aspect of tissues, in addition to the location of these staining patterns in relation to the areas that are essential to growth. The seeds were classified as category A (viable) and category B (non-viable) (Figure 2). Category A was subdivided into: A1 - well-developed embryonic structures, intact and with pink staining, and A2 - less than 50% of the

Table 1. Conditions of priming (hours) at 20 °C and types of cut of kale seeds.

Priming between paper in hours at 20 °C	Preparation of seeds
10 and 14	Whole seeds
10 and 14	Total removal of seed coat
10 and 14	Cut in the distal region to the embryonic axis
10 and 14	Longitudinal cut along the longest axis

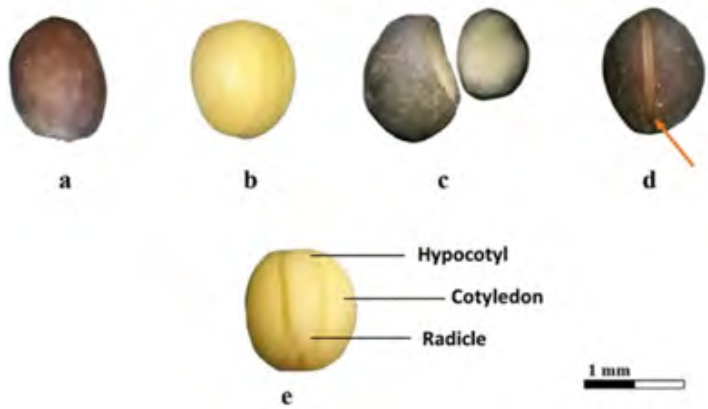

Figure 1. Visualization of kale seed preparations: (a) whole seed; (b) total removal of the seed coat; (c) cut in the distal region to the embryonic axis; (d) longitudinal cut exposing the proximal region (embryonic axis - arrow) and the distal region; (e) seed morphology.

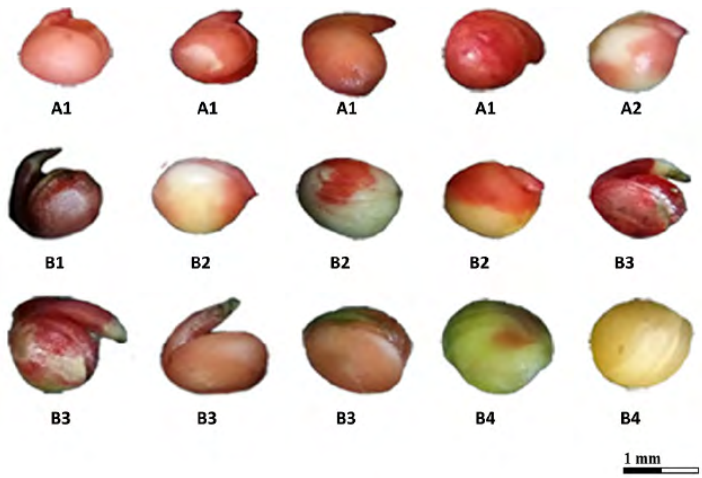

Figure 2. Classes for determination of viability in kale seeds by the tetrazolium test: category A (viable) and category B (not viable).

embryo unstained, without reaching the embryonic axis, and tissue with normal and firm appearance. Category B was subdivided into: B1 - embryo with intense red staining; B2 - more than 50% of the cotyledons unstained; B3 - embryonic axis region unstained; and B4 - embryo completely unstained.

The experimental design used was completely randomized for all tests, with four replications. All statistical analyses were carried out in R software (R Core Team, 2020). Deviations from normality and heterogeneity were not found in this study, according to the Shapiro-Wilk and Bartlett tests, respectively ($p > 0.05$). These analyses were conducted using the package ExpDes.pt. The lots were characterized for physiological quality by analysis of variance and, when significant, Tukey test was applied at 5% significance level.

In order to assess the effect of imbibition time on the mass increment in the seeds, third-degree polynomial regressions were fitted.

The use of 1.0% tetrazolium solution was not efficient for the proper staining of the seeds, resulting in an intense red tone, regardless of the staining time, so this concentration was eliminated from the factorial.

For each lot, the factorial scheme 3x3 (3 concentrations and 3 immersion periods) was used, by means of the generalized linear model with logit link function, employing the *glm* function of the *stats* package. The significance of the simple and interaction effects was tested by deviance analysis and chi-square test ($p \leq 0.05$) with the aid of *anova*

function. For better presentation of the data, from the values fitted by the model, a response surface graph was created in SigmaPlot software.

Pearson's correlation was used to study the association of viability classification in the methodologies studied with physiological parameters. For the graphical representation of these estimates, the corrplot package was used (R Core Team, 2020).

RESULTS AND DISCUSSION

In the characterization of the profile of the kale seed lots (Table 2), the results of the moisture content (U) of the seeds showed variation of at most 0.9 percentage points between the lots. This uniformity is fundamental for the standardization of evaluations and the obtaining of consistent results (Marcos-Filho, 2015).

For the percentage of normal seedlings obtained in the first count (FC) test, there was no significant difference between the lots (Table 2). For germination percentage (G), seeds of lot 2 showed higher germination and differed significantly only from lot 1. It is also observed that the germination values obtained were higher than the minimum standard for the commercialization of kale seeds established by Normative Instruction Nº. 42, which is 80% (Brasil, 2019).

The germination speed index (GSI), in turn, was higher for lot 3, which differed from lot 4. Regarding the results of the vigor tests related to emergence (Table 2), there was no significant difference between the values for normal seedlings in the initial stand (IS), emergence (E) and emergence speed index (ESI).

To establish the most appropriate periods for seed priming in the tetrazolium test, the imbibition curve was defined for two lots (Figure 3).

The initial moisture contents shown by the lot of seeds produced at UFVJM and by the commercial seed lot were 7.37% and 6.75%, respectively. The curves tended to the three-phase imbibition pattern proposed by Bewley and Black (1994) for both lots (Figure 3). Phase I occurred between 14 and 15 hours, when there was a greater increase in wet mass. Phase II lasted approximately 18 hours and showed smaller increments of wet mass gain. Finally, phase III occurred with the beginning of the radicle protrusion 32 hours after the beginning of the imbibition. In kale seeds, phase III of the water absorption curve was reached after 36 hours of imbibition (Kikuti and Marcos-Filho, 2009), while in crambe seeds, the protrusion of the primary root occurred after 42 hours (Silva et al., 2019).

It was verified that with 14 hours there were already protruded seeds even without having reached phases II and III in the imbibition curve. This behavior reveals that hydration for this period was sufficient to reactivate the metabolism of the embryo. However, for efficient evaluation in the tetrazolium test, seeds should have stabilized their

Table 2. Moisture content - U (%), thousand-seed weight - TSW (g), normal seedlings in the first count - FC (%), germination - G (%), germination speed index - GSI, normal seedlings in the initial stand - IS (%), emergence - E (%) and emergence speed index - ESI of kale seeds [(1)].

Lots	Tests							
	U (%)	TSW (g)	FC (%)	G (%)	GSI	IS (%)	E (%)	ESI
1	8.50a	3.4 b	93 a	93 b	26.75 ab	84 a	86 a	11.30a
2	8.25a	3.3 b	98 a	99 a	26.15 ab	89 a	91 a	11.77a
3	7.95a	4.1 a	94 a	97 ab	26.92 a	93 a	96 a	12.33a
4	7.60a	3.0 b	96 a	97 ab	25.86 b	92 a	94 a	12.79a
CV (%)	15.3	9.2	3.9	2.6	1.69	6.8	5.9	7.1
LSD_{Tukey}	2.60	0.67	7.76	5.32	0.94	12.74	11.31	1.79

[(1)] Means followed by the same lowercase letter in the column do not differ from each other by Tukey test at 5% probability level.

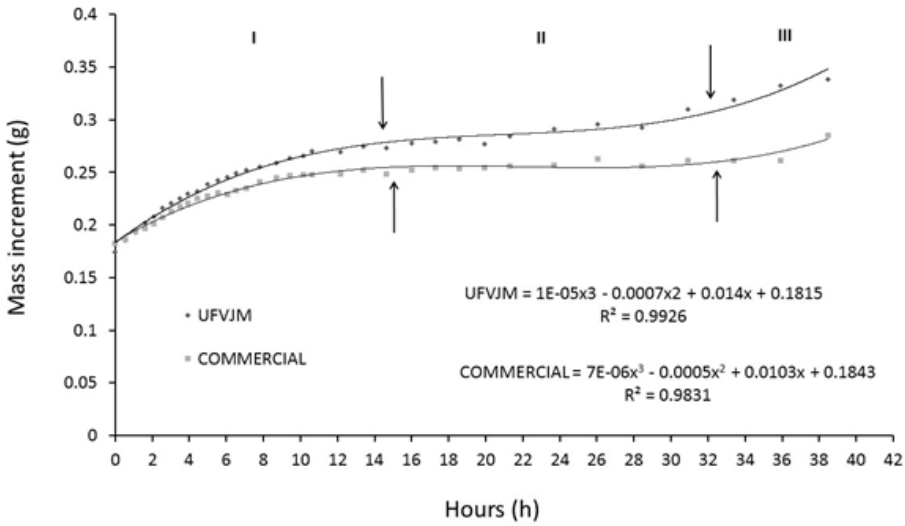

Figure 3. Mass increment (g) of kale seeds (UFVJM) and commercial seeds of kale over 38 hours of imbibition.

water absorption, a behavior similar to that commonly reported in Phase I of the three-phase hydration model (Bewley and Black, 1994), but not protruded the radicle. Thus, the period of 10 hours in water was used for priming, which is sufficient to facilitate the removal of the seed coat.

Regarding the preparation of kale seeds, the longitudinal cut exposing the proximal region (embryonic axis) and the distal region damaged the seeds, which masked the test results, and the presence of the seed coat hindered the penetration of the solution, resulting in uneven or absent staining of the seeds. Similar results indicating the need to remove the seed coat were also obtained by Barros et al. (2005) in zucchini. However, in seeds of forage turnip (Nery et al., 2015), canola (Flores et al., 2015) and carrot (Lima et al., 2018), the longitudinal cut along the longest axis before immersion in the tetrazolium solution was the most indicated.

The use of 1.0% tetrazolium solution was not efficient for the proper staining of the seeds, resulting in an intense red tone, regardless of the staining time. When the seeds were exposed to the staining times of 2 hours and 6 hours, they showed weak and very intense pigmentation for this concentration tested, so it was not possible to correctly visualize the damage caused in the embryonic axis region. Therefore, this concentration was eliminated from the subsequent tests. Several authors (Rezende et al., 2015; Flores et al., 2015; Nery et al., 2015) reported difficulty in interpreting the test when the seeds were exposed to a concentration of 1.0%, because they showed a very intense red staining. This fact brought challenges to the interpretation of the test, especially when associated with prolonged times of exposure.

In order to assess the effect of immersion time and concentrations on seed viability classification by the tetrazolium test, the generalized linear model was fitted with the logit link function. Through deviance analysis, it was possible to verify the existence of significant interaction between concentrations and times of immersion in the tetrazolium solution (Table 3).

Regarding the influence of the tested effects on seed classification, Figure 4 shows that the probability of classifying the seeds as viable decreased with the increase in tetrazolium concentration at the immersion times of 4 and 6 hours. This behavior was verified for the four lots of kale seeds. However, when using the immersion time of 2 hours, reduction in the probability of classifying the seeds as viable was observed only in lot 1. In general, for the four lots there was a higher probability of classifying the seeds as viable with the highest tetrazolium concentration tested and immersion time of 4 hours.

Although the results of Figure 4 are relevant to understand the studied effects on the probability of classification of seeds as viable, this is not enough to indicate a better methodology. For this, studying the association between the percentage of seed viability classification and physiological quality parameters is indispensable. Thus, it can be verified in Figure 5 that no methodology showed a significant association with all characteristics simultaneously. It was also found that the methodology considering the concentration of 0.5% for four hours should be preferred because, in this situation, there are significant correlations with important physiological parameters such as FC, G, E and ESI.

These results show that the use of both the lowest concentration associated with the shortest period and the highest concentration associated with the longest period do not allow reliable analyses because they lead to very weak and very intense staining, respectively, hindering the visual classification and distinction of categories. With these combinations it is not possible to distinguish healthy tissues from injured tissues due to lack of staining and/or intense staining.

One of the factors that should be considered when assessing seed viability is the test run period (França-Neto and Krzyzanowski, 2019). The priming time was reduced from 18 hours (Brasil, 2009) to 10 hours, followed by more 4 hours of immersion in tetrazolium solution for staining. In a study on methodological adjustment with coriander seeds, Silva et al. (2021) found that the time of exposure to the tetrazolium solution can be reduced from 24 to six hours. These results contribute to a greater efficiency in the routine analysis of seed laboratories, allowing quick answers about the situation of seed lots during handling.

Table 3. Deviance analysis for studying the effects of the sources of variation "Concentration" and "Hours of imbibition" on the viability of kale seeds by means of a generalized linear model with the Logit link function.

SV	DF	Deviance	Resid.Dev	pValue
Lots	3	126.8	4252.8	<0.001
Concentration	2	78.86	4174	<0.002
Hours	2	35.34	4138.6	<0.003
Lots x Concentration	6	81.84	4056.8	<0.004
Lot x Hours	6	100.8	3956	<0.005
Concentration x Hours	4	48.29	3907.7	<0.006
Lots x Concentration x Hours	12	63.34	3844.4	<0.007

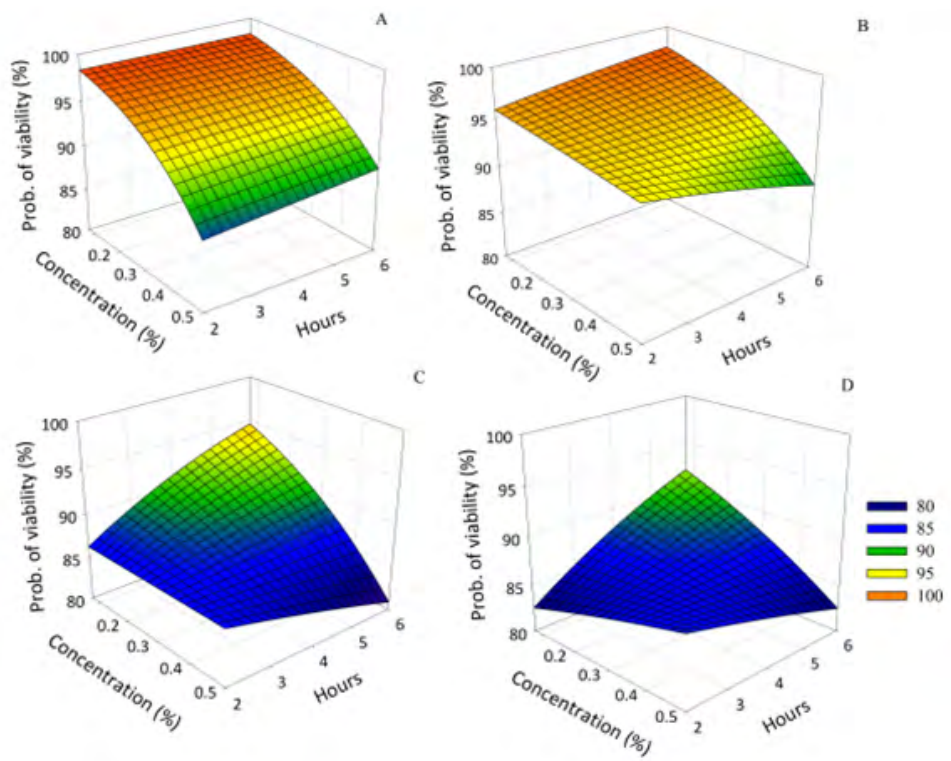

Figure 4. Response surface graph obtained from the fit of the generalized linear model with the Logit link function for the probability of classifying seeds as viable as a function of different concentrations of tetrazolium and immersion times in kale seeds for each lot (lot 1 - A, lot 2 - B, lot 3 - C and lot 4 - D).

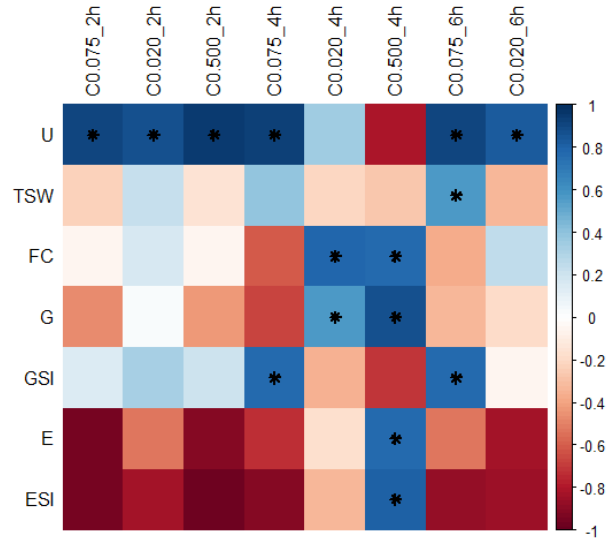

Figure 5. Graphic dispersion of correlations between physiological quality parameters and percentage of viability detected by the tetrazolium test with different concentrations and immersion times in kale seeds. * indicates significant correlations by the t-test ($p \leq 0.05$).

CONCLUSIONS

Priming of kale seeds between paper for 10 hours at 20 °C, followed by the total removal of the seed coat, and use of tetrazolium solution at the concentration of 0.5% for 4 hours at 30 °C are efficient in the evaluation of seed viability.

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REFERENCES

ARMONDES, K.A.; DIAS, D.C.; MARTÍNEZ, P.A.; SILVA, L.J.; HILST, P.C. Condicionamento osmótico e desempenho de sementes de repolho com diferentes níveis de vigor. *Horticultura Brasileira*, v.34, p.428-434, 2016. https://doi.org/10.1590/S0102-05362016003020

BARROS, D.I.; DIAS, D.C.F.D.S.; BHERING, M.C.; DIAS, L.A.D.S.; ARAÚJO, E.F. Uso do teste de tetrazólio para avaliação da qualidade fisiológica de sementes de abobrinha. *Revista Brasileira de Sementes*, v.27, n.2, p.165-171, 2005. https://doi.org/10.1590/S0101-31222005000200024

BEWLEY, J.D.; BLACK, M. *Seeds*: *physiology* of *development and germination*. New York: Plenum Press, 1994. 445p.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Regras para Análise de Sementes. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília, DF: MAPA/ACS, 2009. 399p. https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise__sementes.pdf

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Instrução Normativa nº 42, de 17 de setembro de 2019*. Diário Oficial da União. Edição 182, Seção 1, 2019. p.4.

FANTAZZINI, T.B.; FRANCO DA ROSA, S.D.V.; CARVALHO, G.R.; LISKA, G.R.; CARVALHO, M.L.M.; COELHO, S.V.B.; CIRILLO, M.A.; RIBEIRO, F.A.S. Correlation between historical data of the germination test and of the tetrazolium test in coffee seeds by GAMLSS. *Seed Science and Technology*, v.48, n.2, p.179-188, 2020. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/213051/1/correlation-between-historical-data-of-the-germination-test.pdf

FLORES, M.F.; GRZYBOWSKI, C.R.D.S.; PAZOLINI, K.; POSSENTI, J.C.; PANOBIANCO, M. Criteria for implementation of a tetrazolium test in canola seeds. *Journal of Seed Science*, v.37, n.4, p.222-227, 2015. https://doi.org/10.1590/2317-1545v37n4153255

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C. Tetrazolium: an important test for physiological seed quality evaluation. *Journal of Seed Science*, v.41, p.359-366, 2019. https://doi.org/10.1590/2317-1545v41n3223104

ISTA. *International Seed Testing Association. International rules for seed testing*. Bassersdorf, Switzterland, 2020. 300p.

KIKUTI, A.L.P.; MARCOS-FILHO, J. Condicionamento fisiológico de sementes de couve-flor. *Horticultura Brasileira*, v.27, n.2, p.240-245, 2009. https://doi.org/10.1590/S0102-05362009000200021

LIMA, C.B.; BOAVENTURA, A.C.; VILLELA, T.T. Comparing procedures for performing tetrazolium test on carrot seeds. *Horticultura Brasileira,* v.36, p.240-245, 2018. https://doi.org/10.1590/S0102-053620180216

MAGUIRE, J.D. Speed of germination: aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, v.2, n.2, p.176-177, 1962.

MARCOS-FILHO, J. *Fisiologia de sementes de plantas cultivadas*. Londrina: Abrates, 2015. 660p.

MARCOS-FILHO, J. International Seed Testing Association. *Seed Science and Technology*, v.48, n.1, p.439-451, 2020. https://www.ingentaconnect.com/content/ista/sst/2020/00000048/00000003/art00012;jsessionid=a7aqoqujfaab8.x-ic-live-02

NERY, M.C.; NERY, F.C.; PIRES, R.D.O. Tetrazolium test to evaluated the viability of oil radish seeds. *Bioscience Journal*, v.31, n.3, p.663- 671, 2015. https://seer.ufu.br/index.php/biosciencejournal/article/view/22527

PAIVA, C.T.C.; SILVA, J.B.; DAPONT, E.C.; ALVES, C.Z.; CARVALHO, M.A.C. Qualidade fisiológica e sanitária de sementes comerciais de alface e repolho. *Revista de Ciências Agroambientais*, v.14, p.53-59, 2016. https://periodicos.unemat.br/index.php/rcaa/article/view/1410#:~:text=Em%20sementes%20de%20repolho%20foram,no%20desempenho%20germinativo%20em%20laborat%C3%B3rio.

R CORE TEAM. *R: a language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria, 2020.

REZENDE, R.G.; JESUS, L.L.; NERY, M.C.; ROCHA, A.S.; CRUZ, S.M.; ANDRADE, P.C.R. Teste de tetrazólio em sementes de crambe. *Semina: Ciências Agrárias*, v.36, n.4, p.2539-2544, 2015. https://www.redalyc.org/pdf/4457/445744150046.pdf

RODRIGUES, A.P.; LAURA, V.A.; CHERMOUTH, K.D.S.; GADUM, J. Absorção de água por semente de salsa, em duas temperaturas. *Revista Brasileira de Sementes*, v.30, n.1, p.49-54, 2008. https://doi.org/10.1590/S0101-31222008000100007

ŠAMEC, D.; URLIĆ, B.; SALOPEK-SONDI, B. Kale (*Brassica oleracea* var. *acephala*) as a superfood: Review of the scientific evidence behind the statement. *Critical Reviews in Food Science and Nutrition*, v.59, n.15, p.2411-2422, 2019. https://www.tandfonline.com/doi/pdf/10.1080/10408398.2018.1454400?casa_token=0UpdF5lP2jsAAAAA:36bkNnrymBdOHvlNy7Ya0WOWxBCkX2b-eO_6AQDCX6hZnaF2nvKE7uuMP08JKeSTvbneUFZOpRVBufEF

SILVA, M.F.D.; ARAÚJO, E.F.; SILVA, L.J.D.; AMARO, H.T.R.; DIAS, L.A.D.S.; DIAS, D.C.F.D.S. Tolerance of crambe (*Crambe abyssinica* Hochst) to salinity and water stress during seed germination and initial seedling growth. *Ciência e Agrotecnologia*, v.43, p.1-13, 2019. https://doi.org/10.1590/1413-7054201943025418

SILVA, R.N.O.D.; GADOTTI, G.I.; CARVALHO, I.L.; CARVALHO, I.R.; CAVALCANTE, J.A.; SILVA, J.G.D. Methodological adjustments to the tetrazolium test in coriander seeds. *Journal of Seed Science*, v.43, e202143026, 2021. https://doi.org/10.1590/2317-1545v43251969

TRANI, P.E.; TIVELLI, S.W.; BLAT, S.F.; PRELA-PANTANO, A.; TEIXEIRA, É.P.; ARAÚJO, H.S.; FELTRAN, J.C.; PASSOS, F.A.; FIGUEIREDO, G.J.B.; NOVO, M.C.S.S. Couve de folha: do plantio à pós-colheita. *Boletim Técnico IAC*, v.214, p.42, 2015. https://www.iac.sp.gov.br/publicacoes/arquivos/iacbt214.pdf

VILELA, N.J.; LUENGO, R.F.A. Produção de hortaliças folhosas no Brasil. *Campo & Negócios Hortifruti*, n.146, p.22-27, 2017. https://revistacampoenegocios.com.br/producao-de-hortalicas-folhosas-no-brasil/