UTILIZATION OF HALF-EMBRYO TEST TO IDENTIFY IRRADIATED BEANS

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ABSTRACT

Germination tests were carried out in irradiated and non-irradiated bean seeds which allow to observe characteristically variations on the shoots and roots. The methodology used in this work, is based upon biological changes which occur in two Brazilian beans, ***Phaseolus vulgaris L.***, var. carioca and ***Vigna unguiculata (L.) Walp***, var. macaçar, irradiated in a ^{60}Co source, with doses of 0, 0.5, 1.0, 2.5, 5.0 and 10.0 kGy. The shoots and roots were observed during 3 days of culturing period under specified conditions. The differences observed in these two varieties were analysed immediately after irradiation and after 6 months of storage period at room temperature. Irradiated half-embryos showed markedly reduced root grow and almost totally retarded shoot elongation. Differences between irradiated and nonirradiated half-embryo could be observed after irradiation when differents beans and storage time were varied. The shoots of half-embryos irradiated with more than 2.5 kGy did not undergo any elongation, whereas, the shoots of non-irradiated or those beans irradiated under 1.0 kGy elongated significantly within the 3 day test period.

INTRODUCTION

Beans are a major source of dietary protein in many areas of the world, and they are an important source of nutrients and energy for Latin-American people [1]. For insect disinfestation in beans, irradiation offers an attractive alternative to chemicals. Among existing technologies for food preservation, food irradiation is recognized as a safe and an effective method for a range of specific applications. Radiation processing of beans for the purpose of insect disinfestation with dosages up to 1 kGy is a promising technique for reducing storage loss of the nutritious foodstuffs [2]. These beans are normally infested by several species of insects during storage. Although, substantial quantities of these beans, produced annually in Brazil, are affected by the insect infestation, and efforts to improve the storage time quality for dry beans have included various pretreatment and treatments, including processing by irradiation. The results of over 30 years of research on the toxicological, biological and nutritional quality of irradiated foods have led the World Health Organization to recommend food irradiation as a technique for preserving and improving the safety of food [3]. Since radiation was utilized for food disinfestation, methods were necessary to identify irradiated foods. Considerable efforts have been directed by researchers in order to develop methods for identification of irradiated foods. A half-embryo test to identify irradiated seeds or grains, was utilized since the mid-1980's with this purpose. We propose to check the germination results immediately after irradiation and after 6 month's storage of these irradiated beans and to evaluate the effect of ionizing radiation in roots and shoots.

MATERIAL AND METHODS

Beans. *Phaseolus vulgaris L.*, var. carioca and *Vigna unguiculata (L.) Walp*, var. macaçar, bought in a local market, were utilized.

Irradiation. The beans were irradiated in a ^{60}Co source, with doses of 0, 0.5, 1.0, 2.5, 5.0 and 10.0 kGy.

Germination.. The seeds were placed on distilled water for 3 hr., the seeds were opened and only one side which contained the embryo axis was taken. This side was put into the Petri dish covered with filter paper and a little bit distilled water, to give humidity, and the petri dish closed. Ten half-embryos seeds were put into a black box and cultured at 35 ± 1^0C. Germination, as well as shoots and roots growth, were observed.

Sample Measurements. Based on the growth of half-embryos, the shoots and roots were observed for 3 days of culturing period under the specified conditions The germinated seeds were taking each day and the elongation of shoots and roots were registered in millimeters. Differences found in these two varieties were analysed immediately after irradiation and after 6 months storage period at room temperature.

Statistical Analyses. Ten grains of each variety of beans were studied to evaluate the effect of each irradiation dose.

An estimation of the relative variation was made by determining the coefficient of variation (cv), which is a ratio in percentage of the standard deviation from the mean.

RESULTS AND DISCUSSION

The germination of these two varieties of beans was studied, to determine the influence of ionizing radiation in roots and shoots elongation after incubation. It was observed that the damage by irradiation in shoots and roots has some special characteristics. Figure 1 and 1a, show the roots length (mm) incubated after irradiation and it could be observed that in these two varieties the first period of incubation is not so much representative than after 72h. We can see clearly that with radiation treatment we have less of the half values or less of growth for all doses utilized.

After 6 months storage period, reduction on germination of roots in all samples were observed, but the variety carioca show more sensibility than var. macaçar, as can be seen in figure 2. Roots data after 72 h (Figure 2a) show a small difference in controls and irradiated beans with 0.5 kGy in var. macaçar but for var. carioca we find expressive difference.

Kawamura [4] shows the germination test with wheat and concluded that the critical doses that inhibit root growth vary from 0.15 to 0.5 kGy and also that storage periods of up to 12 months have little effect on irradiation-induced reduction of root length. Furthermore, Fifield [5] shows that germination of wheat was unaffected by radiation dosages of 0.1 and 0.25 kGy. However, 0.5 kGy or more causes a substancial reduction in wheat germination. In germination tests for rice, the critical doses that inhibit roots elongation varied from 0.15 to 0.5 kGy according to Kawamura [6] and also this author can discriminate between irradiated and non-irradiated rice for 12 months or more of storage period after gamma-irradiation.

Figure 3 shows the first day incubation after irradiation and the shoots length, and at this time one can not find greater differences in all varieties and dose response. After 72 h. incubation time, showed in fig.3a., one can find clearly differences in shoot elongation in var. carioca but in var. macaçar at doses of 0.5kGy and 1 kGy they are not so expressive. After 6 months storage period and irradiation expressive difference in both varieties in germination of shoots after incubation at 35°C, were observed, (fig. 4.). After 72 h. incubation, fig.4a. shows, that both varieties had expressive response to irradiation and storage period.

A half-embryo test to identify irradiated grapefruit described by Kawamura [7] shows that shoot elongation and root growth was totally retarded over 1.5 kGy and in this way they can discriminate between irradiated and non irradiated grapefruit. Half-embryo test with lemons and oranges seeds, was proposed by Kawamura [8] as an identification method for irradiated citrus.

For irradiation detection, when compared with the control, after storage period, if the shooting is greater than 50% within 3 days, the seeds are identified as "unirradiated", and if it is less than 50% after 3 days, the seeds are identified as "irradiated". There are a number of other methods available for the identification of a range of irradiated foods [9]. Germination test as a biological method for the identification of irradiated foods was also discussed by Delincée [10].

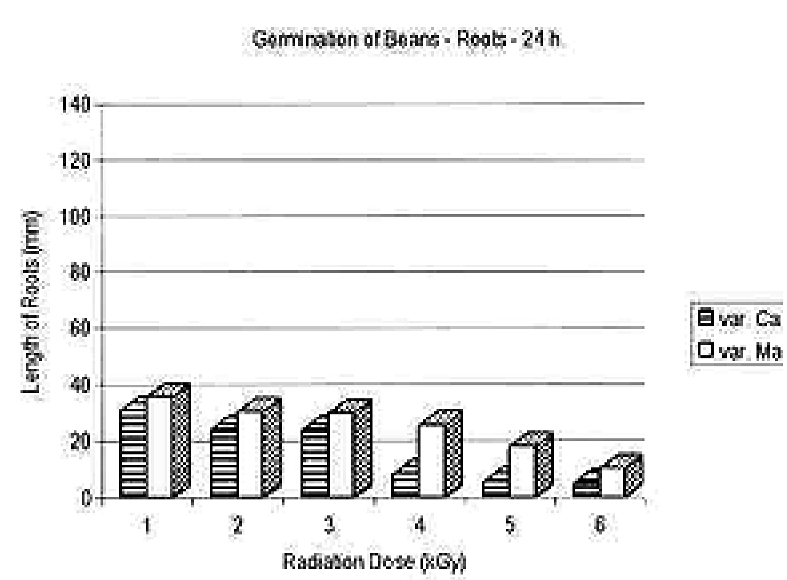

Figure 1. Roots 24 h. after Irradiation

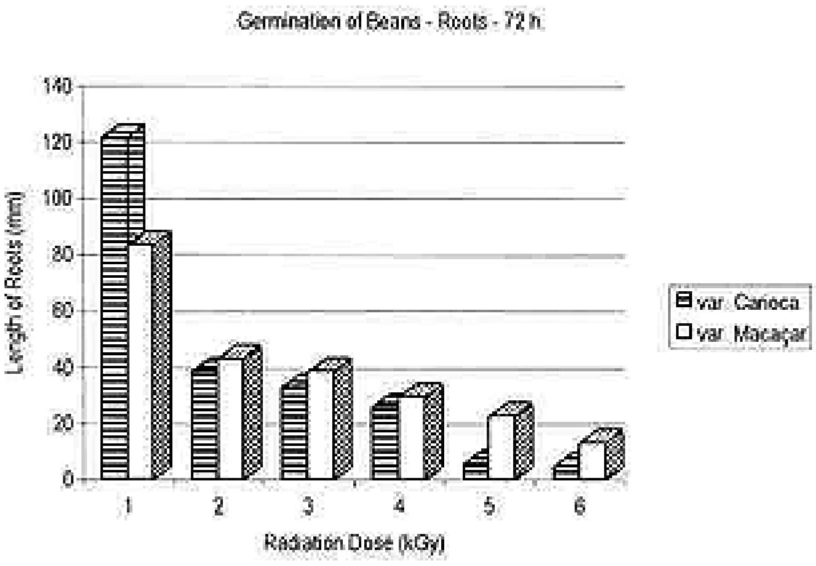

Figure 1a. Roots 72 h. after Irradiation.

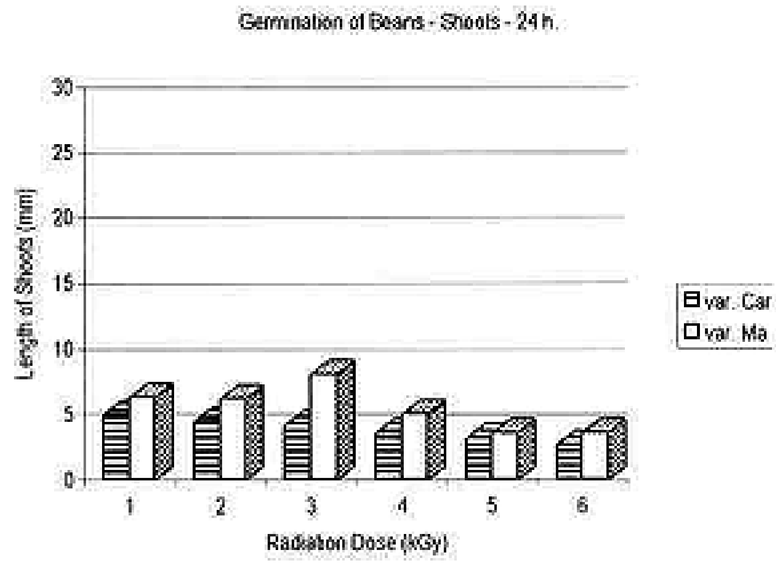

Figure 3. Shoots 24 h. after Irradiation.

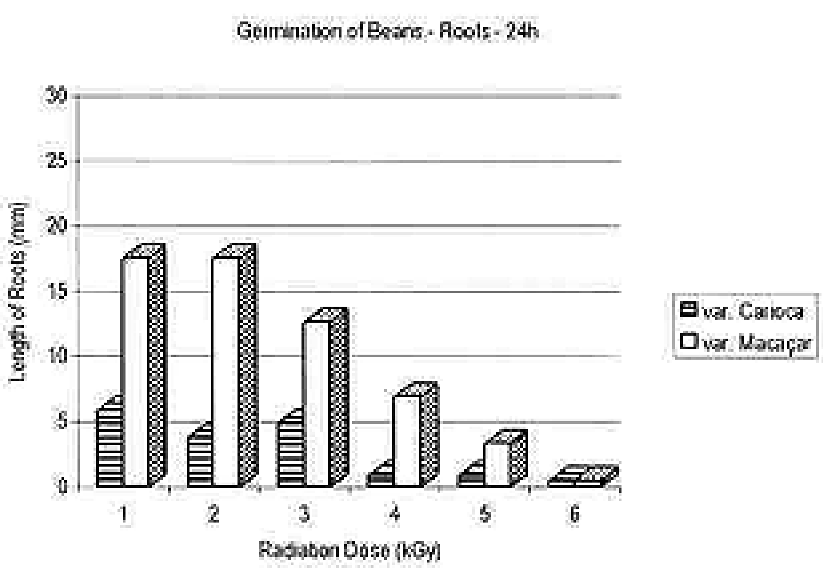

Figure 2. Roots 24 h. for 6 months storage after Irradiation.

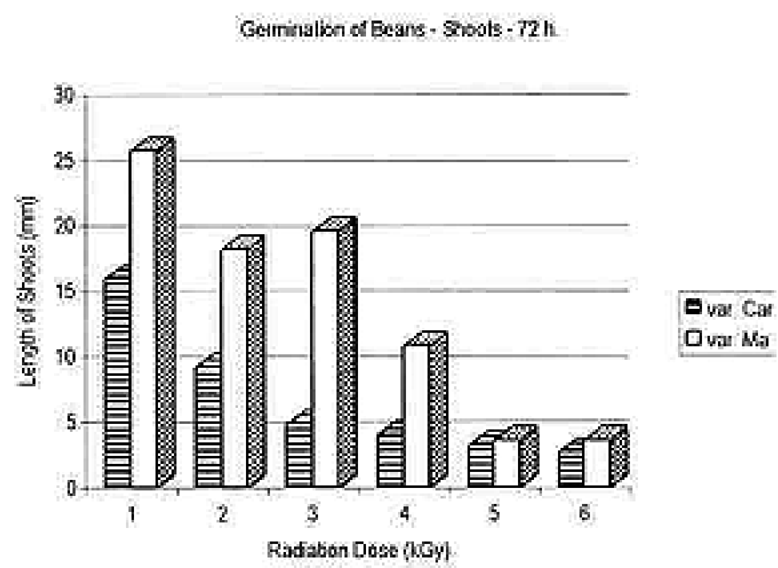

Figure 3a. Shoots 72 h. after Irradiation

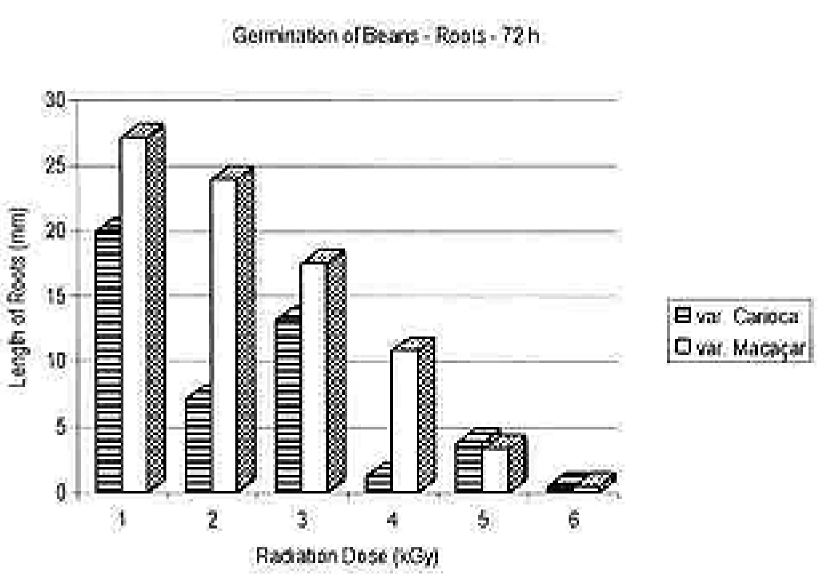

Figure 2a. Roots 72 h. for 6 months storage after Irradiation.

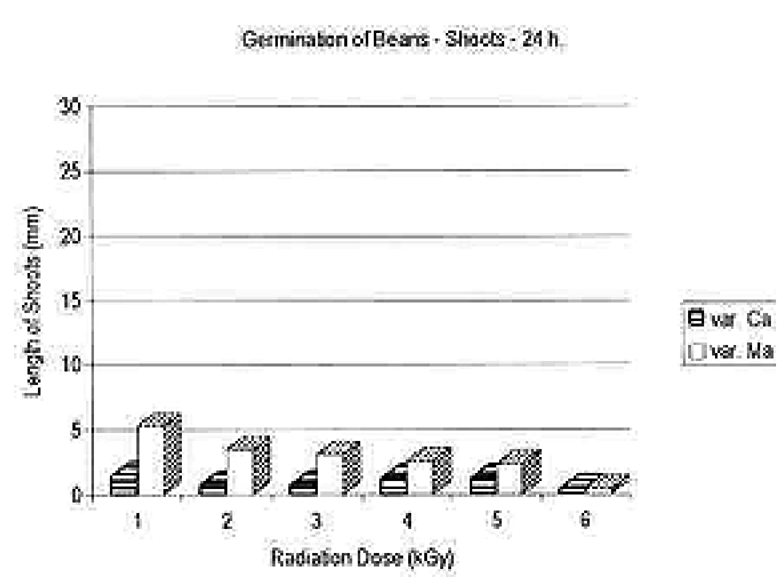

Figure 4. Shoots 24 h. for 6 months storage after Irradiation

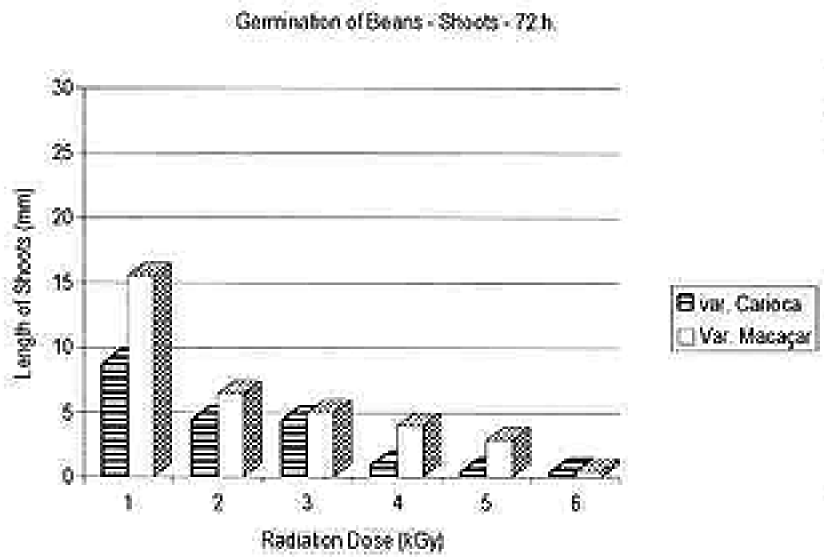

Figure 4a. Shoots 72 h. for 6 months storage after Irradiation

CONCLUSION

In this work, the possibility was shown to test beans, for 6 months storage, were irradiated or not, using a half-embryo test. Whether it was also observed that the two varieties of irradiated beans studied do not grow with the same vigor as the control after this storage period.

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