ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 02, pp.26471-26475, February, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EFFICIENCY OF X-RAY TEST IN THE IDENTIFICATION OF DAMAGE AND ITS RELATION WITH THE PUMPKIN SEEDS QUALITY

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ARTICLE INFO

Article History:
Received 20th November, 2015
Received in revised form
30th December, 2015
Accepted 07th January, 2016
Published online 27th February, 2016

Key words:

Curcubita moschata,
Image analisys,
Physiological quality.

ABSTRACT

The high quality seeds use for the pumpkin fruit production is an important factor because it is directly related to productivity. The objective of this study was to evaluate the efficiency of x-ray test in the physiological and biochemical quality of pumpkin seeds (*Curcubita moschata*). Seeds of three different lots were submitted to x-ray test in Faxitron x-ray device, MX20 model, DC-12 option, with an intensity of 18 kV per 20 seconds. After, the seeds were separated into three different classes: full, damaged and translucent, being these submitted to germination and emergence tests using 10 replicates of 10 seeds from each lot. Tetrazolium test still was performed in the remaining seeds. For the biochemical quality assessment was verified the activity of enzymes catalase and superoxide dismutase from different types of classes and lots of seeds. The use of x-ray technique is effective for detecting abnormalities in pumpkin seeds that negatively affect germination. The enzymes catalase expression and superoxide dismutase is directly related to the seeds quality and through these is possible to separate the lots in the same classes established by x-ray test.

Citation: **Luiza do Nascimento Antônio, Hugo Cesar Rodrigues Moreira Catão, Heloisa Oliveira dos Santos, Raquel Maria de Oliveira Pires, *Barbara Gomes Ribeiro et al, 2016.** "Efficiency of x-ray test in the identification of damage and its relation with the pumpkin seeds quality", *International Journal of Current Research,* 8, (02), 26471-26475.

INTRODUCTION

The seeds use is one of the most expensive parts in the implementation of any culture, including pumpkins, where kg is sold around R$200.00. Currently, the vegetable seed companies put on the market available to the farmer lots with germination over 95%, being the seed quality important factor for the implementation and subsequent success of culture, as well as ensuring a quick and uniform cultivation and trade gains (Marcos Filho, 2015). For pumpkin fruits' commercial production the use of high quality seeds is fundamental factor. Low productivity often is associated, among other factors, the use of seed lots with low germination, reducing vigor and high contamination by pathogens. In pumpkin crop is often the occurrence of empty seeds and damaged morphologically, the latter difficult separation and identification due to the type of processing that suffer, also, by the hard tegument presence, which impedes the embryo viewing without damaging the seeds (Carvalho *et al*., 2009). There are several methods that assess the seeds quality. The germination test is official and carried out in many laboratories for the physiological potential evaluation. In addition to vigor and viability tests, enzyme activity analysis, physical purity and genetic, and non-destructible tests, such as image analysis of the x-rays test (MACHADO and CICERO, 2003). The x-rays test has advantages because it is fast, simple and non-destructive, allows the identification of internal damage seeds, viewing the embryos development phase and evaluation of seeds' internal morphology (COPELAND and MCDONALD, 1985). Thus, the objective of this study was to evaluate the efficiency of x-rays test to identify damage and its relation with the biochemical and physiological quality of pumpkin seeds.

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MATERIAL AND METHODS

The research was conducted at the Seed Center Laboratory of the Agriculture Department - Federal University of Lavras (UFLA), in Lavras, MG - Brazil. Three lots of pumpkin seeds

Curcubita moschata being each lot characterized by seeds produced in three different seasons were used (2003/04, 2008/09, 2013/14) produced in the *Jaíba* region, north of *Minas Gerais*. The seeds were stored in cold storage with average temperature 10 °C and relative air humidity around 40%, until the time of testing. Seeds of three lots were submitted to x-ray test, using the Faxitron x-ray device, MX20 model, DC-12 option, with an intensity of 18 kV per 20 seconds. The seeds were arranged on transparent blades containing 100 seeds per blade. Then, the seeds were classified and separated according to the internal structure into three distinct classes: full, translucent and damaged (Figure 1).

in a dark plastic container, immersed at 1% tetrazolium salt solution during 6 hours at constant temperature 30 °C. After staining, the evaluation was carried out with aid of stereoscopic microscope to determine the viability (BRAZIL, 2009). For enzyme analysis 10 seeds from each treatment were collected and macerated in the PVP presence and liquid nitrogen in melting pot onto ice and then stored at -86°C. Prior to maceration was removed the seed tegument to facilitate the enzymes extraction. For the enzymes extraction was added the extraction buffer (Tris HCL 0.2 M pH 8 + 0.1% β-mercaptoethanol) at a ratio 300 ml per 100 mg of seed powder.

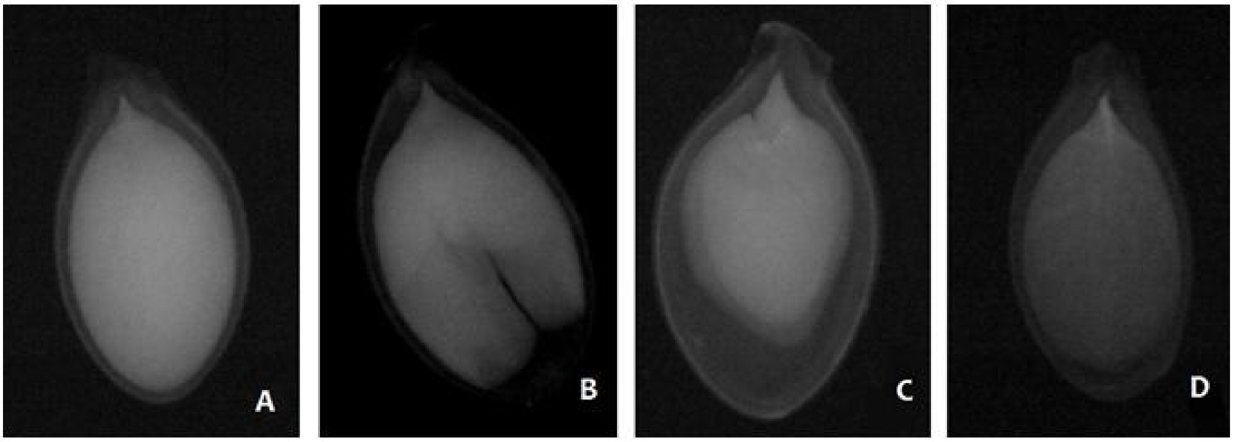

Figure 1. Radiographic images of pumpkins seeds. (A) full seed (B) damaged seed 1 (C) damaged seed 2 (D) translucent seed

The seed water content was determined by oven method at 105 °C per 24 hours (BRAZIL, 2009), using 2 x 50 seeds per lot. After this period, the seeds were taken to desiccators until the cooling of the samples and then, was performed the dry mass of them. The results were expressed as a percentage. Germination test was conducted with 300 seeds of each lot, 100 seeds from each class, with 10 replicates of 10 seeds and the initial quality of each lot with 200 seeds, 8 replicates of 25 seeds. Sowing was made between paper towels type Germitest moistened with distilled water at a ratio of 2.5 $ml.g^{-1}$ of paper. Soon after, they were put to germinate in germinator chamber at a constant temperature at 25 °C and evaluations of normal seedlings were made on two counts. The first on the fourth day and the last on the eighth day after sowing, according the Rules for Seed Analysis (BRAZIL, 2009). The results were expressed as average percentage of normal seedlings of the replicates. Sowing was conducted in plastic trays, 1200 cm^3 containing substrate for growing vegetables. In each container were placed 5 randomly replicates. Daily evaluations were performed from the beginning of the emergency, counting the number of emerged seedlings. It was considered the percentage of normal seedlings at 21 days. To calculate the emergency speed index, according to Maguire (1962), daily readings of the number of seedlings with cotyledons leaves above the soil were carried out. The emergency speed was held along to the emergency test writing down every day and at the same time, the number of emerged seedlings until complete stabilization of the stand. The emergency speed was determined according to the expression of Edmond and Drapala (1958). The remaining seeds of the germination test for each treatment were chopped longitudinally with a scalpel. These were placed

The material was homogenized in vortex and kept in the refrigerator overnight followed by centrifugation at 14000 rpm per 30 minutes at 4 °C. It was applied 60 µL of supernatant onto the gel. The electrophoretic run was performed on polyacrylamide gels system 7.5% (separating gel) and 4.5% (concentrating gel). The run was performed at 120 V per 7 hours. At the end of the run, the gels were revealed for the enzymes Catalase and superoxide dismutase. The gel/electrode system used was Tris-glycine pH 8.9. Gels evaluation was performed on transilluminator and is considered the variation of bands intensity (Alfenas, 2006). The experimental design was completely randomized, factorial (3X3) analyzed three lots and three seed classes (full, damaged and translucent). Data were interpreted by variance analysis for all tests using the statistical program Sisvar® (Ferreira, 2000). To compare the averages, it was used the Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION

The average water content of seeds at the time of testing was 8.3 with a maximum variation of 0.5. It is important to have a water content among lots tested with the lower variation possible, since high water content values accelerates the deterioration process and formation of products which cause immediate damage (Marcos Filho *et al*., 2005). In Table 1, for counting normal seedlings in the germination test it is observed that lot 1 has a lower germination of seeds damaged in relation to translucent and full seeds. Already in the lots 2 and 3 do not observe this difference. Regarding the classes noted that the full seeds, translucent and damaged of the first lot had lower germination compared to other lots of the same class. Lots 2 and 3 had a higher percentage of normal plants. In table 2 in the abnormal seedlings count it was observed that lot 1 the damaged seedlings were lower to the full and translucent classes. Lots 2 and 3 do not differ. Within the classes it is observed that in the full the lot 1 had a higher percentage of abnormal seedlings. In class damaged, showed no difference and in the translucent lot 1 had a high percentage compared to the other two lots.

Table 1. Average results of normal seedlings for three lots of pumpkin seeds and three different classes

Germination (%)			
Classes	Lot 1	Lot 2	Lot 3
Full	68 Ba	98 Aa	90 Aa
Damaged	49 Bb	92 Aa	91 Aa
Translucent	61 Ba	95 Aa	95 Aa
CV(%)			7.00

Average followed by the same capital letters, on the lines, and lower in columns for each treatment and lot, do not differ by Scott-Knott test at 5%.

Table 2. Percentage of abnormal seedlings for three lots and three pumpkin seeds classes

Abnormal seedlings (%)			
Classes	Lot 1	Lot 2	Lot 3
Full	25 Aa	2 Ba	7 Ba
Damaged	12 Ab	5 Aa	7 Aa
Translucent	31 Ba	2 Aa	2 Aa
CV(%)	55.94		

Average followed by the same capital letters, on the lines, and lower in columns for each treatment and lot, do not differ by Scott-Knott test at 5%.

Table 3. Percentage of dead seeds of three lots and three pumpkin seeds classes

Dead (%)			
Classes	Lot 1	Lot 2	Lot 3
Full	7 Aa	0 Aa	3 Aa
Damaged	39 Bb	3 Aa	2 Aa
Translucent	8 Aa	3 Aa	2 Aa
CV(%)			53.47

Average followed by the same capital letters, on the lines, and lower in columns for each treatment and lot, do not differ by Scott-Knott test at 5%

In table 3, for the dead seeds, it is observed that the lot 1 the damaged seeds had a higher percentage, very high differentiating from full and translucent. Already in the lots 2 and 3 do not observe difference among the classes. Regarding the classes, the same result is observed, full and translucent did not differ in relation to the lots and in damaged treatment, lot 1 obtained a high percentage, having a worst rating. In relation to the profiles it was observed that the pumpkin seeds had reduced germination rate due to storage, and similar results have been found by Carvalho *et al.* (2009). Similar results indicate that damaged seeds affect performance of seed lots and were obtained in studies conducted with forest species such as ipe tree (Oliveira *et al.*, 2004), canephora (Oliveira *et al.*, 2003), mastic-white (Machado and Cicero, 2003) and also for pumpkin seeds (Carvalho *et al.*, 2009). In Table 4, comparing lot 1, it is observed that the full seeds and translucent had a better performance in relation to the damaged seeds. In lot 2 it is observed that the full seeds obtained better performance different of damaged and translucent. In lot 3 damaged and translucent seeds were higher than full seeds. Within the classes, full seeds of the first and second lot were higher than third. In the damaged class, it is observed that the seeds of lot 3 were higher than other two lots. And the translucent seeds, it is observed that the lot 2 was lower than lots 1 and 2.

Table 4. Emergence (%) of three lots and three pumpkin seeds classes

Emergence (%)			
Classes	Lot 1	Lot 2	Lot 3
Full	84 Aa	66 Aa	40 Bb
Damaged	47 Bb	37 Bb	75 Aa
Translucent	70 Aa	43 Bb	75 Aa
CV(%)			22.16

Average followed by the same capital letters, on the lines, and lower in columns for each treatment and lot, do not differ by Scott-Knott test at 5%.

Table 5. Emergency speed (days) for three lots and three pumpkin seeds classes

Emergency speed (days)			
Classes	Lot 1	Lot 2	Lot 3
Full	12 Ba	11 Ba	16 Aa
Damaged	13 Ba	10 Ba	12 Bb
Translucent	15 Aa	13 Ba	15 Aa
CV(%)			12

Average followed by the same capital letters, on the lines, and lower in columns for each treatment and lot, do not differ by Scott-Knott test at 5%.

In Table 5, analyzing the data within the lots 1 and 2 do not observe difference. Already on the lot damaged were statistically lower than other classes. Analyzing the full seeds, it is realized that lot 3 was statistically better than lots 1 and 2. In the damaged do not observe difference, and the translucent lot 2 was statistically lower. In table 6, analyzing the lot 1 it was observed that the full seeds were higher than other two classes. Lot 2 the full seeds were statistically higher than other two classes. Already in lot 3 the full seeds were lower than seeds of other classes. In relation to the full class, seeds lots 1 and 2 were higher than lot 3. In the damaged class, seeds lot 3 were higher than those of lot 1 and 2. And the translucent seeds do not differentiate in different lots. The emergency test on the field is important to evaluate the physiological quality of seeds for sowing and marketing purposes, which has been fundamentally based on the germination test. Lots with high homogeneity are well evaluated by the germination test, however if the heterogeneity degree is high, the vigor tests will better assess the performance of these lots at the field level (Spina and Carvalho, 1986). Delay and disuniformity of development can be reflected in product quality and reducing the commercial value as lettuce, cabbage, carrots, cauliflower, eggplant and onion (Kikuti and Marcos Filho, 2007). It is still valid to point out that emergency results on the field can vary widely depending on edaphoclimatic conditions, even for seed lots that have high germination capacity (Chachalis *et al*., 2008), as observed for lot 2. It is observed in lot 1, the full seeds remaining of germination, among all only one was not feasible. Already the damaged and translucent, all were dead, represented by chart 1. In lot 2, it is seen 2 there was no remaining seeds of the full class. But the remaining seeds of the damaged and translucent classes, all were dead. In lot 3, it was observed that all remaining seeds, regardless of class, were dead. Among the available methods for assessing the seeds quality, the tetrazolium test is one of the most efficient, by how quickly and accuracy in determining the viability and vigor (Silva, 2006).

Table 6. Results of emergency speed index (ESI) for three lots and pumpkin seeds and three different treatments

Emergency speed index			
Classes	Lot 1	Lot 2	Lot 3
Full	0.68 Aa	0.77 Aa	0.25 Bb
Damaged	0.36 Bb	0.32 Bb	0.73 Aa
Translucent	0.46 Ab	0.34 Ab	0.52 Aa
CV(%)	8.95		

Average followed by the same capital letters, on the lines, and lower in columns for each treatment and lot, do not differ by Scott-Knott test at 5%.

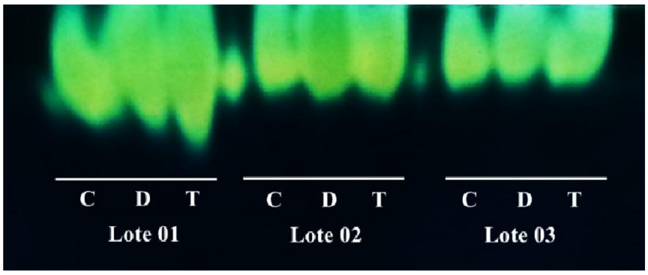

Figure 2. Expression pattern of catalase enzyme in terms of classes and tested seed lots, wherein (C) full seeds, (D) damaged seeds and (T) translucent seeds

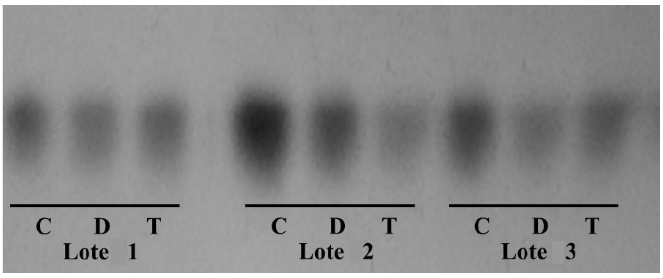

Figure 3. Expression pattern of superoxide dismutase enzyme in terms of classes and tested seed lots, wherein (C) full seeds, (D) damaged seeds and (T) translucent seeds

By electrophoretic analysis for the enzyme catalase may be seen that the first lot, the translucent seeds had a higher deterioration because it has a higher expression of this enzyme. Followed by damaged and after full seeds. Already in lot 2, it is verified that the expression of damaged is higher, followed by translucent and after full seeds. In lot 3, it is seen that three classes have a uniform deterioration, full, translucent and damaged seeds. Oxidative stress caused is combated by a complex antioxidant defense system, related to increased production and metalloenzymes activation, among which is the catalase (CAT), a type of very active peroxidase which removes hydrogen peroxide (H_2O_2), extremely toxic to the cell through its conversion in water $2H_2O+O_2$ (Fagagna, 2008; Scandalios, 2005). During the seeds deterioration process, occurs decrease of this enzyme activity, for its progressive inactivation or reduction and downtime of its synthesis (Marcos Filho, 2005). According Demirkaya, Dietz and Sivritepe (2010), the overall decrease in CAT activity in the seed decreases respiratory capacity, reducing the energy supply (ATP) and assimilated to germination thereof. When evaluated the enzyme expression SOD was possible to observe the reduction in the expression according to the seeds classes within each lot, where observed higher activity of this enzyme in full seeds, can this activity be high due to the protective role that this enzyme plays during the metabolic activation process (Figure 3). Superoxide dismutase (SOD) is a metalloenzymes group able to catalyzing the formation of hydrogen peroxide from superoxide radicals, eliminating them and releasing the cell from oxidative process. This enzyme is found in the cell cytoplasm and mitochondrial matrix (GUELFI, 2001; MCDONALD, 1999). Stewart and Bewley (1980) found in soybeans which viable seeds have the ability to produce SOD in the first hours of imbibition, but ones non-viable showed no activity. Bailly *et al*. (1996) found that the loss of sunflower seed viability, which also has high oil content is associated to decrease in the superoxide dismutase activity. These results are consistent with those found by Goel *et al*. (2003) in cotton seeds and by Sung and Jeng (1994) in peanut seeds.

Conclusion

The use of x-ray technique is effective for detecting abnormalities in pumpkin seeds that negatively affect germination. The expression of catalase and SOD enzymes is directly related to the seeds quality, and through these is possible to separate lots in the same classes established by x-ray test.

Acknowledgements

To Personnel Improvement Coordination of Higher Education – (CAPES), to the National Council for Scientific and Technological Development (CNPq), Foundation for Research of the Minas Gerais State – (FAPEMIG), and the financial support and scholarships awarded to the authors.

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