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A GERMINATION TEST: AN EASY APPROACH TO KNOW THE IRRADIATION HISTORY OF SEEDS

Affaf Khawar[1], Ijaz Ahmed Bhatti[1], Qaiser Mehmood Khan[2], Haq Nawaz Bhatti[1,*] and Munir Ahmed Sheikh[1]

[1]Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan
[2]Environment Biotechnology Division, National Institute of Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan
[*]Corresponding author's e.mail: hnbhatti2005@yahoo.com

Food irradiation is an evolving preserving technique that provides a shield against the spoilage and might have a potential to ensure the food safety and security world wide. In the present study, feasibility to apply germination test to distinguish an un-irradiated and irradiated samples of wheat, maize, chickpea and black eye beans was checked. Samples were irradiated to the absorbed doses ranging from 0-10 kGy using Co-60 gamma irradiator and were germinated in plant growth chamber. Root and shoot lengths were measured at 7th day after gamma radiation treatment. In all the irradiated samples root and shoot lengths were decreased with the increase in radiation absorbed doses. The seeds irradiated to the absorbed doses more than 2 kGy were not germinated. Germination test proved as an easy and simple method to detect irradiation in wheat, maize, chickpea and black eye beans irradiated even at low absorbed doses.
Keywords: Food irradiation, germination test, disinfestations

INTRODUCTION

There is nothing like fresh food but to prevent food from post harvest losses during storage, food processing is required. Although many advanced and developing countries have abundant supplies of safe, fresh and nutritious foods yet microbial contamination is still a matter of deep concern (Mayer-Miebach *et al*., 2005). There are many food-processing methods like canning, dehydration, freezing, salting and fumigation (with phosphine gas, ethyleneoxide, methylbromide etc). The chemical treatments are now highly restricted in advanced countries for environment and health safety purposes (Bhatti and Kwon, 2007). Food irradiation is an alternative to other preservation techniques since irradiated food is wholesome and unlike chemical treatment it does not leave harmful residues. It is an eco-friendly as well as cold pasteurization technique which includes disinfestations, improvement of safety and shelf life of food by the inactivation of pathogens and micro organisms (Suhaj *et al*., 2006).
With a rapid increase of irradiated food the availability of standard methods for detection of irradiated food is important, since diagnostic test can not only prevent illicit trade but also enhance consumer's confidence and international trade (Cutrubinins *et al.,* 2007). When the foodstuff is irradiated physical, chemical and biological changes are produced which are so small that cannot be detected by ordinary methods. Therefore sensitive and reliable analytical techniques are required to detect these very minute changes. These radiolytic specific changes can serve as a diagnostic marker for the identification of irradiated foods (Bhatti and Kwon, 2007).
So far several methods such as electron spin resonance, thermoluminesence and pulsed photo-stimulated luminescence, viscosity measurement, hydrocarbon and 2-alkyl cyclo butanone analysis, ELISA, DNA comet assay, germination test and DEFT/APC count etc. have been tried and standardized for specific food items (Khan *et al.,* 2002; Delincee, 2002). These methods except germination test involve complicated techniques, expensive chemicals and instrumentations, laborious sampling and skilled workers etc. Germination test is simple, inexpensive, less time consuming and is especially applicable for the identification of seeds irradiated at low absorbed doses, sufficient for their disinfestations. It has been tried by many researchers on their native foods. Germination test, also called half embryo test is reported for the evaluation of irradiation in legumes, cereals and even fruit seeds like citrus seeds (Huachaca *et al.,* 2004). In this study the germination test or half embryo test has been employed using wheat, maize, chickpea and black eye beans for

understanding the radiation processing of seeds which may serve as identification marker.

MATERIALS AND METHOD

The seed samples, wheat, maize, chickpea and black eye beans were collected from the grain market, Faisalabad and were irradiated to the absorbed doses of 0.1, 0.3, 0.5, 2, 6 and 10 kGy at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad using CS-137 gamma-radiation source. For germination test at least 20 seeds of un-irradiated and irradiated samples of wheat, maize, chickpea and black eye beans were taken and each set was repeated at least three times. Hence data reported is the average of 60 polled seeds for each sample. The seeds were soaked in distilled water for 10 hours. Moistened 9 cm layer of absorbent cotton with distilled water was placed on petri dish. Germination of seeds was carried out in plant growth chamber (Vindon, England) at controlled conditions of temperature (Chaudhuri, 2002). Optimum temperature required for the germination for each sample was surveyed from the literature. Wheat, maize, chickpea and black eye bean were kept at 28±3°C in the growth chamber for germination test (Nyachiro *et al*., 2002). Root and shoot lengths of un-irradiated and irradiated samples were measured on 7th day after the start of experiment. Data was analyzed statistically using Statistica 99, Version 9.

RESULTS AND DISCUSSION

The effect of gamma irradiation, on germination of seeds (wheat, maize, chickpea and black eye beans) was studied, in order to evaluate the validation of germination test to identify gamma radiation treatment in some selected seeds. The root and shoot lengths reported for each variety is an average of 60 seeds. Root and shoot morphology was evaluated using the lengths of root and shoot measured in mm with a ruler after 7th day from the start of the experiment. The average root and shoot lengths of control wheat seeds were 18mm and 12mm respectively. It was found that these lengths decreased with increase of irradiation doses. At an absorbed dose of 2 kGy the lengths reduced to 2 mm (root) and 0.5 mm (shoot). At 6 and 10 kGy no seed of wheat germinated (Fig.1). The same trend has been reported that showed the reduction in root and shoot lengths of wheat seeds irradiated at different doses (Zhu *et al*., 1993; Kawamura, 1992a; Barros *et al*., 2002; Wang and You, 2000).

In case of un-irradiated maize the average root and shoot lengths of seed were 22 and 15 mm respectively. These lengths reduced to 18 mm (root) and (11 mm) shoot at 0.1 kGy and even smaller at 0.3 kG. At 0.5 kGy radiation absorbed dose the average root and shoot lengths were (6.7 mm) and (2 mm) respectively. There was no protuberance of root or shoot in samples irradiated to the absorbed doses of 2 kGy, 6 kGy and 10 kGy (Fig.2). Mokobia and Rano (2005) in their study on maize, okra and groundnut irradiated at low radiation doses reported the decrease in growth rate in number of germinated seeds with the increase in irradiation doses.

The average root and shoot length elongation of un-irradiated chickpea was measured as 20.5 mm and 6 mm respectively. At 0.1 kGy it was 16 mm (root) and 4 mm (shoot), at 0.3 kGy 11mm (root) and 3.3 mm (shoot), at 0.5 kGy 9 mm (root) and 1.5 mm (shoot), at 2 kGy 5 mm (root) and 1.5 mm (shoot) and at 6 and 10 kGy no germination was observed (Fig. 3). The study carried out by Hameed *et al.* (2008) and Toker *et al*. (2005) also reported that % germination and root/shoot elongation of different varieties of chickpea were inversely proportional to irradiation dose.

Black eye beans showed the same trend of average root and shoot length reduction as in wheat, maize and chickpea. The average value of root length in the un-irradiated control sample was 19.5 mm and that of shoot length was 11.3 mm, which decreased gradually to 5 mm (root) and 3 mm (shoot) at a radiation absorbed dose of 2 kGy. No black eye bean seed irradiated to 6 and 10 kGy was germinated (Fig. 4). Villavicencio and co- workers (1998) also reported root growth that was extremely reduced with increasing irradiation absorbed dose and totally retarded root/shoot elongation in case of two varieties of Brazilian beans, *Phaseolus vulgaris L*. (var. carioca) and *Vigna unguicurlata L.* (var. macar) irradiated to the different absorbed doses 1 to 10 kGy.

In maize and wheat average root/shoot length ratio was increased indicating germination inhibition at all radiation absorbed doses (Fig.5). From the above study, it is evident that there is a significant decrease in root and shoot length of the seeds irradiated even at very low doses of 0.1 kGy and this reduction is inversely proportional to the irradiation doses. This physiological change (germination inhibition) and morphological change (root/shoot elongation) can be used as a radiobiological parameter for irradiation identification.

Same trend has been reported for other food commodities, such as rice peanuts and citrus seeds using half embryo test for irradiation identification successfully (Kawamura *et al*., 1992b; Chiou *et al*., 2006). Selvan and Thomas (1999) found that irradiated onion bulbs did not germinate even placed

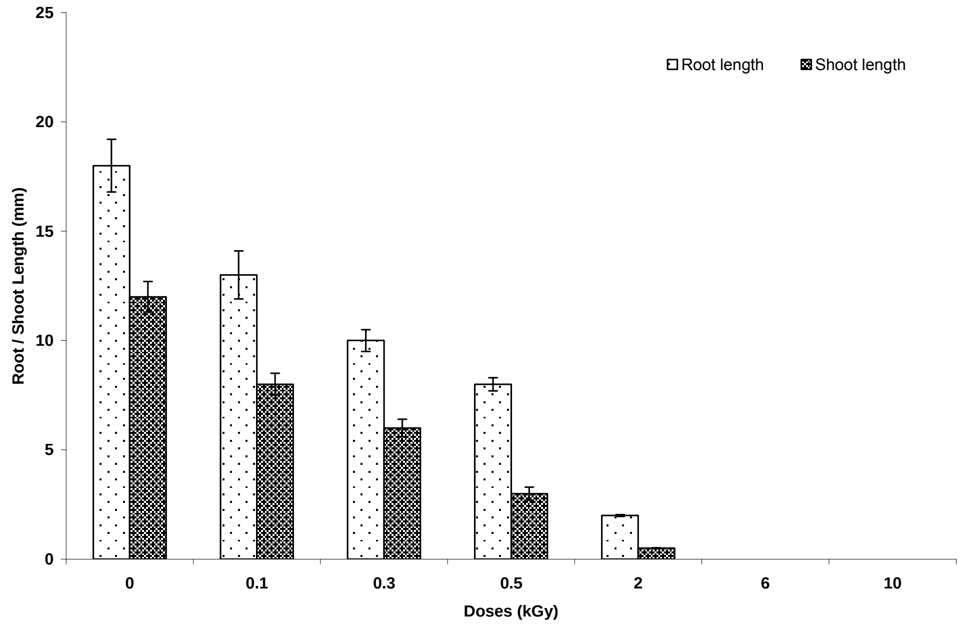

Figure 1. Root length and shoot length of wheat at various irradiation doses (0-10 kGy). Each bar represents mean ± SD.

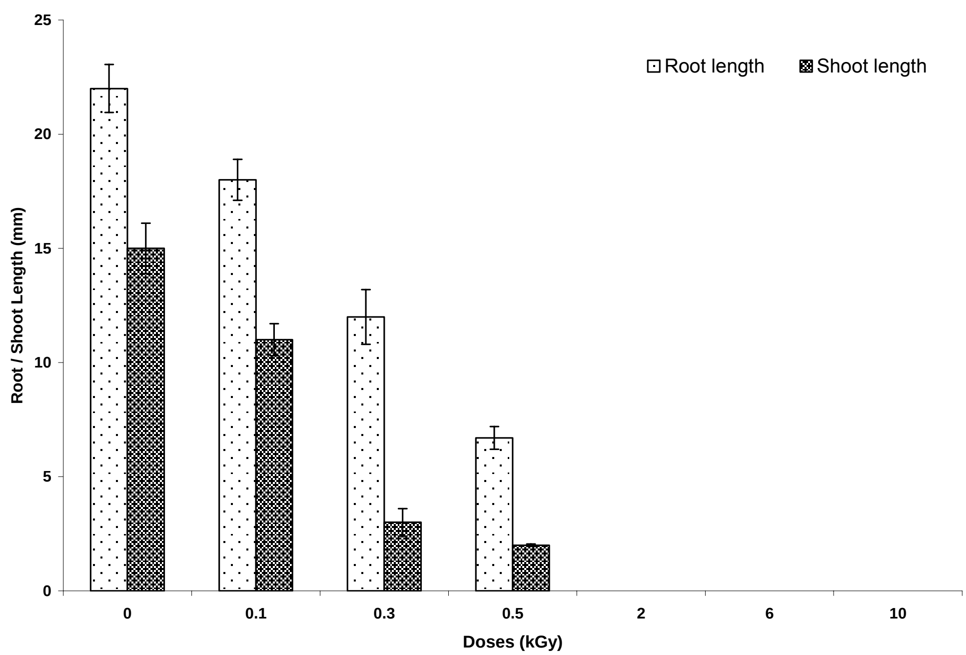

Figure 2. Root length and shoot length of maize at various irradiation doses (0-10 kGy). Each bar represents mean ± SD.

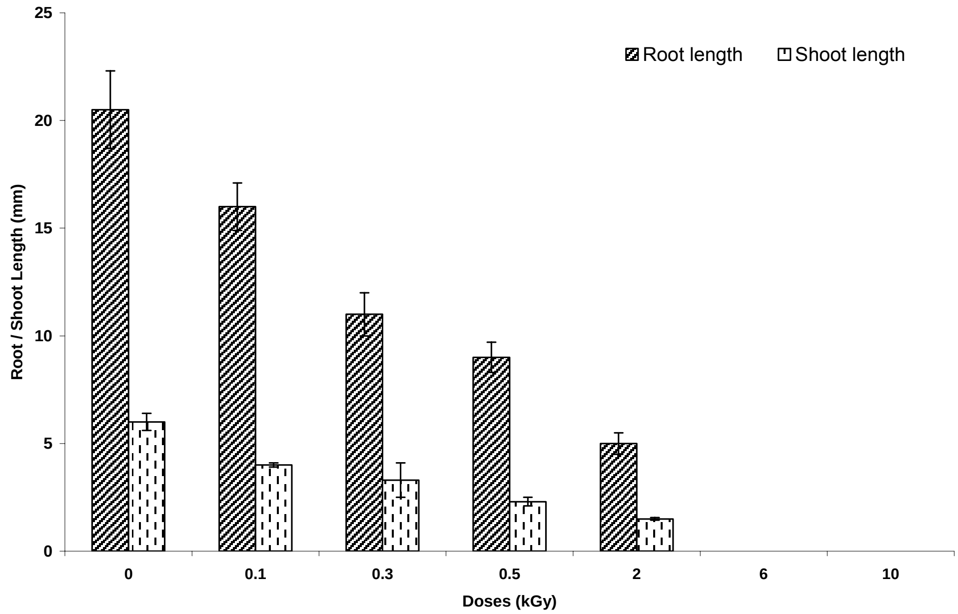

Figure 3. Root length and shoot length of chickpea at various irradiation doses (0-10 kGy). Each bar represents mean ± SD.

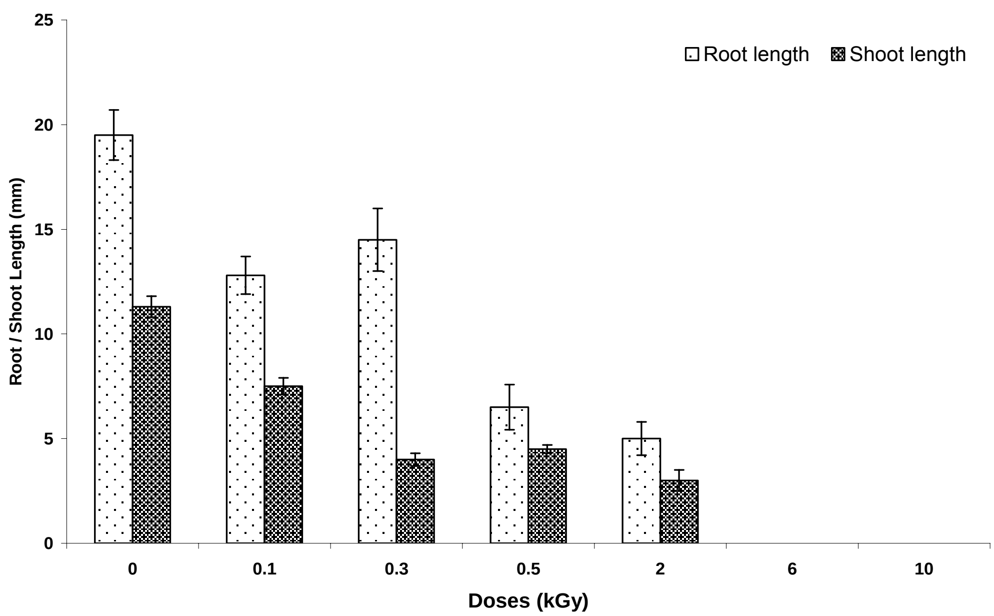

Figure 4. Root length and shoot length of black eye bean at various irradiation doses (0-10 kGy). Each bar represents mean ± SD.

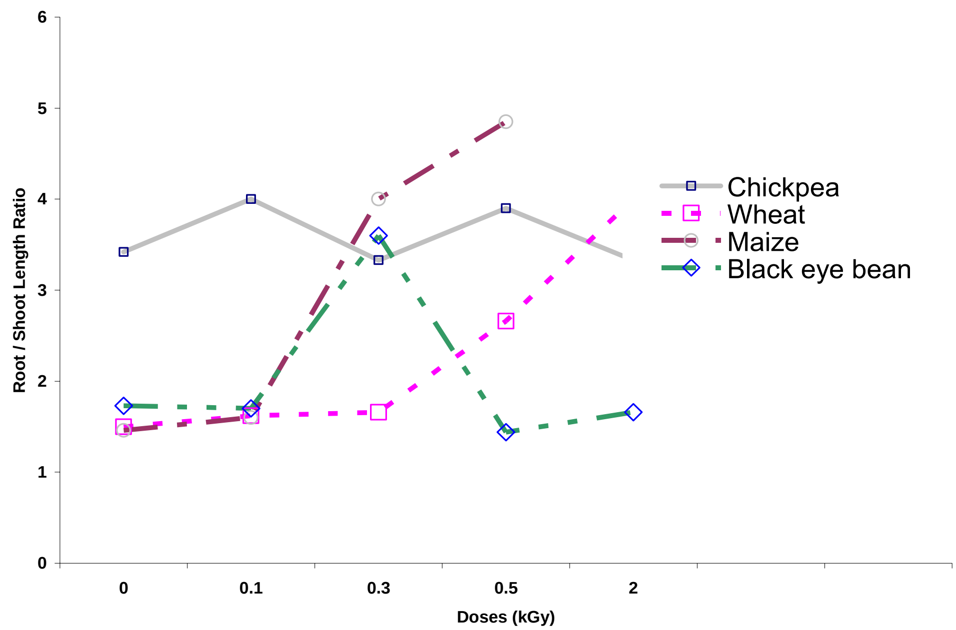

Figure 5. Root/shoot length ratio of chickpea, wheat, maize and black eye bean at various doses (0-10 kGy).

on 0.2% agar (a root promoting media). Chaudhuri (2002) observed drastic reduction of root and shoot growths of lentil seeds at higher doses, even after 12 months of storage. This technique was able to discriminate between irradiated and non-irradiated citrus; studies performed by Kawamura *et al*. (1989b) revealed that root/shoot growth of grape fruit seeds was totally retarded over 1.5 kGy. Kawamura *et al*. (1989a) also demonstrated that irradiated oranges and lemons can be discriminated from non-irradiated ones by half-embryo test of their seeds. However the critical dose that inhibits totally root/shoot growth varied not only among different varieties but also with in a gentotype (Toker *et al*., 2005). From the above discussion it is evident that germination test is a valuable test reported for legumes, cereals and even for different fruit seeds. It is a simple and inexpensive test that can be carried out for the primary and early assessment of irradiation. It is also useful for routine control of irradiation in foods, even at very low doses, required for sprouting inhibition, microorganism infestation, sanitary measures and quarantine treatments.

Gamma-irradiation significantly effect germination even at low irradiation doses required for disinfestations and other purposes. There are many possible reasons for germination inhibition as reported by different authors. Gamma-irradiation generates free radicals in plant produce that may bring metabolic disorders in the seeds leading to growth retardation. It also affects enzyme activity since higher seed vigour is related to higher germination efficiency. It is also reported that high α-amylase activity increased metabolic activity leading to enhanced seed vigour. Hence it may be infused that gamma irradiation may decrease α-amylase activity (Kumagai *et al*., 2000; Stoeva *et al.,* 2001; Afzal *et al*., 2008). In some reports decreased lipase activity with increasing gamma irradiation dose in castor seeds resulted in germination inhibition where as some studies associated increased membrane permeability with seed vigor loss and concluded that gamma irradiation and seed membrane permeability are directly related, hence increased radiation doses increases membrane permeability and reduces seed germination (Thanki *et al.,* 2007; Parera *et al*., 1996; Golovina *et al.,* 1997; Wang and You, 2000).

CONCLUSIONS

The germination test can be successfully used in order to know the irradiation history in wheat, maize, chickpea and black eye bean seed samples even at low absorbed doses. Root and shoot lengths decreased with irradiation absorbed doses in all radiated samples. Moreover high irradiation doses completely inhibit germination. Hence germination test is a simple, rapid and easy method for the irradiation identification in seeds at doses sufficient for disinfestations as no expensive instruments, chemicals and trained persons are required. However it has some limitations also such as it is not applicable for at the spot identification since some weeks are involved for its execution. It is good for primary screening but we cannot say that it is radiation specific because treatment with some chemicals can also results in germination inhibition. After primary screening, for further confirmation other methods that are radiation specific should be employed.

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