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Image analysis to evaluate the physiological potential and morphology of pearl millet seeds[1]

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ABSTRACT - The use of fast and precise procedures for seed analysis is an important factor for making decisions at different stages of seed production. The aim of this study was to evaluate the occurrence of internal damage and associate morphological traits of pearl millet seeds with their physiological potential, and evaluate seed vigor using the Seed Vigor Imaging System (SVIS®) compared to other traditional vigor tests. Eight seed lots of the pearl millet cultivar ADR 300 were analyzed for germination, vigor (first count, accelerated aging, cold test, and SVIS®), internal morphology (X-ray), and morphological parameters (perimeter, area, and roundness). The X-ray test is effective in evaluating the internal morphology of pearl millet seeds, allowing identification of mechanical damage and of deteriorated and malformed tissues, making it possible to establish a relationship between the damage observed and the negative effect on germination. SVIS® analyses on two-day-old seedlings are a reliable method for assessing seed vigor of pearl millet. Parameters of perimeter and area of the seeds are related to their physiological potential.

Index terms: X-ray, *Tomato analyzer*, SVIS®, *Pennisetum glaucum* L.

Técnicas de análise de imagens para avaliação da morfologia e do potencial fisiológico de sementes de milheto

RESUMO - O uso de procedimentos rápidos e precisos para a análise de sementes é um fator importante para a tomada de decisões nas diferentes fases da produção de sementes. Esta pesquisa teve como objetivos avaliar a ocorrência de danos internos e associar características morfológicas de sementes de milheto com o potencial fisiológico e avaliar o vigor das sementes com o uso do *Seed Vigor Imaging System* (SVIS®) em comparação com outros testes de vigor tradicionalmente utilizados. Oito lotes de sementes de milheto da cultivar ADR 300, foram analisados quanto à germinação, vigor (primeira contagem, envelhecimento acelerado, teste de frio e SVIS®), morfologia interna (raios X) e parâmetros morfológicos (perímetro, área e circularidade). O teste de raios X é eficiente para a avaliação da morfologia interna, permitindo a identificação de danos mecânicos, deterioração de tecidos e malformação, possibilitando estabelecer a relação entre o dano observado e o prejuízo causado à germinação. As análises com SVIS® em plântulas com dois dias de germinação são seguras para determinação do vigor de sementes de milheto. Os parâmetros de perímetro e de área estão relacionados com o potencial fisiológico de sementes.

Termos para indexação: Raios X, *Tomato analyzer*, SVIS®, *Pennisetum glaucum* L.

Introduction

Pearl millet (*Pennisetum glaucum* L.) is one of the most widely grown cereal crops in the world; its grain is used as food source for humans, especially in Africa and India. In Brazil, it is often used as a cover crop in the no-till system, for grazing, and for animal feed (Pires et al., 2007; Timossi et al., 2007). In addition, the plant is more tolerant to drought stress than other cereal crops (Ojediran et al., 2010).

In spite of the broad utility of pearl millet, low physical and physiological quality of the seeds sold is currently one of the main problems (Peske and Novembre, 2010), given that production of pearl millet seeds with high physiological potential is fundamental for adequate establishment of seedlings in the field.

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In recent years, various studies directed to evaluation of the internal morphology of seeds have been based on image analysis techniques. Among the techniques available, X-ray analysis has been used to evaluate the internal morphology of seeds, making it possible to study the relationship between the occurrence of problems (mechanical damage, plant tissue malformation, and insect and microorganism damage) and physiological potential. Various studies have already been performed on seeds of different species and have shown the effectiveness of X-ray tests on identification of injuries associated with loss of germination, including studies on sweet corn (Gomes-Junior and Cicero, 2012), rice (Silva et al., 2014), and soybean (Wendt et al., 2014). In addition, X-ray images of seeds can be associated with other computerized image techniques for the purpose of increasing the effectiveness of seed analysis (Gomes-Junior, 2010).

One technique that has been effective for evaluation of seed vigor is seedling image analysis by means of specific software, such as the Seed Vigor Imaging System (SVIS®). This analysis is made by capturing seedling images, allowing determination of indexes of vigor and of uniformity of seedling development, in addition to seedling length. The time necessary to generate results is less than that required to execute the test through manual measurement, providing greater speed and precision in obtaining results (Hoffmaster et al., 2005). Satisfactory results have been achieved in evaluation of seed vigor of various species, such as maize (Mondo et al., 2011), soybean (Marcos-Filho et al., 2009), sweet corn (Alvarenga et al., 2012), wheat (Silva et al., 2012), and dry edible bean (Gomes-Junior et al., 2014).

Identification and separation of seeds with irregular shapes through use of image analysis can also be a viable method for evaluating the physical quality of seed lots, which is possibly associated with germination performance (Wendt et al., 2014). Some free software for image analysis, as for example ImageJ (Schneider et al., 2012) and Tomato Analyzer (Brewer et al., 2006), have tools for analysis of seed morphology based on X-ray images. Among the parameters obtained, perimeter, area, roundness, and diameter can be highlighted. Thus, determination of seed morphological attributes in an automated manner may constitute a fast and efficient procedure for assessing the degree of uniformity of seeds from a lot.

Given the need for new procedures for assessing the physiological potential of pearl millet seeds, the aims of this study were to associate seed morphological traits, evaluated by X-ray images, with physical, germination, and seed vigor parameters; and evaluate seed vigor with use of the Seed Vigor Imaging System (SVIS®) and compare results to those of other traditional vigor tests.

Material and Methods

The study was carried out in the Seed Analysis and Image Analysis Laboratories of the Crop Science Department of the Escola Superior de Agricultura “Luiz de Queiroz” (ESALQ) of the Universidade de São Paulo (USP) in Piracicaba, São Paulo, Brazil. Eight lots of untreated seeds of pearl millet of the cultivar ADR 300 were used, and the following evaluations were carried out:

Seed water content: determined by the laboratory oven method at 105± 3 °C for 24 hours, using two 5.0-g subsamples of seed for each lot (Brasil, 2009), and results were expressed in percentage (wet basis).

1000 seed weight: determined from the mean values of eight replications of 100 seeds, and the value was later calculated for 1000 seeds, according to the procedure described in the Rules for Seed Testing (Brasil, 2009).

Uniformity test (sieve retention): using two 100-g subsamples of pure seeds, shaken manually for one minute in wire sieves with square mesh of 9x9 mm, 10x10 mm, 12x12 mm, and 14x14 mm. After that, the seeds were weighed, obtaining the percentage of retention in each sieve (Brasil, 2009).

Germination test: conducted with four replications of 50 seeds per lot, distributed on sheets of paper towel, moistened with water in the amount of 2.5 times the weight of the dry paper, and kept in a seed germinator at 25 °C. Germination counts were made at three days (first count of germination) and at seven days after sowing (Brasil, 2009). Results were expressed in mean percentage of normal seedlings for each lot.

Accelerated aging: seeds were arranged in a single layer over a suspended screen within a plastic box (11cm x 11cm x 3.5 cm) that contained 40 mL of water. After that, the boxes were kept in a B.O.D. chamber regulated at 41 ºC for 48 hours, in accordance with Garcia and Menezes (1999). After this period, the germination test was carried out and the number of normal seedlings were counted on the fifth day, with the results being expressed in percentage.

Cold test: conducted with four replications of 50 seeds per lot, using plastic trays of 34 cm x 23 cm x 7 cm, with 1 kg of earth and sand mixed at a proportion of 1:3. In each tray, after addition of the substrate, four 50-seed replications were distributed and, after that, covered with 1 kg of the same substrate. Water availability of the substrate was adjusted to 70% of its retention capacity, and the water was previously cooled to 10 ºC, according to Caseiro and Marcos-Filho (2000). After distribution of the substrate, seeds, and water, each tray was placed in a transparent plastic bag to reduce evaporation and, after that, kept in a cold chamber at 10 ºC for seven days. After this period, each tray was transferred to a seed germinator at 25 ºC. The percentages of immersed seedlings were registered on day four, and the results were expressed in mean percentage for each lot.

Evaluation of seed internal morphology (X-rays): performed with eight replications of 25 seeds from each lot. The seeds were placed on transparent double-sided adhesive tape, glued on a sheet of transparent acetate (210 mm x 297 mm), and numbered according to the position occupied on the sheet for their identification at the time of setting up the germination test. The sheet containing the seeds was placed within the digital device "Faxitron X-Ray", model MX-20 DC-12, at a distance of 11.4 cm from the source of emission of the X-rays, considered in this study as the most suitable distance for identification of the seed parts. The X-ray images were saved in a specified file of the computer hard drive. After being X-rayed, the seeds (of each replication), previously identified and numbered, were placed to germinate on paper toweling, distributed in two rows in the upper third of the substrate to allow development of the seedlings in an individualized manner.

After remaining four days in the seed germinator at 25 ºC, the seedlings (normal and abnormal) and the dead seeds were photographed with a Sony camera (DSC-W530) connected to a stereomicroscope, to obtain high-resolution images. The images were stored in the computer for analysis together with the X-ray images. Thus, this allowed changes in the internal morphology of the seeds (mechanical damage, tissue deterioration, and malformation) to be related to possible losses caused to germination. The results were analyzed in a comparative way, seeking to relate the damage detected in image analysis (individual seeds) to possible abnormalities of the seedlings or ungerminated seeds.

The percentage of seeds with damage was determined after X-raying the seeds. First, seeds were individually identified that would probably exhibit compromised performance due to damage identified in the image. The damage initially seen in the X-ray images associated with generation of dead seeds and abnormal seedlings were counted up in regard to the occurrence of mechanical damage, malformation, and tissue deterioration. The results were expressed in percentage of total damage per lot and the contribution of each type of damage within the total damage per lot.

Evaluation of morphological parameters of the seeds: based on X-ray images of the seeds, the perimeter, area, and roundness of the seeds were evaluated. These determinations were made with the assistance of the *Tomato Analyzer* program (Brewer et al., 2006). The results of seed perimeter and area (expressed in mm and mm²), respectively) were used to verify possible relationships between the stated parameters and seed physiological potential. Roundness, which was calculated through equation (1), was used to check for variations in uniformity among the seed lots. The value of 1 is considered the reference value, indicating a perfect circle.

$$(1) \quad C = \frac{A}{P^2} \cdot 4\pi$$

in which C = roundness; A = seed area; P = seed perimeter.

Computerized seedling image analysis (SVIS®): performed on eight replications of 25 seeds from each lot placed to germinate in two rows in the upper third of the surface of the paper toweling, at 25 ºC. Seedlings were evaluated at two and three days of germination to verify the age of the seedlings most suitable for analysis. Having passed through the period of germination, the seedlings from each replication were transferred from the paper toweling to a sheet of black paperboard (30 cm x 22 cm). After that, images were recorded by a scanner (HP, model Scanjet 200), adjusted to a resolution of 100 dpi, arranged in an inverted manner within an aluminum box (60 cm x 50 cm x 12 cm).

The scanned images were analyzed by the software Seed Vigor Imaging System (SVIS®), developed by Sako et al. (2001), calculated by equations (2), (3), and (4). Mean values were obtained of the vigor index, uniformity of development, and seedling length for each lot and cultivar (Hoffmaster et al., 2003).

(2) Vigor index = W_G * growth + Wu * uniformity;

(3) Growth = min (W_h * Lh + Wr * Lr, 1000);

(4) Uniformity = max (1000 – (Wsh *Sh + Wsr * Sr + Stotal + Wsr/h * Sh/r) – Wd*numdead,0).

in which, Lh = Hypocotyl length, Lr = taproot length, Sh = standard deviation of hypocotyl length, Sr = standard deviation of taproot length, Stotal = standard deviation of total seedling length, and Sh/r = standard deviation of the ratio between hypocotyl length and taproot length.

In the configurations of SVIS®, maximum size was established as two inches (5.08 cm) and four and a half inches (11.43 cm) in the analyses made on seedlings at two and three days of germination, respectively. These previously established sizes were based on preliminary tests with samples of seed lots of the cultivar ADR 300. The contribution of the growth and uniformity value to calculation of the vigor index was 70% and 30%, respectively.

Statistical analysis: the experimental design was completely randomized, and the means were compared by the Tukey test ($p \leq 0.05$); the results expressed in percentage were transformed into arc sen $(x / 100)^{1/2}$ in all the evaluations. The X-ray images obtained in the X-ray test were compared to the germination results.

Results and Discussion

The water content of seeds from seed lots of pearl millet ranged from 8.6% to 10.0% (Table 1). Seed water content constituted an important factor both for reliable comparison among seed lots, preferentially variations of up to two percentage points (Marcos-Filho, 2015), and analysis of X-ray images, because it directly affects optical density. Thus, the lower the water content, the greater the optical density, which allows better visualization of the inner parts of the X-rayed seeds (Simak, 1991). For the pearl millet seeds, these water contents proved to be suitable for visualization of the embryonic axis, the endosperm, and the pericarp (Figure 1A).

Table 1. Results of germination (Germ.), first count of germination (1st Count), accelerated aging (AA), cold test (CT), seed water contents before beginning evaluations (WC) and after the accelerated aging test (AA WC) of eight seed lots of pearl millet of the cultivar ADR 300.

Lots	Germ.	1st Count	AA	CT	WC	AA WC
	----------------------------------	-------------------%-------------------			----------------------------	
L1	87 abc	73 b	70 bc	49 abc	8.6	23.2
L2	87 abc	79 ab	77 abc	57 ab	8.7	22.9
L3	81 c	72 b	66 c	49 abc	8.7	22.6
L4	89 abc	83 ab	82 ab	66 a	9.3	22.8
L5	91 ab	86 a	87 a	50 ab	10.0	20.4
L6	80 c	76 ab	72 bc	21 c	9.6	22.7
L7	83 bc	77 ab	73 bc	32 bc	9.6	22.9
L8	92 a	85 ab	68 c	42 abc	9.9	22.7
C.V. (%)	4.38	7.21	7.88	28.9	-	-

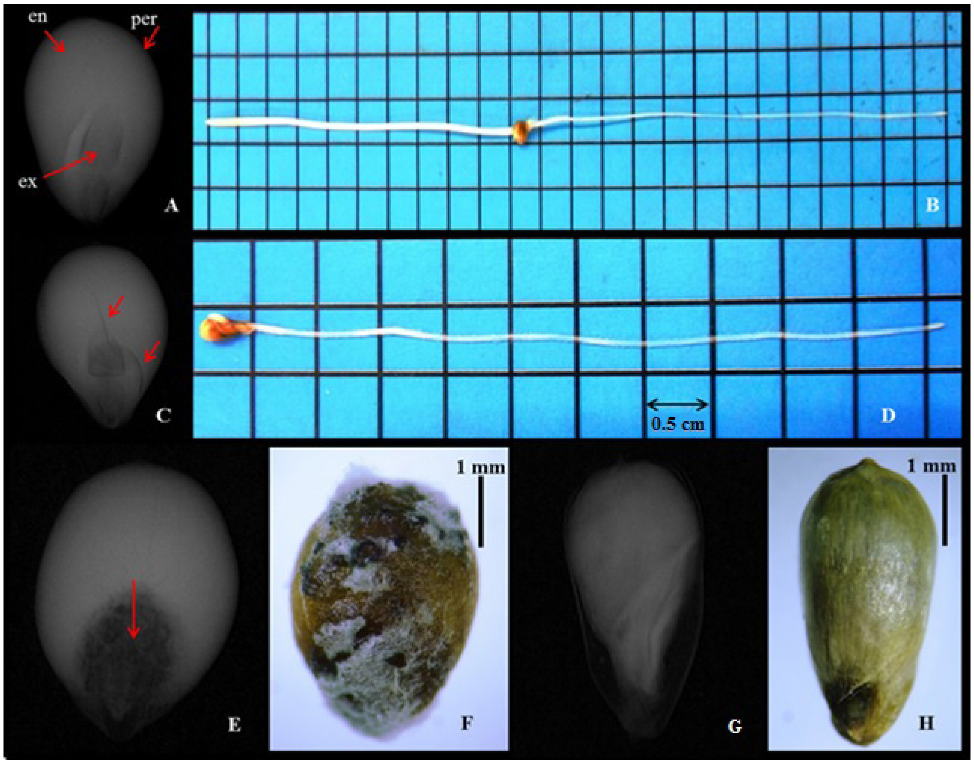

Figure 1. X-ray images of pearl millet seeds without damage (A) highlighting the regions of the embryonic axis (ex), endosperm (en), and pericarp (per); with mechanical damage in the upper region of the endosperm extending to the plumule (C); damage by tissue deterioration throughout the embryonic axis (E); damage by tissue malformation in the endosperm and in the embryonic axis (G); and the respective normal and abnormal seedlings (B and D) and dead seeds (F and H).

The seeds exhibited mechanical damage, damage through tissue deterioration, and malformation (Table 2). Total damage ranged from 2.0% to 13.0%. Seed lots 5, 7, and 8 exhibited the lowest levels of total damage (2.0% to 3.0%); in contrast, lot 3 exhibited the highest percentage of seeds with mechanical damage (7.0%) and with damage through tissue deterioration (9.0%).

Figure 1A shows an X-rayed seed without damage, giving rise to a normal seedling (Figure 1B). In this image, the location of the embryonic axis, the endosperm, and the pericarp can be identified. In contrast, in Figure 1C, mechanical damage in the upper region of the endosperm can be observed, extending to the plumule (Figure 1C), giving rise to an abnormal seedling (Figure 1D).

Damage through tissue deterioration predominantly extended into the embryonic axis can be seen in Figure 1E, resulting in a dead seed (Figure 1F). Malformation can also be identified in Figure 1G and in the lateral part of the endosperm, resulting in a dead seed (Figure 1H). Similar results in which deterioration and malformation of tissues can cause abnormalities in seedlings and death of seeds were also found in rice seeds (Silva et al., 2014).

The results of evaluations of physiological potential of the seed lots are shown in Table 3. The initial water contents of the seeds lots were similar, with maximum variation of 1.4%; after accelerated aging, the variation was 2.8%, which can be considered acceptable for reliability of the results (Marcos-Filho, 1999).

In relation to the results of the germination test, the seed lots exhibited a percentage of normal seedlings above the established minimum of 75% for sale of pearl millet seeds (MAPA, 2008). Lot 8 had a higher percentage of germination than lots 3, 6, and 7.

In regard to vigor evaluated by the first count of germination, accelerated aging, and the cold test, differences among lots were observed. For the first count of germination, values for lots 1 and 3 were lower than lot 5, whereas for the accelerated aging test, lots 1, 3, 6, 7, and 8 had lower performance. In relation to the cold test, the performance of lot

Table 2. Percentage of seeds with total damage and classification regarding mechanical damage, tissue deterioration damage, and malformation damage in eight seed lots of pearl millet, cultivar ADR300.

Lots	Total damage	Mechanical damage	Deterioration damage	Malformation damage
	%			
L1	6.0	3.0	4.0	2.0
L2	6.0	4.0	3.0	0.0
L3	13.0	7.0	9.0	1.0
L4	6.0	5.0	4.0	1.0
L5	2.0	1.0	2.0	1.0
L6	4.0	3.0	3.0	1.0
L7	3.0	1.0	2.0	1.0
L8	2.0	3.0	2.0	0.0

Mean values followed by the same letter in the column do not differ among themselves by the Tukey test ($p \leq 0.05\%$).

Table 3. Results of the vigor index (VI), uniformity index (UI), and seedling length (Length) in SVIS® at two and three days old of seedlings of eight seed lots of pearl millet of the cultivar ADR 300.

Lots	SVIS® 2 Days			SVIS® 3 Days		
	VI	UI	Length cm	VI	UI	Length cm
L1	781 ab	854 ab	3.43 ab	847 b	8507 ab	9.14 a
L2	810 ab	856 ab	3.66 ab	927 a	876 ab	10.28 a
L3	767 ab	818 b	3.48 ab	729 c	809 c	7.15 b
L4	838 a	883 ab	4.06 a	899 ab	861 ab	10.04 a
L5	818 ab	893 a	3.89 a	892 ab	880 a	9.83 a
L6	737 b	832 ab	3.15 b	857 ab	834 bc	9.21 a
L7	779 ab	853 ab	3.50 ab	865 ab	850 abc	9.30 a
L8	807 ab	850 ab	3.69 ab	868 ab	866 ab	9.06 a
C.V.%	7.76	5.21	11.69	5.51	3.35	8.77

Mean values followed by the same letter in the column do not differ among themselves by the Tukey test ($p \leq 0.05\%$).

4 was superior to the performances of lots 6 and 7, which, for their part, were consistent with the results of the accelerated aging tests, exhibiting values lower than the others.

The results obtained through the software SVIS® for seedlings at two and three days old are shown in Table 3. Significant differences for all the parameters evaluated were found for both evaluation times.

The vigor index parameter of the SVIS® analysis at two days of germination allowed identification of more (lot 4) and less (lot 6) vigorous lots. Through seedling length, lots 4 and 5 were more vigorous and lot 6 less vigorous, showing results consistent with results obtained in the accelerated aging and cold tests. Nevertheless, at three days of germination, performance of the lots was different in relation to two days, identified by the length of seedlings of the lots with superior performance (lots 1, 2, 4, 5, 6, 7, and 8) and inferior performance (lot 3), compatible with the results obtained only for the most vigorous lot (lot 5) by first count of germination and accelerated aging.

Seeds that are more vigorous give rise to seedlings with a greater growth rate. This occurs due to greater capacity for transformation and supply of reserves from the storage tissues and from greater incorporation of them by the embryonic axis (Dan et al., 1987). Thus, evaluation of two days old pearl millet seedlings proved to be effective in identifying vigor differences among the lots (especially for the most vigorous lot) in a way comparable to the other vigor tests traditionally used. The index of uniformity of seedling development, both at two days and at three days of germination, showed greater vigor of lot 5 in relation to lot 3.

The advantages of SVIS® in comparison to evaluations of seedling length performed manually with the aid of a ruler include greater accuracy of the results obtained since it minimizes human error as a result of elimination of subjective interpretations (Gomes-Junior, 2010). The time necessary to obtain results decreased with use of the software in relation to the other vigor tests. For example, the final results of the cold test and accelerated aging test of pearl millet seeds are obtained in 11 and 7 days, respectively, whereas in computerized image analysis of seedlings, results can be obtained in two or three days.

The results of the morphological, perimeter, area, and roundness parameters of the seeds exhibited significant differences among the lots, as shown in Table 4. Seed perimeter values show that lots 4 and 5 had higher values in relation to lots 1, 3, 6, and 8, and these results were similar to those obtained from the seed area parameter, in which lot 5 corresponded to the one with greatest area and lots 1, 3, 6, and 8 to those with lowest area. Thus, perimeter and area of the seeds showed a relationship to first count of germination, the accelerated aging and lenght at two days old seedlings by SVIS®. In contrast, this same relationship was not found for roundness, although lot 8 with less roundness also exhibited lower perimeter and area in relation to the other lots (Table 4). The use of roundness would be important to check for the existence of very broad variations concerning seed shape. However, the values were similar among the lots, that is, near 0.8. The results of the sieve retention test identified that the lots used in the present study had predominance of seed retention in similar sieves, the 12x12 mm mesh, an important factor to highlight to allow reliable comparison among the lots.

For the 1000 seed weight variable, lots 2 and 5 exhibited higher values, differing from lots 1, 3, 6, and 7 (Table 4), resulting in classification of the lots in a similar way to the results obtained from seed perimeter and area. These results

Table 4. Morphological parameters of perimeter (Per.), area, and roundness index (Round), percentage of seeds retained in sieves with square mesh of 9x9 mm, 10x10 mm, 12x12 mm, and 14x14 mm, and 1000 seed weight (W1000) of eight seed lots of pearl millet of the cultivar ADR 300.

Lots	Morphological parameters			Sieve (mm)				W1000
	Per.	Area	Round	9x9	10x10	12x12	14x14	
	mm	mm^2	-	------------------%------------------				grams
L1	13.05 c	11.06 cd	0.815 a	10.8	29.8	53.5	5.2	7.36 bc
L2	13.75 ab	12.10 ab	0.804 abc	11.4	30.4	52.5	4.4	8.11 a
L3	13.30 bc	11.33 bcd	0.804 abc	9.7	25.4	57.7	8.0	7.08 c
L4	13.77 a	12.16 ab	0.806 abc	13.5	32.3	46.3	4.3	7.68 ab
L5	13.82 a	12.35 a	0.812 ab	7.6	33.0	48.1	6.4	8.06 a
L6	13.18 c	11.05 cd	0.800 abc	7.3	32.9	52.7	6.6	7.19 bc
L7	13.68 ab	11.88 abc	0.797 bc	6.0	30.7	58.1	5.0	7.28 bc
L8	12.91 c	10.51 d	0.792 c	5.2	24.6	60.8	8.8	7.74 ab
C.V.	2.19	4.95	1.31	-	-	-	-	4.86

confirm the relationship that exists between seed lots of pearl millet with greater seed perimeter, area, and weight and the occurrence of more vigorous seeds. Therefore, the use of these physical parameters can contribute to improving lots in processing, especially through the variability there is in pearl millet seed size. This variability in seed size is one of the main obstacles to suitable establishment of seedlings in the field (Maiti et al., 1990), germination and vigor of pearl millet seeds are directly related to seed size (Gaspar and Nakagawa, 2002).

The results obtained in this study prove the effectiveness of the X-ray test for evaluation of the internal morphology of pearl millet seeds because it is possible to observe mechanical damage, tissue deterioration, and malformation, with direct effects on germination. Furthermore, the study showed that morphological parameters can be associated with the physiological potential of the seeds and that computerized image analysis of seedlings allowed reliable results to be obtained and in a shorter time compared to the traditional tests used. Therefore, this is a promising alternative for quality control programs of companies that produce pearl millet seeds.

Conclusions

The X-ray test is effective in evaluation of internal morphology of pearl millet seeds, allowing identification of mechanical damage, tissue deterioration, and malformation. This makes it possible to establish a relationship between the damage observed and the loss caused in germination.

The use of computerized image analysis of two days old seedlings is reliable for determination of pearl millet seed vigor.

The perimeter and area parameters are related to the physiological potential of pearl millet seeds.

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References

ALVARENGA, R.O.; MARCOS-FILHO, J.; GOMES-JUNIOR, F.G. Avaliação do vigor de sementes de milho super doce por meio da análise computadorizada de imagens de plântulas. *Revista Brasileira Sementes*, v.34, p.488-494, 2012. http://dx.doi.org/10.1590/S0101-31222012000300017

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

BREWER, T.M.; LANG, L.; FUJIMURA, K.; DUJMOVIC, N.; GRAY, S.; KNAAP, E. Development of a controlled vocabulary and software application to analyze fruit shape variation in tomato and other plant species. *Plant Physiology*, v.141, p.15-25, 2006. http://www.oardc.ohio-state.edu/vanderknaap/files/plant_phys_paper.pdf

CASEIRO, R.F.; MARCOS-FILHO, J. Procedimentos para a condução do teste de frio visando a avaliação do vigor de sementes de milho. *Scientia Agricola*, v.57, p.459-466, 2000. http://dx.doi.org/10.1590/S0103-90162000000300014

DAN, E.L.; MELLO, V.D.C.; WETZEL, C.T.; POPINIGIS, F.; SOUZA, E.P. Transferência de matéria seca como método de avaliação do vigor de sementes de soja. *Revista Brasileira de Sementes*, Brasília, v.9, p.45-55, 1987. http://www.abrates.org.br/revista/artigos/1987/v9n3/artigo05.pdf

GARCIA, D.C.; MENEZES, N.S. Teste de envelhecimento precoce para sementes de azevém, aveia preta e milheto. *Ciência Rural*, v.29, p.233-237, 1999. http://www.scielo.br/pdf/cr/v29n2/a08v29n2.pdf

GASPAR, C.M.; NAKAGAWA, J. Influência do tamanho na germinação e no vigor de sementes de milheto (*Pennisetum americanum* (L.) Leeke). *Revista Brasileira de Sementes*, v.24, p.339-344, 2002. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-31222002000100046

GOMES-JUNIOR, F.G.; CICERO, S.N. X-Ray analysis to assess mechanical damage in sweet corn seeds. *Revista Brasileira de Sementes*, v.34, n.1 p.078-085, 2012. https://dx.doi.org/10.1590/S0101-31222012000100010

GOMES-JUNIOR, F.G. Aplicação da análise de imagens para a avaliação da morfologia interna de sementes. *Informativo Abrates*, v.20, p.33-39, 2010. http://www.abrates.org.br/images/stories/informativos/v20n3/minicurso02.pdf

GOMES-JUNIOR, F.G.; CHAMMA, H.M.C.; CICERO, S.M. Automated image analysis of seedlings for vigor evaluation of common bean seeds. *Acta Scientiarum. Agronomy*, v.36, p.195-200, 2014. http://dx.doi.org/10.4025/actasciagron.v36i2.21957

HOFFMASTER, A.L.; FUJIMURA, K.; MCDONALD, M.B.; BENNETT, M.A. An automated system for vigor testing three-day old soybean seedlings. *Seed Science and Technology*, v.27, p.7-24, 2003. http://www.ingentaconnect.com/content/ista/sst/2003/00000031/00000003/art00019

HOFFMASTER, A.F.; XU, L.; FUJIMURA, K.; MCDONALD, M.B.; BENNETT, M.A.; EVANS, A.F. The Ohio State University Seed Vigor Imaging System (SVIS) for soybean and corn seedlings. *Seed Technology*, v.27, p.7-24, 2005. http://europepmc.org/search/?page=1&query=AUTH:%22Hoffmaster+AF%22

MAITI, R.K.; RAJU, P.S.; BIDINGER, F.R. Seedling vigor in pearl millet. I. Role of seed size. *Turrialba*, v.40, p.353-355, 1990. http://www.jstor.org/stable/3628189

Ministério da Agricultura, Pecuária e Abastecimento - MAPA. 2008. Instrução Normativa, 45, de 21 de maio de 2008. 13p. Normas e padrões para produção e comercialização de sementes de espécies forrageiras de clima tropical. http://www.adagri.ce.gov.br/Docs/legislacao_vegetal/IN_30_de_21.05.2008.pdf

MARCOS-FILHO, J. Fisiologia de sementes de plantas cultivadas. Londrina: ABRATES. 2015. 660p.

MARCOS-FILHO, J.; KIKUTI, P.L.A.; LIMA, B. L. Métodos para avaliação do vigor de sementes de soja, incluindo a análise computadorizada de imagens. *Revista Brasileira de Sementes*, v.31, n.1, p.102-112, 2009. http://dx.doi.org/10.1590/S0101-31222009000100012

MARCOS-FILHO, J. Testes de vigor: importância e utilização. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B. (Eds.) Vigor de sementes: conceitos e testes. Londrina: Abrates, p.1-21, 1999.

MONDO, V.H.V.; DIAS, M.A.N.; MCDONALD, M.B. Seed vigor imaging system for two-day-old corn seedling evaluation. *Seed Technology*, v.33, p.191-196, 2011. http://www.jstor.org/stable/23433428

OJEDIRAN, J.O.; ADAMU, M.A.; JIM-GEORGE, D.L. Some physical properties of Pearl millet (*Pennisetum glaucum*) seeds as a function of moisture content. *African Journal of General Agriculture*, v.6, p.39-46, 2010. http://www.researchgate.net/publication/267202055

PESKE, F.B.; NOVEMBRE, A.D.L.C. Condicionamento fisiológico de sementes de milheto. *Revista Brasileira de Sementes*, v.32, p.132-142, 2010. http://www.scielo.br/scielo.php?pid=s0101-31222010000400015&script=sci_arttext

PIRES, F.R.; ASSIS, R.L.; SILVA, G.P.; BRAZ, A.J.B.P.; SANTOS, S.C.; VIEIRA NETO, S.A.; SOUSA, J.P.G. Desempenho agronômico de variedades de milheto em razão da fenologia em pré-safra. *Bioscience Journal*, v.23, p.41-49, 2007. http://www.seer.ufu.br/index.php/biosciencejournal/article/view/6695/4410

SAKO, Y.; MCDONALD, M.B.; FUJIMURA, K.; EVANS, A.F.; BENNETT, MA. A system for automated seed vigour assessment. *Seed Science and Technology*, v.29, p.625-636, 2001.

SCHNEIDER, C.A.; RASBAND, W.S.; ELICEIRI, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, v.9, p.671-675, 2012. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5554542/

SILVA, V.N.; ARRUDA, N.; CICERO, S.M.; MAUS, C.A.; GIACOMELI, R. Morfologia interna e germinação de sementes de arroz de terras baixas produzidas em diferentes regimes hídricos. *Irriga*, v.19, p.453-463, 2014. http://dx.doi.org/10.15809/irriga.2014v19n3p453

SILVA, V.N.; GOMES-JUNIOR, F.G.; CICERO, S.M. Computerized imaging analysis of seedlings for assessment of physiological potential of wheat seeds. *Revista Brasileira de Sementes*, v.34, p.589-596, 2012. http://dx.doi.org/10.1590/S0101-31222012000400009

SIMAK, M. Testing of forest tree and shrub seeds by X-radiography. In: GORDON, A.G.; GOSLING, P.; WANG, B.S.P. (Ed.) Tree and shrub seed handbook. Zurich: ISTA, 1991. p.1-28.

TIMOSSI, P.; DURIGAN, J.; LEITE, G. Formação de palhada por braquiárias para adoção do sistema plantio direto. *Bragantia*, v.66, p.617-622, 2007. http://www.redalyc.org/articulo.oa?id=90866412

WENDT, L.; GOMES-JUNIOR, F.G.; ZORATO, F.M.; MOREIRA, G.C. Avaliação do potencial fisiológico de sementes de soja por meio de imagens. *Pesquisa Agropecuária Tropical*, v.44, p.280-286, 2014. http://www.scielo.br/pdf/pat/v44n3/a11v44n3.pdf