







Morphological, chemical and physiological characterization of *Amaranthus* spp. seeds¹

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ABSTRACT – *Amaranthus* species have great potential for human food due to their nutritional and functional properties, but there are few phytotechnical studies with this crop, making its cultivation unfeasible. Aiming to encourage the production of these species, this study aimed to evaluate and distinguish the morphological, chemical and physiological characteristics of seeds of *Amaranthus* sp. (commercial species), *A. viridis* and *A. hybridus*. The experiments were performed in a completely randomized design, evaluating seeds in size classes (850, 600 and 425 µm) and characterizing them by image analysis using the GroundEye® system, near-infrared spectroscopy (NIR) and radiographic analysis. The first germination count, germination and germination speed index with and without KNO₃ were performed to evaluate the physiological quality of seeds. The results were submitted to analysis of variance and the means compared by the Tukey test. Radiographic analysis showed that the seeds of the three studied species were filled in the size class of 600 and 850 µm, without dormancy, but the commercial species had higher germination speed. GroundEye® and NIR allowed distinguishing the three species according to their geometric characteristics, predominance of color and chemical components.

Index terms: unconventional vegetables, GroundEye®, near-infrared spectroscopy, radiographic analysis.

Caracterização morfológica, química e fisiológica de sementes de *Amaranthus* spp.

RESUMO – As espécies do gênero *Amaranthus* possuem grande potencial para alimentação humana, devido às suas propriedades nutricionais e funcionais, entretanto existem poucos estudos fitotécnicos com a cultura inviabilizando o seu cultivo. Visando incentivar a produção dessas espécies objetivou-se com essa pesquisa avaliar e distinguir as características morfológicas, químicas e fisiológicas das sementes de *Amaranthus* sp. (espécie comercial), *A. viridis* e *A. hybridus*. Os ensaios foram realizados em delineamento inteiramente casualizados avaliando as sementes em classes de tamanho (850 µm, 600 µm e 425 µm) e caracterizando-as por análise de imagens utilizando o sistema GroundEye®, espectroscopia de infravermelho próximo (NIR) e análise radiográfica. Para avaliação da qualidade fisiológica das sementes realizou-se o teste de primeira contagem de germinação, germinação e índice de velocidade de germinação com e sem uso de KNO₃. Os resultados foram submetidos à análise de variância e as médias comparadas pelo teste de Tukey. Pela análise radiográfica foi possível observar que as sementes das três espécies estudadas se apresentam cheias na classe de tamanho de 600 e 850 µm, e não possuem dormência, no entanto a espécie comercial possui maior velocidade de germinação. Por meio do GroundEye® e do NIR foi possível distinguir as três espécies, de acordo com as características geométricas, e predominância de coloração e componentes químicos.

Termos para indexação: hortaliças não convencionais, GroundEye®, espectroscopia de infravermelho próximo, análise radiográfica.

Introduction

Plants of the genus *Amaranthus* have been considered as foods of the future due to their nutritional and functional

properties, such as high content of antioxidants and dietary fibers, and may be an alternative for the diversification of human nutrition (Amaya-Farfan et al., 2005; Silva et al., 2018; Silva et al., 2019). However, the commercialization

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of this genus is still restricted due to lack of knowledge, especially regarding the agronomic management, which limits its production (Ngoroyemoto et al., 2019; Xavier et al., 2018; Xavier et al., 2019).

In this sense, aiming at encouraging the consumption and production of *Amaranthus* spp., knowing the quality of its seeds is crucial. Studies show that the use of seeds with high physiological potential is a factor to be considered for increasing crop yield and, therefore, seed quality control should be increasingly efficient, including tests that rapidly evaluate physiological potential and allow precise differentiation between species (Fessel et al., 2010).

As a result, some techniques have been developed, such as digital imaging as a substitute for subjective human visual evaluation (Venora et al., 2009). The use of seed and seedling images for commercial and technological purposes seeks to facilitate, speed up and automate the categorization of seed characteristics such as size, color, shape, texture, filling, seedling measurement and cultivar identification (Granitto et al., 2005). Image analysis is a fast, objective, compact and non-destructive method. Studies have been performed with X-ray equipment and GroundEye® to evaluate seed quality (Andrade et al., 2016).

Among computer systems, GroundEye® stands out for being equipment that analyzes and extracts more than 300 morphological characteristics of seeds and seedlings (Andrade et al., 2016). Another technique used is the near-infrared spectroscopy (NIR), which determines functional groups of chemical components in seeds, according to the absorbance of the sample. This technique has been employed for identification of constituents in foods such as oil, determination of genetically modified organisms, selection and identification of genotypes, among others (Conceição et al., 2006; Silva et al., 2008; Mittelman et al., 2006; Silverstein and Webster, 1998).

Given the lack of scientific results with *Amaranthus* spp. and aiming at exploring tools such as X-rays, GroundEye® and NIR, this research aimed to evaluate and distinguish the morphological, chemical and physiological characteristics of *Amaranthus* sp. (commercial species), *A. viridis* and *A. hybridus*.

Material and Methods

Plant material

Seeds of three *Amaranthus* species of the germplasm collection of unconventional vegetables of the *Universidade Federal de Lavras* (UFLA) were collected to develop the experiment. These species were identified by the herbarium of the *Empresa de Pesquisa Agropecuária de Minas Gerais*

(EPAMIG) under registration 57999, 58002 and 58003, which refers to *Amaranthus* sp. (commercial species), *A. hybridus* L. and *A. viridis* L., respectively.

The species were multiplied from October 2016 to April 2017 in the UFLA experimental area (21°14' S and 45°00' W; altitude of 919 m) in a randomized block design with three replications. A physical barrier of corn was placed among the species to avoid crossbreeding since polyploidy with interspecific hybridization is common in the genus *Amaranthus*, masking its characteristics (Olusanya, 2017).

Morphological evaluations

After harvesting, seeds of each species were cleaned using a DeLeo® blower with air opening between 1 to 2 cm, eliminating straw and coarse dirt from the field. Subsequently, these seeds were sieved with three meshes (850, 600 and 425 µm), dividing them into three size classes. The remaining impurities were removed manually using a magnifying glass.

Then, seeds were characterized by image analysis using the GroundEye® system, NIR and radiographic analysis. For these analyses, the experiment was conducted in a completely randomized design in a 3 × 3 factorial scheme, in which three species and three size classes were evaluated. Three replicates were used for each treatment and each replication had 100 seeds.

Seeds were placed in the reading device tray of the GroundEye® system v. S120 for image capture. Subsequently, the analysis setting was performed for background color calibration and the CIELab color model, with luminosity index from 0 to 100, dimension a from -17.8 to 42.2 and dimension b from -50.3 to -24.7, was used. After background color calibration, image analysis was performed and seed biometric (area, circularity, circularity by modified circular shape factor – CSFm, shape sphericity, contour deformation, maximum diameter, minimum diameter, perimeter and thinning) and color dominance characteristics were extracted.

These seeds were fixed on transparent acetate sheets with double-sided tape and submitted to radiographic analysis. A radiation intensity of 35 Kv and seed exposure time to X-rays of nineteen seconds were used for equipment calibration. Images were obtained by quantifying the percentage of filled seeds for each sample.

NIR evaluation was carried out with a Bruker® Tensor 27 spectrometer with the software OPUS_Spectroscopy v. 6. This analysis was conducted in eight replicates for each species, 48 scans for each replicate and resolution of 4 cm⁻¹. The data were analyzed by partial least squares-discriminant analysis (PLS-DA) using the software PIROUETTE®.

Physiological evaluations

In order to verify the physiological quality of seeds, a germination test was performed and, at the same time, the germination speed index (GSI) of seeds was evaluated using the mixture of classes 850 and 600 μm for each species, considering that the class 425 μm presented empty seeds with low physical quality, according to the X-ray analysis.

The presence and absence of 0.2% KNO_3 solution to overcome dormancy was evaluated. Thus, for physiological tests, the experiment was conducted in a completely randomized design in a 3×2 factorial scheme, being evaluated species and use of KNO_3 , with four replications for each treatment and fifty seeds per replication.

The germination test was carried out considering the Rules for Seed Testing (Brasil, 2009), using the recommendation for the species *A. hybridus*. Seeds were placed in plastic boxes and BOD incubator. A photoperiod of eight hours of light and sixteen hours of dark, with an alternating temperature of 30–20 °C, was used. Germination was performed on blotting paper by moistening it with a water volume and 0.2% KNO_3 equivalent to 2.5 times the substrate weight. The first count was performed on the fifth day and germination on the fourteenth day after test setting up. GSI of each species was obtained by Maguire (1962) equation, with daily count of normal seedlings (with the emission of the first pair of primary leaves) in the interval from the third to the fourteenth day after sowing.

Seedling, shoot and primary root length were evaluated by images captured by GroundEye® in two evaluations: first count and germination.

Data analysis

The results were analyzed with mean and standard deviation observations and the evaluations were submitted to analysis of variance and means compared by the Tukey test ($p < 0.05$). Experimental precision was analyzed using the coefficient of variation (CV) and statistical analysis was performed using the software SISVAR® (Ferreira, 2011).

Results and Discussion

According to the analysis of variance for geometric characteristics evaluated by GroundEye®, an interaction was observed between the studied factors (species \times size classes). The slicing of species within classes showed that characteristics of the area, contour deformity, maximum diameter, minimum diameter, perimeter and thinning had the highest values for *Amaranthus* sp., regardless of size class (Table 1).

The seed class 425 μm showed the lowest values for all

geometric characteristics, except for thinning and circularity by CSFm in *A. viridis* and *A. hybridus*, possibly because this seed class has a poor formation and low germination potential. Classes 850 and 600 μm did not differ from each other for circularity, circularity by CSFm and shape sphericity, while area, contour deformation, maximum diameter, minimum diameter and perimeter showed the highest values for class 850 μm .

The commercial species (*Amaranthus* sp.), regardless of seed size class, showed mean values of 0.95 (± 0.31) mm^2 of area, 0.35 (± 0.06) cm of perimeter, 0.11 (± 0.02) cm of maximum diameter, 0.09 (± 0.02) cm of minimum diameter and 0.90 (± 0.04) of circularity. On the other hand, *A. viridis* presented mean data of 0.71 (± 0.19) mm^2 , 0.31 (± 0.04) cm, 0.09 (± 0.01) cm, 0 cm, 08 (± 0.01) cm and 0.90 (± 0.01) cm, while *A. hybridus* presented values of 0.69 (± 0.17) mm^2 , 0.31 (± 0.04) cm, 0.09 (± 0.01) cm, 0.07 (± 0.01) cm and 0.87 (± 0.03) cm for area, perimeter, maximum diameter, minimum diameter and minimum diameter, respectively.

Zapotoczny et al. (2006) observed similar results of size and shape of *Amaranthus* sp. seeds (variety MT-3) in the sieve class from 0.80 to 1.00 mm in diameter and that seeds had a mean area of 1.07 mm^2 , perimeter of 0.36 cm, maximum diameter of 0.12 cm, minimum diameter of 0.10 cm, compaction shape factor of 1.00 and circularity of 1.00.

The geometric characterization of *Amaranthus* spp. seeds can be very useful to build machines that would assist in the commercial production of these species. Studies can be carried out from these data to develop seeders, seed cleaners and small seed pelletizing.

Table 2 shows seed colors identified by GroundEye®, according to sieve classes. Seeds of the species *A. viridis* and *A. hybridus* are predominantly black in the three size classes, but 83.10 and 80.50% black in the class 850 μm , respectively. Seeds of the commercial material (*Amaranthus* sp.) are predominantly yellow (57.1%) in this class. There is also a decrease in black color in the smaller seed size class, although it is still the predominant color probably due to the seed maturity stage.

Color is an important factor in separating commercial varieties from spontaneous species. Usually, clear seeds do not have dormancy, whereas black seeds have dormancy and remain in the soil, germinating gradually. Seeds of varying color, such as black, red, gray, pink, yellow, beige and white, are found in the genus *Amaranthus* (Silva et al., 2018; Xavier et al., 2018). According to Vasundhara et al. (2017), color of *Amaranthus* spp. seeds can represent an important source of vegetable pigments that can be used as colorants.

The radiographic analysis showed an interaction between size class and species. Class 425 μm showed the lowest

Table 1. Geometric characteristics of *Amaranthus* spp. seeds at different sieve classes analyzed by GroundEye®.

Characteristic	Sieve (µm)	<i>A. viridis</i>	<i>A. hybridus</i>	<i>Amaranthus</i> sp.	CV (%)
Area (mm ²)	425	0.49 bC	0.51 bC	0.62 aC	2.69
	600	0.76 bB	0.71 cB	0.99 aB	
	850	0.87 bA	0.86 bA	1.25 aA	
Circularity	425	0.89 aB	0.83 cB	0.85 bB	0.76
	600	0.91 aA	0.88 bA	0.92 aA	
	850	0.91 bA	0.90 bA	0.93 aA	
Circularity by CSFm*	425	0.90 aA	0.87 bA	0.88 bB	0.85
	600	0.89 bAB	0.88 bA	0.92 aA	
	850	0.88 bB	0.88 bA	0.92 aA	
Shape sphericity	425	13.63 aB	13.84 aB	13.73 aA	1.41
	600	14.27 aA	14.37 aA	13.58 bA	
	850	14.61 aA	14.63 aA	13.62 bA	
Contour deformation	425	14.67 bC	14.88 abC	15.39 aC	1.48
	600	17.51 bB	17.24 bB	19.21 aB	
	850	18.06 bA	18.25 bA	21.31 aA	
Maximum diameter (cm)	425	0.08 cC	0.08 bC	0.09 aC	1.99
	600	0.10 bB	0.09 bB	0.12 aB	
	850	0.11 bA	0.11 bA	0.13 aA	
Minimum diameter (cm)	425	0.07 bC	0.06 bC	0.07 aC	1.47
	600	0.08 bB	0.08 cB	0.10 aB	
	850	0.09 bA	0.09 bA	0.12 aA	
Perimeter (cm)	425	0.26 bC	0.26 bC	0.29 aC	2.11
	600	0.33 bB	0.32 bB	0.36 aB	
	850	0.35 bA	0.35 bA	0.41 aA	
Thinning	425	0.92 aA	0.90 aA	0.91 aA	1.06
	600	0.88 bB	0.87 bB	0.92 aA	
	850	0.86 bB	0.86 bB	0.92 aA	

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ significantly from each other by the Tukey test ($p < 0.05$).

*CSFm – modified circular shape factor.

percentage of filled seeds, with *A. hybridus* presenting 27.75%, followed by *A. viridis* and *Amaranthus* sp., which did not differ from each other, with a mean of 9.87% (± 2.29) of filled seeds. Classes 600 and 850 µm did not differ among the studied species, with mean values of 97.25 (± 2.16) and 99.08% (± 0.52) of filled seeds, respectively (Table 3).

The germination test showed an interaction between the evaluated factors (use of $\text{KNO}_3 \times$ species). At the first germination count (Table 4), the commercial species (*Amaranthus* sp.) without the use of KNO_3 presented higher percentage of normal seedlings (58%), while using KNO_3 provided the highest percentage in *A. hybridus* and *Amaranthus* sp., which did not differ from each other, with a mean value of 62.00% (± 2.12).

No difference was found at germination within species for

the use or not of KNO_3 nor between species with its use, with a mean of 82.33% (± 4.85) of normal seedlings. However, a difference was observed between species in the absence of KNO_3 , while *Amaranthus* sp. had the highest germination (83.50%) and *A. viridis* presented the lowest value (65%).

Nobre et al. (2015) evaluating four seed lots of the cultivar BRS Alegria (clear seeds) and found germination values ranging from 75 to 85%. The values found in the literature are similar to those of this study.

The use of KNO_3 had a positive effect on the species under study when it was evaluated in the first germination count in relation to the number of normal seedlings. Despite the positive effect of the treatment, *A. viridis* continued with the lowest percentage of normal seedlings and this species may have unknown factors that impair its vigor, as radiographic

Table 2. Dominance of color in *Amaranthus* spp. seeds at different sieve classes analyzed by GroundEye®.

Species	Sieve class (µm)	Black	Yellow	Orange	Celestial	Dark grey	Other
		(%)	(%)	(%)	(%)	(%)	(%)
<i>A. viridis</i>	425	65.93 b	–	–	11.95 a	2.24 b	19.87 a
	600	83.41 a	–	–	8.44 b	5.89 a	2.20 b
	850	83.10 a	–	–	8.68 ab	5.46 a	2.78 b
	CV (%)	3.55	–	–	–	6.78	19.92
<i>A. hybridus</i>	425	67.35 b	–	–	10.71 a	3.17 b	18.75 a
	600	79.74 a	–	–	9.56 a	5.53 a	5.15 b
	850	80.50 a	–	–	11.58 a	4.36 ab	3.55 b
	CV (%)	5.48	–	–	–	11.79	24.5
<i>Amaranthus</i> sp.	425	–	28.84 b	28.75 a	19.24 a	18.89 a	4.27 a
	600	–	50.72 a	10.09 b	16.70 b	18.26 a	4.22 a
	850	–	57.13 a	8.70 b	14.54 c	15.79 a	3.83 a
	CV (%)	–	–	16.42	4.91	9.36	10.51

Means followed by the same letter in the columns, within species, do not differ significantly from each other by the Tukey test ($p < 0.05$).

analysis showed a high percentage of filled seeds in this species for seed sizes of 600 and 850 µm (Tables 3 and 4).

However, KNO_3 is important in the initial germination period of *A. viridis* and *A. hybridus*, as it increased the percentage of normal seedlings by 38.24 and 43.30%, respectively. According to Ellis et al. (1983), the potential of potassium nitrate to increase the germination rate (of some species) may be related to its role as oxidant and electron acceptor. Potassium is also involved in maintaining plant cell balance, promoting respiration and carbohydrate metabolism (Aisha et al., 2007).

Several studies have been conducted with the proposal of using KNO_3 to overcome seed dormancy, such as the research of Ruttanaruangboworn (2017), who evaluated its use in rice seeds and observed better results when KNO_3 was at low concentrations, besides increasing germination speed and uniformity. However, the use of this substance at high concentrations may delay seed soak by phytotoxicity.

The variable GSI showed an interaction between species

and the use of KNO_3 . The slicing showed that *Amaranthus* sp. presented higher GSI (8.69) without the use of this solution, with no difference between species with its use, with a mean of 7.41 (± 1.12). However, no variation was observed with and without the use of KNO_3 , with means of 5.54 (± 1.00), 7.19 (± 0.43) and 8.59 (± 0.13) for *A. viridis*, *A. hybridus* and *Amaranthus* sp., respectively (Table 5). The commercial

Table 3. Percentage of filled seeds of *Amaranthus* spp. according to the sieve class analyzed by X-rays.

Species	Sieve class		
	425 µm	600 µm	850 µm
<i>A. viridis</i>	11.50 bB	96.00 aA	99.25 aA
<i>A. hybridus</i>	27.75 bA	99.75 aA	98.50 aA
<i>Amaranthus</i> sp.	8.25 bB	96.00 aA	99.50 aA
CV (%)	4.00		

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ significantly from each other by the Tukey test ($p < 0.05$).

Table 4. Percentage of normal seedlings in the slicing of KNO_3 within *Amaranthus* species according to first germination count (FGC) and germination (G) evaluation.

Evaluation	Use of KNO_3	Species			CV (%)
		<i>A. viridis</i>	<i>A. hybridus</i>	<i>Amaranthus</i> sp.	
FGC	Without KNO_3	21.00 cB	36.00 bB	58.00 aA	15.38
	With KNO_3	34.00 bA	63.50 aA	60.50 aA	
G	Without KNO_3	65.00 bA	78.50 abA	83.50 aA	11.71
	With KNO_3	77.00 aA	86.50 aA	83.50 aA	

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ significantly from each other by the Tukey test ($p < 0.05$).

species (*Amaranthus* sp.) has faster and more uniform germination, making it more competitive when established in the field compared to other species.

Seedlings were analyzed with the GroundEye® in the first germination count and germination evaluations and the shoot, primary root and total seedling length were measured, with no interaction between factors.

According to Table 6, *Amaranthus* sp. developed the most in this short period, with the highest significant values of the shoot, primary root and total length. It occurred because this species is already commercially cultivated in other countries, showing characteristics of growth and development of a genetically improved cultivar. *A. viridis* and *A. hybridus* presented inferior results when compared to the commercial species, but statistically equal to each other. Significantly superior results regarding the mean of the species were observed in these characteristics when KNO₃ was used.

The germination test (Table 6) showed similar data to the first germination count, but with a general increase in the

seedling length of the species, i.e., *Amaranthus* sp. has the highest values, followed by *A. viridis* and *A. hybridus*. The same trend of the fifth day of evaluation was found on the fourteenth day when evaluating the influence of KNO₃. Seeds soaked with solution were significantly superior to those that did not absorb KNO₃, except for root size at fourteen days, not differing statistically from each other, although numerically higher with the use of this solution.

Most species of economic value of the Rules for Seed Testing (Brasil, 2009), especially large crops, do not require the use of KNO₃, as they do not have dormancy. However, the application of this stimulant is often recommended for seed germination testing of forage grasses, vegetables and ornamental species, showing that the effect of KNO₃ differs significantly between species.

The analysis using the NIR technique, by the visual observation of the spectra, showed that *Amaranthus* spp. seeds submitted to near-infrared had similar bands (Figure 1). The main objective of infrared spectroscopy is to determine functional groups of a given material. It is possible because each group absorbs, at a characteristic frequency, radiation from that region (Silverstein and Webster, 1998). This technique has made great advances in several applications, especially in the area of food science and agricultural products (Small, 2006).

From this observation, it can be inferred that the chemical composition observed among all species is similar since the spectrum shape was not discrepant between the analyzed seeds. However, this technique distinguished the three analyzed species (Table 7).

Table 5. Germination speed index of *Amaranthus* sp. species within KNO₃.

Species	Without KNO ₃	With KNO ₃
<i>A. viridis</i>	4.83 aB	6.25 aA
<i>A. hybridus</i>	6.89 aAB	7.50 aA
<i>Amaranthus</i> sp.	8.69 aA	8.50 aA
CV (%)	19.25	

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ significantly from each other by the Tukey test ($p < 0.05$).

Table 6. Shoot, primary root and total length of seedlings of *Amaranthus* spp. evaluated at the first germination count (FGC) and germination (G) and the influence of KNO₃ use.

Evaluation	Species	Shoot length (cm)	Primary root length (cm)	Total length (cm)
FGC	<i>A. viridis</i>	0.98 b	0.98 b	1.97 b
	<i>A. hybridus</i>	0.97 b	0.92 b	1.89 b
	<i>Amaranthus</i> sp.	1.57 a	1.57 a	3.14 a
	With KNO ₃	1.37 a	1.29 a	2.66 a
	Without KNO ₃	0.98 b	1.02 b	2.01 b
	CV (%)	21.97	16.33	18.49
G	<i>A. viridis</i>	1.58 ab	1.18 ab	2.76 ab
	<i>A. hybridus</i>	1.39 b	1.03 b	2.42 b
	<i>Amaranthus</i> sp.	1.74 a	1.33 a	3.07 a
	With KNO ₃	1.73 a	1.20 a	2.93 a
	Without KNO ₃	1.41 b	1.16 a	2.57 b
	CV (%)	15.56	10.53	12.61

Means followed by the same letter in the column do not differ significantly from each other by the Tukey test ($p < 0.05$).

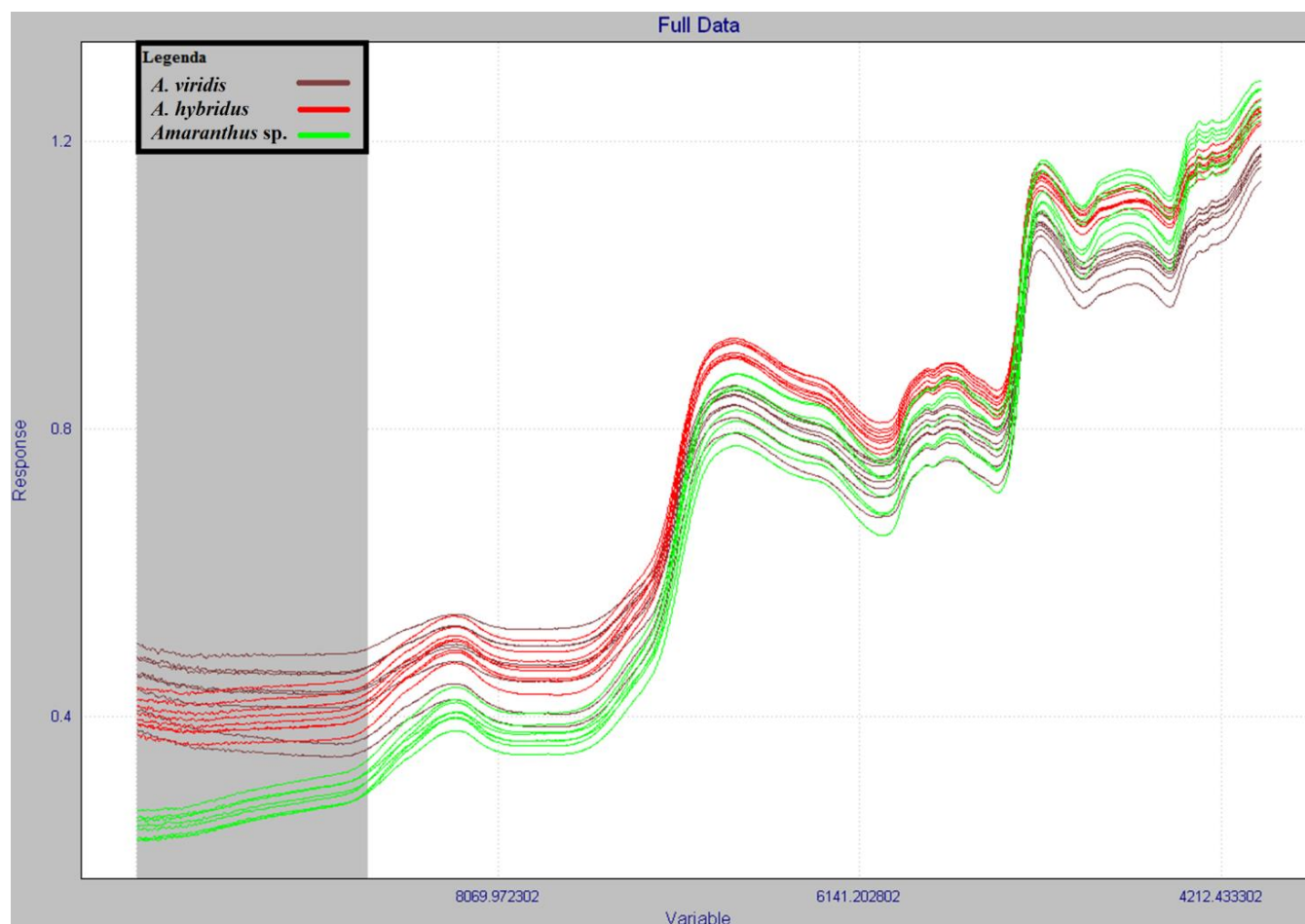


Figure 1. Spectra of *Amaranthus* spp. seeds subjected to near-infrared.

Table 7. *Amaranthus* spp. seeds classified by the near-infrared technique according to their chemical composition.

Species	Classification			Non-classified/Total
	<i>A. viridis</i>	<i>A. hybridus</i>	<i>Amaranthus</i> sp.	
<i>A. viridis</i>	6	0	0	0/6
<i>A. hybridus</i>	0	6	0	0/6
<i>Amaranthus</i> sp.	0	0	6	0/6

A satisfactory percentage of correct answers for calculated r , validated r , calibration error and validation error was verified for the spectroscopy analysis in the model used for the differentiation of seed chemical composition. These results of calibration showed that the analysis had a satisfactory accuracy in differentiating seeds by the chemical constituents of each species. The results of model calibration and validation are shown in Table 8. Five factors (new variables constructed by statistical calculation) were used for cross-evaluation for each species.

Thus, seeds from the three species were correctly classified

Table 8. Performance parameters of the PLS-DA model (discriminant analysis) for differentiating *Amaranthus* spp. seeds.

Statistics	<i>A. viridis</i>	<i>A. hybridus</i>	<i>Amaranthus</i> sp.
Calculated r (%)	98.51	96.80	99.62
Validated r (%)	97.12	92.90	99.11
Calibration error (%)	9.12	13.30	4.59
Validation error (%)	11.24	17.51	6.28

in 100% of the replications (Table 8), with no ambiguity in their classification, i.e., the species had different chemical characteristics that segregated them. This result leads to the conclusion that although the absorption spectrum was not different for both species, different chemical components or at different amounts determined the differentiation between species by the near-infrared technique.

Therefore, there was no crossing between the three species in the field, possibly due to the corn barrier established between plots, even though polyploidy with interspecific hybridization in species of this genus is common, often masking the specific characteristics of each species (Olusanya, 2017).

The same classification pattern was observed for the validation of the model used to classify seeds according to their species (Table 9). All the species were correctly classified

in 100% of the replications.

Figure 2 and Table 10 (adapted from Workman and Weyer, 2012) show the wavelengths that stand out and that were used to differentiate species from each other. For *A. viridis* (Figure 2A), the most pronounced peaks were relative to lengths of 4332, 5219 and 8764 cm^{-1} , which correspond to hydrocarbons/aliphatic compound, amide and hydrocarbon/aromatic compounds, respectively.

For *A. hybridus* (Figure 2B), the main peaks are relative to lengths of 5200, 6912 and 8764 cm^{-1} , which correspond to molecular water, aromatic amine and hydrocarbon/aromatic compounds, respectively. For *Amaranthus* sp. (Figure 2C), the main lengths that contributed to differentiate it from the other species were 4509, 4324 and 4802 cm^{-1} , which correspond to N-H of urea, lipid and N-H/C-N of ovalbumin protein, respectively.

Table 9. Replications used in the validation of the model created from the studied species.

Species	Classification			Non-classified/Total
	<i>A. viridis</i>	<i>A. hybridus</i>	<i>Amaranthus</i> sp.	
<i>A. viridis</i>	2	0	0	0/2
<i>A. hybridus</i>	0	2	0	0/2
<i>Amaranthus</i> sp.	0	0	2	0/2

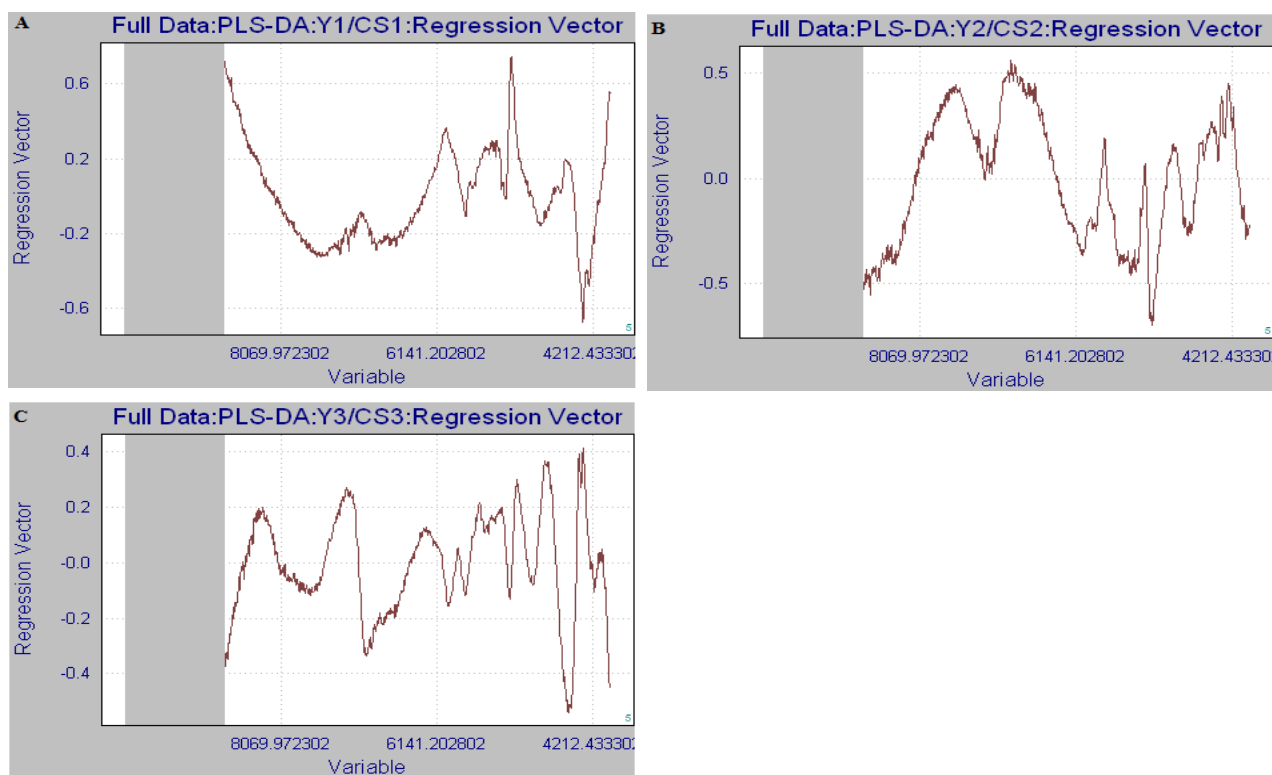


Figure 2. Wavelengths (cm^{-1}) for the studied species. (A) *A. viridis*, (B) *A. hybridus* and (C) *Amaranthus* sp.

Table 10. Chemical functional group and type of chemical compound (Workman and Weyer, 2012) present in *Amaranthus* spp. seeds determined by spectral bands.

Species	Spectral band (cm ⁻¹)	Functional group	Type of compound
<i>A. viridis</i>	4332	C-H Methylene C-H, associated with linear aliphatic R(CH ₂) _N R	Hydrocarbons, aliphatic compound
	5219	C=O Amide(.C=ONH)	Amide
	8764	C-H Aromatic (ArCH)	Hydrocarbon/aromatic compounds
<i>A. hybridus</i>	5200	O-H assigned to molecular water [O-H (.O-H & HOH)]	O-H molecular water
	6912	N-H primary aromatic amine (o-OCH ₃)	Aromatic amine
	8764	C-H Aromatic (ArCH)	Hydrocarbon/aromatic compounds
<i>Amaranthus</i> sp.	4509	N-H combination band from urea (NH ₂ -C=O - NH ₂)	U-N of urea
	4324	CHO - Classic filter instrument	Lipid/oil
	4802	O-H related combination from water change in phase and N-H/C-N combination band from urea (NH ₂ -C=O- NH ₂) from ovalbumin	N-H/C-N from ovalbumin protein

Conclusions

Seeds of *Amaranthus* sp. (commercial species) are light in color and larger when compared to those of *A. viridis* and *A. hybridus*, which are predominantly black.

The mean values of filled seeds were 97.25 (± 2.16) and 99.08% (± 0.52) in the size class 600 and 850 µm, respectively, for the three studied species. The species presented germination ranging from 65 to 83.50%, while GSI varied among species, with values of 5.54, 7.19 and 8.59 for *A. viridis*, *A. hybridus* and *Amaranthus* sp.

GroundEye® and near-infrared spectroscopy (NIR) allowed distinguishing the three species according to their geometric characteristics, color predominance and chemical components.

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