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ORIGINAL ARTICLE

Radiography and biometric analysis of broadleaf vegetable seeds

Análise radiográfica e biométrica de sementes de hortaliças folhosas

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ABSTRACT: Image analysis is an easy to use and non-destructive technique that enables quick decision-making concerning seeds with germination problems or with delays in the analysis period. This work compared seeds of cress, lettuce, endive, chicory, mustard, cabbage and parsley of different classes (full and translucent), separated according to radiographic image, biometrics and germination test of the batches and evaluation of germinability by coat color. The survey was conducted using seeds of broadleaf vegetables subjected to radiographic analysis to obtain a sufficient number of full and translucent seeds for performing the germination test together with the first count and germination speed index. Then, the biometric analysis using the scanned images captured by the Seed Analysis System was performed. In the translucent seeds, except for chicory, it was observed that the germination, vigor and biometric parameters were lower when compared to the full seeds. It was concluded that the analysis of radiographic images is an effective way to categorize the physiological quality of broadleaf vegetables seeds and to demonstrate a connection with their biometrics, but seed coat color cannot be considered as a classification parameter in the studied species.

KEYWORDS
GroundEye®
Morphology
Physiological quality
X-ray

RESUMO: *Análise de imagem é uma técnica de fácil utilização, não destrutiva e que possibilita a rápida tomada de decisão no caso de sementes com problemas na germinação ou demora no período de análise. Neste trabalho relacionou-se as classes de sementes (cheia e translúcida), separadas de acordo com a imagem radiográfica, a biometria e o teste de germinação de lotes de sementes de agrião, alface, almeirão, chicória, mostarda, repolho e salsa, como também a avaliação da capacidade de germinação por cor do tegumento. Esta pesquisa utilizou sementes de hortaliças folhosas, submetidas aos raios X até se obter o número suficiente destas cheias e translúcidas para o teste de germinação. Simultaneamente, foi feita a primeira contagem e o índice de velocidade de germinação. Em seguida, foi realizada a análise biométrica por meio de imagens digitalizadas do Sistema de Análise de Semente. Nas sementes translúcidas, com exceção do almeirão, observou-se que germinação, vigor e parâmetros biométricos foram menores quando comparadas às cheias. Conclui-se que a imagem radiográfica é eficiente para a classificação da qualidade fisiológica de sementes de hortaliças folhosas e demonstra uma relação com sua biometria, porém a cor do tegumento não pode ser considerada como parâmetro de classificação para todas as espécies.*

PALAVRAS-CHAVE
GroundEye®
Morfologia
Qualidade fisiológica
Raios X

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1 Introduction

Nowadays, the process of agricultural production is automated, and for the main agricultural product, seeds, which contribute to increased crop productivity through new technologies, this could not be different. Production and quality programs require speed and accuracy of results (Barbieri et al., 2012); thus, image analysis is currently the technology with the greatest number of researches.

Image analysis stands out as a technique that allows obtaining results quickly in an objective and non-destructive way. Among these techniques there is radiography, image capture and processing by software.

X-ray helps study the seeds' internal morphology. Its principle is X-ray absorption in different amounts by different seed tissues, which depends on their thickness, density and composition, in addition to radiation wavelength (International Seed Testing Association, 2004).

The interpretation of the radiographic images and their comparison with the results of the parallel germination and vigor tests allow associating the integrity of vital seed parts with their physiological potential, in addition to predicting the occurrence of abnormal seedlings through images of immature embryos or aberrations (Mondo & Cicero, 2005; Pinto et al., 2009).

Characteristics of the external morphology can indicate the seed's quality. The GroundEye (Seed Analysis System) equipment and software enables studying the seed's morphology by capturing its image with a digital camera and transferring it to the software, which converts the image pixels into data according to its physical characteristics. GroundEye allows analyzing more than 300 features of a single seed, e.g., geometry (diameter, area, circumference, roundness), color and texture.

Knowledge of the physiological quality of the seeds allows the production of seedlings with uniform size and quality, with advantages for the development of plants, especially in species which require transplantation for being grown, such as broadleaf vegetables (Kikuti & Marcos Filho, 2012).

Broadleaf vegetables are a group of vegetables that have leaves as edible parts. They are sources of vitamins, minerals and fiber as well as carbohydrate and protein in some species. Because of its low-calorie potential, they are recommended in diets, and are easy-to-prepare foods that can be consumed without cooking.

This work compared seeds of cress (*Barbarea verna* (Mill.) Asch.), lettuce (*Lactuca sativa* L.), endive (*Cichorium endive* L.), chicory (*Cichorium intybus* (Linn.)), mustard (*Brassica juncea* L.), cabbage (*Brassica oleracea* L.) and parsley (*Petroselinum crispum* (Mill.) Nym.) of different classes (full and translucent), separated according to radiographic image, biometrics and germination test of the batches and evaluation of germinability by coat color.

2 Material and Methods

The tests were conducted at the Seed Central Laboratory of the Federal University of Lavras, Minas Gerais. The X-ray test and biometric analysis were performed respectively using the HP Faxitron equipment, MX-20, and through image capture by the Seed Analysis System (GroundEye®), S120 version. Seeds from commercial lots of watercress, lettuce, endive, chicory, mustard, cabbage and parsley were used. The seeds were bought in an agricultural establishment.

2.1 Determination of water content

Water content was determined via the oven method, at 130°C for one hour, carried out in duplicate with one gram for each species, according to the *Rules for seed analysis* (Brasil, 2009). The results were expressed as percentages based on the wet weight.

The seeds were subjected to an X-ray test = to obtain different classes according to their radiographic image.

2.2 Radiographic analysis

The seeds were fastened with transparent double-sided adhesive tape adhered to a transparent sheet and subjected to radiographic analysis. Radiation intensity and exposure time were determined through the automatic calibration of the equipment. The images were obtained by the equipment's software and displayed digitally. With these images, it was possible to establish classes according to the internal morphology of the seeds, classifying them as full (seeds that had a well-formed embryo) and translucent (seeds showing a region with a less dense embryo in the radiographic image). The analysis was conducted until a sufficient number of seeds per class for the germination test was reached.

The seeds separated by class and sample (amount of seeds removed before selection by class) were submitted to the germination test, first count and germination speed index and the full and translucent seeds were submitted to biometric analysis.

2.3 Biometric analysis

The seeds were placed on a clear acrylic tray in the GroundEye reading device to capture the images. Ten repetitions of 20 seeds were used per category. Image capture was performed by a professional high-resolution camera (information on the equipment is confidential) contained within the equipment's collection chamber. The background color calibration used was the HSV color space model with 0 to 360 array, 0.00 to 1.00 brightness and 0.00 to 1.00 saturation.

After capturing the images, the seeds were analyzed by the equipment's software, the following biometric parameters having been obtained: area, maximum and minimum diameter, circumference (millimeter and metric unit), and dominant color as percentage.

2.4 Germination test

The germination test was performed using eight repetitions of 25 seeds sown in gerbox acrylic boxes on a blotter paper substrate, moistened with distilled water at a ratio of 2.5 times the dry weight of the paper. They were then kept in a B.O.D-type germination chamber with temperature according to the species (Table 1) and 12-hour daylength. The first count was performed after the sowing period, according to each species (see Table 1), by computing the percentage of normal seedlings (Brasil, 2009). Seeds were defined as dead according to the appearance and consistency of their tissue.

Table 1. Germination temperature, first and final count according to each broadleaf vegetable.

Tabela 1. Temperatura de germinação, primeira contagem e contagem final de acordo com cada espécie de hortaliça folhosa.

Species	Temperature (°C)	First count (days)	Final count (days)
Cress	30	4	7
Lettuce	20	4	7
Endive	20-30	5	14
Chicory	20-30	5	14
Mustard	20-30	5	7
Cabbage	20-30	5	10
Parsley	20-30	10	28

Source: Brasil (2009).

Together with the germination test, the germination speed index (GSI) was carried out, with daily register of the number of normal seedlings, calculated with the formula proposed by Maguire (1962).

The biometric analysis of the seeds using GroundEye showed variations in their coat color. After removal of the seeds by class, they were homogenized, being separated by coat color and then subjected to the germination test.

2.5 Coat color analysis

Seeds of each species were separated with the naked eye in two types of coat color (Figure 1), and then subjected to the germination test, first count and germination speed index, the same way as described above, by color. The nomenclature of each color was designated by GroundEye.

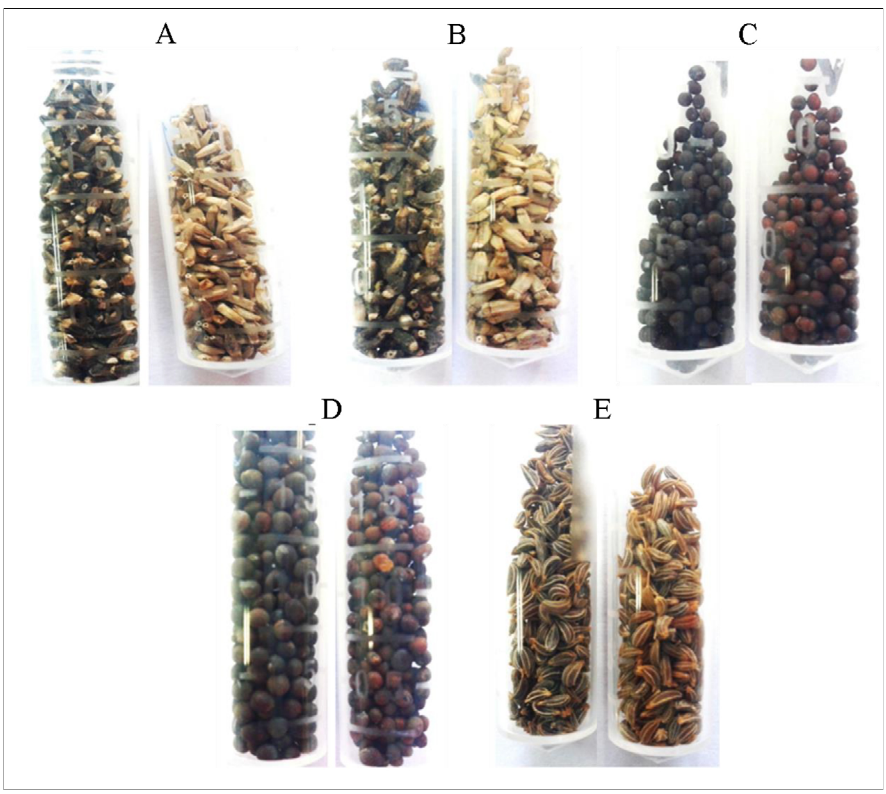

Figure 1. Coat color of broadleaf vegetable seeds: (A) endive; (B) chicory; (C) mustard; (D) cabbage; (E) parsley. The dominant color is on the left side of the image: endive and parsley – dark gray; mustard, cabbage and chicory – black. The secondary color is on the right side of the image: endive – orange; chicory and parsley – yellow; mustard and cabbage – red.

Figura 1. Cor do tegumento de sementes de hortaliças folhosas: (A) almeirão; (B) chicória; (C) mostarda; (D) repolho; (E) salsa. À esquerda da imagem está a cor dominante: almeirão e salsa – cinza escura; mostarda, repolho e chicória – preta. À direita da imagem está a cor secundária: almeirão – laranja; chicória e salsa – amarela; mostarda e repolho – vermelha.

2.6 Statistical procedure

The experimental design was completely randomized with eight repetitions of 25 seeds for the germination test, first count and GSI, and 10 repetitions of 20 seeds for biometric analysis. The data were submitted to analysis of variance using Sisvar® (Ferreira, 2011) and means were compared via the Scott-Knott test at 5% probability ($p > 0.05$). For the biometric parameters, descriptive statistics was also performed using Excel.

3 Results and Discussion

The broadleaf vegetables had water content between 6% and 8%: 8.04% in cress; 8.06% in endive; 8.09% in lettuce; 7.28% in chicory; 6.85% in salsa; 6.46% in mustard; and 5.97% in cabbage. Seed water content influences optical density, thus, the lower the amount of water, the higher the optical density, which enables the proper differentiation of structures in the radiographic images (Simak, 1991).

It was not possible to view different parts of the embryo in these images because the seeds had the same level of radiopacity (Gomes et al., 2014), but their separation in the full and translucent classes may be noted. The seeds were classified as translucent because they had a less dense tissue, which may be seen in the radiographic images as dark spots (Figure 2), since the strength was not enough to block the passage of the X-ray (Gagliardi & Marcos Filho, 2011).

The results of the germination and vigor tests corroborate the analysis of the internal morphology of the seeds, as those for the translucent seeds significantly differed from those for the full seeds, with the exception of endive.

According to the data obtained in the germination test for all species, the full seeds showed no difference from the sample. It was also observed that the germination percentage of the translucent lettuce, cabbage, and parsley seeds was equal to or less than 50%, whereas the difference in the full seeds was 51 percentage points for lettuce, 34 percentage points for cabbage and 45 percentage points for parsley. This low germination rate of the translucent seeds was due to the smaller amount of reserve tissue, which means they have less substance to be translocated, this being an essential aspect for energy supply in seed germination (Nonogaki et al., 2010).

In the first count analysis, lettuce, chicory and mustard did not differ from the sample, but there was a significant difference compared with the translucent seeds. For cabbage, there were two classes and the sample, and the difference between the full and translucent seeds was 35 percentage points, the percentage of the former having been 7% higher than the sample. Parsley had only 2% normal full seedlings, and watercress did not differentiate between the classes and the sample.

In the germination speed index (GSI), lettuce, watercress and parsley showed no statistical differences for the full seeds and sample. Chicory did not differ between the full and translucent seeds. However, significant differences were obtained for mustard and cabbage in relation to the two classes and the sample, as well as a 10.61 rate for full mustard seeds and 7.40 for cabbage seeds.

In the vigor test, cabbage best represented the study of the internal morphology of the seeds, as it achieved the greatest results of vigor for the full seeds, showing that the more well-formed the embryo, the greater the amount of reserve tissue and the more vigorous the seeds.

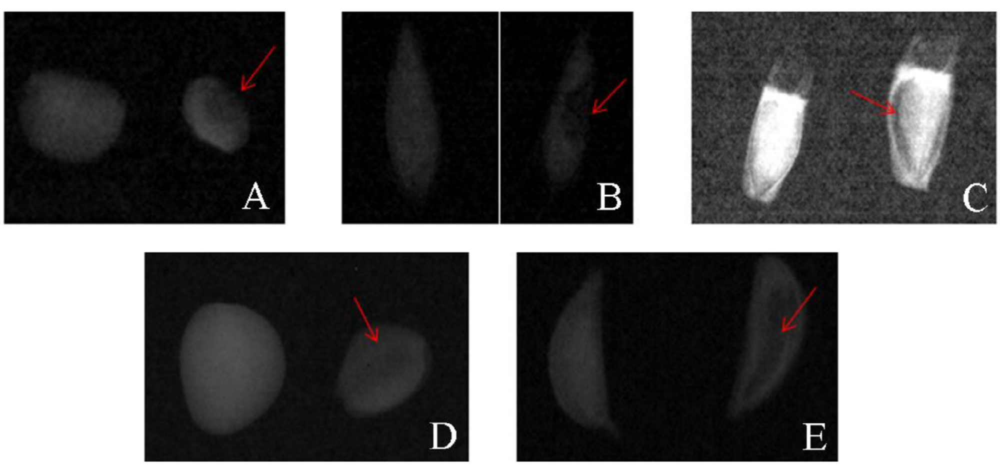

Figure 2. Radiographic images of seeds of broadleaf vegetables: (A) cress; (B) lettuce; (C) chicory; (D) cabbage; (E) parsley. The full seeds are on the left side of the image and the translucent seeds are on the right side.

Figura 2. Imagens radiográficas de sementes de hortaliças folhosas: (A) agrião; (B) alface; (C) chicória; (D) repolho; (E) salsa. À esquerda da imagem estão as sementes cheias, e à direita, as sementes translúcidas.

The low germination rate of the translucent seeds of lettuce, cabbage, and parsley can be explained due to the percentage of dead seeds having been approximately 50%, indicating that the seeds' less dense tissues were damaged by pathogens, which is justified by the higher proportion of fungi during the test's development for the translucent seeds. For rice seeds with modifications in the embryo, the dark spots in the radiographic images indicated tissue deterioration, and also resulted in dead seeds (Silva et al., 2014b).

A graphical representation of germination (A), vigor (B and C) and percentage of dead seeds (D) for broadleaf vegetable seeds separated by their internal morphology is shown in Figure 3.

In the identification of problems associated with the physiological potential of the seeds, their internal morphology was assessed to identify the parts that compose it, being of fundamental importance for the elucidation of doubts about the abnormality of seedlings or the presence of non-germinated seeds in the germination test (Gomes Junior, 2010).

In 'Brasileirinha' pumpkin seeds obtained from fruits at different maturation stages, is was detected through the use of the X-ray technique that the greater the fruit's ripening time, the greater the number of full seeds (Silva et al., 2014a).

Seeds classified as translucent adversely affect the physiological quality of the lot. Therefore, the use of the X-ray technique is an interesting quality control instrument used by companies to assist the decision-making process.

The translucent seeds and lower physiological potential also have lower biometric parameters, except for lettuce which had higher parameters (Table 2). The lettuce seeds were possibly compressed in the post-harvest process, resulting in a flattened and elongated shape.

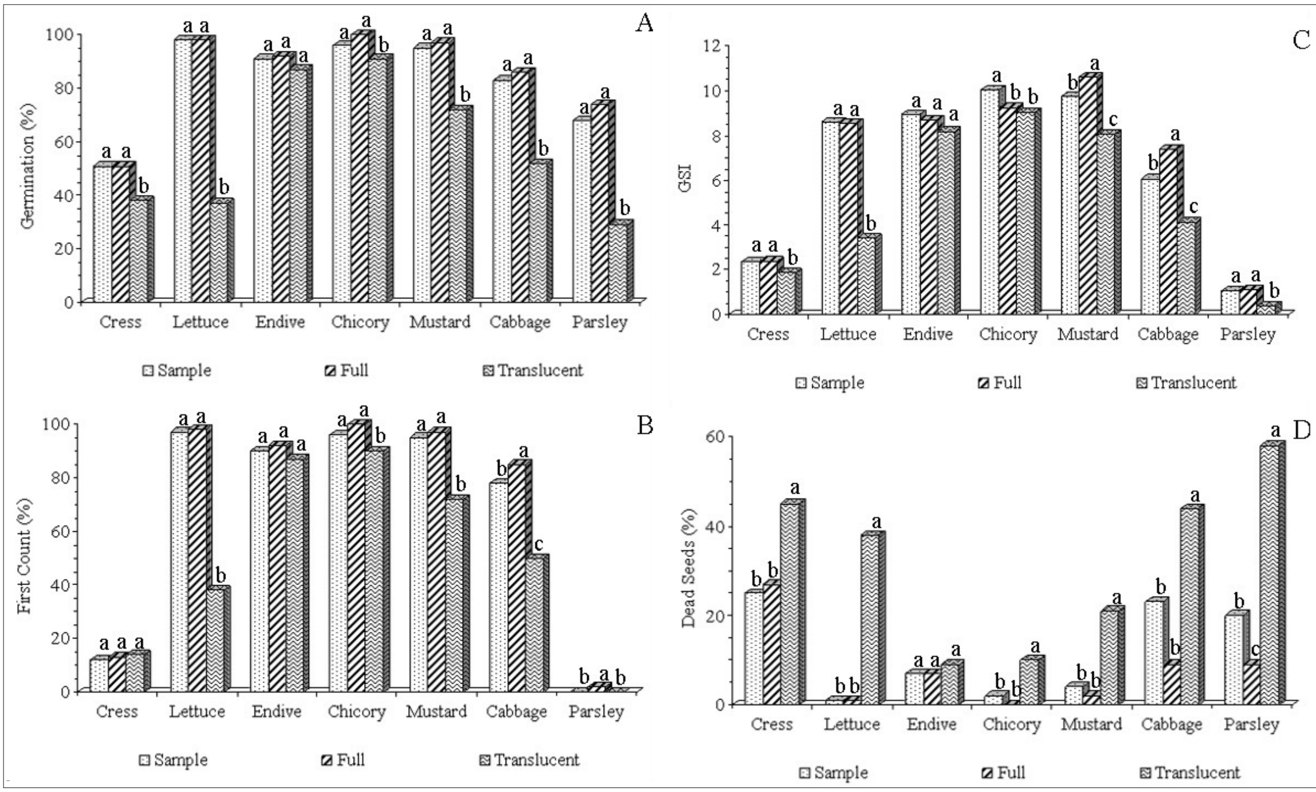

Figure 3. Germination percentage (A), first count (B), germination speed index – GSI (C) and percentage of dead seeds (D) of broadleaf vegetable seeds separated by radiographic image.

Figura 3. Porcentagem de germinação(A); primeira contagem(B); índice de velocidade de germinação – IVG (C); e porcentagem de sementes mortas (D) de sementes de hortaliças folhosas separadas pela imagem radiográfica.

Table 2. Biometric parameters, predominant color (C), larger diameter (LD), smaller diameter (SD), area (A) and perimeter (P) of broadleaf vegetable seeds separated by radiographic image.

Tabela 2. Parâmetros biométricos, cor predominante (C), maior diâmetro (LD), menor diâmetro (SD), área (A) e perímetro (P), de sementes de hortaliças folhosas separadas pela imagem radiográfica.

Species	Classes	C (%)[1]	LD (mm)	SD (mm)	A (mm^2)	P (mm)
	Full seed	60.76 a	1.90 a	1.33 a	2.16 a	5.56 a
Cress	Translucent seed	50.20 b	1.83 b	1.27 b	1.96 b	5.30 b
	CV (%)	18.51	2.36	2.90	3.29	1.98
	Full seed	34.26 a	3.33 a	0.96 a	2.42 a	7.93 a
Lettuce	Translucent seed	39.94 a	3.50 b	0.98 a	2.61 b	8.29 b
	CV (%)	48.28	2.68	2.81	4.66	2.34
	Full seed	41.08 a	3.32 a	1.33 a	3.76 a	8.69 a
Endive	Translucent seed	28.88 b	3.05 b	1.11 b	3.00 b	7.89 b
	CV (%)	16.20	1.84	3.54	3.76	2.34
	Full seed	37.28 a	3.00 a	1.22 a	3.25 a	7.96 a
Chicory	Translucent seed	35.60 a	2.92 b	1.21 a	3.14 a	7.74 b
	CV (%)	15.49	2.37	3.02	4.08	2.70
	Full seed	53.22 a	1.42 b	1.17 a	1.35 a	4.28 a
Mustard	Translucent seed	62.52 a	1.48 a	1.14 a	1.39 a	4.37 a
	CV (%)	19.19	3.28	5.04	7.94	3.81
	Full seed	79.14 a	1.96 a	1.67 a	2.66 a	6.03 a
Cabbage	Translucent seed	41.69 b	1.93 a	1.45 b	2.26 b	5.65 b
	CV (%)	14.21	2.82	3.29	5.46	2.80

Table 2. Continuation...

Tabela 2. Continuação...

Species	Classes	C (%)[1]	LD (mm)	SD (mm)	A (mm²)	P (mm)
Parsley	Full seed	27.46 a	3.24 a	1.26 a	3.38 a	8.51 a
	Translucent seed	24.76 a	2.67 b	1.00 b	2.30 b	6.99 b
	CV (%)	13.54	3.08	4.22	5.01	2.99

Means followed by the same letter in the columns do not differ statistically from each other, Scott-Knott test, $p < 0.05$. [1] Cress, Endive and Salsa: dark gray; Mustard, cabbage and lettuce: black; Chicory: orange.

According to the descriptive analysis of the biometric parameters (Table 3), the low variation of the mean occurred because the standard deviations are relatively small, with diameters ranging from 0.04 to 0.12, area from 0.04 to 0.16 and perimeter from 0.09 to 0.24. This standard deviation occurs because all commercial seeds go through a beneficiation process that groups them by weight and/or size. In this study it was found that the variation in size is related to the amount of reserve tissue.

Seeds containing higher biometric parameters have a higher amount of tissue reservation and consequently better development, with well-formed embryos and better-nourished seedlings (Carvalho & Nakagawa, 2012). As the translucent seeds possibly had their reserve tissue deteriorated, they failed to reach the point of physiological maturity.

Size is indicative of physiological quality in many species, with small seeds within the same lot having lower germination rate and less vigor than medium and large seeds (Popinigis, 1985). Classification by size or weight is a strategy that can be used to standardize the seedlings' emergence and to obtain seedlings with similar size or with greater vigor (Carvalho & Nakagawa, 2012). However, to spread safflower (*Carthamus tinctorius* L.), there is no need to sort the seeds at the time of sowing, since size variation has no influence on the plants' emergency speed and average emergence rate (Abud et al., 2010).

Morphological studies of seeds are conducted to understand their structures and provide information about germination, variability, storage, sowing methods and assist in the identification of the species (Diniz et al., 2015).

Through the biometric analysis it was seen that the seeds of these species showed variations in coat color, and the dominant color, described in Figure 1, was related to seeds classified as full, except for chicory (Table 2).

For watercress and lettuce, it was not possible to obtain differently colored seeds in the tests. Cabbage was the only species in which statistical difference was observed both for germination and for vigor, and seeds with a black coat had the highest values in both tests.

Table 3. Mean, maximum, minimum, and standard deviation values of biometric parameters of broadleaf vegetable seeds separated by radiographic image.

Tabela 3. Valores médio, máximo, mínimo e desvio padrão dos parâmetros biométricos, de sementes de hortaliças folhosas separadas pela imagem radiográfica.

Species	Classes	Values	LD (mm)	SD (mm)	A (mm²)	P (mm)
Cress	Full seed	Mean	1.90	1.33	2.16	5.56
		Minimum	1.83	1.25	2.09	5.42
		Maximum	1.97	1.37	2.22	5.75
		Standard deviation	0.04	0.04	0.04	0.09
	Translucent seed	Mean	1.83	1.27	1.83	5.30
		Minimum	1.76	1.22	1.76	5.12
		Maximum	1.93	1.32	2.06	5.49
		Standard deviation	0.05	0.04	0.09	0.12
Lettuce	Full seed	Mean	3.33	0.96	2.42	7.93
		Minimum	3.24	0.94	2.32	7.71
		Maximum	3.41	1.01	2.57	8.07
		Standard deviation	0.06	0.02	0.08	0.11
	Translucent seed	Mean	3.50	0.98	2.61	8.29
		Minimum	3.37	0.95	2.45	7.99
		Maximum	3.73	1.03	2.88	8.75
		Standard deviation	0.12	0.03	0.15	0.24

Table 3. Continuation...
Tabela 3. Continuação...

Species	Classes	Values	LD (mm)	SD (mm)	A (mm^2)	P (mm)
Endive	Full seed	Mean	3.32	1.33	3.76	8.69
		Minimum	3.22	1.25	3.57	8.33
		Maximum	3.42	1.38	3.93	9.07
		Standard deviation	0.06	0.05	0.13	0.23
	Translucent seed	Mean	3.05	1.11	3.00	7.89
		Minimum	2.97	1.06	2.78	7.66
		Maximum	3.17	1.17	3.20	8.17
		Standard deviation	0.06	0.03	0.12	0.15
Chicory	Full seed	Mean	3.00	1.22	3.25	7.96
		Minimum	2.93	1.18	3.10	7.73
		Maximum	3.06	1.26	3.41	8.36
		Standard deviation	0.05	0.02	0.11	0.19
	Translucent seed	Mean	2.92	1.21	3.14	7.74
		Minimum	2.78	1.11	2.88	7.31
		Maximum	3.04	1.25	3.33	8.03
		Standard deviation	0.08	0.05	0.15	0.23
Mustard	Full seed	Mean	1.42	1.17	1.35	4.28
		Minimum	1.37	1.12	1.25	4.13
		Maximum	1.47	1.24	1.47	4.47
		Standard deviation	0.04	0.04	0.08	0.12
	Translucent seed	Mean	1.48	1.14	1.39	4.37
		Minimum	1.41	1.03	1.22	4.12
		Maximum	1.60	1.31	1.72	4.83
		Standard deviation	0.05	0.07	0.13	0.20
Cabbage	Full seed	Mean	1.96	1.67	2.66	6.03
		Minimum	1.90	1.61	2.50	5.83
		Maximum	2.05	1.72	2.89	6.31
		Standard deviation	0.05	0.04	0.12	0.14
	Translucent seed	Mean	1.93	1.45	2.26	5.65
		Minimum	1.87	1.38	2.09	5.43
		Maximum	2.05	1.55	2.54	5.99
		Standard deviation	0.06	0.06	0.15	0.18
Parsley	Full seed	Mean	3.24	1.26	3.38	8.51
		Minimum	3.13	1.22	3.19	8.17
		Maximum	3.40	1.32	3.65	8.83
		Standard deviation	0.10	0.03	0.12	0.23
	Translucent seed	Mean	2.67	1.00	2.30	6.99
		Minimum	2.53	0.90	2.04	6.62
		Maximum	2.78	1.09	2.55	7.51
		Standard deviation	0.08	0.06	0.16	0.24

LD = larger diameter; SD = smaller diameter; A = area; P = perimeter.

Parsley had no statistical difference in the first count, which was zero. It obtained a difference of 60 percentage points in germination between the observed colorings, and dark gray as the dominant color in the most vigorous. The yellow seeds were those that had the highest number of dead seeds, 49%.

Mustard and chicory showed statistical difference only for GSI, with red for mustard and yellow for chicory having corresponded to the lowest index. Chicory showed no statistical difference for any of the tests.

The graphical representation of germination (A), vigor (B and C) and percentage of dead seeds (D) in the broadleaf vegetable seeds separated by coat color is shown in Figure 4.

The seeds of some species differ in coat color, which is considered a visual morphological index often indicative of information on physiological maturity, in addition to being associated with quality (Castellani et al., 2007). However, this statement only corroborates the germination results of cabbage and parsley seeds, since they were greater for darker colors, suggesting that the seeds reached physiological maturation.

The use of morphological characteristics, such as seed coat color, for the differentiation of the seeds' physiological maturity makes the study of physiological quality faster since the separation of immature seeds may be performed in the processing stage.

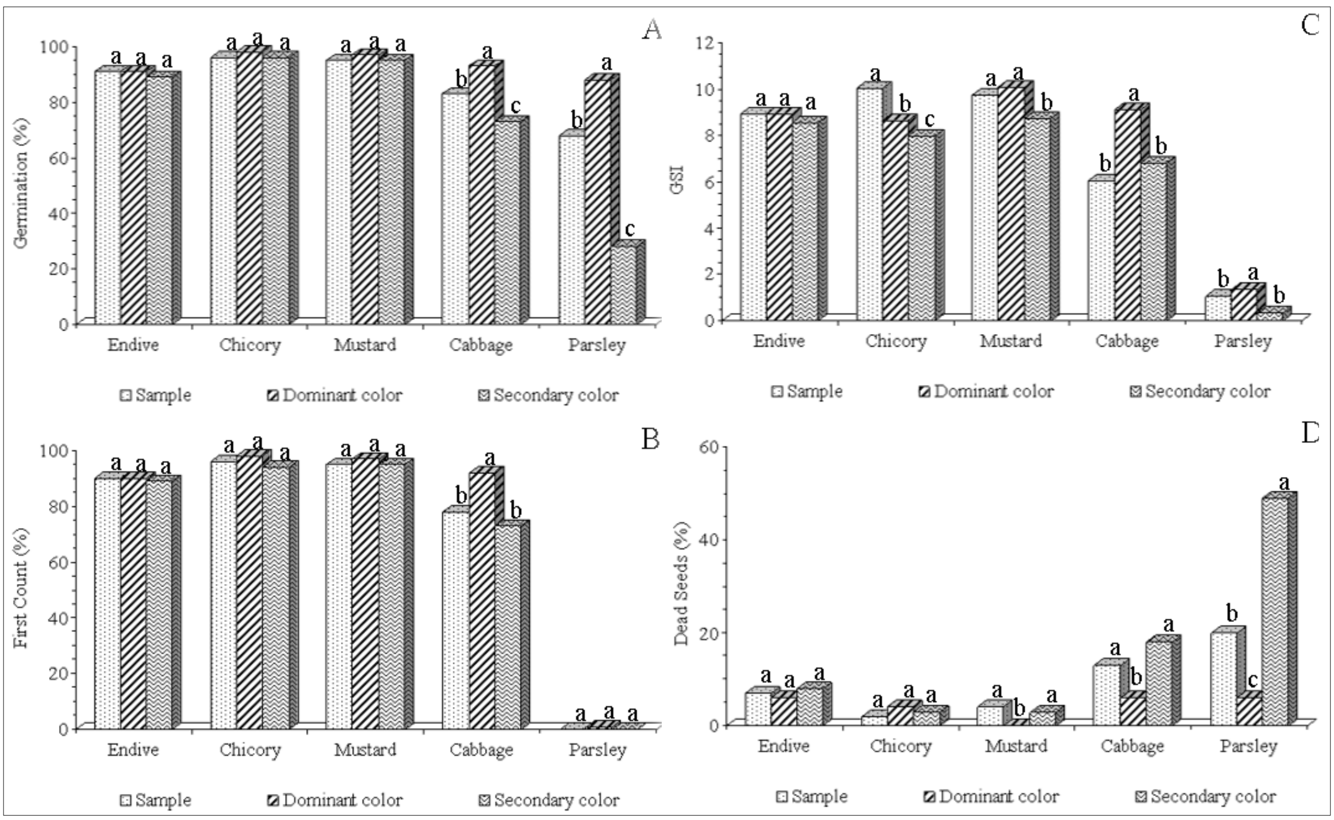

Figure 4. Germination percentage (A); first count (B); germination speed index – GSI (C); and percentage of dead seeds (D) of broadleaf vegetable seeds separated by coat color. Dominant color: endive and parsley – dark gray; mustard, cabbage and chicory – black. Secondary color: endive – orange; chicory and parsley – yellow; mustard and cabbage – red.

Figura 4. Porcentagem de germinação (A); primeira contagem (B); índice de velocidade de germinação – IVG (C); e porcentagem de sementes mortas (D) de sementes de hortaliças folhosas separadas pela cor do tegumento. Cor dominante: almeirão e salsa – cinza escura; mostarda, repolho e chicória – preta. Cor secundária: almeirão – laranja; chicória e salsa – amarela; mostarda e repolho – vermelha.

4 Conclusions

The study of radiographic images is effective for the classification of the physiological quality of the broadleaf vegetable seeds and to demonstrate the relationship between their optical density and biometry; in addition, the seeds' classification by coat color can be considered a parameter for classification of physiological quality for cabbage and parsley only.

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