ABRATES

Journal of Seed Science
ISSN 2317-1545
www.abrates.org.br/revista

ARTICLE

Digital image processing of coated perennial-soybean seeds and correlation with physiological attributes

Journal of Seed Science, v.42, e202042004, 2020

http://dx.doi.org/10.1590/2317-1545v42227516

Amanda Justino Acha[1]*, Henrique Duarte Vieira[1]

ABSTRACT: Perennial soybean (*Neonotonia wightii*) is a *Fabaceae* with potential to be used in consortium with *Poaceae* plants to improve pasture quality. In order to add value to perennial soybean seeds and improve their seed distribution, seeds coated with different materials in coating machine were characterized by digital image analysis and physical attributes related to physiological attributes in order to define the ideal amount and material to be used in the coating. Different material quantities were tested, 150 g, 200 g and 250 g, divided into layers, namely: sand, calcium silicate + sand and limestone + sand. Coating promoted maximum increments of approximately 350% in seed mass and significant increases of up to 230% in area, 154% in maximum diameter, 162% in minimum diameter, 167% in contained diameter and 152% in perimeter. The coating was also efficient in reducing the moisture of the pellets by increasing the layers that cover the seeds. The sand + limestone combination resulted in the largest pellets. The combination of sand + silicate did not interfere with plant speed and formation. Thus, it was considered the appropriate material for the coating of perennial soybean seeds.

Index terms: seed coating, silicate, pellets, digital image analysis, *Neonotonia wightii*.

Processamento digital de imagens de sementes de soja perene revestidas e correlação com atributos fisiológicos

RESUMO: A soja perene (*Neonotonia wightii*) é uma *Fabaceae* com potencial para ser utilizada em consórcio com plantas da família *Poaceae*, a fim de melhorar a qualidade das pastagens. Na busca por agregar valor às sementes de soja perene e melhorar a sua distribuição na semeadura, sementes revestidas com diferentes materiais foram caracterizadas via análise de imagem digital e os atributos físicos relacionados com atributos fisiológicos, a fim de definir a quantidade e o material ideais a serem utilizados no revestimento. Foram testados 150 g, 200 g e 250 g de material divididos em camadas, sendo eles: areia, silicato de cálcio + areia e calcário + areia. O recobrimento promoveu incrementos máximos de aproximadamente 350% à massa das sementes e aumentos significativos de até 230% na área, 154% no diâmetro máximo, 162% no diâmetro mínimo, 167% no diâmetro contido e 152% no perímetro. O recobrimento foi eficiente, também, em reduzir a umidade dos péletes com o aumento das camadas que recobrem as sementes. A combinação de areia + calcário resultou nos maiores péletes. A combinação de areia + silicato não interferiu na velocidade e formação de plantas, sendo considerado o material adequado para o recobrimento de sementes de soja perene.

Termos para indexação: recobrimento, silicato, péletes, análise digital de imagens, *Neonotonia wightii*.

***Corresponding author**
E-mail: amandajacha@gmail.com

Received: 8/16/2019.
Accepted: 11/14/2019.

[1]Universidade Estadual do Norte Fluminense (UENF), Departamento de Produção Vegetal, 28013-602 – Campos dos Goytacazes, RJ, Brasil.

This article is part of the first author's doctoral thesis, which was defended at the Universidade Estadual do Norte Fluminense Darcy Ribeiro.

INTRODUCTION

In pasture management, the practice of intercropping plants with forages of the family *Fabaceae* has generated increased yields and contributed to the sustainability of the system, as it elevates the protein content of forage, improves nitrogen incorporation into the soil and increases protection against erosion. Additionally, this practice favors pest control and the maintenance of rest areas (Tambara et al., 2017).

According to Gama et al. (2013), the perennial soybean (*Neonotonia wightii* (Am.) Lackey) has great potential for use in intercropping due to its ability to incorporate 150 to 300 kg of N $ha^{-1}.yr^{-1}$, in addition to producing an average of 20 to 30 $t.ha^{-1}$ of fresh matter and 6 to 8 $t.ha^{-1}$ of dry matter yearly. This *Fabaceae* species can help in the recovery of degraded pastures in addition to attaining high yields, deep rooting and high defoliation resistance.

Constant improvement of seed-processing technologies is fundamental for the agricultural sector, since the current market is demanding and aware of the importance of quality (Melo et al., 2016). Seed coating is a technique capable of altering the physical traits of seeds which allows the seed producer to add mineral salts, fungicides or insecticides as necessary, thereby adding value to the seed for marketing (Derré et al., 2013).

For high-quality seeds to be supplied to the producer at a fair price, research must be conducted to define the methodology, type and concentration of materials capable of providing high-quality coating without compromising the seed. Many problems have been reported regarding the quality of coated seeds for pastures, which are due mainly to the use of inadequate practices; e.g. uneven coating, contamination by other species and coating that reduces seed germination. Divergences may exist between studies on seed coating due to the material used and thickness of the layer deposited on the seeds (Somrat et al., 2018; Xavier and Vieira, 2018).

Image analysis is a tool used in basic and applied studies that aims at elucidating various aspects of seed behavior and the improvement of methodologies for the evaluation of different seed-quality attributes. This method proposes to increase efficiency and reliability of results, as it reduces human error (Medeiros et al., 2018). The technique consists of obtaining information of recorded objects based on physical traits like color, texture, geometry etc. Because it is a non-destructive method, the seeds can be set to germinate after the image is captured. Thereafter, possible relationships between the physical and physiological traits can be defined based on the analyses (Guedes et al., 2011; Zang et al., 2018). However, information on the use of image analysis in the evaluation of the quality of coated seeds and the quality of the coating process is still limited, considering the broad diversity of existing species and the specific characteristics of each one. Thus, studies are warranted to improve this technique (Silva et al., 2014).

On these bases, the present study proposes to characterize, by digital image analysis, the physical attributes of coated perennial soybean seeds and evaluate their correlation with physiological attributes in order to define the optimum quantity and material to be used in coating.

MATERIAL AND METHODS

The *Neonotonia wightii* seeds were acquired from the BRSeeds® company and subjected to chemical scarification with concentrated sulfuric acid for twenty minutes, except for seeds for intact control treatment. Subsequently, they were washed in abundant water and left to dry at room temperature prior to being coated.

Seed coating

To coat the seeds, it was refined the technique employed by Acha et al. (2016), using a bench-top seed coating machine (N10 Newpack®) with the pan rotating at a speed of 64.5 rpm and the adhesive solution applied at a pressure of four bar, for two seconds. Next, the hot-air blower was activated at a temperature of 50 ºC, for three minutes.

The seed coating process consists of the formation of layers, which are composed of two portions of 12.5 g of filler material and two jets of adhesive material (Cascorez® extra glue, based on polyvinyl acetate [PVA] diluted in deionized water previously heated to 70 ºC, at the 1:2 [v/v] ratio, respectively).

One hundred grams of perennial-soybean seeds which had been previously scarified were placed in the coating pan alongside a portion of filler material (12.5 g). Next, a jet of adhesive solution was applied, and the seed mass was tumbled in the pan for one minute. Afterwards, another jet of glue and another 12.5 g portion of filler material were applied. For the following layers, a jet of adhesive solution was immediately applied, followed by a portion of filler material; then another jet of adhesive solution and then the second portion of the filler material. Subsequently, the hot-air blower was activated, finishing layer formation. This procedure was repeated until the programmed layers were complete.

Treatments

The following materials or combinations were tested as fillers: fine sand – A (sieved through a 0.35 mm square mesh); sand + calcium silicate – AS; and sand + dolomitic limestone – AC (0.25 mm), with the number of layers (grams of material) varying between 6 (150 g), 8 (200 g) and 10 (250 g). The filler materials were mixed at the 1:1 ratio before being deposited in the pan. Material density: fine sand: 2.91 g/cm³; calcium silicate: 2.66 g/cm³; dolomitic limestone: 2.86 g/cm³.

Tests and analysis

After coating, the seeds were evaluated for the physical and physiological traits.

Laboratory – The following variables were analyzed, following the Rules for Seed Testing (Brasil, 2009): one-thousand-seed weight, water content, between-paper germination test (% G) and germination speed index (GSI) (Maguire, 1962).

Greenhouse – The following variables were analyzed: emergence test (% E), performed in trays with coarse sand previously washed in abundant water, and emergence speed index (ESI), which was evaluated over thirty days (Maguire, 1962). Subsequently, at the end of ninety days, shoot and root length and dry matter were measured following the methodology adopted by Acha et al. (2018).

Digital analysis of the seeds – It was performed using GroundEye® S120 software, formerly known as SAS®, a semi-automated seed analysis tool which extracts numerous data from the capture of a high-resolution two-dimensional image. The system provides individual information of each seed and groups (the data into color, texture, shape, morphology and uniformity), providing over three hundred traits. After the variables were refined based on definition and importance, those which best met the needs for the evaluation of coating quality were selected (Table 1), namely,

Table 1. Variables obtained in the digital image analysis using GroundEye®.

Variable	Definition	Formula
Fine-tuning	It is a process of reducing the amount of pixels in an image, which consists in removing all redundant pixels producing a new simplified image. (Gonzalez and Woods, 2008)	Where A is the area, p is the perimeter and 4π is the normalization factor
Irregular contour	Sets the level of tuning of the object	T = Where P stands for perimeter and PC stands for convex perimeter
Contour deformation	Detects and counts the amount of "gaps" between the convex perimeter and the original perimeter of an object	–
Area (cm²)	Corresponding to the amount of space an object's surface has	Where p represents one image pixel and R represents object pixels
Maximum diameter (cm)	It is the longest line that goes through the centroid	–
Minimum diameter (cm)	It is the shortest line that goes through the centroid	–
Contained diameter (cm)	It is the largest diameter of the circumference that fits within the object	–
Perimeter (cm)	It is the measure of the contour of a two-dimensional object, that is, the sum of all sides of a geometric figure	2 (bh) – rectangle 2r – circle

fine-tuning, irregular contour and contour deformation, which relate to the contour; and area (cm^2), maximum diameter (cm), minimum diameter (cm), contained diameter (cm) and perimeter (cm), which pertain to seed size (TBIT, 2014).

Statistical procedure

The seed-coating experiment was undertaken as a completely randomized design with nine treatments in four replicates with 100 g of seed per treatment. The laboratory tests also followed a completely randomized design, whereas a randomized-block design was adopted for the greenhouse part of the experiment. Nine coating treatments and two control treatments (no scarification or scarified) were tested in the laboratory and in the greenhouse, in four replicates per environment, with fifty seeds each.

The data collected after the evaluations were subjected to the Shapiro-Wilk normality test, with no need for transformation and homoscedasticity of the variances evaluated by Bartlett's test. Analysis of variance was performed, and means were compared by Tukey's test at the 5% probability level, using Sisvar® statistical software. Pearson's correlation coefficient (r) was also calculated between the evaluated physical and physiological variables. For all analyzed variables, the control treatments were not included in analysis of variance and test of means; thus, for them, only descriptive analysis was performed.

RESULTS AND DISCUSSION

In the intact and scarified control treatments, one-thousand-seed weight was 5.82 and 5.55 g, respectively. For the different coatings tested, the coating methodology employed was efficient in providing increases of approximately 350, 280 and 200% in seed weight in the treatments with ten layers of sand + limestone, ten layers of sand + silicate and ten layers of sand, respectively, compared to the scarified control treatment (Figure 1). These values are certainly related to the density of materials and their combination; i.e., even though fine sand has the highest density (2.91 g/cm^3) among the tested materials, when combined with dolomitic limestone (2.86 g/cm^3) and calcium silicate (2.66 g/cm^3), it heightens their adherence to the seed, consequently providing higher seed weights.

In terms of seeding uniformity, the increase in seed weight is a positive factor, as it favors precision seeding, allowing the seeds to be used by most mechanical seeders. However, even though the increase in seed weight is an important criterion in the evaluation of coating quality, this criterion should not be evaluated individually, since an increase in seed weight is directly linked to the final cost of the product and to the physiological quality of the seed (Acha et al., 2016).

The water content of the coated seeds is of fundamental importance for the evaluation of their quality and proper storage (Marcos-Filho, 2015). The water content of seeds of the control treatments were 8.69% and 11.59%, for intact and scarified seed, respectively. After being coated, the seed moisture content was reduced in all treatments, being progressive with the increase of the number of layers. The lowest moisture content (4.33%) was observed in the treatment with ten layers of silicate + sand. Thus, it can be stated that the time and temperature for seed drying during coating was efficient in all treatments tested.

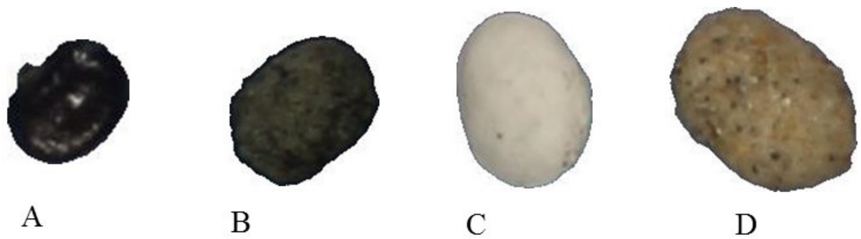

Figure 1. Perennial-soybean seeds. A) Intact seed. B) Sand coating – ten layers. C) Silicate + sand coating – ten layers. D) Limestone + sand coating – ten layers.

Among the variables provided by GroundEye® within the "shape" group, it was selected those that least met the coating-quality criteria referring to the geometric traits, which are important for evaluating, classifying and standardizing the pellets formed by the different treatments. Area, maximum, minimum and contained diameters and perimeter are the variables referring to seed geometry and which are responsible for describing the region occupied by the seed in the image plane. Guedes et al. (2011) evaluated the area, perimeter, maximum and minimum diameters, circularity and roundness of soybean seeds and concluded that digital analysis is valid to determine the physical quality of seeds when compared to manual evaluation methods.

All treatments were able to translate the increases provided in seed weight during coating into pellet size (Table 2). However, the treatments involving eight and ten layers of sand + limestone stood out with the highest means for the selected variables, demonstrating the adhesion power of the sand + limestone + PVA glue combination.

As seen for one-thousand-seed weight, the weight of coated seeds increased by 230, 180 and 190% in the sand + limestone, sand + silicate and sand treatments, respectively, when compared to the uncoated seeds, whose area was 0.036 cm^2. These gains were obtained in the treatments with ten coating layers. Coating also increased the diameters and the perimeter as the number of layers was increased. Thus, the increases provided to seed weight after coating were reflected on pellet size (Table 2).

As coating thickness is increased, the perennial-soybean seeds gain protection against the attack of pests, both in the field and in storage, slowing the seed deterioration process (Gardarin et al., 2010). Thickness is an important factor also when aiming to add fertilizers, fungicides and other agricultural additives, which should be added at a certain distance from the embryo to prevent toxicity. However, it is not a coating criterion to be evaluated separately, and thus other parameters should be used as well.

Considering that the area variable refers to the amount of space occupied by the object's surface, that the diameters are based on the object's circumference and that perimeter is the sum of the entire contour of the two-dimensional object (TBIT, 2014), these variables are directly linked and are positively influenced by one-thousand-seed weight ($r > 0.81$).

GroundEye® was efficient in determining the maximum and minimum diameters of coated *Stylosanthes* sp. and perennial-soybean seeds in the studies of Acha et al. (2016), Silva et al. (2017), Acha et al. (2018) and Xavier and Vieira (2018). These authors obtained significant increases for those variables with the different coating methodologies

Table 2. Digital analysis of coated perennial-soybean seeds by GroundEye® software. Area, maximum diameter (MAXD), minimum diameter (MIND), contained diameter (CONT) and perimeter.

Treatments*	Area (cm²)	MAXD (cm)	MIND (cm)	CONT (cm)	Perimeter (cm)
A6	0.051 d[1]	0.288 e	0.218 e	0.210 e	0.873 d
A8	0.062 bc	0.320 cd	0.238 cd	0.228 cd	0.967 c
A10	0.070 b	0.343 b	0.245 c	0.236 c	1.037 b
AS6	0.053 d	0.299 de	0.220 e	0.213 de	0.880 d
AS8	0.065 bc	0.327 bc	0.243 cd	0.239 bc	0.965 c
AS10	0.066 bc	0.328 bc	0.251 bc	0.246 bc	0.970 c
AC6	0.058 cd	0.308 cde	0.229 de	0.218 de	0.928 cd
AC8	0.079 a	0.365 a	0.263 ab	0.255 ab	1.087 ab
AC10	0.083 a	0.374 a	0.272 a	0.265 a	1.112 a
Intact seed	0.036	0.243	0.168	0.159	0.732
Scarified seed	0.036	0.250	0.163	0.156	0.736
CV%	5.54	2.78	2.78	3.04	2.68

*Control treatments: intact and scarified seeds. Scarified seeds with six, eight and ten layers of sand (A6, A8 and A10); with six, eight and ten layers of sand + calcium silicate (AS6, AS8 and AS10); and with six, eight and ten layers of sand + limestone (AC6, AC8 and AC10).

[1]Means followed by the same letter in the column do not differ significantly between each other according to Tukey's test at the 5% probability level.

applied, which served as base for refining the technique applied in this experiment.

Correlation analysis between the variables area, maximum, minimum and contained diameter, perimeter and one-thousand-seed weight provides precise information about the efficiency of the methodology in producing high-quality coating, which makes it possible to identify the best material and the proportion for adhesion. The analysis in GroundEye® indicates whether coating was able to change the initial seed shape, besides the possibility of rapidly and efficiently classifying and standardizing the pellets regarding their size, reducing the chances of errors made by the evaluator. Accordingly, interpreting these traits is essential for perfecting the coating technique, and this may result in the discard of treatments that do not meet the main objectives of seed coating, which are to change the size, shape and density of seeds.

For a reliable digital analysis of the traits represented by an image, one must make use of techniques that treat and eliminate false aspects that might be erroneously detected and interpreted. Among them, the fine-tuning variable is employed to reduce undesirable pixels; i.e., it is a process designed to reduce the form into a more simplified version (skeleton) while maintaining the essential characteristics of the original object, considering even small imperfections (Gonzalez and Woods, 2008; Artero and Tommsselli, 2009; Russi et al., 2017). It is a variable that indicates how many adjustments were necessary to prepare the image of the object for a perfect analysis. Fine-tuning is negatively correlated ($p < 0.05$) with contour irregularity ($r = -0.97$) and positively correlated with contour deformation ($r = 0.87$). This result was observed when correlating the data of the thinning variable with contour irregularity and contour deformation in all treatments tested.

The data presented in Table 3 confirm this correlation, as was observed that the treatments with eight and ten layers of sand + silicate provided significantly higher fine-tuning values. These treatments led to lower contour irregularity and, consequently, a higher deformation index, when compared to the other treatments.

It is believed that the high thinning index for silicate + sand treatments is associated with the fact that it has low fixation compared to the other materials, probably due to the difference in particle size between silicate and sand, resulting in a higher number of “blurs” in the image.

Considering that contour irregularity defines the level of fine-tuning of the analyzed object (TBIT, 2014), when was interpreted the indices obtained by digital image analysis through GroundEye®, it was observed an inverse relationship between contour irregularity and the fine-tuning values. Thus, lower the contour irregularity values mean a greater need

Table 3. Digital analysis of coated perennial-soybean seeds by GroundEye® software, describing fine-tuning, contour irregularity and contour deformation.

Treatments*	Fine-tuning	Contour irregularity	Contour deformation
A6	0.840 cd[1]	0.029 ab	26.340 e
A8	0.838 cd	0.029 ab	27.816 de
A10	0.817 d	0.032 a	27.655 de
AS6	0.869 ab	0.023 c	33.349 bc
AS8	0.874 a	0.023 c	36.165 ab
AS10	0.880 a	0.023 c	36.665 a
AC6	0.839 cd	0.028 b	28.390 de
AC8	0.836 cd	0.028 b	30.550 cd
AC10	0.845 bc	0.027 b	31.736 c
Intact seeds	0.840	0.29	30.260
Scarified seeds	0.840	0.28	31.162
CV%	1.21	5.03	4.48

*Control treatments: intact and scarified seeds. Scarified seeds with six, eight and ten layers of sand (A6, A8 and A10); with six, eight and ten layers of sand + calcium silicate (AS6, AS8 and AS10); and with six, eight and ten layers of sand + limestone (AC6, AC8 and AC10).

[1]Means followed by the same letter in the column do not differ significantly between each other according to Tukey’s test at the 5% probability level ($p < 0.05$).

to adjust the object represented in the image. It indirectly assists the evaluation of coating quality, as it is associated with the thinning variable.

In terms of contour deformation, the difference in particle size between fine sand and calcium silicate possibly provided a coating in which the materials did not fit perfectly, requiring for greater image corrections. Given this information, in the treatments in which only sand and the sand + limestone combination was used, contour uniformity was superior to that obtained with sand + silicate, suggesting that these particles fit better during coating. This is because sand and limestone share the same particle size (0.25 mm), thus needing less fine-tuning to be better interpreted by the software and forming a coating with fewer “gaps” between the convex perimeter and the original perimeter.

The results obtained for fine-tuning, contour irregularity and contour deformation demonstrate that it is possible to evaluate the aesthetic result of the combinatory or non-combinatory action of the materials tested in coating. These are important criteria to be adopted when aiming at improved pellet finishing quality.

Attesting the quality of the lot of perennial-soybean seeds used, a GSI of 2.5 and a germination percentage of 25.5% were detected in laboratory conditions for the intact seeds. For the seeds scarified in sulfuric acid, GSI was 11.4, and germination percentage was 59.5%. In the greenhouse, the seeds achieved an ESI of 1.7% and an emergence percentage 26.5% (intact seeds); and an ESI of 4.1% and 51.5% emergence (scarified seeds). In both environmental conditions, chemical scarification showed to be efficient, breaking the integument dormancy specific to seeds of *Neonotonia wightii* (Acha et al., 2016).

In examining the influence of the treatments on the speed and formation of normal perennial-soybean seedlings in the laboratory and in the greenhouse (Figures 1 and 2), it was observed that, in laboratory conditions, the maximum GSI and germination values achieved were 9.2 and 66%, respectively. In the greenhouse, the observed ESI and emergence percentage were 4.13 and 56%, respectively. For both conditions, those values refer to the treatment with six layers of sand.

The seeds coated with six layers were the lightest and smallest for all tested filler materials (Table 2). Thus, they possess a smaller barrier to be overcome during germination in comparison to the other treatment groups. In the specific case of treatment A6 (six layers of sand), the layer was more easily broken due to the particle size of sand, which reduces the adhesion of this material when in low amounts, especially in the scarified seeds, which have a smoother surface. For this reason, the A6 treatment resulted in coating with many imperfections and, consequently, a product of lower quality that did not meet the main purpose of seed coating, which is to increase its size.

Seed scarification is known to accelerate the soaking process, which at one point may cause alterations in the conformation and structure of the membrane system, influencing germination (Marcos-Filho, 2015). Considering that the A6 treatment numerically exceeded (G = 66% and E = 56%) the values of normal seedlings achieved by the scarified-control seeds (G = 59.5% and E = 51.5%), it is believe that the treatment with six layers of sand provided an external protection to the seeds by controlling the water input, preventing the scarified seeds from suffering physiological damage that would be reflected in the formation of normal seedlings with the rapid water absorption.

The treatments of perennial-soybean seeds with sand + silicate stood out positively for germination (GSI) and formation of seedlings in laboratory conditions (% G), not differing statistically from the treatment with six layers of sand, which achieved the highest numerical values (Figures 2 and 3). Thus, the sand + silicate combination provided a coating in which the seeds managed to break the created barrier with greater ease, regardless of the amount of material tested in this study.

It is noteworthy that coating should not be an obstacle to root development or to the plant shoots, but rather be water-soluble and allow the passage of oxygen for natural embryo development (Santos et al., 2010). As observed by Xavier et al. (2015) in seeds of *Stylosanthes* cv. Campo Grande, the coating formed by layers of calcium silicate + sand in this study, in *Neonotonia wightii* cv. Comum, easily came apart when in contact with water, regardless of the number of layers tested. As such, this coating stood out positively.

Despite using larger proportions of filler materials and mechanically scarified seeds, unlike the methodology applied in the current study, Xavier and Vieira (2018) also observed positive results in the germination of perennial-soybean seeds coated with calcium silicate alone, combined with sand and combined with activated coal. The authors found no statistical differences between the treatments.

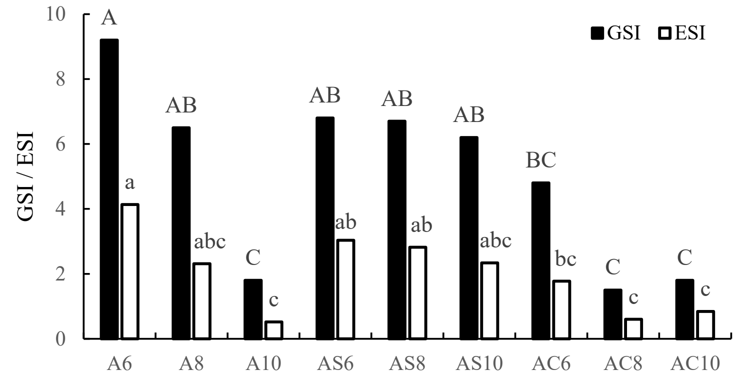

Figure 2. Germination speed index (GSI) of coated perennial-soybean seeds and seedling emergence speed index (ESI). Scarified seeds with six, eight and ten layers of sand (A6, A8 and A10); with six, eight and ten layers of sand + calcium silicate (AS6, AS8 and AS10); and with six, eight and ten layers of sand + limestone (AC6, AC8 and AC10).

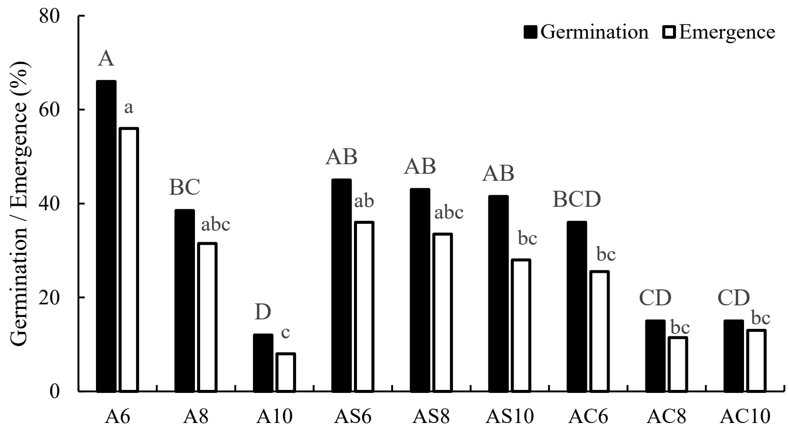

Figure 3. Germination percentage of coated perennial-soybean seeds and seedling emergence. Scarified seeds with six, eight and ten layers of sand (A6, A8 and A10); with six, eight and ten layers of sand + calcium silicate (AS6, AS8 and AS10); and with six, eight and ten layers of sand + limestone (AC6, AC8 and AC10).

The sand + limestone combination used in coating provided greater resistance to the pellets. Regardless the number of layers, this treatment tended to generate the lowest GSI, ESI and germination and emergence percentages (Figures 2 and 3), though not differing statistically from the treatments with ten layers of sand. Considering that the portion and type of material used influence the diffusion of gases and water between the seed and the external environment (Nascimento et al., 2009), it can be stated that because sand and limestone share the same particle size (0.25 mm) and are denser, the increase in the number of sand + limestone layers also causes an increase in particle aggregation capacity and, with their perfect fit, a higher coating resistance is obtained. This makes it difficult for gas exchange.

Therefore, the sand + limestone combination (AC6, AC8 and AC10) and the use of 250 g of sand (A10) cause an impairment in water uptake. Consequently, low percentages of formed seedlings were achieved in comparison to the other treatments (Figures 2 and 3). These results were also observed by Xavier et al. (2015) and Santos et al. (2010) in seeds of *Stylosanthes* and *Brachiaria* grasses, respectively.

The values achieved in the coating treatments under different environmental conditions reveal a numerical decrease in the speed and formation of normal seedlings in the greenhouse compared to the number attained in laboratory. Given the reduced environmental control in a greenhouse (i.e. temperature and luminosity fluctuations, compared to the test conducted in laboratory, and how the emergence test was conducted [in trays with sand as a substrate]), it is believed that these variations delayed germination and seedling formation, since sudden changes in environmental conditions tend to deregulate the seed metabolism (Marcos-Filho, 2015), and the use of sand as a substrate is another barrier to be overcome by the seedling.

The amount and material of filler used in seed coating did not significantly influence ($p < 0.05$) the growth of seedlings formed in the greenhouse after ninety days of sowing (Table 4). However, the treatment with six layers of sand stood out for the numerical gains obtained in shoot dry matter (2.91 g/pl) and root dry matter (5.54 g/pl) in relation to scarified-control treatment (1.36 g/pl and 4.30 g/pl, respectively for shoot and root dry matter). Thus, once again, the protective action of the seed coating is highlighted, where the six layers of sand promote a slower soak to the seed, reducing damage to the cell membrane and thus favoring the emergence and formation of plants.

Despite not significantly differing from the other treatments, the combination of ten layers of sand + limestone provided a 15% gain in shoot length and a 52% gain in root length when compared to the scarified-control treatment, which provided 1.36 cm and 16.97 cm long shoots and roots, respectively. This result is believed to be related to the action of calcium on the plant metabolism, where it acts as an essential element that plays an important role in the division and elongation of plant cells (Ahmad et al., 2016).

It should be emphasized that all coating treatments resulted in higher root length values than the scarified-control treatment (16.97 cm) (Table 4). Xavier et al. (2015) examined the growth of *Stylosanthes* cv. Campo Grande plants originating from seeds coated with different materials, including the sand + calcium silicate and sand + limestone combinations, and observed no significant effects of the tested treatments. However, seed coating provided numerical gains in plant growth, when compared to control treatment.

To classify the coating quality, the evaluation criteria must be associated so that decisions are not made based solely on only one trait. It is emphasized that these criteria should represent the important physical and physiological traits for the evaluation of seed quality. Therefore, the geometric traits (area, maximum, minimum and corrected diameters and perimeter) pertaining to pellet size obtained by GroundEye® were correlated (r) with the physiological variables (germination, GSI, emergence and ESI) evaluated in laboratory and greenhouse conditions (Table 5).

There was an inverse relationship between the physical and physiological variables; i.e., as the area, maximum, minimum and corrected diameter and perimeter increased, there was a significant decrease in germination and emergence percentages as well as in the speed of plant formation in laboratory (GSI) and greenhouse (ESI), for the treatments in which the seeds were coated with sand and sand + limestone. In the treatments involving sand + silicate, however, regardless of pellet size, there was no significant effect on the speed and formation of normal seedlings; i.e.,

Table 4. Plant growth parameters under greenhouse conditions, evaluated ninety days after sowing.

Treatments*	Shoot length (cm)	Dry shoot mass (g/pl)	Root length (cm)	Root dry mass (g/pl)
A6	1.42 ± 0.38[1]	2.91 ± 2.44	18.45 ± 0.97	5.54 ± 5.46
A8	1.28 ± 0.32	0.65 ± 0.31	18.59 ± 1.06	1.20 ± 0.68
A10	0.99 ± 0.21	0.20 ± 0.13	21.19 ± 2.74	0.32 ± 0.26
AS6	1.26 ± 0.32	1.86 ± 0.50	18.80 ± 0.79	3.49 ± 2.40
AS8	1.22 ± 0.39	2.27 ± 1.51	20.17 ± 3.93	2.12 ± 1.54
AS10	1.21 ± 0.29	1.42 ± 1.04	19.52 ± 1.58	2.28 ± 1.88
AC6	0.95 ± 0.66	2.22 ± 2.42	19.21 ± 1.70	2.89 ± 3.00
AC8	1.04 ±0.31	0.28 ± 0.10	17.23 ± 4.95	0.52 ± 0.26
AC10	1.56 ± 0.53	0.69 ± 0.69	25.74 ± 8.27	0.78 ± 0.75
Intact seeds	1.09 ± 0.40	1.36 ± 0.55	18.06 ± 1.34	1.74 ± 0.92
Scarified seeds	1.36 ± 0.32	2.87 ± 1.71	16.97 ± 1.35	4.30 ± 2.27

*Control treatments: intact and scarified seeds. Scarified seeds with six, eight and ten layers of sand (A6, A8 and A10); with six, eight and ten layers of sand + calcium silicate (AS6, AS8 and AS10); and with six, eight and ten layers of sand + limestone (AC6, AC8 and AC10).
[1]Means ± standard deviation (n = 4).

sand + calcium silicate and the tested amounts of this combination did not significantly influence the physiological quality of the seeds when compared to the other treatments.

Despite not being an element essential to plants, calcium silicate is known to cause alterations in chemical composition, cell mechanical resistance, tolerance to abiotic stresses and attack of pathogens and pests, making the seed more vigorous (Rodrigues et al., 2011). Therefore, silicate coating has great potential to be the base for the inclusion of fertilizers, insecticides and/or fungicides, thereby adding greater qualitative value to the seeds.

In coated seeds, the increase in seed size leads to reduced germination and emergence speed because the material deposited on the seed becomes a physical barrier that needs to be overcome (Acha et al., 2016). Thus, in the present study, GroundEye® was efficient in evaluating the quality of the cover through physical evaluations related to the characteristics of the contour and seed size. These variables were used to estimate the physiological quality of perennial soybean seeds coated with the different materials tested. As such, it is a satisfactory piece of equipment for researchers seeking to improve coating techniques and large-scale companies, as it provides high accuracy and speed assessments and is not destructive.

Upon the conclusion of assessments, it is observed that the combination of ten layers of sand + silicate provided satisfactory results for seed coating quality, considering the 280% increase in seed weight, facilitating mechanical sowing, and the increase in seed thickness, with gains in area, diameters and perimeter, which increase seed protection and facilitate the application of agricultural additives. This treatment is thus of great relevance, as it did not significantly interfere with the speed and formation of seedlings in laboratory and greenhouse conditions, in addition to had provided numerical gains in root growth, when compared to the other coating treatments tested.

Table 5. Correlation analysis Person's (r) of the different tested materials, between the physical variables area, maximum diameter (MAXD), minimum diameter (MIND), corrected diameter (CORD) and perimeter; and the physiological variables germination (G), germination speed index (GSI), emergence (E) and emergence speed index (ESI).

Sand	G	GSI	E	ESI
Area	-0.870*	-0.855*	-0.778*	-0.814*
MAXD	-0.869*	-0.857*	-0.788*	-0.818*
MIND	-0.846*	-0.816*	-0.727*	-0.768*
CORD	-0.885*	-0.870*	-0.701*	-0.739*
Perimeter	-0.882*	-0.864*	-0.789*	-0.823*
Silicate + sand	G	GSI	E	ESI
Area	0.215	0.175	-0.329	-0.393
MAXD	0.185	0.123	-0.301	-0.375
MIND	0.271	0.231	-0.294	-0.350
CORD	0.271	0.219	-0.295	-0.351
Perimeter	0.208	0.173	-0.331	-0.396
Limestone + sand	G	GSI	E	ESI
Area	-0.839*	-0.807*	-0.597*	-0.652*
MAXD	-0.842*	-0.813*	-0.597*	-0.651*
MIND	-0.831*	-0.785*	-0.586*	-0.659*
CORD	-0.815*	-0.773*	-0.588*	-0.666*
Perimeter	-0.832*	-0.800*	-0.605*	-0.657*

*Significant according to Pearson's (r) test at the 5% probability level.

CONCLUSIONS

Digital image analysis is efficient in precisely determining the physical traits of coated seeds, regardless of the material used. The correlation between the physical and physiological variables reveals that the progressive increase in the size of seeds coated with sand and sand + limestone negatively influences the speed and formation of normal seedlings in laboratory and greenhouse conditions. The sand + calcium silicate combination in the amount of 250 g, split into ten layers, is the most recommended material/quantity to coat seeds of perennial soybean.

ACKNOWLEDGMENTS

CAPES, FAPERJ, UENF.

REFERENCES

ACHA, A.J.; VIEIRA, H.D.; SOUZA, C.L.M.; SILVA, F.W.A. Methodology of applying different doses of boron and zinc in the coating of perennial soybean seeds. *Journal of Experimental Agriculture International*, v.26, n.5, p.1-9, 2018. https://doi.org/10.9734/JEAI/2018/43968

ACHA, A.J.; VIEIRA, H.D.; FREITAS, M.S.M. Perennial soybean seeds coated with high doses of boron and zinc. *African Journal of Biotechnology*, v.15, n.37, p.1998-2005, 2016. http://dx.doi.org/10.5897/AJB2016.15560

AHMAD, P.; ABDEL LATEF, A.A.; ABD ALLAH, E.F.; HASHEM, A.; SARWAT, M.; ANJUM, N.A.; GUCEL, S. Calcium and potassium supplementation enhanced growth, osmolyte secondary metabolite production and enzymatic antioxidant machinery in cadmium-exposed chickpea (*Cicer arietinum* L.). *Frontiers in Plant Science*, v.7, n.513, p.1-12, 2016. https://doi.org/10.3389/fpls.2016.00513

ARTERO, A.O.; TOMMASELLI, A.M.G. Detecção e afinamento de bordas em direções previamente conhecidas. *Boletim de Ciências Geodésicas*, v.15, n.2, p.157-177, 2009. http://www.redalyc.org/articulo.oa?id=393937708001

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS, 2009. 395p. http:// www.agricultura.gov.br/arq_editor/file/2946_regras_analise__sementes.pdf

DERRÉ, L.O.; CUSTÓDIO, C.C.; AGOSTINI, E.A.T.; GUERRA, W.E.X. Obtenção das curvas de embebição de sementes revestidas e não revestidas de *Urochloa brizantha* e *Urochloa ruziziensis*. *Colloquium Agrariae*, v.9, n.2, p.103-111, 2013. http://revistas.unoeste.br/index.php/ca/article/view/937

GAMA, T.D.C.M.; VOLPE, E.; LEMPP, B.; COSTA GALDEIA, E. Recuperação de pasto de capim-braquiária com correção e adubação de solo e estabelecimento de leguminosas. *Revista Brasileira de Saúde e Produção Animal*, v.14, n.4, p.635-647, 2013. http://revistas.ufba.br/index.php/rbspa/article/view/2735

GARDARIN, A.; DÜRR, C.; MANNINO, M.R.; BUSSET, H.; COLBACH, N. Seed mortality in the soil is related to seed coat thickness. *Seed Science Research*, v.20, n.4, p.243-256, 2010. https://doi.org/10.1017/S0960258510000255

GUEDES, M.A.; MATA, M.E.R.M.C.; DUARTE, M.E.M.; FARIAS, P.A. Caracterização física de grãos de soja utilizando-se processamento digital de imagens. *Revista Brasileira de Produtos Agroindustriais*, v.13, n.3, p.279-294, 2011. http://www.deag.ufcg.edu.br/rbpa/rev133/Art1338.pdf

GONZALEZ, R.C.; WOODS, R.E. *Digital image processing*. 2nd ed. Upper Saddle River, New Jersey: Prentice Hall, 2008. https://pdfs.semanticscholar.org/15bd/427a1a5f9bc57a7f67fb1b1fc85c5bb39f46.pdf

MAGUIRE, J.D. Speed of germination-aid selection and evaluation for seedling emergence and vigor. *Crop Science*, v.2, n.1, p.176-177, 1962. http://dx.doi.org/10.2135/cropsci1962.0011183X000200020033x

MARCOS-FILHO, J. Deterioração de sementes. Fisiologia de sementes de plantas cultivadas. 2nd ed. Londrina, PR: ABRATES, 2015. 660p.

MEDEIROS, A.D.; PEREIRA, M.D.; SILVA, J.A. Processamento digital de imagens na determinação do vigor de sementes de milho. *Revista Brasileira de Ciências Agrárias*, v.13, n.3, p.1-7, 2018. http://doi:10.5039/agraria.v13i3a5540

MELO, L.F.; MARTINS, C.C.; SILVA, G.Z.; BONETI, J.E.B.; VIEIRA, R.D. Beneficiamento na qualidade física e fisiológica de sementes de capim-mombaça. *Revista Ciência Agronômica*, v.47, n.4, p.667-674, 2016. http://dx.doi.org/10.5935/1806-6690.20160080

NASCIMENTO, W.M.; SILVA, J.B.C.D.; SANTOS, P.E.D.C.; CARMONA, R. Germinação de sementes de cenoura osmoticamente condicionadas e peletizadas com diversos ingredientes. *Horticultura Brasileira*, v.27, n.1, p.12-16, 2009. http://cms.horticulturabrasileira.com.br/images/stories/27_1/20092712.pdf

RODRIGUES, F.A.; OLIVEIRA, L.A.K.; KORNDORFER, A.P.; KORNDORFER, G.H. Silício: um elemento benéfico e importante para as plantas. *Informações Agronômicas*, n.134, p.14-20, 2011. http://www.ipni.net/publication/IA-BRASIL.NSF/issue/IA-BRASIL-2011-134

RUSSI, C.C.; SILVA, F. A.; PEREIRA, D.R.; PAZOTI, M.A.; ALMEIDA, L.L.; ARTERO, A.O. Detecção do padrão de motricidade fina em pacientes com doença de Parkinson a partir de imagens digitalizadas. *Colloquium Exactarum*, v.9, n.1, p.33-44, 2017. http://revistas.unoeste.br/index.php/ce/article/view/1971

SANTOS, F.C.; OLIVEIRA, J.A.; VON PINHO, E.V.R.; GUIMARÃES, R.M.; VIEIRA, A.R. Tratamento químico, revestimento e armazenamento de sementes de *Brachiaria brizantha* cv. Marandu. *Revista Brasileira de Sementes*, v.32, n.3, p.69-78, 2010. http://www.scielo.br/pdf/rbs/v32n3/v32n3a08.pdf

SILVA, F.W.A.; VIEIRA, H.D.; BARONI, D.F.; MAITAN, M.Q.; ACHA, A.J. Germination performance of Campo Grande (*Stylosanthes capitata/macrocephala*) stylers seeds coated with different layers of inert material. *Journal of Experimental Agriculture International*, v.18, n.4, p.1-8, 2017. https://doi.org/10.9734/JEAI/2017/36768

SILVA, P.P.; FREITAS, R.A.; CÍCERO, S.M.; MARCOS-FILHO, J.; NASCIMENTO, W.M. Análise de imagens no estudo morfológico e fisiológico de sementes de abóbora. *Horticultura Brasileira*, v.32, n.2, p.210-214, 2014. http://cms.horticulturabrasileira.com.br/images/stories/32_2/201432216.pdf

SOMRAT, N.; SAWADEEMIT, C.; VEARASILP, S.; THANAPORNPOONPONG, S.N.; GORINSTEIN, S. Effects of different binder types and concentrations on physical and antioxidant properties of pelleted sweet corn seeds. *European Food Research and Technology*, v.244, n.3, p.547-554, 2018. https://doi.org/10.1007/s00217-017-2979-y

TAMBARA, A.A.C.; SIPPERT, M.R.; JAURIS, G.C.; FLORES, J.L.C.; HENZ, É.L.; VELHO, J.P. Production and chemical composition of grasses and legumes cultivated in pure form, mixed or in consortium. *Acta Scientiarum Animal Sciences*, v.39, n.3, p.235-241, 2017. http://dx.doi.org/10.4025/actascianimsci.v39i3.34661

TBIT. Tecnologia e Sistemas. *Manual de usuário do sistema de análise de sementes*. Lavras, MG, 2014. 152p.

XAVIER, P.B.; VIEIRA, H.D. Physiological potential of seeds of perennial soybean cv. Comum coated with different materials. *American Journal of Plant Sciences*, v.9, n.1, p.1-12, 2018. http://www.scirp.org/journal/ajps

XAVIER, P.B.; VIEIRA, H.D.; AMORIM, M.M. Physiological potential of *Stylosanthes* cv. Campo Grande seeds coated with different materials. *Journal of Seed Science*, v.38, n.4, p.117-124, 2016. http://dx.doi.org/10.1590/2317-1545v38n4164209

ZHANG, C.; SI, Y.; LAMKEY, J.; BOYDSTON, R.; GARLAND CAMPBELL, K.; SANKARAN, S. High-throughput phenotyping of seed/seedling evaluation using digital image analysis. *Agronomy*, v.8, n.5, p.1-14, 2018. https://doi.org/10.3390/agronomy8050063