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SARS software for analysis of radiographic images of *Urochloa decumbens* (Stapf) RD Webster seeds

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ABSTRACT: Recently is growing the need for non-invasive, fast, and accurate technologies that can predict seed quality. Between these technologies, X-ray image analysis stand out for evaluation of the internal morphology of the seeds. Thus, the aim of the present study was to evaluate the efficiency of a specialized software for analyzing digital radiographs of *Urochloa decumbens* seeds called SARS (*Sistema de Análise de Radiografias de Sementes* - Seed Radiograph Analysis System). Five comercial seed lots of *U. decumbens* cv. Basilisk were used. The seed lots were produced in the 2018/2019 crop season. Radiographic images of the seeds were analyzed in SARS, through which physical characteristics were obtained. The seeds were then subjected to germination test, in which variables related to the physiological quality were evaluated. It was possible to observe that the seeds with greater germination and vigor showed strong and significant correlations with some of the physical variables obtained using SARS. Thus, high correlation of seedling length and relative seed density is important for validating the seed radiographic image analysis method. SARS proved to be an efficient tool for analyzing digital radiographs of *U. decumbens* seeds. It can generate descriptors which support morphometric and internal analysis of the seeds. Physical parameters obtained by using the technique have close relationship with the germination and vigor of the seeds.

Index terms: forage species, image analysis, radiographic images of seeds.

RESUMO: Recentemente está crescendo a necessidade de tecnologias não invasivas, rápidas e precisas que possam estimar a qualidade das sementes. Entre essas tecnologias, destaca-se a análise de imagens de raios X para avaliação da morfologia interna das sementes. Assim, o objetivo deste estudo foi avaliar a eficiência do *software* SARS na análise de radiografias digitais de sementes de braquiária, denominado SARS (Sistema de Análise de Radiografias de Sementes) na avaliação da qualidade física das sementes. Cinco lotes comerciais de sementes de *Urochloa decumbens* cv. Basilisk foram utilizados. As sementes foram produzidas na safra de 2018/2019. As imagens radiográficas das sementes foram analisadas por meio do SARS, por meio das quais foram obtidas características físicas. As sementes foram submetidas ao teste de germinação, no qual foram mensuradas variáveis relacionadas à qualidade fisiológica. Foi possível observar que as sementes com maior potencial de germinação e vigor apresentaram correlações fortes e significativas com algumas das variáveis físicas obtidas pelo SARS. Assim, a alta correlação do comprimento de plântulas e a densidade relativa das sementes é importante para validar o método de análise de imagens radiográficas de sementes. O SARS mostrou-se uma ferramenta eficiente para análise de radiografias digitais de sementes de braquiária, gerando descritores que dão suporte a análise morfométrica e interna das sementes, que podem ser associados à sua qualidade fisiológica. Os parâmetros físicos obtidos pela técnica têm estreita relação com a germinação e o vigor das sementes.

Termos para indexação: espécie forrageira, análise de imagens, imagens radiográficas de sementes.

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INTRODUCTION

Brazil is considered the world's largest producer, consumer, and exporter of tropical forage seeds (Vigna et al., 2011). *Urochloa* (syn. *Brachiaria*) *decumbens* (Stapf) RD Webster, popularly known in Brazil as 'Brachiaria' is a grass of African origin and is among the different species of great importance in the forage seed market, being much sought after due to its rusticity and wide adaptation to various environmental conditions (Cardoso et al., 2014).

With the growing demand for forage and increased competition in the international market, it is necessary to select high-quality seeds for the establishment of pastures in the fields, constituting a fundamental part of the production process. Seed quality is composed of several attributes, and physiological potential is one of the most important. This information is obtained through analyses of germination and vigor of seeds (Marcos-Filho, 2015).

A limitation to the evaluation of the physiological potential of seeds is the long time required to perform the tests or the technological restrictions associated with the analyses (Elmasry et al., 2019) physiological, biochemical and molecular determinations. Whilst proven to be effective, these approaches can be criticized as being destructive, time consuming, labor intensive and requiring experienced seed analysts. The scientific community, including public and private agents, has sought non-invasive technologies that can better predict seed quality quickly and nondestructively (Grasso et al., 2018).

With this, some studies have recently shown the efficiency and speed of the application of radiographic images in the evaluation of internal morphology of seeds, identification of morphological and embryonic abnormalities, damage caused by insects or mechanical damage, endosperm of seeds and diagnosis on physical characteristics of interest, mostly related to germination and vigor of seeds (Abud et al., 2018; Borges et al., 2019; Leão-Araújo et al., 2019; Ribeiro et al., 2021). Although it is a consolidated technique for seed analysis, its use is still restricted, due to the need to purchase expensive equipment, associated with the need for trained analysts who can understand the results (Hughes et al., 2017).

In this context, there is the possibility of performing these evaluations more accurately using recent advances in digital image processing techniques. These analyses are performed based on the pixel density measured in gray scale, which is related to the tissue density of the internal structures of the seeds when irradiated by X-rays (Abud et al., 2018).

Several techniques involving digital image processing are available with great potential to be applied in the analysis of seed radiographs. However, there are no reports of a specialized system of analysis of radiographic images for seeds of *U. decumbens* forage grasses. Automating the analysis and extraction of information from images that may be related to seed quality, performed quickly and efficiently, can contribute to improving laboratory tests, reducing the time and cost of the analysis.

Considering the importance of using automatic and non-destructive techniques to evaluate seed quality, the aim of this study was to evaluate the efficiency of a software specialized in analysis of digital radiographs of *U. decumbens* seeds, called SARS (*Sistema de Análise de Radiografias de Sementes*), in the evaluation of the physical quality of seeds.

MATERIAL AND METHODS

Samples from five commercial seed lots of *Urochloa decumbens* cv. Basilisk (2018/2019 crop season) were fixed in four groups of 50 seeds on adhesive paper. The sheets were placed on a Faxitron MX-20 cabinet X-ray system (Faxitron X-ray Corp. Wheeling, IL, USA) to obtain radiographic images. The device setting has been adjusted to the voltage of 20 kV. The radiation period used was 10 seconds, with focal length adjusted to 27.8 cm from the seeds. The calibration of the image control was defined at 16383 (width) x 2296 (center). Digital radiographic images were stored in TIFF (Tagged Image File Format) files and then analyzed.

SARS is a free and easy-to-use tool for X-ray analysis of seeds. It is based on automated evaluation of quality of seeds from digital image processing, which generates information about their physical characteristics. This study involved a software developed to perform the processing of radiographic images of *Urochloa decumbens* seeds and is being improved for evaluation of other species. An algorithm was developed using digital image processing techniques and designed to optimize and automate image analysis. Radiographs were analyzed using the SARS software, which is a tool developed in MATLAB language to automatically perform radiographic image analysis.

In the analysis procedure by SARS, the analyst informs the number of seeds contained in the sample, loads the metric (a coin of 0.10 cents) and determines the value for calibration of the focal length between the seeds and the X-rays. From this, the program converts the values of pixels into millimeters.

Then, the user is directed to choose the image to be analyzed. After this choice, there is pre- image processing step to remove the noises, in addition to extending the contrast to separate the regions of interest from the seeds and the image background. Then, the images are segmented, and the regions of interest (seeds) are delimited. Finally, the thresholding technique based on the Otsu's method is used (Gonzalez and Woods, 2009), which allows separating the embryonic area (embryo and nutritive tissue) from the seed coat. Subsequently, the selections of binary images are redirected to the original in the representation and description step, generating 12 variables (Table 1). Shortly after processing, the analysis results are contained in a pivot table and automatically saved to a file in XLS format.

Seed descriptors are calculated in the recognition and interpretation step. The software can analyze the seeds in three categories: 1 - Well-formed, 2 - Malformed and 3 - Empty. In addition, the system is also able to identify irregularities present in the embryo, among seeds with good formation from a convex grid, generating percentage values of these irregularities that determined whether the seeds are affected by some type of damage, such as mechanical damage.

Tests were set up after obtaining radiographic images using the same seeds to determine their physiological potential. Seed moisture content was previously determined by the oven method at 105 ± 3 °C for 24 hours, based on the Rules for Seed Testing (Brasil, 2009). Two samples of 0.5 g seeds were used for each lot.

The seeds were later distributed in transparent Gerbox boxes (11 x 11 x 3 cm) on two sheets of paper for germination (Germitest®) and moistened with KNO_3 solution (0.2%). The boxes were kept in germinator under alternating temperature of 20 and 35 °C, for fourteen and eight hours, respectively (Brasil, 2009). Germination counts (root protrusion) were performed daily, and normal seedlings were counted until the twentieth day after the test was set up. In the final count, the seeds were classified as germinated, represented by those with primary

Table 1. List of descriptors generated by the analysis of radiographic images of seeds.

Abbreviation	Name descriptor	Description
Area	Area (mm²)	Height of the smallest rectangle that encompasses the selection
Perim.	Perimeter (mm)	Length in millimeters of the polygon boundary
Width	Width (mm)	Width of the smallest rectangle that encompasses the selection
Length	Length (mm)	Length of the smallest rectangle that encompasses the selection
Circ.	Circularity (mm)	Circularity = 4πArea/Perimeter²
IntDen	Integrated Density (gray mm.pixel^{-1})	Sum of the values in gray scale of the region
RelDen	Relative Density (gray mm.pixel^{-1})	Mean of values in gray scale of the entire segmented region
Solid.	Solidity	Solidity = area of the polygon/ area of the convex hull of the region
WellForm	Well-Formed (%)	Percentage of well-formed seeds
MalForm	Malformed (%)	Percentage of malformed seeds
Empty	Empty (%)	Percentage of empty seeds
MecDam	Mechanical damage (%)	Percentage of seeds with mechanical damage

root longer than 2 mm and not germinated, represented by those that showed no root protrusion until 21 days of evaluation. The seedlings formed were characterized as normal (those with all well-developed structures) and abnormal (underdeveloped or showing alterations).

The germination speed index was determined together with the germination test, and the counts were performed daily, calculated by the formula proposed by Maguire (1962).

In addition, seedling length was also measured. This analysis was performed from the images of the normal seedlings obtained after the last count of the germination test; seedlings and seeds were scanned, and the images were processed in ImageJ® software. The images were used to measure the length of shoots and primary root of the seedlings, expressed in mm.seedling^{-1}. In addition, the growth, uniformity, vigor and corrected vigor indices were obtained. The uniformity index is calculated from the size of the seedling analyzed (Xi), average seedling size (x), total number of seedlings evaluated (n) and number of non-germinated seeds or dead seedlings (ndead). These indices were proposed by Sako et al. (2001), except for the corrected vigor index, which is based on the vigor index and is expressed by the following equation: CVI = (0.70 * G + 0.30 * U) * (germ/100), where CVI is the corrected vigor index, G is the growth rate, U the uniformity index, and germ is the germination percentage, proposed by Medeiros and Pereira (2018).

The experiment was conducted in a completely randomized design, with five lots and four replications. The data were subjected to analysis of variance (ANOVA) and the means obtained were analyzed by Tukey test ($p \leq 0.05$). Pearson's correlation coefficients (r) were calculated between all physiological characteristics and variables obtained from radiographic analyses. The significance of the correlation coefficients was determined by the t-test ($p \leq 0.05$). R software (R Core Team, 2019) was used for statistical analysis.

RESULTS AND DISCUSSION

Figure 1 shows the software screen with an radiograph containing 50 seeds, which was loaded and processed by SARS. In the image it is also possible to observe the percentage of well-formed, malformed, empty seeds, seeds with mechanical damage and seeds damaged by predation. The settings used in the X-ray device were adequate to generate images with good contrast between the regions of interest of the seeds and the image background. According to Figure 1, SARS software estimated descriptors for each seed, including the percentage of well-formed, malformed, empty, and mechanically damaged seeds.

For the characteristics related to seed size and shape, significant differences were observed between the lots tested with grouping at three or four levels. The variables area and perimeter ranked the lots in four levels, which show values from 5.46 to 6.11 mm² and 40.96 to 50.58 mm. Circularity, in turn, grouped the lots into two levels, ranging from 0.41 to 0.47mm. Length and width separated the lots into three levels, with values between 0.55 and 0.60 mm and between 1.28 and 1.37 mm. Solidity ranged from 0.92 to 0.94 mm (Table 2).

These variables have been used in seed research and have high potential for quality analysis of seed lots, as they may be related to physiological attributes such as germination and seedling length (Abud et al., 2018; Medeiros et al., 2018). The variables relative and integrated density are calculated from the gray values of each pixel of the image and can demonstrate the integrity of the seed tissues and the resistance to X-ray passage. Gray values vary according to the resistance to X-ray passage through seeds and the presence of damage, since photons in an X-ray beam can be transmitted, dispersed (Compton dispersion) or absorbed (photoelectric collision) when they collide over an object (Kotwaliwale et al., 2014; Medeiros et al., 2018).

According to Marchi and Gomes-Junior (2017), it is important to evaluate the internal part of the seed occupied by the embryo, the presence of mechanical damage and stains that indicate tissue deterioration (malformation). These variables were obtained and calculated using SARS software. However, the descriptors are directly linked to relative density, since the lowest levels of gray resulting from radiographic images come from deteriorated or less filled seeds.

Figure 1. SARS screen showing the processing of a digital radiographic image containing 50 seeds.

Table 2. Mean values of variables obtained through automated radiographs analysis of images of five seed lots of *U. decumbens* using SARS.

Lot	Area (mm^2)	Perimeter (mm)	Circularity	RD ($gray.pixel^{-1}$)	ID ($gray\ mm^2.pixel^{-1}$)	Length (mm)
1	5.57 bc	40.96 c	0.47 a	71.20 b	395.30 c	0.55 c
2	5.46 c	43.77 bc	0.41 b	74.42 ab	415.25 bc	0.55 c
3	5.76 bc	43.39 bc	0.45 a	76.56 a	440.57 ab	0.57 b
4	5.88 ab	46.77 ab	0.45 a	75.69 a	438.68 ab	0.57 b
5	6.11 a	50.58 a	0.46 a	77.01 a	464.13 a	0.60 a
FC	11.99*	9.05*	7.01*	6.51*	19.68*	72.58*
CV (%)	2.6	5.45	3.63	2.44	2.75	0.8

Lot	Width	Solidity	Well-formed	Malformed	Empty	Mechanical damage
	(mm)			(%)		
1	1.28 b	0.94	33 b	22 a	7	10 ab
2	1.29 b	0.93	47 a	9 b	4	10 ab
3	1.29 b	0.93	48 a	5 b	6	5 b
4	1.33 ab	0.92	48 a	5 b	8	9 ab
5	1.37 a	0.92	47 a	3 b	8	12 a
FC	5.66*	2.85^{ns}	98.89*	29.45*	0.98^{ns}	3.06*
CV (%)	2.31	0.85	2.87	32.83	56.2	33.52

Lowercase letter = column grouping for each evaluation by Tukey test ($P < 0.05$); *= significant by F test ($p < 0.05$); FC = calculated F value; CV = coefficient of variation.

These characteristics are important because they are linked to the good initial establishment of seedlings in the field (Finch-Savage and Bassel, 2016).

It was possible to rank the lots in up to three levels for the variables relative density and integrated density. The well-formed, malformed, and empty variables (Table 2) showed values ranging from 33 to 48%, 3 to 22% and 4 to 8%, respectively. In addition, damaged seeds ranged from 5 to 12%, meaning that lots 3, 4 and 5 showed high tissue integrity in embryonic filling of seeds.

The quality of a seed can be directly linked to its internal structure (Du et al., 2019). The X-ray technique has been increasingly used because it is a non-destructive method, capable of accessing information about internal seed structures and being indicated by the International Seed Testing Association (ISTA, 2019). However, even with promising results, manual analysis of radiographic images interpreted by analysts can be slow, error-prone, and impractical on a large scale (Medeiros et al., 2020). Based on this, the results obtained by SARS indicate its potential as an easy-to-use tool for analysis of radiographic images of *Urochloa decumbens* seeds, enabling a faster and more accurate analysis than that performed by a human analyst. It is important to highlight that these results demonstrate the efficiency of this tool for studies on the physical characteristics and embryonic integrity of seeds.

The voltage, exposure time, focal length and contrast settings of the X-ray system applied in this study together with the low variation in the moisture content of the lots (10.04% to 10.60%) are correlated with the visualization of the internal morphology of the seeds (Figure 2). Thus, it was possible to observe the main internal structures of the seeds through radiographic images (Figure 2A), as well as identifying well-formed, malformed, or empty seeds (Figure 2B[1,2,3]) and mechanically damaged seeds (2B[4]). Thus, it is important that the seeds used in X-ray tests have low moisture content, resulting in an adequate optical resolution of radiographic images with good level of detail of their internal structures. Similar results have been found in studies conducted with X-ray analysis of *Senna multijuga* seeds (Marchi and Gomes-Junior, 2017) and *Crotalaria juncea* seeds (Arruda et al., 2016). In addition, there are other factors that may affect the detailing of the internal components in the generated radiograph that are related to the constitution of seeds.

For root protrusion, lots 3, 4 and 5 were superior and lots 1 and 2 were inferior (Table 3). In addition, lots 3, 4 and 5 were superior in terms of formation of normal seedlings, while lot 2 was intermediate and lot 1 was inferior; the same occurred with the germination speed index (Table 3). Considering root protrusion, normal seedlings, and germination speed index, only lots 3, 4 and 5 could be marketed in Brazil according to official standards of production and commercialization of seeds of forage grasses (Brasil, 2009). Germination speed and seedling length are influenced by seed vigor, and lots with fast and uniform germination have practical advantages for the establishment of the crop in the field (Finch-Savage and Bassel, 2016).

Differences between lots were also observed when analyzing seedling growth. It was observed that seedling length (Table 3) made it possible to separate the lots into three levels, high vigor (lot 3), intermediate vigor (lot 2) and low vigor (1, 4 and 5). The growth index classified the lots into three groups, and lot 3 had the highest value, lot 2 had intermediate value and lots 1, 4 and 5 had the lowest results. The lots were also separated into superior performance in the uniformity index (Table 4) (lots 3, 4 and 5), intermediate performance (lot 2) and superior performance (lot 1).

Some authors point out that uniformity in seedling growth (Table 4) is a characteristic that necessarily needs to be considered in the evaluation of seed lots, to provide information on the degree of deterioration, seedling growth potential and seedling emergence uniformity (Leão-Araújo et al., 2017; Medeiros et al., 2018; Silva et al., 2017).

The vigor and corrected vigor indices (Table 4) were considered sensitive parameters to detect differences between the lots in at least three levels of vigor, and lot 3 classified as the best, lots 2, 4 and 5 as intermediates and lot 1 was the worst. The corrected vigor index, proposed by Medeiros and Pereira (2018), can be considered a seed quality index (germination, growth, and uniformity of seedlings). It can be used efficiently to classify seed lots according to their physiological potential, as observed in the present study.

The results obtained for the five lots in general showed significant differences in the physiological potential of the seeds, that is, there were differences in germination and vigor (Tables 3 and 4). In addition, significant differences

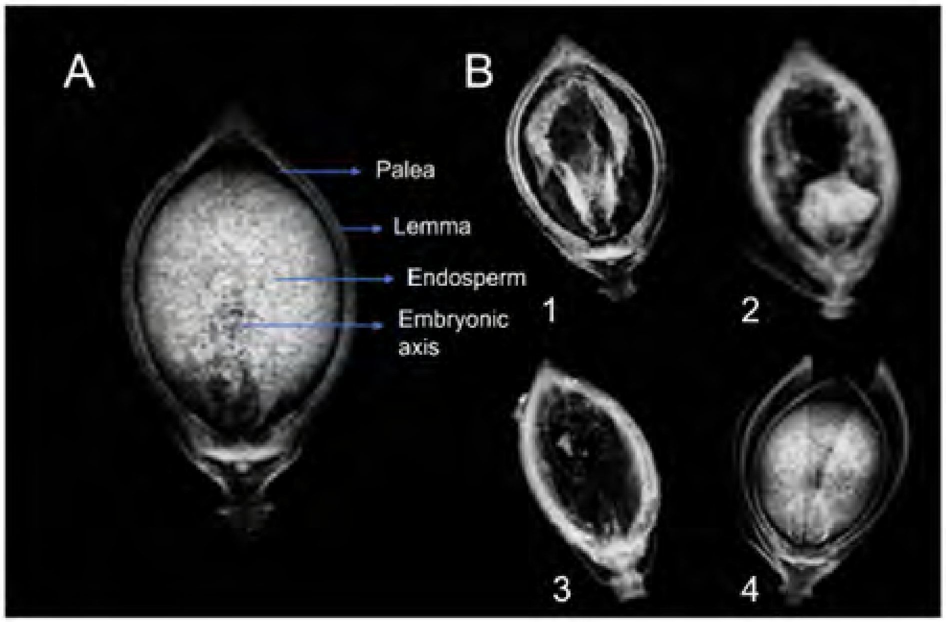

Figure 2. Radiographs of *Urochloa decumbens* seeds enabling visualization of their basic structures (A) and seeds with embryonic malformation or mechanical damage (B). 1, 2 – Embryonic malformation; 3 – tissue deterioration and 4 - cracks.

Table 3. Mean values for physiological variables obtained through tests of germination, GSI and total length of seedlings originated from five seed lots of *Urochloa decumbens*.

Lot	Root Protrusion	Normal Seedlings	GSI	Seedling Length
	(%)		(index)	(mm)
1	31 c	29 c	3.20 c	17.36 c
2	57 c	56 b	6.04 b	31.51 b
3	72 a	70 a	7.15 ab	52.14 a
4	75 a	75 a	8.14 a	24.51 bc
5	82 a	80 a	8.27 a	25.11 bc
FC	56.06*	69.46*	38.56*	54.43*
CV (%)	8.43	7.98	10.23	11.95

Lowercase letter = column grouping for each evaluation by Tukey test ($p < 0.05$); *= significant by F test ($p < 0.05$); FC = calculated F value; CV = coefficient of variation.

between lots were also detected when considering the physical parameters analyzed from X-ray images, such as area, perimeter, circularity, relative density, length, width, and well-formed, malformed, and empty seeds (Table 2).

The seed lots with higher germination and vigor showed strong and significant correlations with some of the physical variables obtained by SARS analysis (Figure 3). These variables included relative density, integrated density, length, width, solidity, area, perimeter, well-formed seeds, and malformed seeds.

The significant correlations show efficiency of the technique of automated analysis of radiographic images through SARS to obtain the physical characteristics of *Urochloa* seeds and a possible relationship of these physical parameters

Table 4. Mean values for total growth and growth and vigor indices obtained through the analysis of images of seedlings originated from five seed lots of *Urochloa decumbens*.

Lot	Growth	Uniformity	Vigor	Corrected Vigor
			(Index)	
1	373.70 c	270.25 c	342.67 c	101.74 c
2	678.15 b	477.34 b	617.91 b	343.51 b
3	1122.13 a	638.31 a	976. 98 a	674.45 a
4	527.45 bc	693.84 a	577.37 b	442.70 b
5	540.48 bc	738.28 a	599.82 b	480.77 b
FC	54.43*	55.62*	44.20*	38.85*
CV (%)	11.95	9.11	10.97	16.44

Lowercase letter = column grouping for each evaluation by Tukey test ($p < 0.05$); *= significant by F test ($p < 0.05$); FC = calculated F value; CV = coefficient of variation.

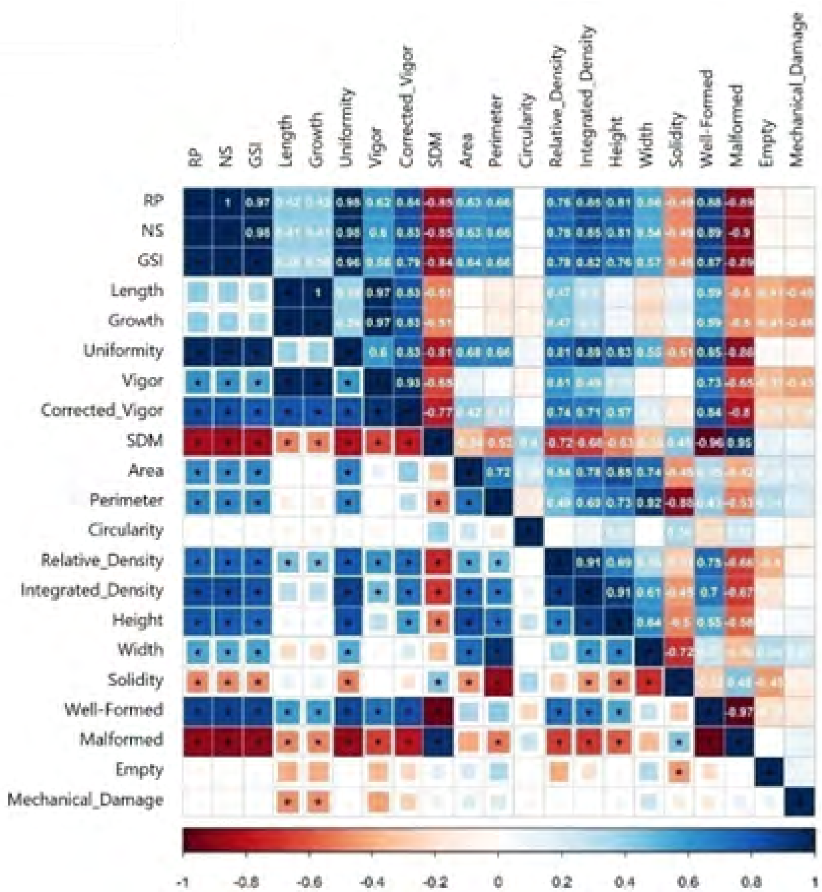

Figure 3. Correlation analysis of the physical variables obtained through radiographic analysis of seeds with SARS and physiological analyses.

with the physiological potential. The correlation between seedling length and relative density of seeds is important to validate the radiographic image analysis method to evaluate the physiological potential of seeds (Abud et al., 2018; Ribeiro et al., 2021). The analysis of correlation between these variables may indicate that the use of this method ensures efficiency to estimate the development of *Urochloa decumbens* cv. Basilisk seedlings.

The parameters relative density, integrated density, length, width, solidity, area, perimeter, well-formed seeds, and malformed seeds are related to seed shape and show efficiency in seedling performance (Figure 3). Mechanical damage and low seed filling lead to a lower value of radiolucency (dark), because the gray values are lower and consequently the relative density is reduced. Thus, seeds with low relative density tend to show low physiological potential.

It is important to highlight that the use of methodologies for qualitative evaluation of seeds in Brazil has improved in recent years, being considered good and faster tools that are able to reduce costs in seed storage and seedling production programs (Medeiros et al., 2018). Seed-producing companies have used tests such as germination and seedling length in decision-making processes to establish strategies for marketing seed lots of some species (Marcos-Filho, 2015).

However, these tests may have limitations related to the time spent to obtain the results, errors inherent to the subjectivity of the evaluations, besides being destructive (Huang et al., 2015; Rahman and Cho, 2016). According to the Rules for Seed Testing (Brasil, 2009), it takes 21 days of evaluation to perform the germination test in the species *Urochloa decumbens*. However, the evaluation of seed vigor in seven days was recently proposed by Jeromini et al. (2018) through computerized analysis of seedlings, but these authors concluded that the proposal was not adequate to evaluate the vigor of *Urochloa brizantha* seeds.

In view of the results obtained in this study, it was possible to observe that the SARS software proved to be efficient in the evaluation of physical characteristics such as area, perimeter, width, solidity, circularity, integrated density, and relative density, and that there is relationship between these characteristics and the physiological potential of the seeds, so it can be used to indicate quality differences between seed lots of *Urochloa decumbens* cv. Basilisk. Although it does not dispense conventional germination and vigor tests, as dead seeds can also have intact tissue, radiographic analysis can be used in quality control programs mainly for the disposal of low-quality seed lots. It means time and resources saving, therefore, SARS software is a promising tool and new perspectives can be created for its application in the forage sector with its improvement, thus ensuring greater efficiency in analysis and decision making in the seed industry.

CONCLUSIONS

Automated analysis of *Urochloa decumbens* seeds is efficient in obtaining data on tissue integrity. The variables obtained from SARS software are directly correlated with seed germination and vigor. However, among the variables obtained, the relative and integrated densities were those that stood out to predict the physiological potential of the seeds.

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