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The use of X-ray to evaluate *Brachiaria brizantha* seeds quality during seed processing[1]

Raios X na avaliação da qualidade de sementes de *Brachiaria brizantha* durante o beneficiamento

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ABSTRACT - The X-ray test permits the visualization of the internal structures of seeds in a non-destructive way making thus possible the diagnosis of causes of seed low germination and purity. The objective of this project was to evaluate the quality of *Brachiaria brizantha* seeds by means of the X-ray test and the effect of the processing stage. The seeds were sampled before processing and after going through the air and screen machine, and the first and the second gravity tables. Seeds were evaluated as to water content, purity, weight of a thousand seeds, germination, and the X-ray test. The treatment replications were distributed according to a complete random design with four replications. The X-ray test permit to verify the completely full seeds as well as deteriorated, hollow, mechanically damaged seeds and empty spikelets. The processing procedure caused no damage to the seeds. The analysis by means of X-ray is a quality control procedure resulting in information that can be used in the adjustments of processing machines.

Key words: Forage grasses. Image analysis. Marandu. Germination

RESUMO - O teste de raios X permite a visualização das estruturas internas das sementes de modo não destrutivo e individualizado, possibilitando o diagnóstico de causas da baixa germinação e pureza de um lote. O objetivo do trabalho foi avaliar a qualidade das sementes de *Brachiaria brizantha* utilizando-se o teste de raios X e o efeito das etapas de beneficiamento. As sementes foram amostradas antes do processamento e após a saída da máquina de ar e peneiras, primeira e segunda mesa gravitacional. As sementes foram avaliadas quanto ao teor de água, pureza, peso de mil sementes, germinação e pelo teste de raios X. O delineamento experimental utilizado foi o inteiramente casualizado com quatro repetições. O teste de raios X permite a identificação de sementes cheias, com tecido deteriorado, mal formadas, com danos mecânicos e espiguetas vazias de *B. brizantha*. O beneficiamento não causou dano mecânico nas sementes de *B. brizantha*. A análise de raios X pode ser utilizada para o monitoramento da qualidade, fornecendo informações que podem ajudar no ajuste das máquinas de beneficiamento.

Palavras-chave: Gramíneas forrageiras. Análise de imagem. Marandu. Germinação

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INTRODUCION

Brachiaria brizantha (Hochst. ex A. Rich) Stapf, cv. 'Marandu', occupies more than 60% of the pasture area in Brazil and represents 90% of the volume of the marketed forage seeds (VERZIGNASSI *et al*., 2012).

Due to the harvesting method prevailing in Brazil, which consists in the sweeping of the seeds from the soil surface, the lots of grass seeds have high amounts of soil particles, stones, empty spikelets, partially filled caryopses, pieces of straw and weed seeds (BRASIL, 2009; MELO *et al*., 2016; NERY *et al*., 2009).

To remove impurities and improve seed quality the lots have to be processed which consists in submitting the seed lots to a number of sequential machine-based operations which contribute to homogenize the seed lot and thus increase the seeds physiological and physical quality (MELO *et al*., 2016; PEREIRA; ALBUQUERQUE; OLIVEIRA, 2012).

For grass seeds processing, screen-and-air machines are used. These machines separate particles based on differences in physical characteristics, such as size and specific weight thus making possible the removal of undesirable particles (MELO *et al*., 2016; NERY *et al*., 2009).

On the other hand, processing is the most important source of mechanical injury undergone by seeds during the production process it is responsible for 50% of the damages suffered by seeds due to the several drops during their moving from deposits into machines and vice-versa this resulting in reductions in seed germination, vigor and purity (CARVALHO; NAKAGAWA, 2012).

Some tests have been used in laboratory for the identification of mechanical damage in seeds such as the tetrazolium test for soybean (PACHECO *et al*., 2015), common bean (FORTI; CICERO; PINTO, 2008) and cotton (CERVI; MENDONÇA, 2009); the sodium hypochlorite for soybean, rapid green for corn and sweet-corn, iodine for corn (MARCOS FILHO; CICERO; SILVA, 1987) and X-ray test for castor bean (KOBORI; CICERO; MEDINA, 2012), rice (MENEZES *et al*., 2012), brow hemp *(*ARRUDA; CICERO; GOMES-JÚNIOR, 2016*), bean (*MONDO *et al*., *2009)*, sweet-corn (GOMES-JÚNIOR; CICERO, 2012) and *Jatropha curcas* (PINTO *et al*., 2009). But data concerning laboratory tests efficient to detect mechanical damage in forage grass seeds have not been found in the literature.

X-ray technique can be used to evaluate forage grass seeds quality since the radiation is absorbed in several degrees, depending on seed coat thickness, density, structure composition and the radiation wave length thus resulting in an image that indicates the seed internal morphology. The test principle is based on the difference of seed tissues density. The denser the tissue is, the clearer is the image and wholes, damages or difference in the tissues are more distinct because differences in coloration (INTERNATIONAL SEED TESTING ASSOCIATION, 2004).

This way, the X-ray test may be considered a simple, quick and non-destructive method viewing to detect empty, damaged or badly formed seeds thus helping to control seed lots quality (MONDO *et al*., 2009). So, the objective of this project was to evaluate *B. brizantha* seed lots quality during seed processing by means of the X-ray test.

MATERIAL AND METHODS

B. brizantha seeds were collected with the help of a soil sweeping machine during the cropping year of 2013/14 in producing fields of 'Monte Alegre de Minas', state of Minas Gerais, Brazil. The seeds were processed in a Seed Processing Unit by passing them through screen of the air and screen machine (ASM) and screen machine and two 2.40 m long and 1.25 m wide gravity tables. The speed of vibration of the two gravity tables was of 1.750 rpm and the transversal and longitudinal inclinations were of 17 and 12, respectively.

Seed samples were taken during processing phases and from the different machines which resulted in 10 treatments such as described next: T1 - check treatment, composed by the non processed seeds; T2 - lower screen of the ASM, mesh 12 and opening of 26 mm; T3 - intermediate screen of the air and screen machine with mesh eight and opening of 18 mm; T4 - bottom of the ASM, that is the material which was retained by the screens; T5 - the light material from the first gravity table, removed by an aspirator at the entrance of this machine; T6 - upper discharge spout of the first gravity table, collected at a distance of 40 cm starting from the higher extremity of the table exit when the lateral inclination is considered; T7 - intermediate discharge spout of the first gravity table in the intermediate segment of 50 cm from the table exit; T8 - upper discharge spout of the second gravity table in the 55 cm long segment starting from the higher table spout; T9 - intermediate discharge spout of the second gravity table, in the 40 cm long intermediate segment; T10 - lower discharge spout of the second gravity table, in the 30 cm long segment of the lowest extremity (Figures 1 and 2).

The discharge divisions of the two gravity tables were adjusted so as to increase the concentration of undesirable material in the lower discharge spout and due to this they were of different sizes.

Figure 1 - Seed processing procedure with sample collecting points indicated

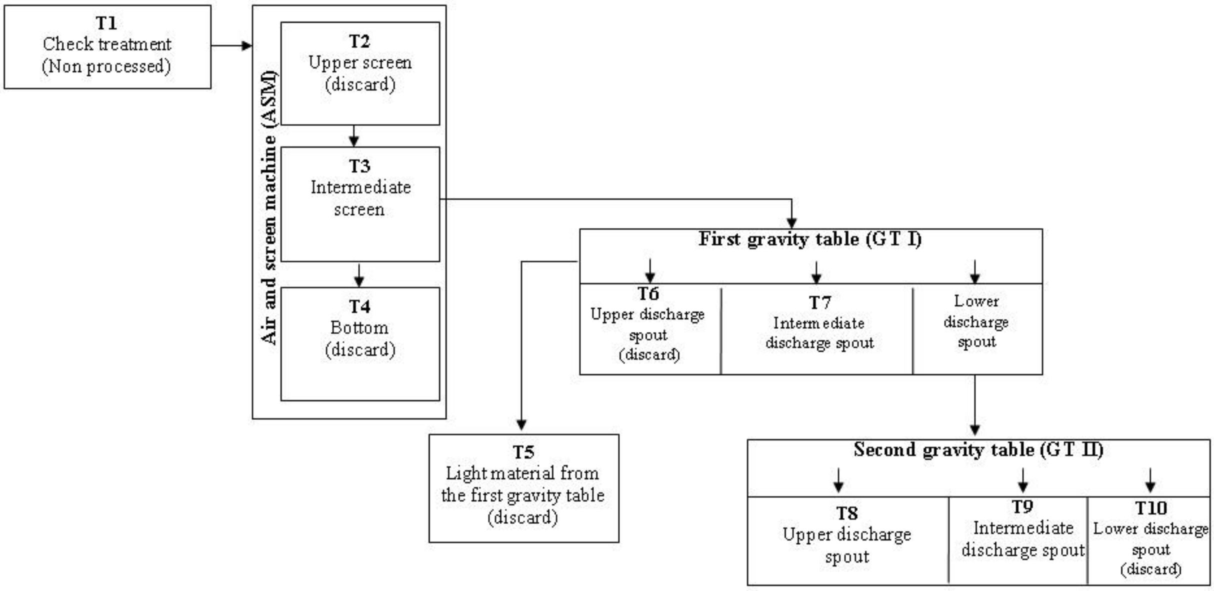
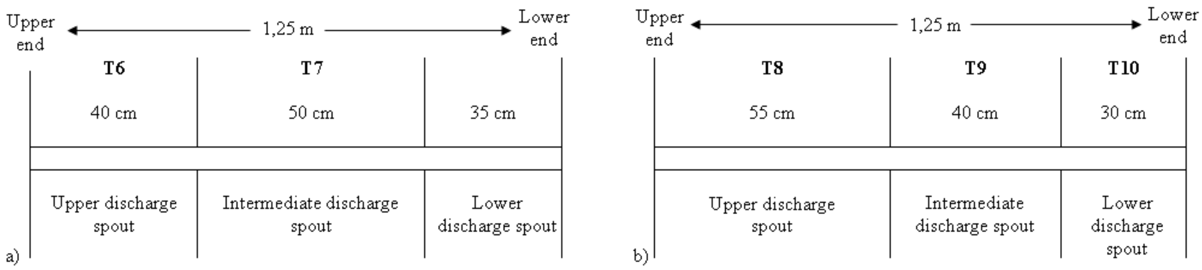


Figure 2 - Seed sample collecting points in the first (a) and second (b) gravity tables



After the machines were stabilized, the seeds from each treatment (processing phase) were sampled at regular intervals of five minutes between replications, with 20 simple samples of each treatment (with a mean weight of 100 g) from the different discharge spouts. These samples were then grouped and homogenized in compound samples which were reduced to get the mean samples of 500 g each (BRASIL, 2009).

Following that, the samples were taken to the Seed Analysis Laboratory, a unit of the Plant Production Department on the campus of the Paulista State University (UNESP), in Jaboticabal, state of São Paulo, Brazil. Then, the seeds were passed through a seed divisor so as to get the working samples and the seed quality was then evaluated by means of the following tests and procedures:

Seed water content - determined by the oven method at 105 ± 3 °C for 24 hours and the results expressed in percentage (BRASIL, 2009).

Physical purity - Two subsamples of 2 g were used weighed in a precision scale (0.001 g) and getting the components separated with the help of a pneumatic blower. To get the pure seed fraction, the separation process was complemented by manual picking and the results expressed in percentage (BRASIL, 2009). The subsamples weight was larger than that recommended by the Rules for Testing Seeds so as to guarantee a bigger representatively of the present components and also to get the adequate number of seeds for all the tests expected to be run.

One thousand seeds weight - eight 100 seed samples taken from the pure seed fraction were weighed in a 0.001 g precision scale and the results expressed in grams (BRASIL, 2009).

Germination test - carried with four 100 seed samples sown on top of two filter paper sheets wet with an amount of water equal to 2.5 times the sheet weight and placed inside transparent plastic boxes (11.0 X 11.0 X 3.5 cm) with the lid on. The test was carried under alternate temperatures of 20 - 35 °C and eight hours photoperiod. Normal seedlings were considered those whose plume grew out of the coleoptile and thre primary root was at least 1 cm long. Countings were made on the 7th and the 21rst day after sowing (BRASIL, 2009).

X-ray test - carried at the Image Analhysis Laboratory of the Plant Production Department of the “Luiz de Queiroz” Superior School of Agriculture, a unit of the São Paulo State University (USP), in Piracicaba, state of São Paulo, Brazil, where 200 seeds of each treatment were radiographed in a Faxitron X-ray, model MX-20 DC 12, coupled to a Core 2 Duo computer (3.16 GHz, 3 GB of memory RAM, hard disk of 160 GB) and Multi Sync monitor LCD1990SX of 17 inches). For the radiographies, the seeds were positioned at a distance of 14.3 cm of the X-ray emitting source. For the correct positioning of the seeds during their exposition to the X-rays, a double-face adhesive tape applied to a 1.0 mm thick transparent acetate film. From the radiographic images, the seeds were analysed on the computer screen and classified as totally full and without damages, malformed seeds or immatures, having deteriorated tissues, with mechanical damages and empty spikelets (CARVALHO; ALVES; OLIVEIRA, 2010; MENEZES *et al*., 2012; PINTO *et al*., 2009).

The experiment was set according to a completely random design with four replications. The experimental data were submitted to the normality test and homoscedasticity. After that they were submitted to the analysis of variance and the means compared by the Tukey test, at the level of 5% of probability. The values from the X-ray test were transformed in $\sqrt{(x + 0,5)}$.

RESULTS AND DISCUSSION

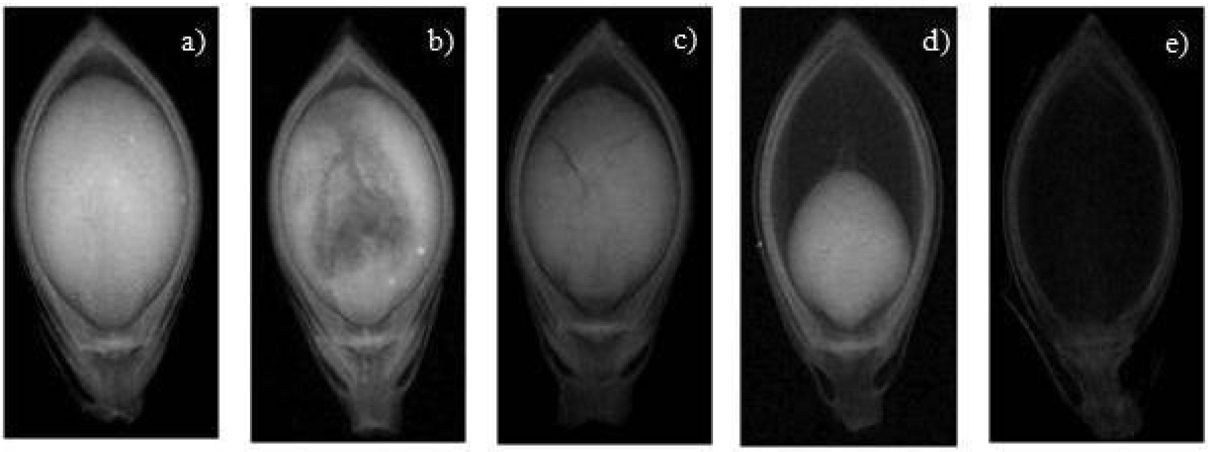
The X-ray test permitted to identify and classify five predominant morphological types of *B. brizantha* seeds (Figure 3): a) full seeds, with the internal cavity fully occupied by the caryopsis; b) with damaged or deteriorated tissue when the caryopsis showed dark stains; c) with mechanical damage due to ruptures in the caryopsis; d) ill-formed or immature seeds, when the internal cavity was partially filled by the caryopsis since it did not complete its development; e) empty spikelets, with no caryopsis inside.

It was observed that the material from the different processing phases showed physical purity varying from zero to 90.7% (Table 1). So, the samples coming from the upper screen and from the bottom of the ASM (T2 and T4); drifting from the first gravity table (T5) and lower discharge spout of the second gravity table (T10) showed no seeds and this did not permit to evaluate the other quality characteristics of the samples of these treatments (Tables 1 and 2).

The seeds of the other processing phases showed water contents between 8.9 and 9.2% (Table 1). The small difference between those two values was important to guarantee trustworthy results of the germination and one thousand seeds test (BRASIL, 2009; MELO *et al*., 2016; STEINER *et al*., 2011).

It was verified that the first processing operation of pre-cleaning made by the ASM was not enough to improve the physical and physiological characteristics of the lot since the check treatment (T1) seeds and those retained on top of the intermediate screen of the ASM (T3) showed similar purity results (38.7 and 45.8%), germination (66.2 and 77.2%) and one thousand seed weight (8.9 and 8.5 g), that is, values which did not differ significantly.

Figure 3 - Full seeds (a), with deteriorated tissue (b), with mechanical damage (c), ill formed (d), and empty spikelets (e) of *Brachiaria brizantha* submitted to the X-ray test



This similarity between values of samples from the ASM was also detected by the X-ray test when the composition of the samples was evaluated in terms of percentage of full seeds, seeds with deteriorated tissue, mal-formed seeds and empty spikelets (Tables 1 and 2). So, the ASM removed only the larger impurities.

Table 1 - Physical purity (P), water content (WC), germination (G), and one thousand seed weight (OTSW) of *Brachiaria brizantha* after seed processing

Seed processing	P	WC	G	OTSW (g)
	(%)			
T1 – Check treatment (non processed)	38.7 c	8.9	66.2 b	8.9 b
T2 – Upper screen of ASM1	0.0 e	-	-	-
T3 – Intermediate screen of ASM	45.8 c	9.0	77.2 ab	8.5 b
T4 – Bottom of ASM	0.0 e	-	-	-
T5 – Light material of GT I1	0.0 e	-	-	-
T6 – Upper discharge apout GT I	4.2 e	9.2	86.7 a	10.2 a
T7 – Intermediate discharge apout GT I	90.7 a	8.9	80.5 a	9.9 a
T8 – Upper discharge spout GT II	77.9 b	9.0	50.5 c	7.9 c
T9 – Intermediate discharge spout GT II	19.1 d	8.9	37.5 d	7.1 d
T10 – Lower discharge spout GT II1	0.1 e	-	-	-
F tratament	651.90**		54.02**	82.00**
d.m.s.	7.53		11.37	0.54
C.V. (%)	6.88		7.56	4.16

** Significant at the level of 1% of probability by the F test. Means in the same column, followed by the same letter, are not significantly different at the level of 5% of probability, according to the Tukey test. ASM - air and screen machine, GT I - first gravity table, GT II - second gravity table. [1] With the exception of the purity analysis, all the other quality evaluations were not made for those treatments due to the lack of seeds

Table 2 - Percentage of full seeds, with deteriorated tissue, mal-formed and empty spikelets of *B. brizantha* submitted to the X-ray test after seed processing phases

Seed processing	Full seeds	Deteriorated tissue	Mal-formaded	Empty
	(%)			
T1 – Check treatment (non processed)	77.0 b	7.5 ab	9.0 ab	4.5 abc
T2 – Upper screen of ASM1	-	-	-	-
T3 – Intermediate screen of ASM	72.5 b	7.5 ab	11.0 b	8.5 c
T4 – Bottom of ASM	-	-	-	-
T5 – Light material of GT I1	-	-	-	-
T6 – Upper discharge apout GT I	95.0 a	2.5 a	1.5a	0.0 a
T7 – Intermediate discharge apout GT I	87.0 a	6.0 ab	6.0 ab	0.0 a
T8 – Upper discharge spout GT II	48.5 c	12.5 b	36.0 c	2.5 ab
T9 – Intermediate discharge spout GT II	17.0 d	6.5 ab	70.0 d	6.5 bc
T10 – Lower discharge spout GT II1	-	-	-	-
F tratament	168.09**	3.37*	167.56**	10.53**
d.m.s.	9.38	1.67	8.58	1.50
C.V. %	9.50	39.34	25.86	56.12

**, *, [ns] Significant at the level of 1%, 5% and not significant of probability by the F test. Means in the same column, followed by the same letter, are not significantly different at the level of 5% of probability, according to the Tukey test. ASM - air and screen machine, GT I - first gravity table, GT II - second gravity table. [1] With the exception of the purity analysis, all the other quality evaluations were not made for those treatments due to the lack of seeds

The performance during the pre-cleaning phase depends on the size and number of screen, because the seeds are light and to separate them from the impurities can vary with the characteristics of the lot (NERY *et al*., 2012), possibly the physical quality and seed physiology could have been improved by increasing screen of larger and smaller size than the seeds, before passing through the gravity tables.

The improving of the *B. brizantha* seeds characteristics took place only when they were submitted to the first gravity table (GT I) in which the seeds coming from the first upper spout (T6) showed the maximum one thousand seed weight (10.2 g) and the maximum germination (86.7%) (Table 1). But the seeds from that spout are usually discharged by seed companies since they have low purity (4.2%) due to the presence of heavy impurities such as stones and large soil particles, this being the main quality problem of this fraction of seeds.

The high germination shown by the seeds from the upper spout (T6) may be ascribed to the high percentage of full seeds and to the very low presence of seeds with deteriorated tissue, mal-formed and to the absence of empty spikelets, such as verified by the X-ray test (Table 2). The seeds of superior quality present in this fraction could be separated by means of a strickter and more precise regulation of this machine or by repassing them through other machines during processing, such as another gravity table. This procedure was used to increase the purity of seed lots such as mungo-bean and mombaça-grass (ARAÚJO *et al*., 2011; MELO *et al*., 2016). On the other hand, it should be considered the economical viability of this procedure since it depends on the size of the original lot and of the commercial value of those seeds.

The seeds from the intermediate discharge spout of the gravity table (T7) showed purity of 90.7% and germination of 80.5%. So, it was the only sample within those coming from the processing phases which could be commercialized in the national market since it is in agreement with the quality standards as established by the Brazilian Ministry of Agriculture for the commercialization and sowing of *B. brizantha* seeds 60% of germination and 60% of pure seeds for seed classes S1 and S2 and of 80 and 60% for certified seeds, respectively (BRASIL, 2008).

The maximum quality of seeds from the intermediate discharge spout of the gravity table (T7) was evaluated by means of the X-ray test since it is observed that the sample has 87% of full seeds and minimum percentages of seeds with deteriorated tissue and mal-formed which totalize only 12% in addition to not having empty spikelets in is composition (Table 2).

The seeds from the upper discharge of the second gravity table (T8) showed the second best purity percentage (77.9%) (Table 1). But, as to germination, one thousand seed weight, full seeds, and mal-formed seeds, the values of 50.5%, 7.9 g, 48.5%, and 36%, respectively, were superior only to those from the intermediate discharge spout of the same machine (T9). The radiographic analysis of the seeds from the upper discharge spout of the second gravity table (T8) showed that that was the processing phase which resulted in the highest percentage of seeds with deteriorated tissue (12.5%). The low germination of the seeds of this phase may be due to the fact that half (48.5%) of the pure seeds were deteriorated or mal-formed (Tables 1 and 2).

The X-ray test permitted to verify that even the pure seeds separated during the purity analysis in values of 19.1% from the sample of the intermediate discharge spout from the second gravity table (T9) still had empty spikelets (6.5%) thus confirming reported (OLIVEIRA; CICERO, 1993) difficulties for purity analysis of small grass seeds. It was observed that 70% of the seeds of this processing phase were mal-formed or immature. These facts may explain the minimum values verified for the weight of one thousand seeds (7.1 g) and germination (37%) since, after the evaluation of the seeds internal morphology, it was verified that only 17% of them had wholly formed caryopsis.

The seeds of the intermediate discharge spout of the second gravity table (T9) showed low germination due to their having low reserves and to being mal-formed as it was shown by their low one thousand seed weight. This processing phase was not capable of removing all the seeds which were mal-formed. The occurrence of immature and mal-formed seeds was probably caused by the fact that they were taken from the soil surface by sweeping; these seeds underwent natural shedding and so dropped onto the soil (BONOME *et al.*, 2006).

The test results exhibited by the seeds from the upper, intermediate, and lower discharge spouts of the second gravity table (T8, T9, and T10) show that this machine was not efficient to improve both the physical and physiological quality of the seeds so as to comply with the standards established for the commercialization of *B. brizantha* seeds (BRASIL, 2008). So, for the seeds from the upper and intermediate discharge spouts of the second gravity table (T8 and T9) to be commercialized it is necessary to make a more refined adjustment of that machine, so as to improve the removal of undesirable material.

The mal-formed and empty seeds of *B. brizantha* were not detected in the analysis of samples without the use of X-ray. Thus, the results of this test may be used as a reference for the adjustment of processing machines viewing the improvement of seed lots quality.

The mechanically injured seeds were observed in all phases of processing and they varied between 0.5 to 2.0% so that it was not possible to say that those injury levels were caused by seed processing. Probably those injuries were caused by the mechanical harvesting by sweeping since this method expose them to impacts against hard surfaces which cause rupture of tissues (QUEIROZ *et al*., 2012), such as those shown in Figure 3. So the radiographic analysis was found an efficient means to identify mechanical injuries in *B. brizantha* seeds and can be used as a quick method of quality evaluation.

CONCLUSIONS

1. The X-ray test permits to identify full seeds, seeds with deteriorated tissues, mal-formed seeds, seeds with mechanical damage and empty spikelets of *B. brizantha*;
2. Seed processing in this situation does not cause mechanical damage to *B. brizantha* seeds;
3. The X-ray test may be used to monitor *B. brizantha* seeds supplying information that may help in the adjustment of processing machines.

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