

# Using X-Rays to evaluate fissures in rice seeds dried artificially<sup>1</sup>

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**ABSTRACT** - The objective of the present study was to evaluate the efficiency of X-Rays in identifying fissures in artificially dried rice seeds and the relationship between damage and seed performance in the germination test. Irrigated rice seeds of the IRGA 417 and IRGA 420 cultivars were harvested with 23.3 and 24.5% water content respectively and submitted to stationary drying treatments at 32, 38, 44 and 50 °C. X-Rays were taken of subsamples of 100 seeds for each treatment, using an MX-20 X-ray equipment. The X-Rayed seeds were classified from 1 to 3, where 1 corresponded to seeds without fissures, 2 to seeds with non-severe fissures and 3 to seeds with severe fissures. The same X-Rayed seeds were planted and on the seventh day the seedlings (normal or abnormal) and dead seeds were photographed and evaluated to verify any relationship between the fissures and physiological potential. Higher drying temperature increased the percentage of fissures in the two cultivars, which can adversely affect their germination. Seeds with fissures can be identified using X-Rays.

Index terms: *Oryza sativa* L., image analysis, seed quality, X-Ray images, germination.

## Utilização de raios-X na avaliação de fissuras em sementes de arroz submetidas à secagem artificial

**RESUMO** – O presente trabalho avaliou a utilização de raios-X na identificação de fissuras em sementes de arroz, após a secagem e a relação desses danos com a germinação de sementes. Utilizaram-se sementes de arroz irrigado, cultivares IRGA 417 e IRGA 420, colhidas com teores de água de 23,3 e 24,5%, respectivamente, submetidas aos tratamentos de secagem estacionária, sob as temperaturas de 32, 38, 44 e 50 °C. Subamostras de 100 sementes provenientes de cada tratamento foram radiografadas em aparelho de raios-X (MX-20). As sementes radiografadas foram classificadas em: 1 - sementes sem fissuras; 2 - sementes com fissuras não severas e 3 - sementes com fissuras severas. As mesmas sementes radiografadas foram colocadas para germinar e após sete dias foram avaliadas, sendo fotografadas as respectivas plântulas ou as sementes mortas, para o estabelecimento da relação entre fissuras e desempenho fisiológico. O aumento da temperatura de secagem proporcionou aumento da porcentagem de sementes com fissuras das duas cultivares, sendo que as fissuras severas afetaram negativamente a germinação. As imagens de raios-X permitem identificar fissuras em sementes de arroz submetidas à secagem artificial e correlacioná-las com a presença de plântulas normais e anormais no teste de germinação.

Termos para indexação: *Oryza sativa* L., análise de imagens, qualidade de sementes, imagens de raios-X, germinação.

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## Introduction

Rice seeds are harvested as close as possible to physiological maturation with a high water content and this requires immediate drying.

Seed drying causes the evaporation of peripheral water and the consequent irregular contraction of the superficial layers due to the compression caused by atmospheric pressure. The warming up of the seed increases the internal pressure causing an expansion of the internal layers. At higher temperatures, shear stress develops, which can cause the formation of fissures in the seeds (Kunze, 1977; Chen and Kunze, 1983).

In order to examine the internal fissures in rice seeds with the naked eye, the glumellas have first to be removed. The development of fissures can reduce the capacity to regulate water and gas exchange as well as increase sensibility to the phytotoxic effects of fungicides and insecticides (Soave and Moraes, 1987; Carvalho and Nakagawa, 2000).

Image analyses have been used as a method to verify damage or to analyze seed quality in many species. It is a promising technique owing to its rapidity and non-destructiveness, and it allows physiological tests to be made on the material being examined.

X-Rays stand out among the various methods of image analysis since they permit a visualization of the mechanical damage caused by drying, insects and other adverse factors, before and after harvesting, including cracks and fractures in the seeds (ISTA, 1993; Poulsen et al., 1998). This technique results in a permanent image on a film as it moves over the seed. The image can show a greater or lesser degree of radiopacity and radioluminescence, due to the absorption level of the X-Rays by the seeds, which is determined by tissue composition, thickness and density and also by the wave length of the ionizing radiation (ISTA, 1993).

Morphological aspects of the seeds associated with viability can be evaluated by an X-Ray test (Copeland and McDonald, 1985) and seed anatomy has been correlated with germination, and seedling vigor or morphology, although the relationship between them depends on the species (Simak, 1991). Among the species where this relationship has been observed are seeds of tomato (Van der Burg et al., 1994), corn (Cicero et al., 1998 and 2003; Carvalho et al., 1999a and 1999b; Cicero and Banzatto Junior, 2003; Mondo and Cícero, 2005), *Peltophorum dubium* (canafistula) (Oliveira et al., 2003), *Tabebuia impetiginosa* (ipê-roxo) and *Tabebuia serratifolia* (ipê-amarelo) (Oliveira et al., 2004), and

soybeans (Obando Flor et al., 2004), demonstrating it is an efficient technique for evaluating seed quality.

The X-Ray test could be a useful tool for studying the physical quality of rice seeds and the objective of the present study was to evaluate the efficiency of the X-Ray test in the identification of fissures in artificially dried rice seeds and the relationship with seed germination.

## Material and Methods

The study was made at the Image Analysis Laboratory, in the Agricultural Department of the Escola Superior de Agricultura "Luiz de Queiroz" /USP, in Piracicaba – São Paulo state. The early-maturing irrigated rice cultivars, IRGA 417 and IRGA 420, which had a low stature, fine, long seeds, high initial vigor and a high amylose content, were used.

The seeds of the IRGA 417 cultivar were harvested with a 23.3% and those of IRGA 420 with 24.5% water content. Twenty kilo batches of each cultivar were used and manually cleaned before being divided into four equal samples to be dried at temperatures of 32, 38, 44 and 50 °C.

Drying was done in an Intecnial SAC-18 stationary drier for samples, starting with the highest temperature, lasting around 2.5 hours, and finishing with the lowest temperature, lasting four hours. After drying, the seeds remained under the normal environmental conditions of Santa Maria, Rio Grande do Sul state, at around 85% RH (the mean for May). After the drying treatments, seed quality was evaluated with the following tests:

*Water content:* measured before and after drying. This was determined at  $105 \pm 3$  °C during 24 hours, in an oven with circulating air, with two repetitions for each experimental unit, in accordance with the Rules for Seed Analysis - RSA (Brasil, 2009).

*Germination and first count of germination tests:* four repetitions of 100 seeds for each batch were used, sown in rolls of paper towel and kept in a germinator at 25 °C. The paper substrate was moistened with distilled water at a ratio of 2.5 times the dry substrate weight. The evaluations were made at 5 and 14 days after the start of the test, in accordance with the RSA (Brasil, 2009), with results expressed as a percentage of normal seedlings. The first count of germination test was made together with the germination test, counting the number of normal seedlings 5 days after the test began. The results were expressed as a percentage of normal seedlings.

*Cold test without soil:* four repetitions of 100 seeds for each batch, distributed in rolls of paper towel, moistened with a quantity of water 2.5 times the dry substrate weight. The rolls were placed inside plastic bags, sealed with sticky tape and kept in chambers at 10 °C for seven days. After this period, the rolls were transferred to a germinator at 25 °C for a further seven days, as described by Barros et al. (1999). The results were expressed as a percentage of normal seedlings.

*Root length:* the mean root length of ten normal, randomly-selected seedlings was measured. The seeds were sown in paper towels moistened with distilled water at a ratio of 2.5 times the substrate weight in four repetitions of 20 seeds and taken to a germinator at 25 °C. Sowing was done on a line traced on the upper third of the paper substrate in a longitudinal direction, as described by Nakagawa (1999). Evaluations were made seven days after sowing, using a ruler graduated in millimeters (mm). The mean root length was calculated by adding up the seedling means for each repetition and dividing by the number of seedlings measured, expressing the results in millimeters (mm).

To detect fissures after drying, the seeds were X-Rayed by placing the repetitions of each treatment in individual, numbered cells of an acrylic plate, with a transparent sticky tape in the bottom to fix the seed in a suitable position.

X-Ray images were obtained by placing the acrylic plate with the seeds directly on the X-Ray film (Kodak Min-R 2000, size 20x24 cm), 35 cm from the radiation source. The radiation intensity and exposure time, which permitted the best visualization of the internal fissures of the rice seeds, was 14 kV for 360 seconds, as described by Menezes et al. (2005), using Faxitron MX-20 X-Ray equipment. The development of the X-Rays was done in a Hope 319 Micromax X-Ray automatic processor. Later, the X-Ray images were captured individually by an Umax

PowerLook 1100 Scanner for amplification and examined using a computer.

The X-Rayed fissures were classified from 1 to 3 according to criteria set out by Cicero et al. (1998), for corn seed and were modified for the rice seeds: 1 = seeds without visible fissures; 2 = seeds with non-severe fissures; 3 = seeds with severe fissures.

The relationship between detected fissures and physiological performance was evaluated by numbering and X-Raying the seeds from each treatment and then submitting them to the germination test (Brasil, 2009). Sowing was done with groups of 25 seeds on the upper third of the paper substrate, and the seedlings or seeds were evaluated on the seventh day after the test began. All the abnormal seedlings and seeds which did not germinate were then photographed using a digital Nikon D1 camera, linked to a computer. The images could be examined simultaneously and comparisons made on the computer screen by observing the X-Ray with its respective classification and the resulting seedling or ungerminated seed.

The experimental design was completely random, with four repetitions, in a factorial 2x4 (cultivars x temperature) and 3x4 (damage levels x temperatures) scheme, to evaluate the physiological quality and relate fissures to germination performance, respectively. An analysis of variance was done for the damage levels and the means were compared using the Tukey test at the 5% probability level. The data collected as percentages were transformed into arcsine.

## Results and Discussion

The water content of the rice seeds after drying at the highest temperature was 13% and 12.6% for the IRGA 417 and IRGA 420 cultivars, respectively (Table 1). All the samples were dried in the 24 hours following harvesting, as recommended by Fornasieri Filho and Fornasieri (1993).

Table 1. Water content of rice seeds of IRGA 417 and IRGA 420 cultivars before and after stationary drying at different temperatures.

Cultivars	Water Content %	Drying Temperatures			
		32 °C	38 °C	44 °C	50 °C
IRGA 417	23.3	13.5	13.1	13.2	13.0
IRGA 420	24.5	13.6	13.2	13.0	12.6

The water contents at both harvest and after drying were within the ranges recommended for handling seed batches in order to preserve the physiological quality, as described by Peske et al., (1998) and Bragantini, (1999).

The percentage of fissures in the seeds of the cultivars is shown in Figure 1 and shows an increase in the percentage seeds with fissures with increases in the drying temperature. The highest temperatures resulted in higher rates of water vapor loss from seeds per unit of time, without leaving an interval for water redistribution inside the seeds (continuous drying). This situation established humidity gradients between the internal and external parts of the seeds and also between batches, depending on the drying temperatures, and this was sufficient to produce increasing percentages of fissures, as also observed by Kunze and Hall (1965); Kunze and Choudhury (1972) and Chen and Kunze (1983). The IRGA 420 cultivar showed a higher percentage of fissures compared to the IRGA 417, and such cultivar differences were also observed by Kunze and Choudhury (1972) and Kunze (1985).

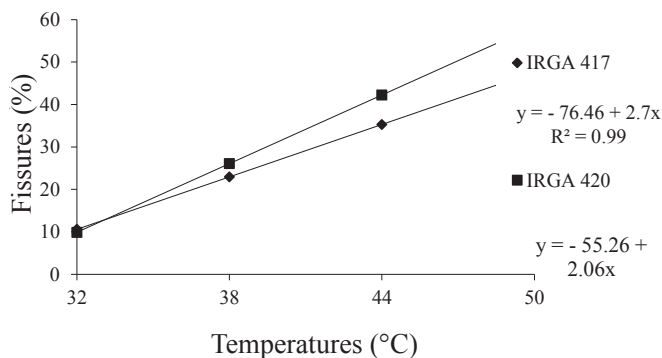


Figure 1. Percentage of seeds with fissures in the rice cultivars IRGA 417 and IRGA 420 as a function of different drying temperatures.

The percentage of fissures before the drying treatments was not determined but it is known that keeping rice in the field after physiological maturation exposes the seeds to alternate natural processes of drying and rehydration, which can result in fissures. However, the percentage of fissures originating from the field is generally lower than that arising during or after drying at high temperatures.

The results of the percentage germination, first count of germination test, accelerated aging and cold test without soil of the IRGA 417 and IRGA 420 cultivars are shown in Figure 2.

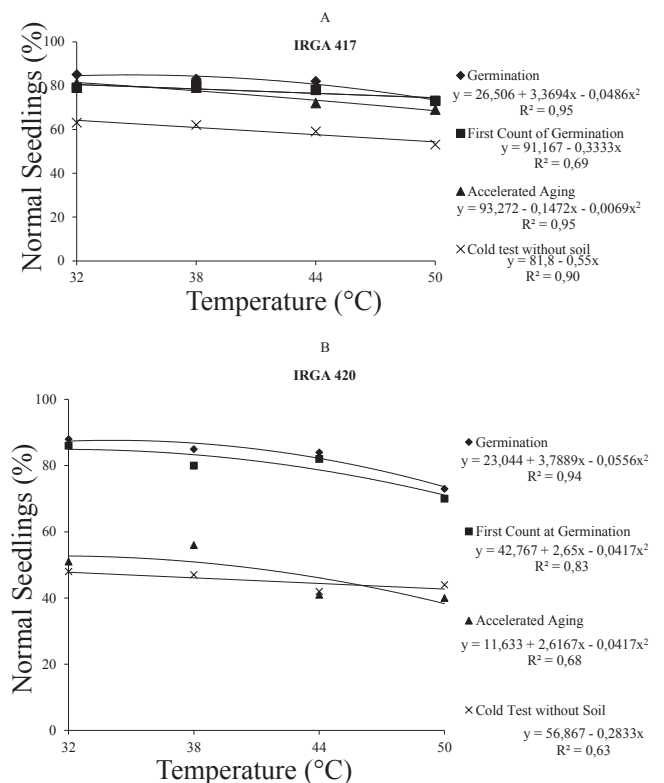


Figure 2. Percentage germination, first germination count, accelerated ageing and cold test without soil for the IRGA 417 (A) and IRGA 420 (B) cultivars after drying at different temperatures.

The first three temperatures for germination caused similar effects to the seeds of both cultivars, with very close absolute values, but at 50 °C the final percentage was lower. The high drying temperatures can cause reductions in the speed and percentage of germination, produce abnormal seedlings and cause seed death (Peske et al., 1998).

The results of the first count of germination and accelerated aging tests showed a reduction in seed vigor with increasing temperatures. The cold test without soil gave similar results for the drying treatments, differing, however, from Saravia et al. (2007), who considered it adequate for evaluating rice seed quality.

The initial physiological quality of the cultivar batches was influenced by the seeds having only been cleaned manually because of the large 20 kg quantities harvested, which did not permit a complete cleaning.

The percentages of seeds with different levels of fissures which produced abnormal seedlings or ungerminated seeds can be seen in Table 2. An increase in temperature



had an adverse effect on the seeds since it increased the percentage of seeds with fissures, which did not produce normal seedlings.

Table 2. Percentage of seeds with different levels of fissures which originated from abnormal seedlings and ungerminated seeds, after stationary drying at different temperatures.

Temperature (°C)	Damage Level		
	2	3	Total (2+3)
IRGA 417			
32	8.4b*	7.0b	15.4c
38	18.7ab	4.2b	22.9bc
44	36.4a	9.6b	46.0ab
50	22.3ab	34.9a	57.2a
CV (%)	30.9	27.3	36.8
IRGA 420			
32	14.6b	0.0b	4.6c
38	31.7ab	0.0b	31.7bc
44	43.4a	12.5ab	55.9ab
50	39.8ab	38.1a	77.9a
CV (%)	28.2	29.9	34.1

\*Means followed by the same letter in each column for each cultivar do not differ among themselves according to the Tukey test at the 5% probability level. Damage level: 2 – not severe; 3 - severe.

For the IRGA 417 at 32 °C, there was a lower percentage of seeds producing abnormal seedlings or ungerminated seeds, but there was no difference for either cultivar for non-severe damage or any noticeable effects on germination for temperatures between 32 and 38 °C. However, fissures caused damage to seed batches since their presence was generally accompanied by physiological damage. Damage level 2 varied in the quantity and position of the fissure but it was not vital for the seed; this type of damage was also associated with the seed physiological quality and seedling formation, that is, producing normal or abnormal seedlings, or rarely a dead seed.

At temperatures of 32, 38 and 44 °C, a similar percentage of seeds with damage level 3 was observed, with no variations in the number of abnormal seedlings and ungerminated seeds produced. At 50 °C, there was a significant increase in the number of severe fissures, which associated with other drying effects at high temperatures, adversely affected seed physiological quality and resulted in dead seeds.

Considering the total number of seeds with fissures produced by drying, the negative effect of temperature on germination and seedling formation could be seen more clearly. At 50 °C, there were more seeds with fissures, resulting in a significantly higher number of abnormal seedlings and dead seeds than at 32 and 38 °C.

The results for the IRGA 420 cultivar were similar to those obtained from the IRGA 417. Seeds dried at lower temperatures showed lower percentages of fissures and, consequently, a lower percentage of abnormal seedlings and ungerminated seeds. The temperatures of 32 and 38 °C did not produce severe fissures in the IRGA 420 but at 50 °C, however, there was a higher percentage of seeds with severe fissures and a higher percentage of abnormal seedlings and dead seeds, as had been observed in the IRGA 417.

Figures 3 to 5 illustrate the levels of fissures, and the seedlings and ungerminated seeds produced by both cultivars as a result of stationary drying at different temperatures. Figure 3 shows that seed 16 from the IRGA 417 cultivar, dried at 32 °C, did not show a visible fissure (ranked 1) and produced a normal seedling. Seeds classified as 1 can also produce normal weak or abnormal seedlings due to physiological or metabolic damage, since the drying temperatures can damage vegetable tissues. This damage causes cell membrane disintegration and protein breakdown (Daniell et al., 1969). The physiological damage can also cause changes in sub-cellular systems, including the chromosomes (Roberts, 1981).



Figure 3. Cultivar IRGA 417, X-Ray of seed without any visible damage (A), normal seedling (B).

Figure 4 shows seed 39 of the IRGA 417 cultivar, dried at 38 °C, with non-severe fissures (classification 2). The fissures did not reach the embryo and permitted germination but they did affect root growth, resulting in an abnormal seedling. Level 2 damage can result in weak, normal seedlings, with retarded growth during the germination test.



Figure 4. Cultivar IRGA 417, X-Ray of seed with non-severe damage (A), abnormal seedling, with the root system poorly developed, being disproportional to the total seedling size (B).

Figure 5 shows seed 21 of the IRGA 420 cultivar, dried at 50 °C, with severe, level 3 fissures. The fissures occurred in various directions in the seed and reached the embryo. This type of fissure was more evident at a drying temperature of 50 °C and resulted in seed death.

The drying temperature influences the quantity and type of fissure in rice seeds, affecting germination, principally when the fissures are associated with physiological damage, presumably caused by the high drying temperatures (Daniell et al., 1969).

The non-severe fissures resulted in abnormal or normal weak seedlings, probably due to an interruption or reduction in endosperm nutrient translocation to the embryo. However, severe fissures reached the embryo and stopped germination. X-Ray images permitted the identification of fissures and their relationship to seedlings (normal or

abnormal) and dead seeds, at the end of the germination test. This represents a good alternative for evaluating heat damage, as has been shown for mechanical or insect damage in the seeds of corn, soybeans, tree species (Cicero et al., 1998, Carvalho et al., 1999b, Cicero and Banzatto Junior, 2003 and Oliveira et al., 2003).



Figure 5. Cultivar IRGA 420, X-Ray of seed with severe damage (A), dead seed (B).

## Conclusions

X-Ray images permit the identification of fissures in artificially dried rice seeds and their correlation with the production of normal and abnormal seedlings in the germination test.

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