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Image analysis of the seeds and seedlings of *Vigna radiata* L.[1]

Análise de imagens de sementes e plântulas de *Vigna radiata* L.

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ABSTRACT - In agriculture it is essential to use high-quality seeds capable of expressing their maximum vigour. Image analysis has been used to assess the quality of seed batches. The aim of this study was to characterise seedling growth in the mung bean and the external morphology of the seeds using image analysis, and identify the morphological characteristics associated with high physiological potential. The trial was conducted using two accessions of the mung bean (yellow and green). The 1000-seed weight and germination test were carried out in an initial characterisation. Using image analysis, the following parameters were evaluated in the seeds: area, perimeter, colour, circularity, length and width. In the seedlings, the length and dry matter of the shoots and root system were evaluated together with the total dry matter. A germination of 100% and 89.5%, and 1000-seed weight of 54.91 g and 35.49 g were found for the yellow and green mung beans. In the yellow mung bean, the largest seeds showed a direct relationship with the other morphological parameters of the seeds in addition to a strong relationship between the length of the shoots and the root system. In the green mung bean, there was a direct relationship between seed area and perimeter, length, width and colour; however, the best seedling performance was found in the smaller seeds. The use of image analysis makes it possible to identify seeds of greater physiological potential, which allows more-vigorous batches of mung bean seeds to be selected.

Key words: Physiological potential. Morphological characteristics. Physical attributes.

RESUMO - Na agricultura é indispensável a utilização de sementes de alta qualidade, capazes de expressar o seu máximo vigor. Técnicas de análise de imagens têm sido utilizadas na avaliação da qualidade de lotes de sementes. Objetivou-se caracterizar a morfologia externa das sementes e crescimento de plântulas de feijão-mungo por meio de análise de imagens, para identificar características morfológicas associadas ao alto potencial fisiológico. O ensaio foi realizado utilizando-se dois acessos de feijão-mungo (amarelo e verde). Para caracterização inicial realizou-se o peso de mil sementes e o teste de germinação. Por meio de análise de imagens, avaliou-se os seguintes parâmetros para sementes: área, perímetro, cor, circularidade, comprimento e largura. Para plântulas foram avaliados comprimento e massa seca de parte aérea, sistema radicular e total. Verificou-se germinação de 100 e 89,5% e peso de mil sementes 54,91 g e 35,49 g para feijões mungo amarelo e verde. Para o mungo amarelo verificou-se que as maiores sementes apresentaram relação direta com outros parâmetros morfológicos das sementes, além de alta relação comprimento de parte aérea e sistema radicular das plântulas. Para o mungo verde, houve relação direta entre área da semente e os parâmetros perímetro, comprimento, largura e cor, no entanto, o melhor desempenho de plântulas foi observado para as sementes menores. O uso da técnica de análise de imagens possibilita identificar sementes com maior potencial fisiológico, o que permite selecionar lotes mais vigorosos de sementes de feijão-mungo.

Palavras-chave: Potencial fisiológico. Características morfológicas. Atributos físicos.

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INTRODUCTION

The mung bean (*Vigna radiata* L.) is an annual, erect or semi-erect legume. It is traditionally grown in Asia, where India stands out as the world's largest producer (SILVA *et al*., 2018; VIEIRA *et al*., 2011). In China, Japan and the USA, among other countries, it is widely consumed in the form of bean sprouts, which are obtained after the seeds germinate (ARAUJO *et al*., 2011). In Brazil, cultivation of the mung bean has been increasing due to the inclusion of bean sprouts in the diet, (KEATING, 2011; OLIVEIRA *et al*., 2013; VIEIRA *et al*., 2003).

In agriculture, in order to achieve a higher yield per area, in addition to suitable cropping techniques, it is essential to use high-quality seeds, capable of expressing their high vigour by means of their physical, sanitary, genetic and physiological attributes (SILVA *et al*., 2018). The physiological quality of seeds is determined via tests carried out in the laboratory and which estimate seed vigour; however, these procedures generally require long periods of analysis (MEDEIROS; PEREIRA; SILVA, 2018).

Image analysis of seeds and seedlings is one potential technique, widely used in research, which relates characteristics of seed morphology to seedling performance, thereby expressing seed vigour with greater precision and less subjectivity, affording quick results and where, in most tests, the analysis is non-destructive (MEDEIROS *et al*., 2019; NORONHA *et al*., 2019; VASCONCELOS *et al*., 2018).

Through image analysis it is possible to obtain such variables as area, circularity, length, width, perimeter and colour of the seed coat, among others. These variables can be evaluated using software to characterise the physical aspects of the seeds. In addition, a relationship with the physiological characteristics is possible, allowing the selection of seed batches presenting metabolic processes that may afford a faster growth rate and greater uniformity during the germination process (LIMA *et al*., 2018; VASCONCELOS *et al*., 2018).

Promising results using different techniques of image analysis were found in research carried out with different species of varying sizes and morphological characteristics, such as radiographic images related to the internal morphology of broccoli seeds (ABUD; CICERO; GOMES JUNIOR, 2018), the analysis of radiographic images related to the physiological quality of leucaena seeds (MEDEIROS; PEREIRA; SILVA, 2018), the determination of physiological quality during the imbibition process in moringa seeds (NORONHA; MEDEIROS; PEREIRA., 2019) and the image analysis of soya seeds and classification of the seedlings by vigour (MEDEIROS *et al*., 2020).

Given the limited information available on the quality of mung bean seeds, in addition to the possibility of using techniques that reduce the subjectivity of the evaluations, the aim of this study was to characterise the external morphology of the seeds and seedling growth in yellow and green mung beans using image analysis to identify the morphological characteristics associated with high physiological potential.

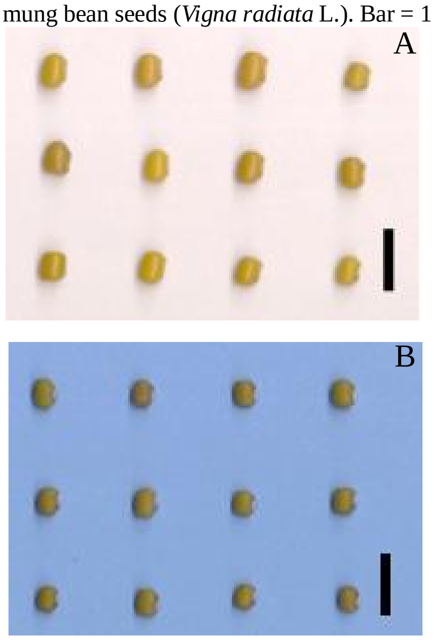
MATERIAL AND METHODS

The experiment was conducted at the Seed Analysis Laboratory of the Department of Plant Sciences, Federal University of Ceará (UFC). Two mung bean accessions from the cowpea Active Germplasm Bank (UFC) were used in the research, one with a green seed coat (2008 harvest) and the other yellow (2010 harvest), which were kept in a cold chamber at 10 °C and an RH of 45%.

In an initial characterisation of the seed batch, the 1000-seed weight was determined as per the Rules for Seed Analysis (BRASIL, 2009).

In the image analysis, 200 seeds were initially distributed in two replications of 100 seeds, on sheets of ethylene vinyl acetate (EVA), white for the yellow mung bean and blue for the green mung bean, so as to ensure high contrast with the colour of the seed coat (Figure 1), as defined in preliminary tests. The images were captured at a resolution of 300 dpi by a scanner (HP Scanjet 2004) mounted in an inverted position inside an aluminium box. The images were stored for later analysis.

Figure 1 - RGB (Red-Green-Blue) images of yellow (A) and green (B) mung bean seeds (*Vigna radiata* L.). Bar = 1 cm



The ImageJ® software (Image Processing and Analysis in Java) was used for the morphological characterisation of the seeds. The variables obtained from the seeds were: **area** – selected area obtained in pixels and later converted into one square-centimetre units (cm^2); **RGB** (Red-Green-Blue) seed-coat colour – obtained from the average grey-scale colour of the pixels of the individual images of each seed; **perimeter** – the length of the outer limit of the selected area, in centimetres (cm); **circularity** – obtained with the equation R = 4π x {(area)/(perimeter2)}, where values close to 1.0 indicate a perfect circle and values close to 0 suggest an elongated shape; **length** - measured longitudinally in the central region of the seeds, in centimetres (cm); **width** - measured transversely from the hilum to the dorsal region in the central area of the seeds, in centimetres (cm).

The seeds were then submitted to the germination test following the same order as the images for the morphological characterisation, using ten replications of 20 seeds in a Biochemical Oxygen Demand (BOD) chamber at an alternating temperature of 20-30 ºC, and evaluated on the seventh day, as per the recommendations of the Rules for Seed Analysis (BRASIL, 2009).

At the end of the germination test, the seedlings were transferred to the blue EVA sheets (Figure 2), defined in preliminary tests as the best contrasting colour for measuring the length of the seedlings. As with the seeds, image analysis employing the ImageJ® software was used to obtain the following variables: shoot length (**SL**), root system length (**RL**), and total length (**TL**= SL+RL) in centimetres (cm) using the 'freehand line' tool.

To determine the shoot (SDW) and root dry weight (RDW), each part of the seedlings was then placed in individual Kraft paper bags and kept at a temperature of 65 ºC for 48 hours. The dry matter was then weighed using a precision scale (0.001 g), and the results expressed in milligrams (mg) per seedling.

A completely randomised experimental design (CRD) was used. After obtaining the variables for each seed and seedling individually, the seeds were classified according to their area and then distributed into four size classes (Table 1) by means of frequency distribution analysis using the R v3.6. software. The remaining variables were classified as a function of seed area.

By means of multivariate principal component analysis, the mean values were calculated and transformed to a mean of zero and variance of one to avoid overestimating or underestimating the weight of the variable under study due to differences in the measurement scale. The principal component analysis was calculated using an n x p matrix, for which 'n' is the number of classes (4) and 'p' is the mean result for each variable (11) within each class. From the correlation matrix, the eigenvalues (values representing the variability retained by each new component) and eigenvectors (linear combination of the parameters under evaluation) were

Figure 2 - Images of yellow mung bean seedlings seven days after the start of the germination test, showing the measurement of seedling length (marked in yellow to obtain the length of the individual shoots)

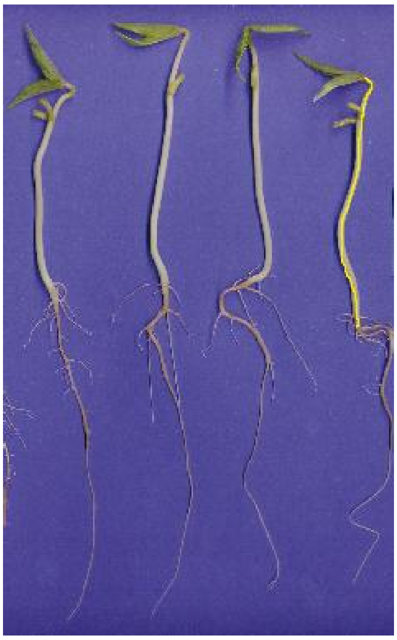


Table 1 - Size classes based on the frequency distribution of seed area in the two mung bean accessions

Class	Seed area (cm^2)			
	Yellow mung bean	Relative frequency	Green mung bean	Relative frequency
I	0.095 - 0.128	46	0.077 - 0.092	19
II	0.129 - 0.139	54	0.093 - 0.099	39
III	0.140 - 0.150	55	0.100 - 0.107	62
IV	0.151 - 0.194	45	0.108 - 0.145	80

calculated. The results were expressed on a two-dimensional graph (biplot) using the JMP® Pro 14.0 software. Pearson's correlation coefficient was estimated at 5% probability for the relationship between the biometric characteristics of the seeds and seedlings.

RESULTS AND DISCUSSION

The 1000-seed weight for the yellow and green mung beans was 54.91 and 35.49 g respectively. According to Brasil (2009), this information gives an idea of the size of the seeds, as well as their maturation stage and health. In addition, it aids in such operations as sowing, marketing and seed analysis, where knowledge of this aspect of the seeds is necessary.

The germination percentage found for the yellow and green mung bean was 100% and 89% respectively, showing that the batches presented high performance in relation to seed viability.

The seeds were distributed into four classes by area, in ascending order, where class IV (CIV) contained the largest seeds. Principal component analysis (CPA) allows the original information contained in the *p* variables ($p = 11$) to be condensed into two or more orthogonal latent variables called the principal components, which are a linear combination of the original variables created from the two largest eigenvalues of the covariance matrix (HAIR *et al*., 2005). Consequently, the initial set of eleven variables were now characterised by two new variables, which allowed their two-dimensional placement on the graph (order of accessions of the principal components) (Table 2).

The principal component analysis for the yellow mung bean can be seen in Figure 3. The greater the seed area, the higher the values seen for the other morphological parameters such as perimeter, length, width and circularity. It was found that the colour of the seed coat (RGB) in the yellow mung bean was darker in seeds with the greatest area (CIV). Some of the reported causes of darkening in vegetables are an accumulation of reactive oxygen species and changes in the cell wall after harvest (CHEN *et al*., 2018).

On the seventh day after sowing, the morphological characteristics of the seedlings of the yellow mung beans from the larger seeds also showed greater shoot length, root system length and root dry weight (Figure 3).

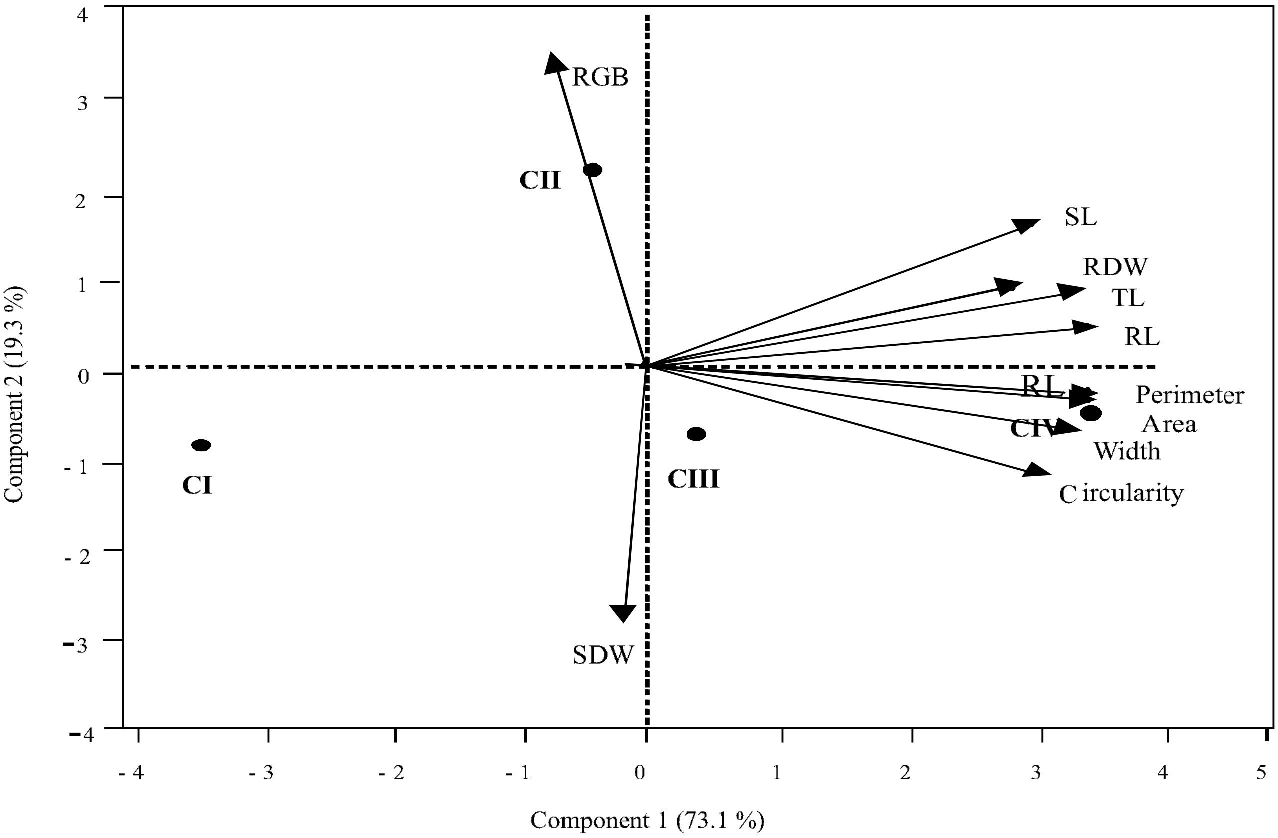
Principal Component 1 (CP1) had an eigenvalue of 8.05, which explains 73.1% of the total variance (Table 2). The component comprised the linear sum of the results for area (A), perimeter (P), circularity (C), seed length (DL), seed width (SW), root system dry weight (RDW), shoot length (SL), root system length (RL) and total seedling length (TL), subtracting the results for the colour of the seed coat (RGB) and shoot dry weight (SDW) (Table 2).

Table 2 – Principal components for the morphological characteristics of the seeds and seedlings of mung bean accessions

Principal components (PC)	Yellow mungo bean		Green mungo bean	
	PC1	PC2	PC1	PC2
Eigenvalue	8.05	2.16	7.66	2.31
Contribution PC (%)	73.1	19.6	69.7	20.9
Accumulated contribution (%)	73.1	92.8	69.7	90.7
Area	0.35	-0.08	0.36	0.10
RGB	-0.07	0.67	0.30	0.32
Perimeter	0.35	-0.06	0.36	0.09
Circularity	0.31	-0.24	-0.13	0.61
Seed length (DL)	0.35	-0.05	0.35	0.14
Seed width (SW)	0.34	-0.14	0.36	0.04
Shoot dry weight (SDW)	-0.02	-0.55	0.02	0.64
Root dry weight (RDW)	0.29	0.17	-0.15	0.10
Shoot Length (SL)	0.31	0.31	-0.34	0.18
Root system length (RL)	0.35	0.08	-0.34	0.12
Total length (TL)	0.34	0.16	-0.35	0.14

Values for Principal Components 1 (PC1) and Principal Components 2 (PC2) highlighted in bold, represent the contribution (weight) of each original variable to each component

Figure 3 - Two-dimensional graph for the linear combination of the new components adopted as a function of the original morphological parameters, for the different categories of seed area in the yellow mung bean. Area (A); perimeter (P); circularity (C); seed length (DL); seed width (SW); seed-coat colour (RGB). For the seedlings: shoot dry weight (SDW); root system dry weight (RDW); shoot length (SL); root system length (RL); total seedling length (TL)



According to this component (CP1), each of the variables has a similar weight in calculating the component, considering the morphological parameters of the seeds and seedlings, except for RGB and SDW (Table 2). As such, any one variable could be used as a basis for selecting high quality seeds.

An eigenvalue of 2.16 was obtained for the second principal component (PC2), which therefore explained 19.3% of the total variance (Table 2). The variables that were most relevant in calculating this component were the colour of the seed coat (RGB) and shoot dry weight (SDW) (Figure 3).

As such, based on the morphological characteristics of the yellow mung bean seeds obtained via image and multivariate analysis, it was possible to predict seedling performance. Information on seed morphometry is essential for adopting a morphological standard of quality which can aid in various stages of the production process, including sowing, machine regulation and even the marketing of seeds classified by size.

Furthermore, classifying seeds based on morphological standards makes it possible to identify seeds with greater physiological potential, allowing the selection of more-vigorous batches.

Selecting more-vigorous seeds based on a morphological characteristic, such as size or specific weight, can be carried out during processing. This may have an impact on the marketing of the seeds by adding value to more vigorous batches. Research has already been carried out on processing the green mung bean to confirm the best processing flowchart based on the physical characteristics of the batches (ARAÚJO *et al*., 2011).

Using simple techniques, such as the one adopted to carry out this research, it is possible to obtain essential information for selecting seeds of high physiological quality from the analysis of RGB images. A system of image analysis can be easily installed in routine and research laboratories using equipment for capturing images in RGB with a camera or scanner, and free software for the analysis.

Figure 4 shows the principal components obtained for the green mung bean. It can be seen that the first two principal components explained 90.7% of the variance contained in the original variables (Table 2). PC1 and PC2 contributed 69.7% and 20.9% respectively.

For the green mung bean it was found that the greater the seed area (CIV) the greater the increases seen in the other morphological parameters of the seeds, such as length, width and perimeter. Seeds of this class have a lighter coloured coat. However, the more-circular seeds were distributed in class II (CII) (Figure 4) and showed no direct relationship between an increase in area and the other morphological seed variables.

The morphological parameters of seedlings of the green mung bean showed different behaviour to that seen for the yellow mung bean, with classes I (CI) and II (CII), which represent the seeds with the smallest area, affording better seedling performance, with higher values for shoot length, root length and total length, in addition to root system dry weight (Figure 4).

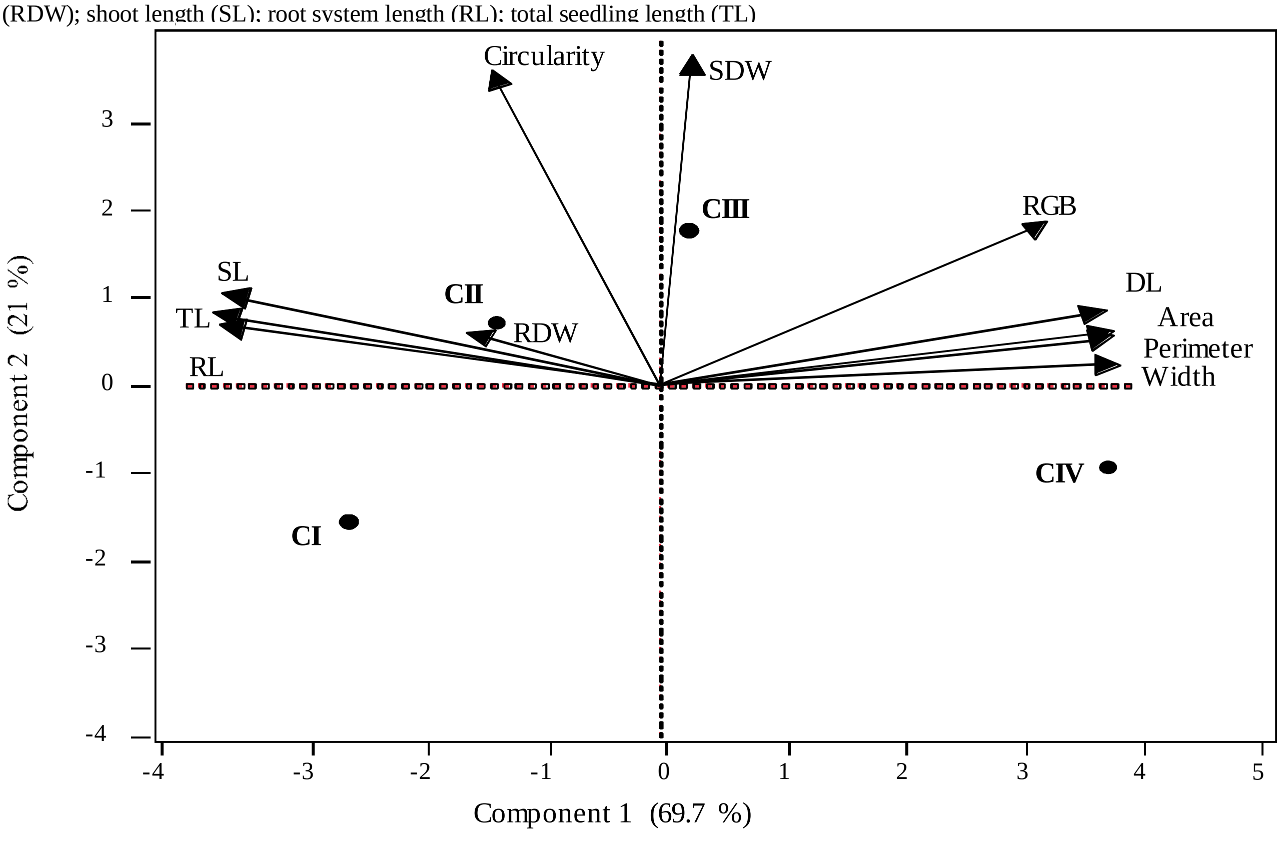
In many species, the size of the seeds may influence the speed of water absorption, as can be seen in *Phaseolus vulgaris* (LOPES; BENNETT; BRANDÃO, 2018). However, in various species this factor has no metabolic effect.

Furthermore, the chemical composition of the seeds can also alter the imbibition speed during the germination process. Seeds of *V. radiata* consist of approximately 62% carbohydrates and 27% proteins, are rich in free amino acids and have significant amounts of oleic and linoleic acid (CHEN *et al*., 2019).

The chemical composition of the seed coat can change the imbibition speed during the germination process thereby affecting seed vigour. In soya seeds, there are structural differences between seed coats of different colour, where seeds with a black coat are thicker than those of genotypes with a yellow coat, in addition to differences in tissue permeability, with light-coloured seeds being more permeable (HUTH *et al*., 2016; MERTZ *et al*., 2009).

Based on the results of this research, compared to behaviour seen in soya seeds, it is suggested that imbibition speed was not affected as a function of seed area in the yellow mung bean, as the seed probably contains less

Figure 4 - Two-dimensional graph relative to the linear combination of the new components adopted as a function of the original morphological parameters, for the different categories of seed area in the green mung bean. Area (A), perimeter (P); circularity (C); seed length (DL); seed width (SW); seed-coat colour (RGB). For the seedlings: shoot dry weight (SDW); root system dry weight (RDW); shoot length (SL); root system length (RL); total seedling length (TL)



lignin, resulting in similar performance for the seedlings. However, in the green mung bean, tissue hydration was greater in the smaller seeds, resulting in greater seedling vigour for this class. The lignin content of mung bean seeds is an important characteristic; however, no information about its quantification is available in the literature.

For the green mung bean, PC1 had an eigenvalue of 7.66, which explains 69.7% of the total variance. The component comprised the linear sum of the results for area (A), seed-coat colour (RGB), perimeter (P), seed length (DL), seed width (SW) and shoot dry weight (SDW), subtracting the results for circularity (C), root dry weight (RDW), shoot length (SL), root system length (RL) and total seedling length (TC) (Table 2).

It was found that except for the variables of circularity, SDW and RD, all the other variables have similar relevance in calculating this component, considering the morphological parameters of the seeds and seedlings (Table 2).

An eigenvalue of 2.31 was obtained for the second principal component, explaining 20.9% of the total variance. The variables that were most relevant in calculating the component were seed-coat colour (RGB), circularity (C) and shoot dry weight (SDW) (Table 2).

For the yellow mung bean, it could be seen that using principal component analysis, seed class IV obtained the best values for most of the results, except for RGB and shoot weight (Figure 3). For the green mung bean, class IV had the best results for the morphological characteristics of the seeds only. In the seedlings, the best results were seen for class I, where the smaller the seed, the more vigorous the respective seedlings (Figure 4).

The analysis of seed images is an important tool that can be used together with correlation analysis to further increase the speed of the results, as some characteristics can be selected indirectly due to showing a positive or negative correlation.

For the yellow mung bean, shoot length and root system length were positively correlated with the physical variables of the seeds, except for circularity and RGB (Figure 5). This indicates that to obtain more vigorous seedlings, larger seeds should be selected.

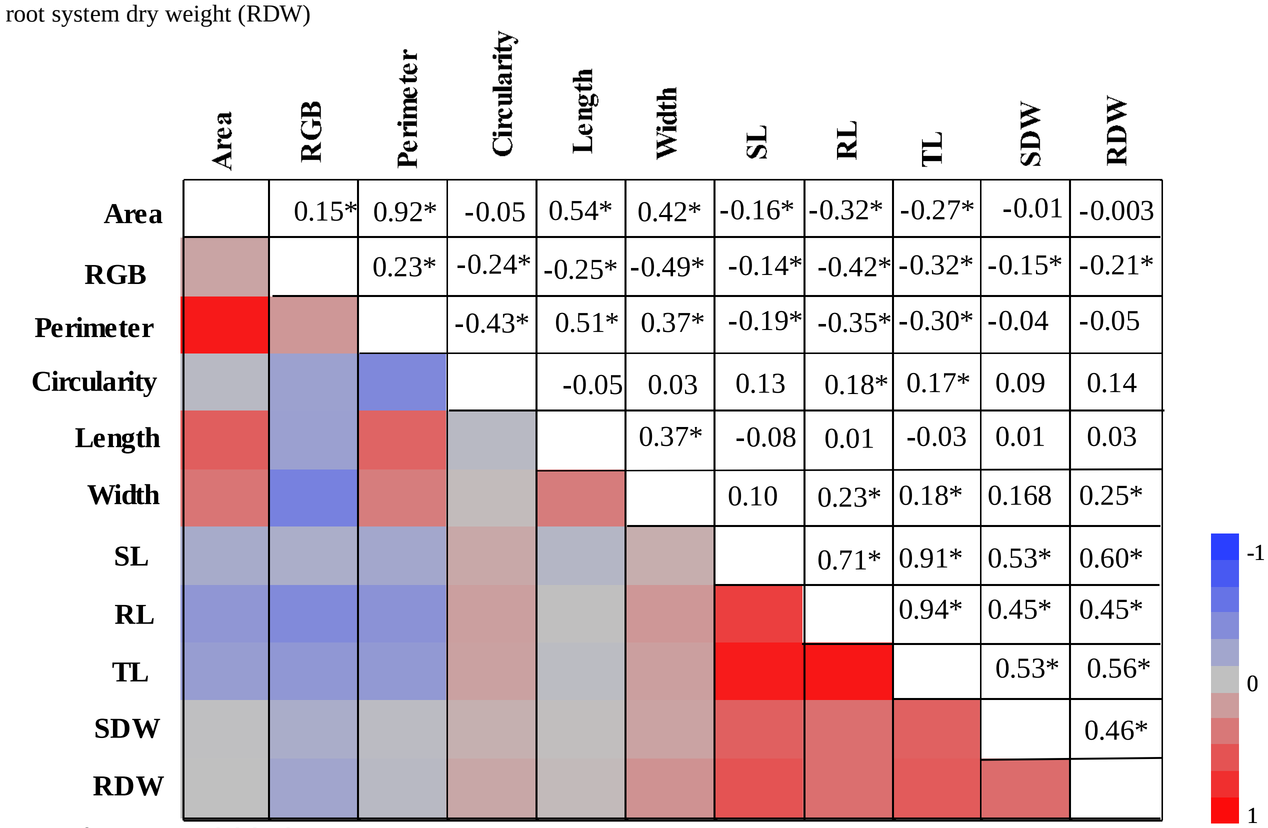
Figure 5 - Pearson correlation coefficients (r) between the morphological parameters of the seeds and seedlings of the yellow mung bean. Seed coat colour (RGB); shoot length (SL); root system length (RL); total seedling length (TL); shoot dry weight (SDW); root system dry weight (RDW)

	Area	**RGB**	**Perimeter**	**Circularity**	**Length**	**Width**	**SDW**	**RDW**	**SL**	**RL**	**TL**
Area		-0.07	0.99*	-0.03	0.83*	0.67*	0.05	0.09	0.08	0.15*	0.15*
RGB			-0.07	0.02	-0.06	0	0	-0.03	0.01	-0.01	0
Perimeter				-0.17*	0.86*	0.65*	0.05	0.10	0.08	0.16*	0.16*
Circularity					-0.24*	0.10	-0.07	-0.08	-0.05	-0.09	-0.10
Length						0.43*	0.09	0.08	0.07	0.17*	0.17*
Width							-0.03	0.03	-0.06	0.10	0.06
SDW								0.23*	-0.02	0.03	0.02
RDW									0.05	0.10	0.10
SL										0.22*	0.62*
RL											0.90*
TL											

-1 0 1

* = significant at 1% probability by F-test

Figure 6 - Pearson's correlation coefficient (r) between the morphological parameters of the seeds and seedlings of the green mung bean. Seed coat colour (RGB); shoot length (SL); root system length (RL); Total seedling length (TL); shoot dry weight (SDW); root system dry weight (RDW)



* = significant at 1% probability by F-test

Just as in the yellow mung bean, the correlation analysis between the variables in the green mung bean confirms the result obtained with the principal component analysis (Figure 6). The morphological characteristics of the seeds showed a significant and positive correlation with seed area, except for circularity. For the seedlings, there was a significant and negative correlation between seed area and the morphological parameters of SL, RL and TL. From this it can be inferred that the larger the seed area, the lower the values of the seedling parameters.

The morphological characteristics of the accessions under evaluation showed varying physiological performance depending on seed area, the larger seeds showing better performance in the yellow mung bean. However, in the green mung bean, the smaller seeds showed greater vigour, evidenced by better seedling performance.

CONCLUSION

Image analysis is efficient for evaluating the external morphology of mung bean seeds. The physiological potential of the seeds can be estimated from the relationship between the morphological characteristics of the seeds and seedlings.

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