

NON-DESTRUCTIVE IDENTIFICATION OF PHYSICAL DAMAGE IN MUNG BEAN (*Vigna radiata* L.) SEEDS BY X-RAY IMAGE ANALYSIS

IDENTIFICAÇÃO NÃO DESTRUTIVA DE DANOS FÍSICOS EM SEMENTES DE FEIJÃO MUNGO (*Vigna radiata* L.) POR ANÁLISE DE IMAGEM DE RAIOS X

Thiago Pulici Martins MACHADO¹; André Dantas de MEDEIROS^{1,*};
Daniel Teixeira PINHEIRO¹; Laércio Junio da SILVA¹;
Denise Cunha Fernandes dos Santos DIAS¹

¹ Universidade Federal de Viçosa, Campus Viçosa, Departamento de Agronomia, Viçosa, MG, Brasil. andre.d.medeiros@ufv.br*.

ABSTRACT: Global demand for pulses such as the mung bean has grown in the last years. For successful production of these crops it is necessary to use high quality seeds. Methodologies based on X-ray image analysis have been used as a complementary tool to evaluate the physical quality of seeds due to their speed and potential for automation. The aim of this study was to evaluate the efficiency of X-ray analysis for non-destructive evaluation of the physical quality of *Vigna radiata* seeds and to relate the variables obtained with their physiological potential. For this, seeds from eight lots were X-rayed and subsequently subject to germination test. In total, 18 physical and physiological parameters were determined. The X-ray image analysis was efficient for evaluating the internal morphology of *Vigna radiata* seeds and allowed the identification of various damage types. However, it was not possible to relate the physical variables to the seed quality as the lots presented similar germination percentage. Physical variables such as solidity and circularity are related to percentage of root protrusion and length of seedling hypocotyl. Low relative densities indicate deteriorated tissues, related to severe morphological damage and non-viable seeds.

KEYWORDS: Radiographic images. Physical quality of seeds. Pulses.

INTRODUCTION

Pulses are legume dried seeds, such as beans, peas, chickpeas and lentils. They are an important nutritional source for billions of people around the world, although their functional attributes and benefits have not yet been fully explored (SINGH, 2017). Global demand for these species has increased, including for mung bean (*Vigna radiata* L.).

Mung bean are classified as a specie of tropical climate. It is consumed as grain, flour or shoot, and can reach production of up to 1.276 kg ha⁻¹ (LI et al., 2017). The use of high-quality seeds for planting is necessary to reach the maximum productive potential of this crop. Thus, seed lots need to be genetically pure, composed of seeds free from physical damage and diseases, and with high viability, so that they can complete the germination process and produce normal seedlings (FINCH-SAVAGE; BASSEL, 2016).

The mung bean has small and predominantly green seeds. The quality of mung bean seeds can be evaluated through germination and vigor tests, such as emergence, accelerated aging and others (ARAÚJO et al., 2011), which require a relatively long time for the results to be obtained. Based on the need to optimize the

analyses of seed lots quality, the rapid tests are suggested. These tests include electrical conductivity, pH of the exudate and potassium leakage, that can be used efficiently to evaluate the seed vigor of different species (GUOLLO et al., 2016; SZEMRUCH et al., 2015; ZUCARELI et al., 2013). It is important to emphasize that, despite providing fast and reliable results, the main disadvantage of these rapid tests is that they are destructive, not allowing reuse of the seeds for other evaluations.

Therefore, the use of the X-ray technique is a viable alternative for evaluating seed vigor (RAHMAN, CHO, 2016). The X-ray technique is based on the absorption of these rays by the tissues, which are transposed to the image (radiography) in gray shades. In addition to being non-destructive, this technique has been efficient in identification of internal seed characteristics (RAHMAN; CHO, 2016), such as nutrient accumulation (CAPPA et al., 2015; RAMOS et al., 2016); modifications of embryo reserves (NIELSEN; DAMKJÆR; FEIDENHANS'L, 2017), percentage of the space occupied in the internal cavity (NORONHA; MEDEIROS; PEREIRA, 2018) and physical damage that directly reflect on seed germination and vigor (ABUD; CICERO; GOMES JUNIOR, 2018; MEDEIROS et al., 2018).

In this sense, Melo et al. (2010) demonstrated the efficiency of the image analysis technique for quantifying coleopteran damages in *Vigna unguiculata* seeds. Also, for common bean seeds, the X-ray image analysis is an efficient tool to evaluate mechanical damage and those caused by insects, and can be included in the quality control programs of seed companies (FORTI; CICERO; PINTO, 2008; MONDO et al., 2009).

Although sensitive and relatively simple, visual analysis of X-ray images may generate errors based on its subjectivity. In addition, they are time-consuming and require skilled labor for a more careful analysis. Thus, methodologies that enable automation of these analyses would contribute to optimizing the evaluations.

The ImageJ® software, a pioneering open image analysis tool (SCHNEIDER; RASBAND; ELICEIRI, 2012), has been used for semi-automated analysis of radiographic images of seeds of various species (ABUD; CICERO; GOMES JUNIOR, 2018; MEDEIROS et al., 2018; NORONHA; MEDEIROS; PEREIRA, 2018). Considering that each species has its own seed characteristics that influence the generation of the image, the variables to be analyzed through image analysis must be adjusted.

In light of the above, the aim of this study was to evaluate the efficiency of X-ray analysis for non-destructive evaluation of the physical quality of mung bean seeds, and to relate the obtained variables to the seed physiological potential.

MATERIAL AND METHODS

The research was conducted in the Seed Analysis Laboratory and in the X-ray Laboratory of the Agronomy and Entomology Departments, respectively, at the Federal University of Viçosa.

Mung bean seeds were produced in 2018 in the experimental area of the EPAMIG, in Viçosa, Minas Gerais, Brazil (20°45'15"S and 42°52'26"W, altitude 680 m). The experimental area has a low slope and the soil is classified as dystrophic red-yellow Latosol. Seeds of two morphological groups (Group I: small bright; Group II: large matte) were used in the experiment, each represented by four lots. The seeds were evaluated using the following tests:

Physical analyses

1.1. Degree of humidity: It was determined by the oven method at 105 ± 3 °C for 24 hours (BRASIL, 2009). For this purpose, two subsamples

of 5 g of seeds for each lot were used. The results were expressed as percentage (wet basis, w.b.).

1.2. X-ray image analysis: Five repetitions of 40 seeds were fixed in an ordered way on transparent paper with doubled-sided tape to allow later individual identification in subsequent analysis. The seeds were positioned to ensure that the embryonic axes were x-rayed laterally to the cotyledons. The seeds were then irradiated with X-ray equipment (MX-20 Faxitron X-ray Corp. Wheeling, IL, U.S.A.). The images of each repetition were generated in 5 seconds, using 23 kV of energy in the beam, 20.9 cm away from the shutter. The digital images were adjusted with the contrast scale centered at 6067 points and 15263 points wide, using Faxitron software. The images were then saved in TIFF (Tagged Image File Format) format and analyzed.

The X-ray images were first visually and then semi-automated analyzed using the ImageJ® software (SCHNEIDER; RASBAND; ELICEIRI, 2012), according to methodology proposed by Medeiros et al. (2018).

The parameters obtained in the visual analysis were:

No damage: Percentage of seeds without visual damages.

Damage: Percentage of seeds presenting any type of damage.

Predation: Percentage of seeds that showed insects inside the internal cavity and/or signs of predation.

Malformed: Percentage of seeds with embryonic anomalies.

Fungi: Percentage of seeds with characteristic signs of fungal damage.

Mechanical damage: Percentage of cracks, scratches and/or kneading seen on the radiographs of the seeds.

The parameters obtained in the semi-automated analysis were:

Seed area: Expressed in mm², indicates the approximate size of the longitudinal section of the seed, determined based on the number of pixels occupied by the seed in the image, which was converted using the ratio of 80.2040 pixels/mm, scale adjusted by previous test on the appliance.

Perimeter: Expressed in mm, was determined using the scale of 80.2040 pixels/mm.

Circularity: Calculated by the formula:

$$C = (4\pi * \text{area})/\text{perimeter}^2$$

Which numerically represents how close to a perfect circle an object is. The closer to zero, the

more irregular is the shape. $c = 1$ is equivalent to a perfect circle.

Height: Height of the smallest rectangle surrounding the seed selected, expressed in mm.

Width: Width of the smallest rectangle surrounding the seed selected, expressed in mm.

Relative density: Expressed in units of gray per pixel. It ranges from absolute black "0" to

absolute white "255". Intermediate values are considered gray. The relative density is the mean of the values of all pixels selected in the image (Figure 1). This variable is related to the tissue density and expresses the resistance to the passage of X-rays. The higher the value, the higher the resistance.

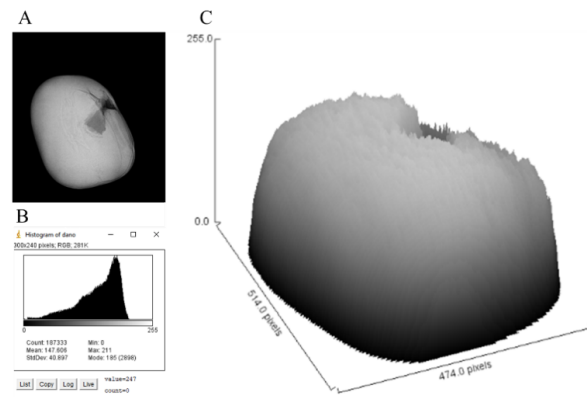


Figure 1. Mung bean seed X-ray image analysis.

Radiographic image (A), gray histogram (B), 3D representation of the gray levels per pixel of one seed (C).

Integrated density: Expressed in $\text{gray} \cdot \text{mm}^2 \cdot \text{pixel}$. It also represents the density of the seed tissues, but ponders the value according to the seed area. Thus, this variable is more sensitive to variations related to seed size.

Finally, visual information about the seed internal morphology such as insect bites, mechanical damage and anatomical irregularities was recorded.

Physiological analyses

Germination test: After the x-ray test, the seeds were subjected to the germination test. Five replicates of 40 seeds were distributed on paper towel (Germitest[®]), in the same sequence or position adopted for the X-ray test which allowed subsequent identification of the respective seedlings. The paper towel was moistened with a volume of water equivalent to 2.5 times the weight of the dry paper. The rolls were kept in a B.O.D.-type chamber with controlled temperature and photoperiod (20-30 °C, 8 hours of light and 16 in the dark). The percentage of root protrusion, normal seedlings and hard seeds (BRASIL, 2009) were evaluated on the seventh day. Normal seedlings were considered those with primary roots and hypocotyl without damage and major twisting, with secondary roots and primary leaves. Germination speed index and root protrusion speed index were calculated according to the formula proposed by Maguire (1962).

The shoot and root seedling length were determined manually, using a millimeter ruler. These measurements were used to calculate the uniformity index (CASTAN; GOMES-JUNIOR; MARCOS-FILHO, 2018) and the corrected seed vigor (MEDEIROS; PEREIRA, 2018) using the R package SeedCalc (SILVA; MEDEIROS; OLIVEIRA, 2019).

The experimental design was completely randomized, with five repetitions. The normality of the errors was verified by the Shapiro-Wilk test and the homoscedasticity by the Bartlett test. Transformation of the data ($\arcsin(x/100)^{1/2}$) was required only for the percentage of hard seeds. The variance analysis (ANOVA) was performed and the means were compared by the Tukey test ($p \leq 0.05$). Finally, the Pearson correlation coefficients (r) were calculated (t test, $p \leq 0.05$). The software R (CORE TEAM, 2018) was used for statistical analyses.

RESULTS AND DISCUSSION

The moisture level of the seeds ranged from 12 to 13% and from 10 to 11%, between the seed lots of Group I and Group II, respectively. Thus, this low variation of seed humidity between lots ($< 1\%$) allowed a safe comparison between them, without compromising the physiological and X-ray analysis. Moreover, this range of seed moisture combined to the configurations of the X-ray equipment used

were efficient to generate visually clear radiographs, enabling identification of the main structures that

make up the seed, as well as the identification of physical damages (Figure 2).

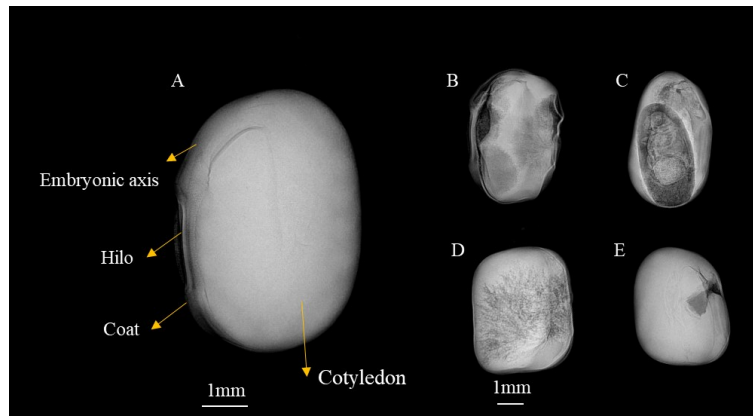


Figure 2. Radiographs of mung bean seeds: seed structures (A), malformed seed (B), presence of insect (C), seed infected by fungi (D) and mechanical damaged seeds (E).

The X-ray technique enabled identification of critical structures of the seeds (Figure 2A), such as the embryonic axis. However, because the seed tissues had relatively uniform densities on all surfaces, it was not possible to access more detailed information about the embryonic axis constituents, such as primordial leaf and central cylinder.

Also, it was observed malformed (Figure 2B) and physically damaged seeds (Figures 2C, 2D e 2E). Seeds damaged by insects generally had low density centers, indicating the site of predation. In some cases, it was possible to visualize the insect inside the seed (Figure 2C). Also, other types of damage have also been identified, such as cracks in the cotyledons and in the embryonic axis (Figure

2E), which may occurred in the process of seed harvesting or processing. Furthermore, the presence of fungi within the seeds was easily identified, characterized by low density circular zones with apparent mycelial branches by X-rays (Figure 2D).

The visual analysis of the X-ray images allowed to identify that the seeds of the lots had a low incidence of damage, not exceeding 16% for seeds of the Group I and 19.5% for the Group II (Table 1). However, within each group, variation was observed in the percentage of damages, 6,5% to 16% and 4,5% to 19%, for Group I and Group II, respectively. Also, a higher proportion of fungi and mechanical damages were observed for the lots of both groups (Table 1).

Table 1. Percentage of damage occurrence in seeds of different lots of mung bean, with damage-type stratification.

Damage occurrence	Group I				Group II			
	L1	L2	L3	L4	L5	L6	L7	L8
No damage	84	92.5	93.5	87.5	86.5	95.5	80.5	88.5
Damage	16	7.5	6.5	12.5	13.5	4.5	19.5	11.5
Predation	1	2	0.5	5.5	0.5	0	0.5	0
Malformed	2.5	2	3	4	5	3	4.5	3.5
Fungi	8	2	3	3	6.5	2	13.5	8.5
Mechanical damage	9	5	4	4.5	1.5	0	1.5	0

Thus, visual evaluation of the radiographs is a simple way to identify damaged seeds, as well as to infer the possible cause of the damage. However, in order to make the X-ray analysis process faster and less subjective, semi-automated image analysis was performed using ImageJ® software. The

parameters obtained by this analysis are shown in Table 2.

Table 2. Mung bean seeds physical parameters obtained through semi-automated analysis of radiographic images of eight seed lots.

Lot	Area mm ²	Per.	Width mm	Height	Circ.	Rel. density gray pixel ⁻¹	Int. density gray mm ² pixel ⁻¹
Group I							
1	11.84 b	15.45 b	4.23 b	3.43 ab	0.64 a	157.30 b	1868 b
2	11.79 b	17.38 a	4.28 b	3.38 b	0.52 b	161.65 a	1909 b
3	12.87 a	18.37 a	4.52 a	3.51 a	0.50 b	160.82 ab	2076 a
4	11.94 b	17.04 ab	4.31 b	3.41 b	0.55 ab	158.47 ab	1898 b
Fc	10.49*	9.40*	13.08*	6.13*	6.55*	3.74*	14.49*
CV	2.92	5.18	1.81	1.46	10.01	1.46	2.84
Group II							
5	18.0 a	22.13	5.65 b	3.95	0.49	161.45	2908 ab
6	18.6 a	22.97	5.82 a	3.95	0.48	160.80	2995 a
7	16.14 b	21.32	5.17 d	3.86	0.47	161.92	2619 c
8	16.93 b	21.67	5.36 c	3.9	0.48	162.57	2755 bc
Fc	19.89*	1.34 ^{ns}	50.6*	3.17 ^{ns}	0.07 ^{ns}	0.19 ^{ns}	11.39*
CV	3.15	6.23	1.67	1.41	14.05	2.31	3.91

*, ^{ns} = significant and not significant according to the F test ($p < 0.05$); Means followed by the same letters within the column, for each group, do not differ according to the Tukey test ($p < 0.05$); Fc = F calculated; CV = coefficient of variation. Per = Perimeter; Circ. = Circularity; Rel. density = Relative density; Int. density = Integrated density.

It was observed that the seeds of Group I had area ranging from 11 to 12 mm², and the seeds of the Group II had slightly larger areas (~ 16 to 18 mm²) (Table 2). The lots showed variation within each group for most descriptors, except perimeter, height, circularity and relative density for the lots of the Group II. This variation may be related to the influence of environmental factors in the field during the seed production. Information on phenotypic characteristics of seed size and shape may contribute significantly to genetic improvement programs, with the potential to act simultaneously with the molecular profile technologies (TANABATA et al., 2012), and may often be related to physiological variables (MEDEIROS et al., 2018; WHAN et al., 2014).

It could be observed that the relative density of the seeds differed between lots 1 and 2 (Group I) and the integrated density differentiated lot 3 (Group I) from the others (Table 2). In Group II, for lot 2 was obtained the highest integrated density. The relative density of the seed is related to the levels of radioluminescence (dark) and radiopacity (light), translated to the image in gray values. These values can indirectly reflect seed deterioration, mechanical damage and embryonic filling (MEDEIROS et al., 2018). The integrated density, in turn, indicates the gray level by the seed area, which may be of interest when the seed area is associated with aspects of physiological quality, evidencing more clearly the relationship between the integrity and the size of the seed tissues.

Recent researches using parameters based on gray levels in radiographs has shown relationship with germination and seedling length, evidencing the ability of these variables to accurately infer the physical and physiological quality of broccoli and leucaena seeds (ABUD; CICERO; GOMES JUNIOR, 2018; MEDEIROS et al., 2018). However, the relationship between gray level and physical and physiological parameters, evidenced in previous researches, may not be generalized for other species, due to the characteristic of the seeds of the species under study, such as oil content, seed thickness, among other characteristics.

Table 3 shows the parameters related to the seed physiological quality. In the Group I, it was observed that only for normal seedlings and viability there was no significant difference between the seed lots. For the other variables, higher values were observed for lots 1 and 2, indicating the superiority of these lots in relation to seed germination and vigor.

For lots 1 and 2 were obtained the higher values for shoot, root and total seedling length, as well as uniformity and vigor indexes. With regards to the radicular protrusion velocity (RPS), lots 1 and 2 also showed higher values. Then, the seeds of these two lots can be considered of greater vigor, since rapid seed germination and adequate seedling development is an indication of seed vigor (FINCH-SAVAGE; BASSEL, 2016; OLIVEIRA et al., 2017). However, it is important to note that for these

lots, there was a lower proportion of hard seeds, which contributes to higher water uptake and activation of the metabolism in the germination process (BEWLEY et al., 2013). In contrast, there were no significant differences for most of the variables of physiological quality comparing the

seed lots in Group II. For root and total length variables and corrected vigor, it was observed superiority for lot 6, which, as for lots 1 and 2 in Group I, showed a lower proportion of hard seeds (Table 3).

Table 3. Germination and vigor of eight lots of mung bean seeds.

Lot	NS	RP	Viability %	HS	GSI index	RPS	HL	RL	TL	Unif. index	CVI
Group I											
1	88	93 a	99	5 b	4.84 a	16.66 a	5.87 a	8.51 ab	14.38 a	795 a	718 a
2	86	94 a	99	5 b	4.42 ab	17.11 a	5.63 a	8.67 a	14.30 a	812 a	713 a
3	78	82 b	99	17 a	4.04 b	13.65 b	4.89 b	7.46 b	12.35 b	761 ab	568 b
4	79	82 b	99	17 ab	4.15 a	14.05 b	4.89 b	7.60 ab	12.49 b	719 b	576 b
Fc	3.64 ^{ns}	8.67*	0.11 ^{ns}	4.79*	5.34*	17.70*	9.55*	4.79*	7.22*	5.20*	20.50*
CV	7.30	5.65	1.65	50.05	7.88	6.13	6.88	7.83	6.90	5.19	6.37
Group II											
5	86	96	98	2 ab	4.32	15.88	4.93	8.75 b	13.68 b	779	705 b
6	88	99	99	0 b	4.45	16.80	4.83	10.83 a	15.66 a	819	841 a
7	86	96	99	4 a	4.31	16.54	4.97	9.03 b	14.00 b	813	729 b
8	87	97	99	2 ab	4.20	15.96	4.58	8.74 b	13.31 b	821	717 b
Fc	0.10 ^{ns}	1.40 ^{ns}	0.34 ^{ns}	4.72*	0.64 ^{ns}	0.92 ^{ns}	1.09 ^{ns}	13.87	7.13*	1.81 ^{ns}	13.04
CV	5.67	2.58	1.84	5.32	6.60	6.35	7.79	6.45	6.12	4.01	5.22

*, ns = significant and not significant according to the F test ($p < 0.05$); Means followed by the same letters within the column, for each group, do not differ according to the Tukey test ($p < 0.05$); Fc = F calculated; CV = coefficient of variation, NS = Normal seedling; RP = Root protrusion; HS = Hard seed; GSI = germination speed index; RPS = Root protrusion speed index; HL = Hypocotyl length; RL = Root length; TL = Total length; Unif. = Uniformity index; CVI = Corrected vigor index.

In this study, there were no significant correlations ($p < 0.05$) between the majority of the physical and the physiological parameters, with the exception of perimeter and circularity, which

correlated with hypocotyl length in a moderate way ($r = -0.72$ and $r = 0.77$, respectively), evidencing a possible association between these variables (Figure 3).

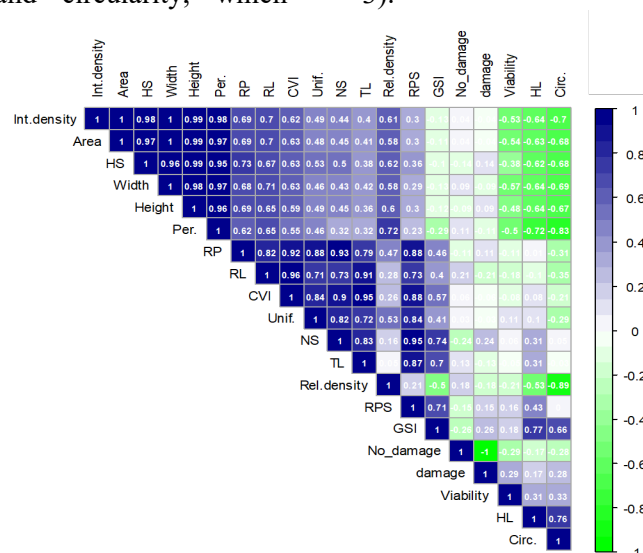


Figure 3. Coefficients of correlation between physical and physiological parameters associated with *V. radiata* seed quality. NS = Normal seedling; RP = Root protrusion; HS = Hard seed; GSI = germination speed index; RPS = Root protrusion velocity; HL = Hypocotyl length; RL = Root length; TL = Total length; Unif. = Uniformity index; CVI = Corrected vigor index; Per. = Perimeter; Circ. = Circularity; Rel. density = Relative density; Int.density = Integrated density.

The low correlation observed between the physical and physiological variables may be related to the great homogeneity observed between the lots. For example, there was no difference between the seed lots regarding the number of normal seedlings. Although significantly different for Group I, it was observed a small difference between lots comparing the values of the relative density for both groups (Table 1). This variable is correlated with seed physiological quality of some other species (ABUD; CICERO; GOMES JUNIOR, 2018; MEDEIROS et al., 2018). It means that in future experiments, seed lots with more contrasting physiological quality should be used.

Previous researches have shown that the analysis of seed radiograph is efficient to verify whether or not there is tissue malformation, but does not necessarily establish a direct relationship with physiological processes, i.e., seeds classified as well formed by the X-ray technique may give rise to abnormal seedlings and even dead seeds (SEVERIANO et al., 2018; SILVA et al., 2014). In

the present research, although it was observed difference between the physical parameters comparing the seed lots, it did not reflect on the seed quality. In contrast, research with broccoli and leucaena seeds showed a close relationship between variables related to tissue integrity, as well as descriptors of shape and size, with physiological attributes (ABUD et al., 2018; MEDEIROS et al., 2018).

Although correlations were low, it was possible to observe direct relationships between seed damage and abnormal seedlings formation (Figure 4). In general, seedlings originated from seeds with more translucent radiographic images (lower density) and with physical damage (cracks and/or scratches) produced less vigorous seedlings. On the other hand, seeds with more opaque coloration (higher density) produced more vigorous seedlings, with more developed shoots and roots and a greater proportion of adventitious roots (Figure 4A).



Figure 4. Radiographs of *V. radiata* seeds and their respective seedlings.

It is important to emphasize that the locals in which the damage occurred influenced the seedling formation. When present in cotyledon regions, damage generally did not cause severe abnormalities in seedlings, but resulted in seedlings with lower vigor (fewer secondary roots, shorter roots and less developed shoots) (Figure 4A). Similarly, when present near or directly in the embryonic axis, physical damages affected not only the root protrusion, but also caused abnormalities in the seedlings (Figure 4B).

These results are consistent with those observed in bean (SOOD et al., 2016) and melon

seeds (AHMED et al., 2018), confirming the efficiency of the X-ray technique to evaluate mechanical damage to seeds. Also, the seed energy reserves are directed to the embryonic axis during the process of germination and establishment of seedlings (FINCH-SAVAGE; BASSEL, 2016), then it was expected that damaged seeds would generate abnormal seedling. The damage directly on or near the axis tends to result in a damaged seedling, whereas damage in the cotyledons only compromises the amount of reserves that will be mobilized for the development of the embryonic axis.

CONCLUSIONS

The X-ray image analysis is efficient for evaluating the internal morphology of *Vigna radiata* seeds and for damage identification.

Image analysis is not efficient to make inferences regarding the seed vigor when is compared seed lots with similar germination percentage.

Variables obtained from the automated image analysis, such as solidity and circularity, are related to percentage of root protrusion and seedling hypocotyl length.

Low relative densities in the images indicate deteriorated tissues, related to severe morphological damage and non-viable seeds.

RESUMO: A demanda mundial por leguminosas como o feijão mungo tem crescido nos últimos anos. Para o sucesso da produção destas culturas é necessário a utilização de sementes de alta qualidade. Metodologias baseadas na análise de imagens de raios X têm sido utilizadas como ferramenta complementar para avaliação da qualidade física de sementes em função da sua rapidez e potencial de automatização. O objetivo deste trabalho foi avaliar a eficiência da análise de raios X para avaliação não destrutiva da qualidade física de sementes de *Vigna radiata*, e relacionar as variáveis obtidas com o seu potencial fisiológico. Para tal, sementes de oito lotes foram radiografadas e submetidas ao teste de germinação. Por meio dessas avaliações foram determinadas 18 variáveis, distribuídas entre físicas e fisiológicas. A análise de imagens de raios X foi eficiente para a avaliação da morfologia interna das sementes *Vigna radiata* e permitiu a identificação de vários tipos de danos. Porém, não foi possível relacionar as variáveis físicas com a qualidade das sementes, pois os lotes apresentaram porcentagens de germinação semelhantes. Variáveis físicas como solidez e circularidade estão relacionadas com a percentagem de protrusão radicular e o comprimento de hipocótilo das plântulas. Densidades relativas baixas indicam tecidos deteriorados, tendo relação com danos morfológicos graves e sementes inviáveis.

PALAVRAS-CHAVE: Imagens radiográficas. Qualidade física de sementes. Pulses.

REFERENCES

- ABUD, H. F.; CICERO, S. M.; GOMES JUNIOR, F. G. Radiographic images and relationship of the internal morphology and physiological potential of broccoli seeds. *Acta Scientiarum. Agronomy*, v. 40, n. 1, p. 1–9, 2018. <http://dx.doi.org/10.4025/actasciagron.v40i1.34950>
- AHMED, M. R.; YASMIN, J.; COLLINS, W.; CHO, B. K. X-ray CT image analysis for morphology of muskmelon seed in relation to germination. *Biosystems Engineering*, v. 175, p. 183–193, 2018. <https://doi.org/10.1016/j.biosystemseng.2018.09.015>
- ARAÚJO, R. F.; ZONTA, J. B.; ARAUJO, E. F.; HEBERLE, E.; ZONTA, F. M. G. Teste de condutividade elétrica para sementes de feijão-mungo-verde. *Revista Brasileira de Sementes*, v. 22, n. 1, p. 123–130, 2011. <http://dx.doi.org/10.1590/S0101-31222011000100014>
- BEWLEY, J.D., BRADFORD, K.J., HILLHORST, H.W.M, NONOGAKI, H. *Seeds: Physiology of Development, Germination and Dormancy*. 3rd. ed. New York: Springer Science, 2013. 392 p.
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Brasília, DF: MAPA/ACS, 2009. 399 p.
- CAPPA, J. J.; YETTER, C.; FAKRA, S.; CAPPA, P. J.; DETAR, R.; LANDES, C.; PILON-SMITS, E. A. H.; SIMMONS, M. P. Evolution of selenium hyperaccumulation in *Stanleya* (Brassicaceae) as inferred from phylogeny, physiology and X-ray microprobe analysis. *New Phytologist*, v. 205, n. 2, p. 583–595, 2015. <http://dx.doi.org/10.1111/nph.13071>

CASTAN, D. O. C.; GOMES-JUNIOR, F. G.; MARCOS-FILHO, J. Vigor-S, a new system for evaluating the physiological potential of maize seeds. **Scientia Agricola**, v. 75, n. 2, p. 167–172, 2018. <http://dx.doi.org/10.1590/1678-992x-2016-0401>

FINCH-SAVAGE, W. E.; BASSEL, G. W. Seed vigour and crop establishment: extending performance beyond adaptation. **Journal of Experimental Botany**, v. 67, n. 3, p. 567–591, Feb. 2016. <http://dx.doi.org/10.1093/jxb/erv490>

FORTI, V. A.; CICERO, S. M.; PINTO, T. L. F. Análise de imagens na avaliação de danos mecânicos e causados por percevejos em semente de feijão. **Revista Brasileira de Sementes**, v. 30, n. 1, p. 121–130, 2008. <http://dx.doi.org/10.1590/S0101-31222008000100016>

GUOLLO, K.; POSSENTI, J. C. ; FELIPPI, M. ; QUIQUI, E. M. ; DEBASTIANI, A. B. ; TEDESCO, J. L.; MENEGATTI, R. D. Evaluation of the physiological quality of forest seed species through the pH test of the exudate. **Australian Journal of Basic And Applied Sciences**, v. 10, p. 1-8, 2016. Available at: <https://ssrn.com/abstract=2792786>. Accessed on: 14 Jun. 2018.

LI, L.; YANG, T.; LIU, R.; REDDEN, B.; MAALOUF, F.; ZONG, X. Food legume production in China. **The Crop Journal**, v. 5, n. 2, p. 115–126, 2017. <https://doi.org/10.1016/j.cj.2016.06.001>

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

MEDEIROS, A. D.; ARAÚJO, J. O.; ZAVALA-LEÓN, M. J.; SILVA, L. J.; DIAS, D. C. F. S. Parameters based on x-ray images to assess the physical and physiological quality of *Leucaena leucocephala* seeds. **Ciência e Agrotecnologia**, v. 42, n. 6, p. 643–652, 2018. <http://dx.doi.org/10.1590/1413-70542018426023318>

MEDEIROS, A. D.; PEREIRA, M. D. SAPL ®: a free software for determining the physiological potential in soybean seeds. **Pesquisa Agropecuária Tropical**, v. 48, n. 3, p. 222–228, 2018. <http://dx.doi.org/10.1590/1983-40632018v4852340>

MELO, R. D. A.; FORTI, V. A.; CICERO, S. M.; NOVEMBRE, A. D.; DE MELO, P. C. T. Use of X-ray to evaluate damage caused by weevils in cowpea seeds. **Horticultura Brasileira**, v. 28, n. 4, p. 472–476, dez. 2010. <http://dx.doi.org/10.1590/S0102-05362010000400016>

MONDO, V. H. V.; JUNIOR, F. G.; PUPIM, T. L.; CICERO, S. M. Avaliação de danos mecânicos em sementes de feijão por meio da análise de imagens. **Revista Brasileira de Sementes**, v. 31, n. 2, p. 27–35, 2009. <http://dx.doi.org/10.1590/S0101-31222009000200003>

NIELSEN, M. S.; DAMKJÆR, K. B.; FEIDENHANS'L, R. Quantitative in-situ monitoring of germinating barley seeds using X-ray dark-field radiography. **Journal of Food Engineering**, v. 198, p. 98–104, 2017. <http://dx.doi.org/10.1016/j.jfoodeng.2016.11.011>

NORONHA, B. G. de; MEDEIROS, A. D. de; PEREIRA, M. D. Avaliação da qualidade fisiológica de sementes de *Moringa oleifera* Lam. **Ciência Florestal**, v. 28, n. 1, p. 393-402, 2018. <http://dx.doi.org/10.5902/1980509831615>

OLIVEIRA, D. F. A.; OLIVEIRA, D. F. A.; MELO, S. M. B.; AVELINO, A. P.; MACÊDO, C. E. C.; PACHECO, M. V.; VOIGT, E. L. The deterioration of *Moringa oleifera* Lam. seeds in the course of storage involves reserve degradation. **Acta Physiologiae Plantarum**, v. 39, n. 12, p. 269, 2017. <http://dx.doi.org/10.1007/s11738-017-2572-9>

R CORE TEAM. **R Development Core Team. R: A Language and Environment for Statistical Computing**. 2018. Available at: <https://www.r-project.org/>. Accessed on: 1 Jan. 2018

- RAHMAN, A.; CHO, B.-K. Assessment of seed quality using non-destructive measurement techniques: a review. **Seed Science Research**, v. 26, n. 04, p. 285–305, 2016. <https://doi.org/10.1017/S0960258516000234>
- RAMOS, I.; PATACO, I. M.; MOURINHO, M. P.; LIDON, F.; REBOREDO, F.; PESSOA, M. F.; CARVALHO, M.L.; SANTOS, J.P.; GUERRA, M. Elemental mapping of biofortified wheat grains using micro X-ray fluorescence. **Spectrochimica Acta Part B: Atomic Spectroscopy**, v. 120, p. 30–36, 2016. <https://doi.org/10.1016/j.sab.2016.03.014>
- SCHNEIDER, C. A.; RASBAND, W. S.; ELICEIRI, K. W. NIH Image to ImageJ: 25 years of image analysis. **Nature Methods**, v. 9, n. 7, p. 671–675, 1 jul. 2012. Available at: <http://www.nature.com/articles/nmeth.2089>. Accessed on: 26 Apr. 2018. <https://doi.org/10.1038/nmeth.2089>
- SEVERIANO, R. L.; PINHEIRO, P. R.; JUNIOR, F. G. G.; DE MEDEIROS, A. D.; PEREIRA, M. D. X-ray test on passion fruit seeds submitted to different aryl removal methods. **Comunicata Scientiae**, v. 9, n. 3, p. 356–362, 2018. <https://doi.org/10.14295/cs.v9i3.2706>
- SILVA, L. J.; MEDEIROS, A. D. de; OLIVEIRA, A. S. SeedCalc, a new automated R software tool for germination and seedling length data processing. **Journal of Seed Science**, v. PRELO, 2019. <https://doi.org/10.1590/2317-1545v42n2217267>
- 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://dx.doi.org/10.1590/S0102-05362014000200016>
- SINGH, N. Pulses: an overview. **Journal of Food Science and Technology**, v. 54, n. 4, p. 853–857 2017. <https://doi.org/10.1007/s13197-017-2537-4>
- SOOD, S.; MAHAJAN, S.; DOEGAR, A.; DAS, A. Internal crack detection in kidney bean seeds using X-ray imaging technique. **2016 International Conference on Advances in Computing, Communications and Informatics (ICACCI)**, p. 2258–2261, 2016. <https://doi.org/10.1109/ICACCI.2016.7732388>
- SZEMRUCH, C.; DEL LONGO, O. T.; FERRARI, L.; RENTERIA, S.; MURCIA, M.; CANTAMUTTO, M. Á.; RONDANINI, D. P. Ranges of vigor based on the electrical conductivity test in dehulled sunflower seeds. **Research Journal of Seed Science**, v. 8, n. 1, p. 12–21, 2015. <https://doi.org/10.3923/rjss.2015.12.21>
- TANABATA, T.; SHIBAYA, T.; HORI, K.; EBANA, K.; YANO, M. SmartGrain: High-throughput phenotyping software for measuring seed shape through image analysis. **Plant Physiology**, v. 160, n. 4, p. 1871–1880, 2012. <https://doi.org/10.1104/pp.112.205120>
- WHAN, A. P.; SMITH, A. B.; CAVANAGH, C. R.; RAL, J. P. F.; SHAW, L. M.; HOWITT, C. A.; BISCHOF, L. GrainScan: a low cost, fast method for grain size and colour measurements. **Plant Methods**, v. 10, n. 1, p. 23, 2014. <https://doi.org/10.1186/1746-4811-10-23>
- ZUCARELI, C.; BRZEZINSKI, C. R.; ABATI, J.; HENNING, F. A.; RAMOS JUNIOR, E. U.; NAKAGAWA, J. Lixiviação de íons potássio, cálcio e magnésio para determinação do vigor em sementes de milho doce. **Informativo ABRATES**, v. 23, n. 3, p. 56–60, 2013. Available at: <https://www.embrapa.br/soja/busca-de-publicacoes/-/publicacao/981118/lixiviacao-de-ions-potassio-calcio-e-magnésio-para-determinacao-do-vigor-em-sementes-de-milho-doce>. Accessed on: 14 Jun. 2018