X-Ray analysis to assess mechanical damage in sweet corn seeds[1]

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ABSTRACT – The X-Ray test is a precise, fast and non-destructive method to detect mechanical damage in seeds. In the present study, the efficiency of X-Ray analysis in identifying the extent of mechanical damage in sweet corn seeds and its relationship with germination and vigor was evaluated. Hybrid 'SWB 551' (*sh2*) seeds with round (R) and flat (F) shapes were classified as large (L), medium (M1, M2 and M3) and small (S), using sieves with round and oblong screens. After artificial exposure to different levels of damage (0, 1, 3, 5 and 7 impacts), seeds were X-Rayed (15 kV, 5 min) and submitted to germination (25 °C/5 days) and cold (10 °C/7 days) tests. Digital images of normal and abnormal seedlings and ungerminated seeds from germination and cold tests were jointly analyzed with the seed X-Ray images. Results showed that damage affecting the embryonic axis resulted in abnormal seedlings or dead seeds in the germination and cold tests. The X-Ray analysis is efficient for identifying mechanical damage in sweet corn seeds, allowing damage severity to be associated with losses in germination and vigor.

Index terms: *Zea mays* L., image analysis, germination, vigor.

Análise de raios-X para a avaliação de injúrias mecânicas em sementes de milho doce

RESUMO – O teste de raios-X é um método preciso, rápido e não destrutivo para a avaliação da ocorrência de injúrias mecânicas em sementes. Avaliou-se a eficiência deste teste na identificação de injúrias mecânicas em sementes de milho doce e a sua relação com a germinação e o vigor. Sementes do híbrido 'SWB 551' (*sh2*) com formato esférico (R) e achatado (F) foram classificadas como grandes (L), médias (M1, M2 e M3) e pequenas (S) por meio de peneiras com perfurações circulares e oblongas. Após serem submetidas artificialmente a diferentes níveis de injúrias (0, 1, 3, 5 e 7 impactos), as sementes foram radiografadas (15 kV, 5 min) e submetidas aos testes de germinação (25 °C/5 dias) e de frio (10 °C/7 dias). As imagens digitais das plântulas normais, anormais e sementes não germinadas provenientes dos testes de germinação e de frio foram analisadas paralelamente às respectivas imagens radiográficas das sementes. Injúrias incidindo sobre o eixo embrionário resultaram no desenvolvimento de plântulas anormais ou na morte das sementes nos testes de germinação e de frio. A análise de raios-X é eficiente na identificação de injúrias mecânicas em sementes de milho doce, permitindo estabelecer as relações entre a severidade das injúrias e os efeitos negativos causados à germinação e ao vigor.

Termos para indexação: *Zea mays* L., análise de imagens, germinação, vigor.

Introduction

The evaluation of mechanical damage in endospermic seeds, such as corn, has been reported in the literature by several authors using the fast green (Chowdhury and Buchele, 1975; Borba et al., 1994; Peterson et al., 1995; Menezes et al., 2002), amaranth solution (Oliveira et al., 1998; Brandão-Junior et al., 1999; Fessel et al., 2003) and iodine tests (Oliveira et al., 1998; Brandão-Junior et al., 1999; Cicero and Silva, 2003; Marchi et al., 2006). However, since these tests do not allow evaluation

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of germination after treatment and do not precisely indicate the injuries that promote seedling abnormalities or embryo death, these methods are considered disadvantageous (Cicero et al., 1998; Cicero and Banzatto-Junior, 2003). This occurs because, in addition to not precisely identifying internal seed damage, they do not consider the extension and localization of the external damage (Cicero et al., 1998).

Based on the tetrazolium test, Chowdhury and Buchele (1975) identified internal seed damage in corn that was possibly related to embryo death. However, a better understanding of this topic was only possible after research by Cicero et al. (1998), which showed transversal ruptures in the endosperm, either restricting nutrients translocation to the embryonic axis or occurring directly in the embryonic axis, negatively affecting germination. Similarly, Carvalho et al. (1999), evaluating pre-harvest stress cracks in corn seeds by X-Ray analysis, observed that when injury is located inside or perpendicular to the embryonic axis the physiological potential of the seed is affected. Continuing the work of Cicero et al. (1998), the efficiency of the X-Ray test regarding the adverse effects of mechanical damage on corn seed vigor was demonstrated by Cicero and Banzatto-Junior (2003). It was observed that a longitudinal fracture occurring superficially (pericarp) in the middle region of the seed, without reaching the embryo, did not affect seedling development in the cold test. Furthermore, transversal ruptures in the endosperm restricted nutrient translocation to the embryonic axis or a deep fracture affected the embryonic axis, resulting in an abnormal seedling or dead seed.

Use of the X-Ray test has increased in seed technology, since it allows fast analysis of seed morphology and detects possible damage and embryo abnormalities without destroying the seed (Bino et al., 1993). The objective of the present study was to evaluate the efficiency of the X-Ray test in identifying mechanical damage in sweet corn seeds and determine its relationship with germination and vigor.

Material and Methods

This research was carried out at the Image Analysis and Seed Analysis Laboratories of the Crop Science Department, "Luiz de Queiroz" College of Agriculture/University of São Paulo (LPV/ESALQ/USP) in Piracicaba, SP, Brazil, between June 2006 and July 2008.

Round (R) and flat (F) sweet corn seeds, hybrid *sh2* 'SWB 551', were classified as large (L), medium (M1, M2 and M3) and small (S), using sieves with round and oblong screens. The following classes were established: RL (22/64" = 8.7 mm), RM1 (20/64" = 7.9 mm), RM2 (18/64" = 7.1 mm), RS (16/64" = 6.4 mm), FL (22/64" x 3/4" = 8.7 mm x 19.0 mm), FM3 (20/64" x 3/4" = 7.9 mm x 19.0 mm) and FS (16/64" x 3/4" = 6.4 mm x 19.0 mm).

Mechanical damage: seeds were submitted to mechanical damage using a BC Impact Simulator (Model 2000, Dow AgroSciences®) in which 965 kPa compressed air launched seeds against a steel plate at a distance of 10 cm. Five levels of mechanical damage (0, 1, 3, 5 and 7 consecutive impacts against the steel plate) were evaluated. Seed moisture content (w.b.) at the moment of mechanical damage was 11% in seeds from RL, RM1, RM2, RS, FL, FM3 and FS seed classes and 20% in the FL, FM3 and FS seed classes.

X-Ray test: fifty seeds selected for the germination test and another fifty selected for the cold test were placed in individual cells of an acrylic plate. Transparent tape was placed under the seeds to fix them in a suitable position. The seeds were then numbered for identification according to their position in the plate. The plates were positioned directly over the radiographic film (Kodak MIN-R EV, size 18 x 24 cm) at a distance of 57.2 cm from the radiation source. Seeds were X-Rayed (15 kV, 5 min), using a MX-20 model of FAXITRON X-Ray equipment. Film was developed in a Hope X-Ray processer (Micromax model 319). The X-Ray film images were digitized using an Umax Scanner (PowerLook 1100 model) for amplification and visualization on a Core 2 Duo 6400 computer (2.13 GHz, 2GB RAM memory, 350 GB SATA II Hard Disk and 21-inch monitor).

Seed germination: germination was evaluated using paper towel rolls moistened on a weight basis of 2.5:1 (water: paper). Two hundred seeds previously numbered and X-ayed were distributed in groups, often on the upper third of the substrate and covered with another sheet of paper towel. Rolls were placed in a germinator at 25 °C and evaluations were made five days after sowing, according to the Rules for Seed Testing (Brasil, 2009). Normal seedlings (whose seeds showed mechanical damage), abnormal seedlings and dead seeds were photographed with a Nikon digital camera (model D1), connected to a Pentium 4 computer (3.0 GHz, 1 GB RAM memory, 160 GB Hard Disk and 21-inch monitor).

Cold test: two hundred seeds were placed on sheets of paper towels and identified as in the germination test, but with a water:paper ratio of 2.7:1. Before rolling, seeds were covered with a fine layer of earth from an area that had recently been cultivated with corn. The rolls were placed in plastic boxes (32.5 x 21.5 x 11.5 cm), sealed and kept in a cold chamber at 10 °C for seven days. After removal from the plastic boxes, the rolls were transferred to a germinator and kept at 25 °C for five days. Afterwards, normal seedlings, abnormal seedlings and dead seeds were photographed, according to (Cicero and Banzatto-Junior, 2003).

Evaluation of mechanical damage: X-Ray images of each seed were analyzed individually and given scores according to the severity and location of the mechanical damage (Table 1), based on the modified classification of Cicero et al. (1998). The X-Ray image of the seed was observed on the computer screen jointly with the photographic image of the seedling or dead seed.

Data were not submitted to statistical analysis. The results were analyzed as a comparison between the damage detected by X-Ray image analysis of individual seed and the occurrence of seedling abnormalities or dead seed.

Table 1. Criteria for scoring (X.Y) mechanical damage in the embryo and endosperm of sweet corn seeds evaluated by the X-Ray test.

Embryo (X)	Score	Endosperm (Y)	Score
Absence of damage	1	Absence of damage	1
Mechanical damage to the scutellum but without affecting the central area (nonsevere damage)	2	Little damage with no loss of the endosperm (nonsevere damage)	2
Damage on the plumule, coleoptile, central area of the scutellum, radicle or seminal root region (severe damage)	3	Loss of part of the endosperm (severe damage)	3

Results

The percentage of damaged seeds, considering the sum of values corresponding to scores 1.2 to 3.3, did not exceed 11.3% for either the germination or cold tests (Table 2). On the other hand, the percentage of damaged seeds that resulted in abnormal seedlings and dead seeds was 5.8% and 9% for germination and cold tests, respectively.

The effect of the mechanical damage (number of impacts) was demonstrated by the increased percentage of scored seeds 1.2 to 3.3 (Table 3). Based on the results shown in Tables 2 and 3, higher percentages of scored seeds with mechanical damage were observed when non-severe damage occurred in the endosperm (score 1.2) and severe damage in the embryo and endosperm (score 3.3).

Table 2. Percentage of normal (N) and abnormal seedlings (A) and dead seeds (D) in the germination and cold tests for each score attributed to the embryo (X) and endosperm (Y).

Score (X.Y)	Germination			Cold test		
	N	A	D	N	A	D
1.1	67.7	20.0	3.2	53.4	21.6	13.7
1.2	1.4	1.1	0.4	1.2	0.8	0.8
1.3	0.6	0.2	0.3	0.3	0.5	0.7
2.1	0.2	0.3	0.0	0.1	0.1	0.0
2.2	0.5	0.6	0.1	0.4	0.6	0.5
2.3	0.4	0.7	0.1	0.2	0.6	0.9
3.1	0.0	0.2	0.0	0.0	0.0	0.1
3.2	0.1	0.5	0.3	0.1	0.4	0.7
3.3	0.1	0.5	0.5	0.0	0.7	1.6
Total (1.2 to 3.3)	3.3	4.1	1.7	2.3	3.7	5.3

Table 3. Percentage* of seeds from each level of damage (number of impacts) for each score attributed to the embryo (X) and endosperm (Y).

Score (X.Y)	Number of impacts				
	0	1	3	5	7
1.1	100.0	92.6	91.4	88.0	86.4
1.2	0.0	2.1	2.6	3.0	3.6
1.3	0.0	1.1	1.0	1.5	1.7
2.1	0.0	0.2	0.3	0.5	0.7
2.2	0.0	0.9	1.0	1.8	2.0
2.3	0.0	1.0	1.1	1.8	2.1
3.1	0.0	0.1	0.1	0.2	0.2
3.2	0.0	0.9	1.0	1.2	1.0
3.3	0.0	1.1	1.5	2.0	2.3

*Based on 3,200 seeds for each level of damage, corresponding to the sum of all seed classes in the germination and cold tests.

No influence of the increasing number of impacts on the seed physiological potential was observed (Tables 4 and 5). The minimum percentage of normal seedlings in the germination and cold tests was observed in the three impacts treatment, which was 78% and 53% respectively (Table 4). High percentages of dead seeds were observed in the cold test, ranging from 10% to 27%. Analyzing each seed class individually (Table 5), the lowest germination was obtained for RL class (76% on average) and the highest for the FS class. For the cold test, except for the RM2 and RS classes, that showed the lowest values (62% and 56% on average, respectively), all seed classes showed similar percentages of normal seedlings, ranging from 66% to 71%.

Table 4. Percentage* of normal (N) and abnormal seedlings (A) and dead seeds (D) in the germination and cold tests for each level of damage (number of impacts).

Number of impacts	Germination			Cold test		
	N	A	D	N	A	D
0	81	18	1	70	14	16
1	79	20	1	68	18	14
3	78	21	1	53	20	27
5	85	14	1	74	16	10
7	84	15	1	62	19	19

*Based on the X-Rayed seeds of all seed classes.

Based on the X-Ray analysis of 12,800 seeds submitted to impacts, eleven principal types of mechanical damage were classified, with their respective percentage of occurrence (Table 6). Thus, seeds showing only mechanical damage characterized as non-severe in the embryo and severe in the endosperm (score 2.3), represented by loss or rupture of part of the endosperm, were the most frequent (0.98%), exemplified by Figure 1. However, seed damage characterized by loss of all or part of the radicle, reaching or not the base of the scutellum or endosperm, was represented by scores 3.1, 3.2 and 3.3, which occurred in 0.26% of the seeds (Figure 2). A transversal fracture in the plumule without affecting the scutellum and endosperm (score 3.1), shown in Figure 3, was the less frequent type of mechanical damage, observed in only 0.05% of the seeds.

Table 5. Results of germination (G) and cold (C) tests for each level of damage (number of impacts) for the seed classes*.

Number of impacts	RL		RM1		RM2		RS		FL		FM3		FS	
	G	C	G	C	G	C	G	C	G	C	G	C	G	C
0	76	74	82	78	78	71	85	70	81	74	80	64	82	62
1	76	61	81	70	85	63	85	69	71	64	82	69	78	75
3	72	52	77	61	75	47	82	34	75	55	84	59	83	65
5	78	76	78	77	84	64	86	59	89	82	87	80	95	79
7	79	67	77	71	85	63	81	48	87	75	84	58	93	54
Average	76	66	79	71	81	62	84	56	81	70	83	66	86	67

*RL: round large, RM: round medium, RS: round small, FL: flat large, FM: flat medium and FS: flat small.

Table 6. Description and occurrence (percentage) of the main types of mechanical damage observed by the X-Ray test in sweet corn seeds (Sh_2) submitted to mechanical damage.

Description of damage	Occur.* (%)	Example (Fig.)
1. Loss or fracture of part of the endosperm, without reaching the embryonic axis and damage in the scutellum but not its central region (*score 2.3*)	0.98	1
2. Loss of part of the endosperm, without reaching the embryo (*score 1.3*)	0.73	-
3. Longitudinal fractures throughout the embryonic axis (*score 3.2*)	0.40	4
4. Fractures throughout the seed reaching the embryo and endosperm (*scores 3.2 and 3.3*)	0.33	-
5. Fractures only in the endosperm, without reaching the scutellum (*score 1.2*)	0.30	-
6. Longitudinal seed fracture – with loss of almost 50% of seed physical integrity (*score 3.3*)	0.30	-
7. Loss of all or part of the radicle, reaching or not the base of the scutellum or endosperm (*scores 3.1, 3.2 and 3.3*)	0.26	2
8. Transversal seed fractures (without the plumule or radicle parts) – with loss of almost 50% of seed physical integrity (*score 3.3*)	0.24	-
9. Fractures in the endosperm reaching the scutellum, but with no affect on the embryonic axis (*score 2.2*)	0.17	-
10. Diagonal fracture through the endosperm affecting the plumule (*score 3.2*)	0.09	-
11. Transversal fracture in the plumule without affecting the scutellum and endosperm (*score 3.1*)	0.05	3

*Occurrence (percentage) based on 12,800 seeds submitted to mechanical damage, corresponding to total of evaluated seeds in the germination and cold tests in all seed classes.

Figure 1. X-Ray image of sweet corn seed (a) - FS class, 1 impact treatment, score 2.3 - and normal seedling from germination test (b).

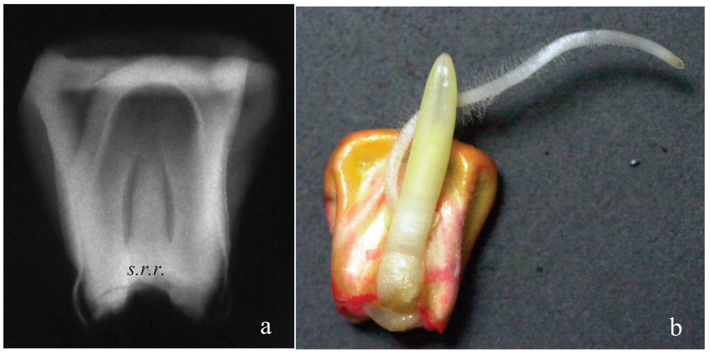

Figure 2. X-Ray image of sweet corn seed (a) - FM3 class, 3 impacts treatment, score 3.1 - and abnormal seedling from germination test (b). *s.s.r.*: seminal root region.

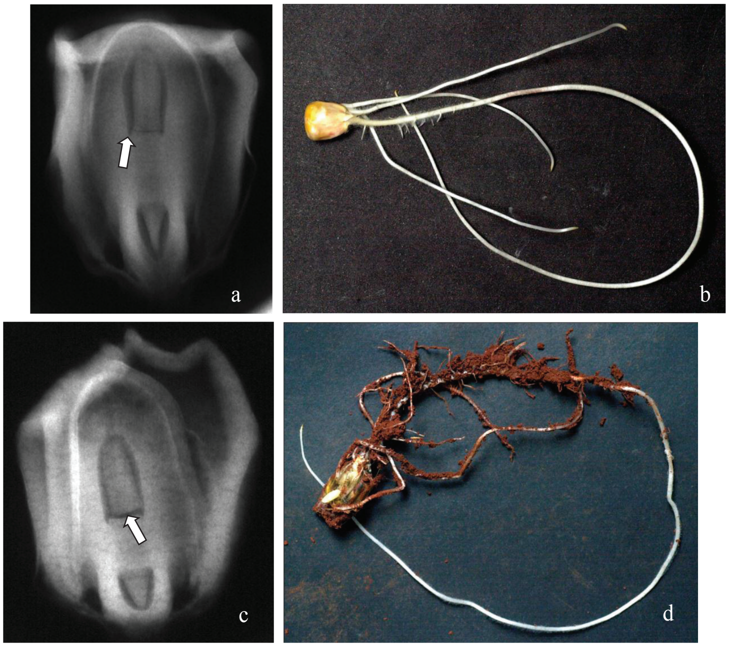

Figure 3. X-Ray images of sweet corn seeds (a, c) - FM3 class, 7 impacts treatment, score 3.1 - and abnormal seedling from germination (b) and cold tests (d).

Considering all types of damage showed in Table 6, the total percentage was 3.85%, with 1.67% classified as severe in the embryo (scores 3.1, 3.2 and 3.3). Among these types of severe damage, those characterized by longitudinal fractures throughout the embryonic axis (shown in Figure 4) were the most frequent, 0.4%.

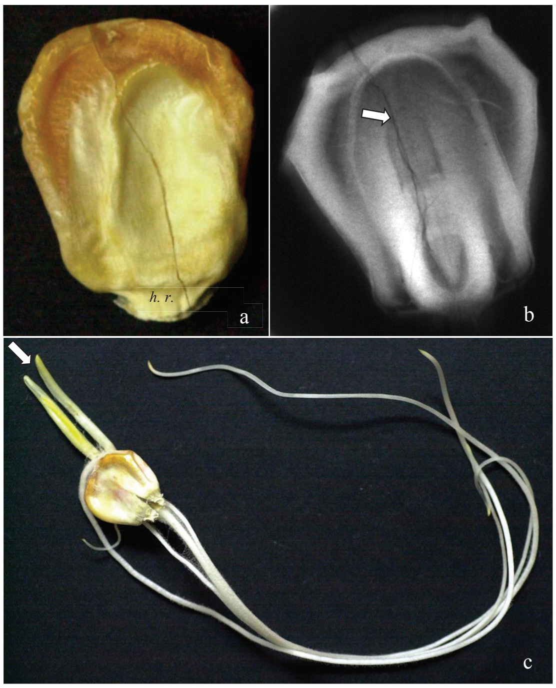

Figure 4. Ventral view of sweet corn seed (a) - FL class, 1 impact treatment - X-Ray image (b) - score 3.2 - and abnormal seedling from germination test (c). *H.r.*: hilar region.

Discussion

Although the percentage of damaged seeds increased with the number of impacts (Table 3), there was no effect on seed physiological potential. The results in Table 4 can be explained by the higher percentage of seeds with non-severe damage. Also, the low percentage of seeds with severe damage in the embryo (scores 3.1, 3.2 and 3.3) (Table 2) could be an effect directly related to the seed structure. According to Wolf et al. (1952), although the embryonic axis represents less than 2% of the dry matter in corn seed, it is also protected by the coleoptile and coleorhiza (in the shoot and root meristems, respectively) as well as by the scutellum, endosperm reserves, aleurone layer and the pericarp.

Furthermore, in the case of sweet corn seed, due to the irregular surface of the pericarp and the lower density in relation to corn, the probability of mechanical damage affecting the embryonic axis tends to decrease. The low percentage of seeds classified as showing severe damage in the embryo (scores 3.1, 3.2 and 3.3), obtained from treatments with a higher level of damage (5 and 7 impacts) could explain why there were no losses in the seed physiological potential. It should be pointed out that the high percentage of abnormal seedlings and dead seeds in the germination and cold tests (Table 2), resulting from seeds that did not present mechanical damage (score 1.1), may be an indication of the low physiological potential, since only 68% and 53% of seeds generated normal seedlings in the germination and cold tests, respectively. Also, the high percentage of dead seeds observed in the cold test indicates that the impacts had no influence on the vigor (Tables 4 and 5), since those seeds not submitted to impacts showed a percentage of normal seedlings similar to seeds submitted to 5 impacts.

The efficiency of the X-Ray test in identifying mechanical damage was decisive for clarifying what kind of damage is harmful for the seed physiological potential. For example, a seed with no severe damage in the embryo and severe damage in the endosperm (score 2.3) can develop into a normal seedling, as shown in Figure 1. Arrow 1 indicates that the damage affected the superior part of the endosperm but did not extend to the embryonic axis. Arrow 2 indicates a loss of part of the endosperm, with the scutellum also affected, but the embryonic axis remained intact.

On the other hand, when mechanical damage occurred in the embryonic axis, the negative effects on germination and vigor were observed. However, as the percentage of damage in the embryonic axis was very low, losses in the physiological potential of the seed sample did not occurr (Table 4). Figure 2 represents the principal occurrence of seeds with damage in the embryonic axis. Observe in Figure 2a that damage severely affected the embryonic axis in the radicle region (score 3.1); thus, there was complete rupture and radicle loss, resulting in the development of an abnormal seedling in the germination test with no primary root (Figure 2b). However, it is important to stress that damage did not occur in the seminal root region (*s.r.r.*), justifying the growth of a rudimentary seminal root.

When mechanical damage reached the embryonic axis, including the plumule region, there was no shoot development or it was precarious. Arrows in Figures 3a and 3c indicate damage only at the base of the plumule; this apparently insignificant damage, and impossible to detect externally, was fundamental for abnormal seedling development in the germination and cold tests (Figures 3b and 3d). At the same time, the radicular system developed vigorously in these seedlings, indicating that the reserves were normally transferred from the endosperm to the root apical meristem, since damage did not occur in any other part of the seed except the plumule.

Photographic analysis of the ventral part of the seed shown in Figure 4a indicates that mechanical damage occurred longitudinally, causing a crack from the apex to the base of the seed (hilar region: *h.r.*). The X-Ray image of this seed (Figure 4b) helped explain the vigorous development of the radicular system, although with irregular shoot development. Considering the path of injury beginning at the seed apex, it can be seen that as it crossed the endosperm and scutellum, the crack reached the entire length of the plumule (arrow, Figure 4b) and crossed the seminal root region without reaching the radicle. The crack passed the cavity between the radicle and the coleorhiza without reaching the radicle and finally crossed the basal part of the scutellum. Consequently, an abnormal seedling from the germination test showed splitting of the coleoptile (arrow, Figure 4c) and rudimentary shoot development, but with vigorous development of the radicular system.

The images shown in this research clearly demonstrate that the X-Ray test is an important procedure for evaluating mechanical damage in sweet corn seeds, as well as establishing a relationship between damage and loss of physiological potential. The most frequent observations were associated with non-severe damage in the endosperm (score 1.2), observed in 2.9% of the seeds evaluated. Nevertheless, 1.4% and 1.2% of these seeds produced normal seedlings in the germination and cold tests, respectively. Losses in germination and vigor were observed only when damage occurred directly in the embryonic axis, which was also observed by Cicero et al. (1998), Carvalho et al. (1999) and Cicero and Banzatto-Junior (2003) in corn seeds. The low percentage of seeds with severe damage, even when submitted to higher numbers of impacts, can explain why there was no immediate negative effect on the physiological potential. However, it was demonstrated that seeds submitted to only one impact showed severe damage (affecting the embryonic axis) as well as those submitted to seven impacts.

Conclusions

X-Ray analysis is efficient for identifying mechanical damage in sweet corn seeds, allowing the severity of the damage to be associated with losses in germination and vigor.

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