EFFECTS OF ELECTRON BEAM TREATMENT IN SOYBEAN GRAINS ARTIFICIALLY INOCULATED BY *PHAKOPSORA PACHYRHIZI*

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Abstract

Interactions between plants and pathogenic fungi have always been of extreme interest to humanity, since the worldwide economy is built around the purchase and sale of species vegetables among countries, who can suffer serious damages by pathogenic organisms contamination. Soybean is the most important culture of grains in Brazil, which is the second biggest exporter of this grain and suffers from the attack of the fungi Phakopsora pachyrhizi that causes the Asian Soybean Rust. The infection for P. pachyrhizi causes fast blackout and the premature fall of leaves, hindering the full formation of them. The technology of irradiation processing for nutritive purposes is a process that is safe and effective for reducing the microbial load, insect infestation, and extending the shelf life of perishable products. This study aims to verify the artificially inoculated soybeans behavior when treated by electron beam from a linear accelerator at different radiation doses while observing whether the fungi population was reduced or not. The grains of soybean were irradiated at IPEN-CNEN/SP in an electron accelerator of Radiation Dynamics Inc. USA, 1.5 MeV-25mA at doses 0; 1.0; 2.5; 5.0; 7.5 and 10.0 kGy.

I. INTRODUCTION

The interactions among plants and pathogenic fungi always had been of extreme interest to humanity. This is due, in large part, to the fact that a large part of worldwide economy is based around the purchase and sale of vegetable species among countries, who can suffer serious damages by pathogenic assault. The devastation from cultivated specie due on the act of fungi can result in the widespread hunger from the people that depend directly on the cultivation of this specie and, in the worst cases, can change the habit of a complete nation[1].

The soybean is the most important grain in Brazil, who is the second largest worldwide exporter, and generates income of billions of dollars directily and indirectly[2]. Among the principal factors that limit the increase of yields are the illnesses caused by for fungi, bacteria, and virus that, generally, are too difficult to control. The principal disease from soy at the moment is the Asian soybean rust[2,3].

The Asian soybean rust is caused by Phakopsora pachyrhizi Sydow & Sydow fungi and was identified in South American for the first time on March of 2001, in Paraguay, and caused a reduction of yield between 10-80%[3,4,5,6,7].

The *P. pachyrhizi* has high humidity dependence, and needs at least six hours in high humidity conditions to start the contamination. Mild temperature conditions are ideal, but this is not a limitation, because this fungus can be developed between 15°C and 30°C. The dissemination is hard to control, because the uredospore dispersion is by the wind or grains, but the development occurs only in the soy plant. The infection for *P. pachyrhizi* causes fast blackout and premature fall of leaves, hindering the full formation of them[7].

Irradiation with ionizing radiation is one of the most effective means to disinfect dry food ingredients[8,9]. The treatment by irradiation can inhibit cellular life division in microorganisms, resulting in a molecular structural modification[10]. Vegetative cells are more sensitive to ionizing radiation than fungi or bacterial spores[10,11]. This study aims to study the soybean's behavior, when artificially inoculated with the soybean rust, when treated by electron beam from a linear accelerator in different radiation doses, and to observe whether the fungi population was reduced or not.

II. EXPERIMENTAL

II.A. Addition of *P. pachyrhizi* spores

Once *P. pachyrhizi* uses soy leaves as a substrate for its growth and soybeans work as a vehicle for its transportation through cultures, the effect of ionizing radiation was also studied in decontaminated leaves. Decontamination was performed using a 0.4% sodium hypochlorite solution. After this, soy leaves were inoculated with *P. pachyrhizi* spores released by EMBRAPA Paraná- Brazil.

II.B. Samples

The Brazilian soybean grains were purchased from a supermarket in São Paulo, Brazil. Uncontaminated soy leaves were developed in our laboratory. Soybeans and soy leaves artificially contaminated were packed in sterile polyethylene bags, labeled and identified with its respective irradiation doses.

II.C. Irradiation

Samples were irradiated at IPEN-CNEN Electron Accelerator, a Dynamitron Machine (Radiation Dynamics Co. model JOB-188, New York, USA), with 0.550 MeV electrons, scanned over 100 cm width and with a linear support speed of 6.72m/min. Applied doses were 0 (control), 1.0, 2.5, 5.0, 7.5 and 10.0 kGy; dose rate was between 2.23 to 22.37 kGy/s and electrical current between 0.3 to 3.2mA. CTA (Cellulose Tri-Acetate) dosimeters were used for the measurement of radiation dose.

The dosimeters were calibrated, followed by a comparison with calorimetric values (standard), and the energy measurement following the ASTM E10.01 Progress Report of set/1993 protocol. In both cases, the variation was less than 5%.

II.D. *P. pachyrhizi* growth

After irradiation process, contaminated soy leaves were placed in sterile Petri dishes (identified with its respective irradiation doses) with 10mL of autoclaved distilled water and incubated at 25ºC for 5 days. As irradiation process became soy leaves dark, a new sterile fresh leaf were added into each Petri dish to confirm fungi growth. They were incubated at 25ºC for 5 days again.

II.E. Germination test

The germination test was carried out as an adaptation of Kawamura (1996).

II.F. DNA Comet assay

The DNA Comet Assay was carried out as described by Cerda *et al.* (1997). Comets were classified as showed in figure 1.

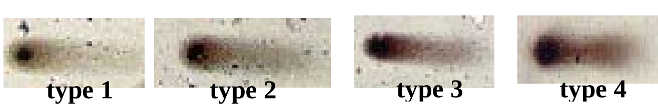


Figure 1. DNA Comets classification

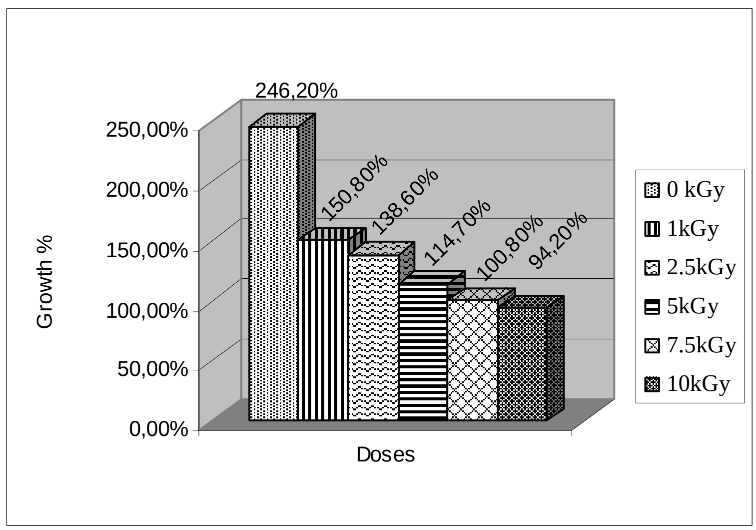
III. RESULTS AND DISCUSSION

Incubated Petri dishes containing soy leaves artificially contaminated, processed by e-beam at doses of 1.0, 2.5 and 5.0 kGy could not inhibit *P. pachyrhizi* growth. Satisfactory results from the e-beam treatment to control *P. pachyrhizi* growth was reached with doses higher than 7.5kGy. The viability test shows that when the radiation doses increase, the fungi colony formation decrease. Using 7.5 and 10.0kGy, no evidence of fungi colonies were found in the samples analyzed by the microbiological viability test.

Using DNA Comet Assay, it was possible to detect DNA degradation due to irradiation treatment. In addition, a germination test determined the influence of ionizing radiation in root elongation after incubation, as well as seed viability. It was observed that the radiation damage increased with increasing radiation doses.

Decreasing percentages of root growth were correlated with increasing radiation doses. It was observed that radiation treatment with high doses had a negative influence in root growth (figure 2).

Figure 2. Root growth from different radiation doses



Analyzing the comet assay results, we could observe that a slight DNA damage of soybeans appeared after radiation treatment with doses over 1.0kGy. It was also observed that this degradation increased with the radiation dose applied, based on the higher DNA migration found. Frequently, non-irradiated soybeans exhibited comets type 1 and 2 only, with very slight amounts of type 3 as observed in figure 3.

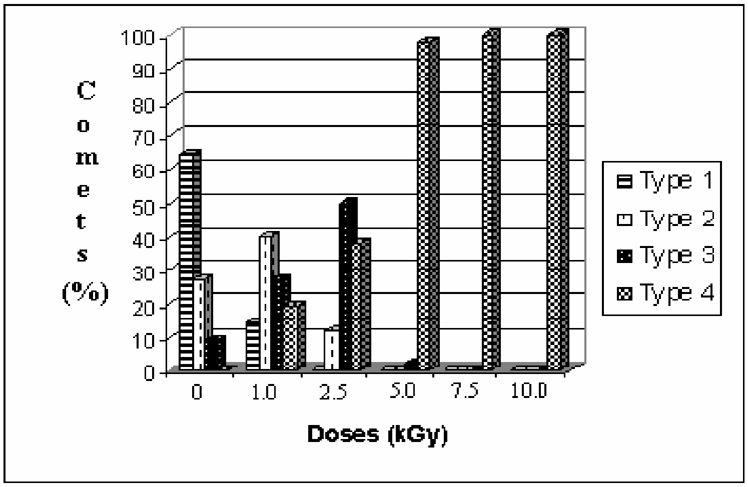


Figure 3. Differents types of comets from different doses

With increasing doses, from 1.0 to 2.5kGy, DNA fragmentation became obvious, increasing the amounts of type 3 comets. At higher doses, from 2.5 to 10.0kGy, type 4 comets were also found. The viability response to electron beam treatment was, in principle, not satisfactory in our machine, for the soybeans grains tested here because the minimum energy of our e-beam machine (0.55Mev) was sufficient to damage the soybean DNA by electron penetration. As well as the doses applied being effective in inhibiting the fungi growth, the seeds were also found to have lost viability with doses higher than 5.0kGy.

As accordance with the common knowledge in the scientific community, fungus contamination needs high radiation doses to guarantee phytosanitary control in food. A current concern is that, with high doses application in food, the nutritional qualities could also decrease. E-beam radiation treatment could be a safety-alternative process to minimize the nutritional losses due the lower electron penetration[14,15]. The penetration power of the electron beam depends on the intensity of the electrical field in which it is accelerated. New concepts of e-beam machines promote the control of the accelerating field in the adjustment of the electrons penetration power so as to not exceed the seed shell thickness that normally ranges from 0,025mm to 0,5mm[16].

In the last decade, experiments using e-beam for seed treatment were carried out efficiently in Germany to control a series of diseases in seeds using the purely physical nature based on the effect of low energy electron beams to bombard the seeds. In these test the seeds were in a continuous gravity-driven flow drop off, and subjected to the bombardment of two electron beam emitted by two contraposed accelerators, with the energy ranging from 105 to 145KeV. This exposure permitted a uniform exposure inside the thickness of seed shell[16,17].

Additional experiments will be carried out to compare different machine potencies.

IV. CONCLUSION

The aim of this work was to use an e-beam in our experiment and to evaluate the superficial efficiency of that treatment. Only superficial effects were studied in the well-established dose range (7.5kGy) for the case of *P. pachyrhizi*. The goal was to eliminate any fungi contamination and yield no nutritional damage, or a minimal damage in DNA, after the radiation treatment in the grains. We are working with more experiments and applying different energies to observe the presence of any DNA degradation after e-beam treatment in the grains.

V. AKNOWLEDGMENTS

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