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Dormancy and evaluation of the physical-physiological quality in sweet potato [*Ipomoea batatas* (L.) Lam.] seeds by image analysis

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ABSTRACT: For the germination of sweet potato (*Ipomoea batatas*) seeds to occur, it is necessary to overcome dormancy, which makes it difficult to assess their physiological potential. The aim of this research was to define a methodology to overcome the dormancy of sweet potato seeds and use image analysis to determine their physical and physiological quality. Four genotypes of sweet potato seeds were used, namely UFVJM-5, UFVJM-22, UFVJM-38 and UFVJM-65. To overcome dormancy, chemical scarification with 98% H_2SO_4 for 10 and 20 minutes, hot water at 95 °C for 10 and 20 minutes, and mechanical scarification with electric grinder were tested. To evaluate the physical quality, the seeds were analyzed with X-rays and classified as intact, empty and malformed seeds. Using the GroundEye® system, the characteristics of color dominance, seed geometry and seedling length were quantified. Treatments with mechanical scarification with grinder and 98% sulfuric acid, for an immersion period of 20 minutes, are efficient to break dormancy in sweet potato seeds. There are genetic divergences between sweet potato genotypes, promoting the formation of different groups. The characteristics of color and geometry of the seeds are the ones that most contribute to genetic diversity of genotypes.

Index terms: GroundEye®, *Ipomoea batatas*, sulfuric acid, X-ray.

RESUMO: Para que a germinação das sementes de batata-doce (*Ipomoea batatas*) ocorra, é necessária a superação da dormência, o que dificulta a avaliação do potencial fisiológico das sementes. Objetivou-se nesta pesquisa definir metodologia para superação da dormência de sementes de batata-doce e o uso da análise de imagens para determinar a qualidade física e fisiológica das sementes. Foram utilizados quatro genótipos de sementes de batata-doce, UFVJM-5, UFVJM-22, UFVJM-38 e UFVJM-65. Para a superação da dormência foi testado a escarificação química com H_2SO_4 a 98% por 10 e 20 minutos, água quente a 95 °C por 10 e 20 minutos e escarificação mecânica com esmeril elétrico. Para avaliação da qualidade física, as sementes foram analisadas com raios X e classificadas em sementes cheias, vazias e malformadas. Por meio do sistema GroundEye®, foram quantificadas as características de dominâncias de cor, de geometria das sementes e comprimento de plântulas. Os tratamentos com escarificação mecânica com esmeril e ácido sulfúrico a 98%, pelo período de imersão de 20 minutos, são eficientes para a superação de dormência em sementes de batata-doce. Existem divergências genéticas entre os genótipos de batata-doce, promovendo a formação de grupos diferentes. As características de cor e geometria das sementes são as que mais contribuem para diversidade genética dos genótipos.

Termos para indexação: GroundEye®, *Ipomoea batatas*, ácido sulfúrico, raios X.

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INTRODUCTION

Sweet potato [*Ipomoea batatas* (L.) Lam.], belonging to the Convolvulaceae family, is a tuberous vegetable of great economic and social importance, produced worldwide (Villavicencio et al., 2007). It occupies the third position in the export of vegetables by Brazil and is the fourth most produced vegetable by the Brazilian population (Anuário Brasileiro de Horti & Fruti, 2021). For being a source of high nutritional value and containing energy, minerals, and vitamins, it is considered very important in the diet (Gonçalves-Neto et al., 2011).

In sweet potato breeding programs, seeds are mainly used (Mwanga et al., 2017). However, these same authors report that the integument of sweet potato seed is very thick, hard and impermeable, which precludes germination, requiring chemical and physicochemical methods to make it possible. Exogenous dormancy is caused by tissues of the seed itself (extraembryonic), such as integument or parts of the fruit, and may be associated with physical, mechanical, and chemical factors. Physical dormancy, specifically, is caused by the impermeable nature of the integument of both seeds and fruit, which totally or partially restricts water diffusion to the embryo (Grzybowski et al., 2019).

Different tests are used to provide important information for evaluating the physiological quality of seeds; however, most of them require time and are subject to different types of interpretations with unwanted variations. Following technological advances, new methodologies that allow faster and more accurate responses regarding aspects of seed quality are being developed (Ribeiro et al., 2021).

Image analysis is a technique that stands out for the greater accuracy, standardization, objectivity and speed in obtaining consistent results (Medeiros et al., 2018a; Acha and Vieira, 2020). Different characteristics can be measured through specific software programs for image analysis, and with the advantage of being non-destructive evaluation methods that allow the subsequent use of seeds, in addition to being a methodology of simple execution and reproduction (Abud et al., 2018).

The X-ray device makes it possible to evaluate, in a few seconds, the internal morphology of the seeds and identify the main changes caused by various factors (Trujillo et al., 2019). From the images generated, several parameters can be obtained from the radiograph that can be correlated with results of other tests, such as germination and vigor tests, facilitating the classification of seed genotypes in terms of seed quality (Medeiros et al., 2018b).

Image analysis of the external morphology of the seed has been used for more accurate classifications of characteristics such as seed shape, size, texture, and color (Venora et al., 2007). This method has been applied by some computer systems, including GroundEye®, which has been indicated due to its potential, standing out for being a national device available in the market.

Thus, evaluating different methodologies to overcome dormancy in sweet potato seeds and using image analysis through X-ray and GroundEye® systems to determine the physical and physiological quality of sweet potato seeds are fundamental.

MATERIAL AND METHODS

Sweet potato seeds of four genotypes were used: UFVJM-5, UFVJM-22, UFVJM-38 and UFVJM-65, defined as genotypes 5, 22, 38 and 65, respectively, all from the 2017 season, produced in the Olericulture Sector of *Universidade Federal de Lavras* (UFLA).

Characterization of genotypes

Moisture content: obtained by the oven method, at 105 °C for 24 hours (Brasil, 2009). Four replications of ten seeds of each genotype were used and the results were expressed in average percentage based on wet weight.

Germination test: performed according to the Rules for Seed Testing (Brasil, 2009). Four replications of 25 seeds were placed in transparent acrylic boxes (11x11x3.5cm) and put into BOD chamber at 25 °C, using paper towel substrate for germination. The results were expressed as percentage of normal seedlings on the seventh day (first count) and on the 21st day.

Germination speed index (GSI): determined by the daily count of normal seedlings (Maguire, 1962).

Emergence test: performed with four replications of 25 seeds. The seeds were sown in plastic trays containing sand and earth, in a ratio of 2:1, and kept in a growth room at 25 °C, with constant light. After the beginning of the emergence, daily evaluations were carried out, computing the initial stand (IS) on the seventh day, and the test was ended after the percentage of emergence stabilized for three consecutive days, when the number of normal seedlings emerged was evaluated. The emergence speed index (ESI) was also determined by the daily count of emerged seedlings (Maguire, 1962).

Seed dormancy overcoming treatments

Three treatments were used for overcoming seed dormancy. For the treatment with hot water at 95 °C, the seeds were put into a 100-mL beaker, containing distilled water, and kept in a water bath at 95 °C for 0, 10 and 20 minutes, with four replications of 25 seeds. For chemical scarification with sulfuric acid (H_2SO_4), the seeds were put into 100-mL beakers, containing 98% H_2SO_4 solution, and kept for 0, 10 and 20 minutes, with four replications of 25 seeds. After the specified periods, the seeds were washed for 1 minute in running water, with the aid of a sieve. Mechanical scarification was performed manually in an electric grinder, for about 30 seconds, on only one side of the seed, until the integument was abraded, avoiding damage to the embryo. After the end of the treatments mentioned, germination tests were performed as described above, evaluating germination, first count and GSI.

In order to aggregate information, the X-ray and GroundEye® systems were used to add morphological characteristics of the seeds and characteristics of the seedlings of sweet potato genotypes.

Image analysis

For physical evaluation through X-rays, the seeds were fixed in transparent acetate sheets with double-sided tape, placed on the lower shelf of the device, and exposed to X-rays with 24 kV for 10.1 seconds. This analysis was conducted with four replications of 25 seeds of each genotype. The images obtained were analyzed for the percentage of intact, empty and malformed seeds for each sample. Seeds whose embryo was intact and with all complete structures were classified as intact seeds, empty seeds were those that had less than 50% of the tissues, and malformed seeds were those that showed structural damage above 50% (Figure 1).

The GroundEye® S120 system was used to evaluate the characteristics of seeds and seedlings. Images of four replications of 25 seeds of each sample were captured before and after overcoming seed dormancy with one of the best-defined treatments, that is, immersion in sulfuric acid for 20 minutes, and these images were analyzed. In the configuration of the analysis to calibrate the background color for the seeds, the YCbCr model was used, with luma color from 0 to 1, blue color from 0.09 to 0.20 and red color from -0.50 to 0.50. Calibration of the background color for the seedlings was performed using the CIELab model, with a lightness index from 0 to 100, dimension “a” from -18.1 to 41.9 and dimension “b” from -60.2 to -16.5. After calibration, the images were analyzed. The characteristics of the seeds quantified were color dominance (black, blue, celestial blue, dark gray, purple and red), percentages of color characteristics (brightness, intensity, luma, lightness and saturation) and geometry (area, tapering, circularity, maximum diameter, minimum diameter and perimeter).

For analysis of the seedlings, the dormancy overcoming test was performed in the seeds that showed one of the most satisfactory results in the data analysis, that is, scarification with sulfuric acid for 20 minutes. Subsequently, the seeds were placed to germinate (Brasil, 2009). After this period, images of the seedlings were captured and later evaluated. For standardization, nine seedlings of each genotype were evaluated. In the GroundEye® system, the mean values of seedling characteristics such as root length (RL), hypocotyl length (HL), root length to hypocotyl length ratio (RL/HL) and total length (TL) were extracted.

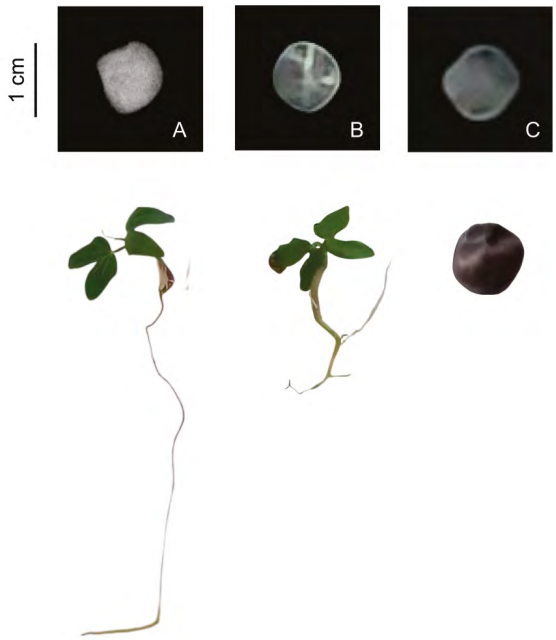

Figure 1. Intact seed and normal seedling after germination (A), malformed seed and abnormal seedling after germination (B), empty seed and ungerminated seed (C) of sweet potato.

Statistical analysis

Statistical analysis consisted of using univariate and multivariate statistical analysis techniques. Univariate analysis was carried out with analysis of variance (ANOVA) of a 5 x 4 factorial scheme with an additional treatment, totaling 21 treatments subjected to the F test and, when significant, their means were grouped by Tukey test at 5% probability level. Multivariate analysis consisted of using cluster analysis (dendrogram) and principal component analysis (PCA). To avoid scaling problems among the variables, they were standardized to make their means equal to zero and their variance equal to one.

The clusters were obtained using the UPGMA (*Unweighted Pair Group Method with Arithmetic Mean)* algorithm through the Euclidean distance, while the cut-off point of the dendrogram was defined based on the criterion of Mojema (1977). For the principal component analysis, the principal components were obtained from the matrix of variance and covariance of the data. The criterion for choosing the principal components was the sum of eigenvalues exceeding 80% of the data variability; for details see Johnson and Wichern (2002). All statistical analyses were performed with R software (R Core Team, 2019), through the following packages: *factoextra*, *ExpDes.pt* and *MultivariateAnalysis*.

RESULTS AND DISCUSSION

There was variation in the initial moisture content of the seeds from 6.19% to 10.38% (Table 1), which coincides with the values recommended in literature for sweet potato seeds, around 7-10%, to avoid losses of seeds due to fungi, rots or insects (Rossel et al., 2008). Moisture content of 7% was also observed for *Ipomoea grandifolia* seeds (Araújo et al*.,* 2016). According to Bewley and Black (1994), moisture content is one of the determining factors in dormancy imposed by the impermeability of the seed integument, because it becomes progressively hard and impermeable as the moisture content decreases.

Regarding the percentage of normal seedlings obtained by the germination test, a low percentage was observed for all sweet potato genotypes. The genotypes UFVJM 38 and UFVJM 65 showed higher germination, the genotype UFVJM 22 showed intermediate germination and the genotype UFVJM 5 showed lower germination compared to the others (Table 1).

Table 1. Results of moisture content – U (%), first germination count – FC (%), germination – G (%), germination speed index – GSI, emergence – E (%), initial stand – IS (%), and emergence speed index – ESI of four sweet potato genotypes, for the characterization of the seed profile.

Genotypes	Tests						
	U (%)	FC (%)	G (%)	GSI	E (%)	IS (%)	ESI
UFVJM 5	6.49b	6a	3b	1.42b	10a	1a	0.46a
UFVJM 22	10.38a	4a	6ab	0.73c	2a	1a	0.21a
UFVJM 38	7.41ab	8a	10a	0.84bc	11a	1a	0.48a
UFVJM 65	8.65ab	12a	11a	1.75a	8a	5a	0.66a
CV (%)	22.36	10.84	19.79	5.71	9.24	15.87	15.07

Means followed by the same lowercase letter in the column do not differ statistically by the Tukey test at 5% probability level. CV: coefficient of variation.

Regarding the germination speed index, the genotype UFVJM 65 showed the best performance, followed by the genotypes UFVJM 5 and UFVJM 38, and UFVJM 22 had the lowest value.

The low percentage of normal seedlings obtained by the germination tests is consistent with the value reported by Voll et al. (2003), who worked with *Ipomoea grandifolia* seeds and found 5% germination, and with the value reported by Van Rheenen (1963), who studied sweet potato seeds without previous treatment and observed germination of 10%. Nunes (1968) also obtained a low germination percentage in sweet potatoes, 11.3%.

The other tests of vigor, first germination count, initial stand, emergence and emergence speed index showed no significant differences between genotypes (Table 1). The values presented in Table 1 reinforced the need to test methodologies for overcoming dormancy in sweet potato seeds.

Regarding the percentage of normal seedlings obtained by the germination test (Table 2), it was found that for the treatment with mechanical scarification the genotype UFVJM 65 showed the best performance (value of 38%), followed by UFVJM 5, differing from UFVJM 22 and UFVJM 38. In addition to the treatment with mechanical scarification, the treatment with chemical scarification (H_2SO_4) for 20 min also promoted a higher germination percentage mainly for the genotype UFVJM 65. Azania et al. (2003) also found that the treatment with chemical scarification with sulfuric acid for 20 minutes in *Ipomoea grandifolia* and *Ipomoea quamocli* seeds led to germination of 34% and 35%, respectively.

For the germination percentage, the mean of the treatment with mechanical scarification was higher than the others (Table 2). The efficiency in the mechanical scarification process depends on the exposure time, intensity of the force applied on the seeds and homogeneity of the sample, because friction in small seeds or less lignified integument can cause cracks that are harmful to the embryo and to germination (Pazuch et al., 2015).

There was no significant difference between dormancy overcoming treatments for the first germination count test in the genotype UFVJM 5. For the genotypes UFVJM 38 and UFVJM 65, higher germination percentage was observed in the treatment with mechanical scarification, but for the genotype UFVJM 22, the treatment with chemical scarification (H_2SO_4) for 20 min did not differ from that with mechanical scarification, and both were superior. In general, the results of the first count indicated superiority of the genotype UFVJM 65 in the treatments that produced more relevant results of dormancy overcoming.

The treatment with mechanical scarification was the best for all genotypes, when compared to the other treatments for dormancy overcoming and for the germination speed index. However, in the treatment with mechanical scarification the genotype UFVJM 65 had the best values, while in the treatment with chemical scarification (H_2SO_4) for 20 min the genotypes UFVJM 38 and UFVJM 65 showed the best performance.

Table 2. Mean values, in percentage (%), of the germination (G), first count (FC) and germination speed index (GSI) for four genotypes of sweet potato seeds subjected to different dormancy overcoming treatments.

Treatments	G (%)			
	Genotypes			
	UFVJM 5	UFVJM 22	UFVJM 38	UFVJM 65
Control	5bA	2bA	10bA	2dA
Mechanical scarification	29aAB	25aBC	16abC	38bA
Chemical scarification (H_2SO_4) for 10 min	2bB	2bB	11bAB	17cA
Chemical scarification (H_2SO_4) for 20 min	9bC	26aB	29aB	52aA
Hot water for 10 min	0bA	0bA	2bA	0dA
Hot water for 20 min	0bA	0bA	1bA	0dA
CV (%)	20.68			
	FC (%)			
Control	4aB	0bB	4cB	14bcA
Mechanical scarification	4aD	17aC	34aB	64aA
Chemical scarification (H_2SO_4) for 10 min	0aA	0bA	2cA	7cdA
Chemical scarification (H_2SO_4) for 20 min	5aB	10aAB	16bB	15bA
Hot water for 10 min	0aA	0bA	0cA	0dA
Hot water for 20 min	0aA	0bA	0cA	0dA
CV (%)	18.14			
	GSI			
Control	0.60cA	0.43dA	0.62cA	0.87cA
Mechanical scarification	7.69aB	8.51aB	7.90aB	11.61aA
Chemical scarification (H_2SO_4) for 10 min	0.41cB	0.41dB	1.41cAB	2.02cA
Chemical scarification (H_2SO_4) for 20 min	2.85bAB	2.39cB	3.75bA	3.88bA
Hot water for 10 min	0.15cB	4.32bA	0.40cB	0.77cB
Hot water for 20 min	0.58cA	0.43dA	0.62cA	0.90cA
CV (%)	17.96			

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically by the Tukey test at 5% probability level. CV: coefficient of variation.

For chemical scarification with H_2SO_4, *Ipomoea potatoes* seeds should remain immersed for a time between 10 and 40 minutes according to Rossel et al. (2008). Despite the recommendation of this exposure time, in the present study, the highest germination values were obtained at the maximum exposure time of 20 minutes (Table 2), since the authors observed through pre-tests that longer times caused disintegration of the seeds.

The recommendation to overcome the dormancy of dispersal units in different species of *Ipomoea*, according to the Rules for Seed Testing (Brasil, 2009), is mechanical scarification and chemical scarification with concentrated sulfuric acid (H_2SO_4). It should be highlighted that the efficiency of sulfuric acid in overcoming dormancy is proven to be responsible for the appearance of cracks around the hilar cleft and porosity of the integument, allowing water absorption and consequently germination, as observed in seeds of *Colubrina glandulosa* Perkins (Lopes et al., 2021).

The use of hot water was not efficient for overcoming the dormancy of sweet potato seeds. An injury may have occurred to the embryo, since during germination the reserves present inside the seeds must be degraded and subsequently mobilized to different parts of the embryo, assisting in seedling growth (Souza, 2009).

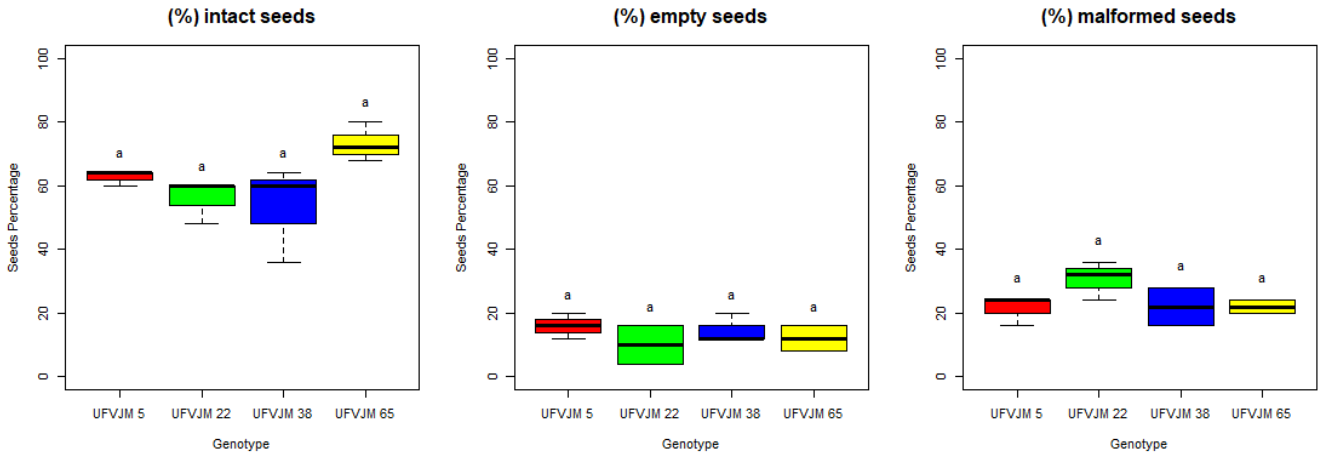

Figure 2. Percentage of intact seeds, empty seeds and malformed seeds obtained by X-ray technique of the internal morphology of seeds of four sweet potato genotypes. Means followed by the same lowercase letter do not differ statistically by the Tukey test at 5% probability level.

There was no difference between genotypes in radiographic analysis for intact, empty and malformed seeds (Figure 2). The highest percentage of intact seeds was observed in the genotype 65, which also obtained a higher germination value. Medeiros et al. (2020), using the X-ray technique, reported that even if the physical and physiological variables are not fully correlated, there may be a strong association between them, which can lead to advances in the procedures of pre-selection of seed genotypes by farmers and by the seed industry.

Based on the recommendations of the Rules for Seed Testing (Brasil, 2009), the treatment of scarification with immersion of seeds in sulfuric acid (H_2SO_4) for 20 minutes was also one of the treatments that showed the most satisfactory results in overcoming the dormancy of sweet potato seeds, so it was the treatment chosen to be used in the GroundEye® system.

The GroundEye® system evaluated the characteristics of the genotypes UFVJM 5, UFVJM 22, UFVJM 38 and UFVJM 65 of sweet potato seeds with and without the treatment of chemical scarification in sulfuric acid (H_2SO_4) for 20 min for color dominance, percentages of color characteristics and seed geometry (Figure 3). Based on the dendrogram and on the PCA analysis, it was possible to observe differences considered high between genotypes and the characteristics analyzed.

For the characteristics color dominance - black, blue, celestial blue, dark gray, purple and red (Figure 3A), the first component explains approximately 49% of the variance of the data matrix. It was possible to observe that the colors black and celestial blue contrasted with the others. There was greater influence of the black color in the genotypes UFVJM 5 and UFVJM 38, without treatment for overcoming dormancy. In turn, the colors celestial blue and blue can be observed with higher predominance in the genotypes UFVJM 22 (with and without treatment) and UFVJM 38 with the use of chemical scarification treatment with sulfuric acid for 20 min. In the UFVJM 65 genotype, there was greater influence of purple color with treatment and of red color without treatment.

The dendrogram of the genotypes showed that the colors related to the color dominance in the seeds were able to separate only the genotype UFVJM 65 with chemical scarification with sulfuric acid for 20 min from the others (Figure 3A).

Visually, the sweet potato seeds have a dark color, coinciding with the identification of the GroundEye® system. This result is similar to that found by Mwanga et al. (2017), who obtained sweet potato seeds with dark and sometimes black color. However, Xavier et al. (2019), using the GroundEye® system to identify the color of *Amaranthus hybridus* seeds, did not correlate the visual color of the seeds with that obtained by the device.

It was possible to observe the following color characteristics: brightness, intensity, luma, lightness and saturation (Figure 3B). The first component explains 77% of the data variability. In PC2, saturation contrasts with the other variables.

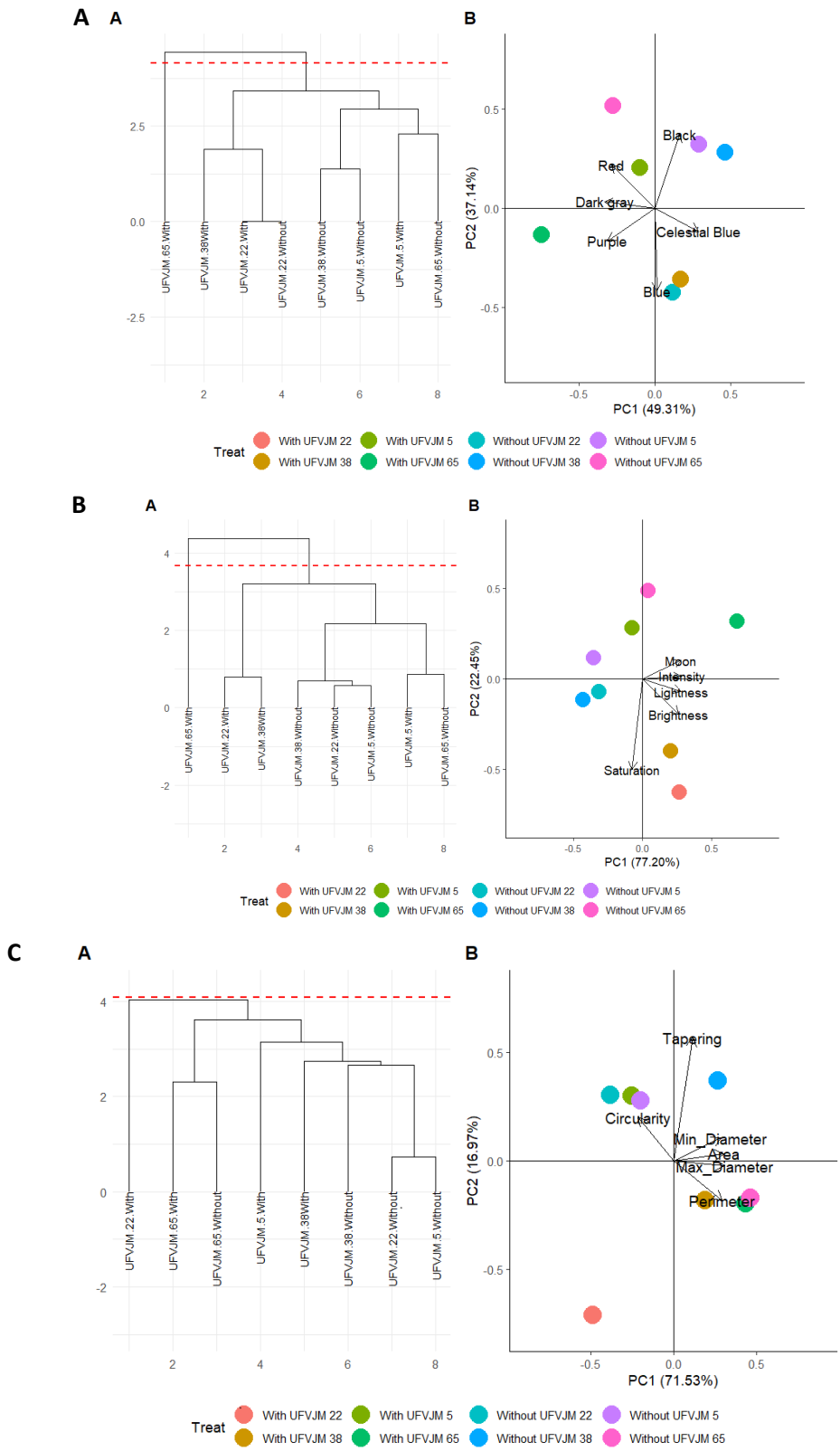

Figure 3. Dendrogram of genotypes (a) and Principal Component Analysis (b) for the treatments, with and without chemical scarification (H_2SO_4) for 20 min, according to the legend: A - dominance of colors: Black, red, dark gray, celestial blue, blue and purple; B - color characteristics: Intensity, lightness, luma, saturation and brightness; and C - characteristics of the geometry of sweet potato seed: tapering, area, maximum diameter, minimum diameter, perimeter and circularity.

It is possible to observe that the color characteristic brightness was more present in the genotypes UFVJM 22 and UFVJM 38, with treatment of dormancy overcoming, while luma and intensity influenced more the genotype UFVJM 65, mainly with treatment. It is also possible to observe that the genotypes without treatments were close in the PCA graph, except for UFVJM 65, indicating that there is a discrepancy for the genotypes in relation to the color characteristics with and without treatment.

These color differentiation factors refer to the vividness or paleness of a color and are associated with its purity, because a material with low saturation or non-existent saturation has darker color, as observed for the color characteristic called saturation (Figure 3B).

Another characteristic of extreme importance, especially for the selection of materials for genetic improvement, is seed geometry.

According to Abud et al. (2018), who studied broccoli seeds, the area of the seed may reflect its vigor, since larger areas coincide with more reserves available for germination and seedling growth.

For the seed geometry characteristics area, tapering, circularity, maximum diameter, minimum diameter and perimeter (Figure 3C), the first component explains 72% of the data variability. In PC2, circularity contrasts with the other variables.

For the seed geometry characteristics, it was found that the variability of the genotype UFVJM 22 is more influenced by the geometry characteristic tapering.

The circularity is inversely proportional to the tapering, and both range from 0 to 1. Circularity refers to the circular shape factor least sensitive to elongation with less dependence on the smoothness of the contour, but for this characteristic, values close to 1 mean greater circularity (Ferreira et al., 2018).

For tapering, as the value moves away from 1, the seed is considered thinner (Viana et al., 2016), as observed in the genotype UFVJM 22, with the treatment of chemical scarification with sulfuric acid for 20 minutes.

The maximum diameter and minimum diameter correspond to the length and width, respectively. Sweet potato seeds are characterized as small, measuring approximately 0.3 cm in diameter (Huaman, 1992).

Based on the information obtained by the GroundEye® system, the following characteristics of seedlings were quantified after germination of sweet potato seeds: root length (RL), hypocotyl length (HL), root length to hypocotyl length (RL/HL), and total length (TL) (Figure 4).

Figure 4 showed that total seedling length (TL) was higher in the genotype UFVJM 38 than in the others. For hypocotyl length (HL), the genotypes UFVJM 5 and UFVJM 38 performed better than the others. For primary root length (RL), there was no difference between genotypes, while for the primary root length to hypocotyl ratio (RL/HL) the genotype UFVJM 5 was inferior to UFVJM 22 and UFVJM 65.

The X-ray technique was efficient for evaluating the internal morphology of sweet potato seeds, and the results obtained in the present study confirm that it is possible to identify and subsequently eliminate the malformation of seeds and can assist in the seed production process and optimize breeding programs, making it possible to visualize radiographs and improving the physical quality and consequently the physiological quality of the studied genotypes.

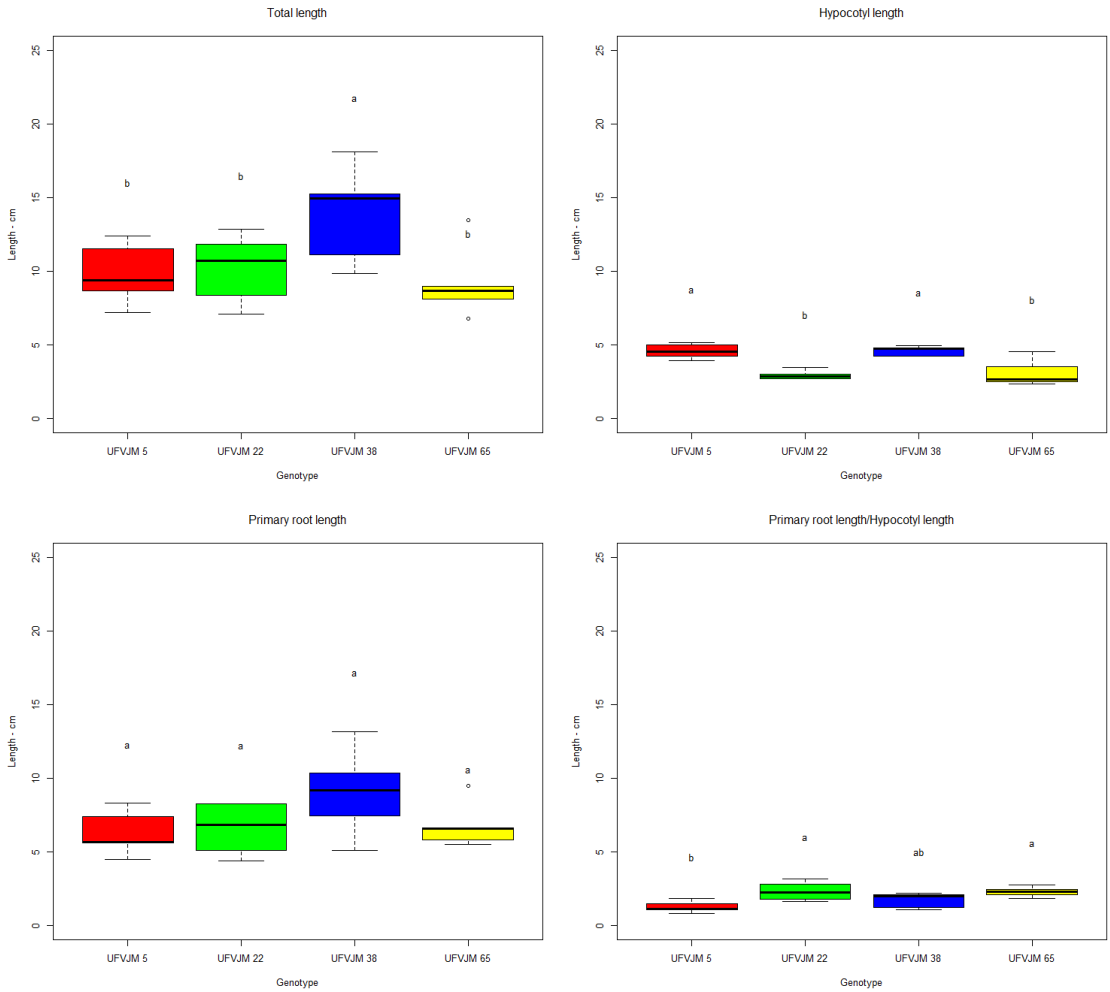

Figure 4. Mean values of total seedling length, hypocotyl length, primary root length and primary root length to hypocotyl length ratio obtained by the GroundEye® system of seedlings of four sweet potato genotypes. Means followed by the same lowercase letter do not differ statistically by the Tukey test at 5% probability level.

CONCLUSIONS

Treatment with mechanical scarification and treatment with 98% sulfuric acid for the immersion period of 20 minutes are the most efficient for overcoming dormancy in sweet potato seeds.

There are genetic divergences between sweet potato genotypes, promoting the formation of different groups. The characteristics of color and geometry of the seeds are the ones that most contribute to genetic diversity of the genotypes.

X-ray is efficient for selecting sweet potato seed genotypes with higher percentage of intact seeds.

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