Utilization of half-embryo test to identify irradiated beans

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SUMMARY

Germination tests were carried out in irradiated and non-irradiated food seeds which allows 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 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. These methodology can be improved in irradiated beans analysis.

RIASSUNTO

Sono stati effettuati tests di germinazione su semi alimentari irradiati e non per osservare variazioni delle caratteristiche di radici e germogli. La metodologia del lavoro è fondata sul metodo usato per verificare le modifiche biologiche ottenute su due fagioli brasiliani, *Phaseolus vulgaris L.* var. carioca, e *Vigna unguiculata (L.) Walp* var. macaçar, irradiate con sorgente di ^{60}Co a dosi di 0, 0.5, 2.5, 5.0 e 10.0 kGy. I germogli e le radici sono stati osservati durante tre giorni di cultura in condizioni appropriate. Le differenze osservate nelle due varietà sono state controllate dopo l'irradiazione e dopo sei mesi di conservazione a temperatura ambiente. Gli embrioni irradiati mostrano una marcata riduzione della crescita delle radici e un quasi completo ritardato allungamento dei germogli. Differenze tra embrioni irradiati e non si possono osservare dopo irraggiamento quando qualità di semi e tempi di conservazione sono differenti. I germogli di semi irradiati con più di 2.5 kGy non mostrano alcuno sviluppo, mentre quelli irradiati con meno di 1.0 kGy si sviluppano in modo significativo entro 3 giorni. Questa metodologia risulta applicabile nell'analisi di semi irragiati.

KEY WORDS: Irradiation; Beans; Half-Embryo test

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. One of the factors limiting an expanded consumption of beans is the development of a textural defect when beans are stored under high relative humidity and high temperature, conditions prevailing in tropical countries. The defects (hard-to-cook and hard-shell) are linked to structure of the seed and related with several compounds including phenolics, phytates and fiber presented in beans.

The irradiation can decrease bean cooking time without reduction in the biological value of proteins [1]. For insect disinfestation in beans, irradiation offers an atractive 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 tecnique for reducing storage loss of the nutritious foodstufs [2]. The beans are normally infested by several species of insects during storage. Although, substantial quantities of the 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, were necessary methods to identify irradiated foods.

Considerable efforts has been directed by researches 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 in this research to check the germination results after irradiation and after 6 month's storage of the irradiated beans evaluating 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 São Paulo city (Brazil) 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 took only one side which contained the embryo axis.
This side was put into the petri dish covered with filter paper and a little bit distilled water, to give humidity, and closed the petri dish. Ten half-embryos seeds were put into a black box and cultured at 35 ± 1°C. 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 alongation of shoots and roots were registered in milimeters. Differences founded in these two varieties were analysed 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, shows the roots length (mm) incubated after irradiation were 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

Fig. 1. Roots lenght of irradiated beans after 24 h of incubation

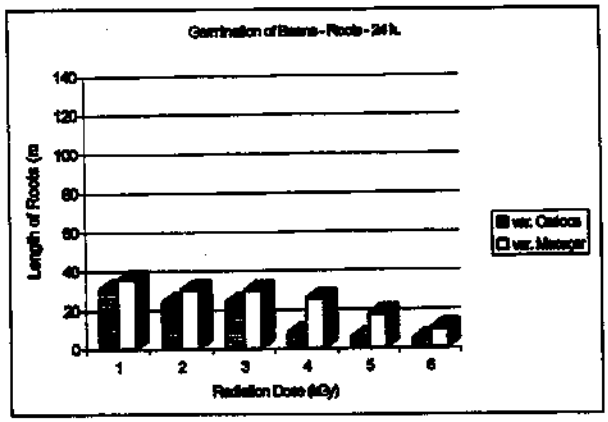


Fig. 1a. Roots lenght of irradiated beans after 72 h of incubation

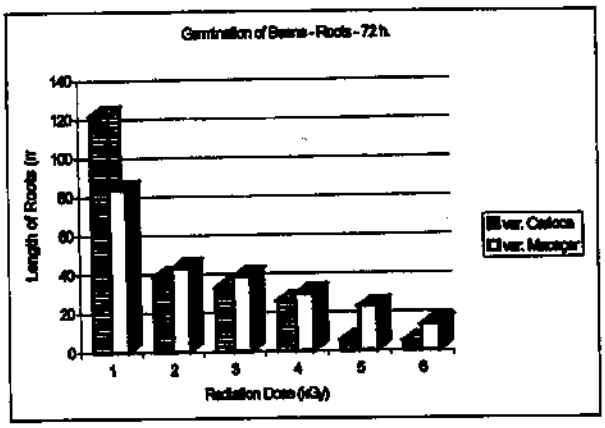


Fig. 2. Roots lenght of irradiated and stored beans for 6 months after 24 h of incubation

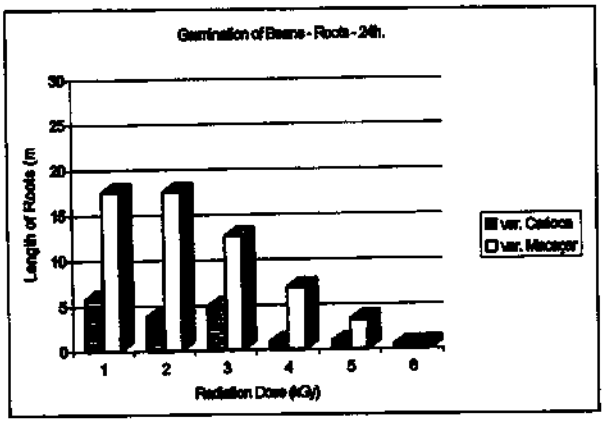
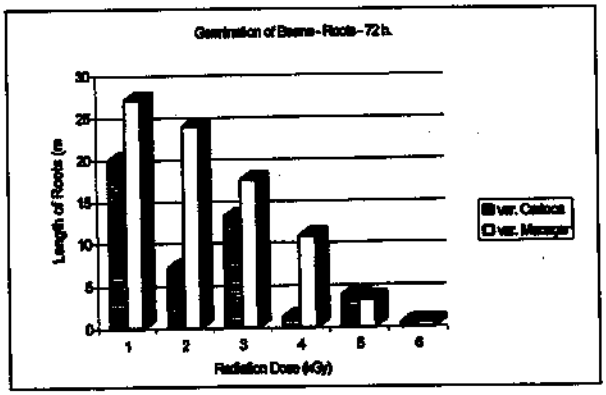


Fig. 2a. Roots lenght of irradiated and stored beans for 6 months after 72 h of incubation



grow in all doses utilized. After 6 months storage period, were observed reduction on germination of roots in all samples but the variety carioca show more sensibility than var. macaçar, as can see in figure 2. Incubaton roots data after 6 months storage period and irradiation. 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 germination test with wheat and concluded the critical doses that inhibits roots elongation varies 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, at 0.5 kGy or more causes a substancial reduction in wheat germination. In Germination test 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, at this time can not find greater differences in all varieties and doses response. After 72 h. incubation time, showed in fig.3a., can find clearly differences in shoot elongation in var. carioca but in var. macaçar at doses 0.5kGy and 1 kGy they are not so expressive. After 6 months storage period and irradiation was observed expressive difference in both varieties in germination of shoots after incubation at 35°C, showed in 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 grow was totaly 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.

Fig. 3. Shoots lenght of irradiated beans after 24 h of incubation

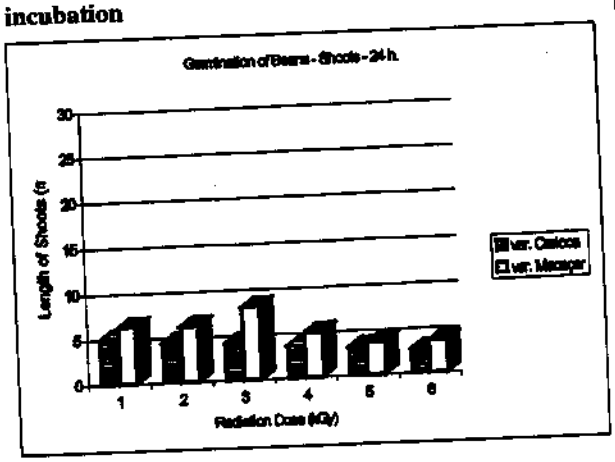


Fig. 3a. Shoots lenght of irradiated beans after 72 h of incubation

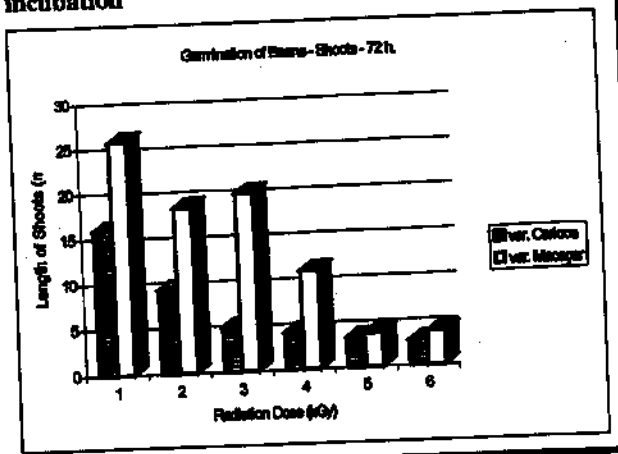


Fig. 4. Shoots lenght of irradiated and stored beans for 6 months after 24 h of incubation

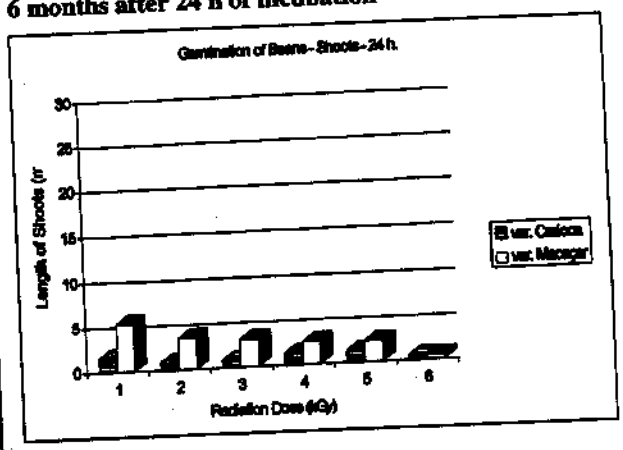
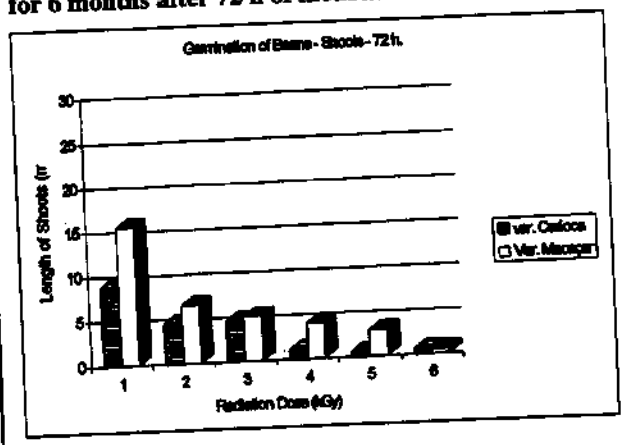


Fig. 4a. Shoots lenght of irradiated and stored beans for 6 months after 72 h of incubation



For irradiation detection, when compaired 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 methods available for the identification of a range of irradiated foods [9]. Germination test is also a biological method for the identification of irradiated foods as showed by Delincée [10].

CONCLUSION

In this work, it was shown the possibility to tested if beans, storage 6 months, were irradiated or not, using a half-embryo test, and also it was 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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