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Detection of radiation treatment of beans using DNA comet assay

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Abstract

A simple technique of microgel electrophoresis of single cells (DNA Comet Assay) enabled a quick detection of radiation treatment of several kinds of leguminous beans (azuki, black, black eye, mung, pinto, red kidney and white beans). Each variety was exposed to radiation doses of 0.5, 1 and 5 kGy covering the permissible limits for insect disinfestation. The cells or nuclei from beans were extracted in cold PBS, embedded in agarose on microscope slides, lysed between 15 and 60 min in 2.5% SDS and electrophoresis was carried out at a voltage of 2 V/cm for 2–2.5 min. After silver staining, the slides were evaluated through an ordinary transmission microscope. In irradiated samples, fragmented DNA stretched towards the anode and the damaged cells appeared as a comet. The density of DNA in the tails increased with increasing radiation dose. However, in non-irradiated samples, the large molecules of DNA remained relatively intact and there was only minor or no migration of DNA; the cells were round or had very short tails only. Hence, the DNA comet assay provides an inexpensive, rapid and relatively simple screening method for the detection of irradiated beans.

Keywords: Beans; Irradiation; Detection of irradiated food; DNA comet assay

1. Introduction

In the countries having warm and humid climates, such as Pakistan, the beans and pulses are subjected to molding and insect pest infestation during storage. Radiation-induced insect disinfestation can be used to prolong the storage time of beans using low doses (0.1–1 kGy) of radiation (Anon, 1991; ICGFI, 1993). This radiation treatment of beans and pulses has been authorised in Pakistan with a maximum dose of 1 kGy (ICGFI Clearance Database; www.iaea.org/icgfi/). The development of detection methods for irradiation treatment of food is useful for administrative control to facilitate international trade and to build consumer confidence. Among a large number of analytical methods developed for detection of irradiated foods during the last decade, the European Committee for Standardization (CEN) has adopted five methods, such as electron spin resonance (ESR) method for foods containing bones or cellulose, thermoluminescence method for foods contaminated with silicate mineral debris and gas chromatography of foods containing fat (Delincée, 1998a). However, these methods require expensive and sophisticated equipment, involve lengthy and laborious sample preparation procedures or can only be applied to specific food items. A rapid and simple test involving inexpensive instrumentation should, therefore, be developed, which can be used as a routine test by food control laboratories.

Most of the plant foods including beans contain DNA. The long double-stranded DNA molecule is a

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sensitive target to ionising radiation, leading to denaturation, base modification and fragmentation either by single- or double-strand breaks. These radiation-induced changes in DNA can be a basis for detection of irradiation in a number of foods (Delincée et al., 1993; Cerda et al., 1997). A sensitive technique to detect DNA fragmentation is the microgel electrophoresis of single cells or nuclei commonly called "Comet Assay" (McKelvey-Martin et al., 1993; Fairbairn et al., 1995; Rojas et al., 1999). The application of this technique to screen irradiated food was first described by Cerda and co-workers (1993). Previously, we carried out a study for the detection of several types of pulses using thermoluminescence (Khan et al., 1998). The aim of the present work is to detect radiation treatment of several types of beans by a fast screening test using DNA comet assay and to optimise the working conditions for this purpose.

2. Materials and methods

2.1. Radiation treatment of beans

Black, black eye, mung, pinto, red kidney, tamarind, azuki and white beans were purchased from local shops in Karlsruhe and stored at room temperature. About 200 g of bean sample was packed into small polyethylene pouches in such a way that a uniform dose of radiation could be administered to them.

The beans were treated with ionising radiation using a 10 MeV electron beam (Circe III linear accelerator, Thomson-CSF Linac St. Aubin, France). GAF DM-1260 (radiochromic film, International Speciality Products, Wayne) was used for the measurement of radiation doses (McLaughlin et al., 1991) by evaluating them at 405 nm for change in optical density with the help of filter photometer (Ciba Corning Halstead, Essex, UK). The radiation doses applied to the beans (0.5, 1 and 5 kGy) covered the range of advisory technological dose limits for insect disinfestation and shelf-life extension according to the guidelines of the International Consultative Group on Food Irradiation (ICGFI, 1993).

2.2. Preparation of single cell suspension

For each bean sample, a small amount (300–900 mg) was crushed finely. Each sample was placed into a small beaker containing about 3–5 ml (or more for viscous suspension) cold phosphate buffer saline and stirred (500 rpm) for 5–7 min on an ice tray. The suspensions were filtered first through 200 μm and then through 100 μm nylon sieves. The beakers containing the filtrate were placed into ice for a selected time period of 15–45 min (see Table 1) for the purpose of sedimentation of undesired material. The supernatant was used as a cell suspension.

2.3. DNA comet assay

The DNA comet assay for these cell suspensions was carried out as previously described (Cerda et al., 1997; Koppen and Cerda, 1997; Khan and Delincée, 1998). Different lysis times were selected for various beans (Table 1), and also the time required for silver staining (20–60 min) varied for the given beans. The microscope slides were evaluated under a standard transmission microscope (e.g. objective $\times 10$ or magnification of 100) for the migration patterns of the DNA. The names of beans and optimised parameters used for DNA assay in the present study are summarised in Table 1.

3. Results and discussion

3.1. Black, black eye, pinto and white beans

The present study on black, black eye, pinto and white beans showed that each kind of irradiated bean sample could be discriminated for radiation treatment at a glance at the microscope slides as compared to unirradiated samples. The unirradiated samples of beans

Table 1
Some optimised parameters of the DNA Comet Assay for beans

No.	Name of food (Botanical name)	Amount used (mg)	Sedimentation time (min)	Lysis time (min)
1	Azuki beans *(Phaseolus angulasis)*	700	30	35
2	Black beans *(Phaseolus vulgaris)*	900	45	30
3	Black eye beans *(Vigna sinensis)*	300	25	30
4	Mung beans *(Phaseolus aureus)*	300	20	25
5	Pinto beans *(Phaseolus vulgaris)*	600	30	25
6	Red kidney beans *(Phaseolus vulgaris)*	800	30	60
7	Tamarind beans *(Tamarindus indica)*	500	15	15
8	White beans *(Phaseolus vulgaris)*	800	25	35

were in the form of round or conical stains of DNA, and of nearly the same size and shape, whereas irradiated samples showed nice comets. Samples irradiated to different doses showed the comets of different shapes and sizes, which were dose dependent. Therefore, a rough estimate about the radiation dose could also be made by analysing the slides. Only in irradiated samples of black beans were the migration patterns different, i.e. showing bigger and wider comets but not much longer in size than the beans irradiated at lower doses. Suitable lysing conditions were found and applied (as summarised in Table 1) so that no false intact cells were found in the irradiated samples. In an earlier study, using a lysing time of only 10 min, it was found that red lentils irradiated to 1 kGy could be detected using comet assay. However, using the same conditions, gram samples irradiated to 1 kGy could not be detected but for samples irradiated to 2 kGy, detection by comet assay was possible (Khan and Delincée, 1998). Longer lysing times now ensured more clear-cut results. Our present results are similar to those obtained for *carioca* var. and *macacar* var. of Brazilian beans irradiated to 1 and 10 kGy where changes in DNA migration pattern could be observed at 1 kGy dose (Villavicencio et al., 1998). In the present study, image analysis was not carried out, however, the shapes and sizes of comets were consistent with the radiation doses.

3.2. Mung beans

In unirradiated samples, large variations in the sizes of the round stained spots were observed and the comets of the irradiated samples also showed similar variations in their sizes for the same dose. Such behaviour could be due to polyploidy, which has been observed already in lentil seeds (Koppen and Cerda, 1997). The unirradiated cells were stained heavily in the form of nice round shapes. The comets for 0.5 and 1 kGy could easily be distinguished on the basis of DNA material in the tails. The tails of the comets were densely loaded with distinct thinly stained narrow neck for 1 kGy; however, in 0.5 kGy, the necks were continued from head to tail. The fact can be illustrated from the photographs of typical comets for the control and 0.5 kGy irradiated samples of mung beans as shown in Fig. 1.

3.3. Red kidney beans

No variations in size or shape of comets were observed for the same doses of radiation. The unirradiated samples contained very distinct and pronounced round intact cells of nearly the same sizes. The samples irradiated to 0.5 kGy could be discriminated form the non-irradiated ones due to different migration patterns of DNA (Fig. 2). The lengths of comets for samples irradiated to 5 kGy were longer than for 1 kGy. However, as compared to the other beans, a relatively longer lysis time of more than 60 min was used for this bean sample. A longer lysis time was needed, since 15–30 min was not enough due to insufficient lysis of cell walls and appearance of false intact cells even in the case of samples irradiated to 5 kGy. Such a longer lysis time has also been used in DNA comet assay of grape-fruits (Delincée, 1998b).

3.4. Azuki beans

The presence of crude debris in the background with stained nuclei was a problem in these bean samples. Although, the debris were interfering with stained DNA, discrimination between black spots of debris and stained cells as intact or comets was still possible and unirradiated and irradiated samples could be distinguished easily with some experience with comet assay. The shapes and sizes of comets were dose dependent and different doses could be recognised. The debris can be minimised or wiped out using relatively longer sedimentation time, but the longer sedimentation will also decrease the amount of cells.

3.5. Tamarind beans

Sufficient amount of DNA material could not be extracted from both the control and irradiated samples of tamarind beans. The irradiated samples showed a few comets (2–3 in number) along with a large amount of

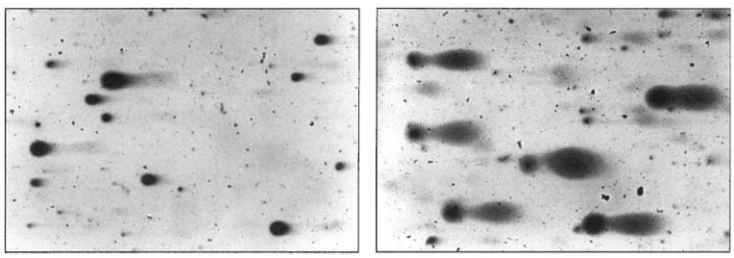

Fig. 1. Typical DNA comets from unirradiated and irradiated mung beans. (Silver staining; anode to the right).

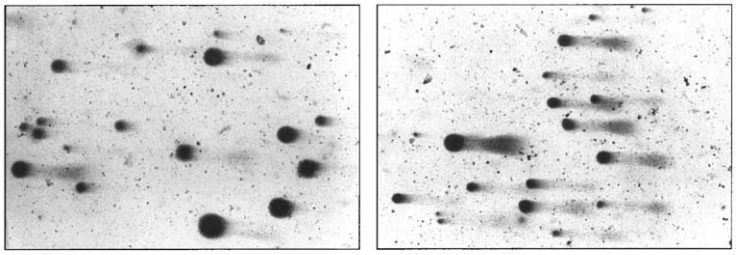

Fig. 2. Typical DNA comets from unirradiated and irradiated red kidney beans. (Silver staining; anode to the right).

interfering crude debris. The unavailability of sufficient DNA precluded the use of comet test for these samples. Further, improved assay conditions as compared to those used in the present study may help in further studies.

It can be concluded that, using suitable experimental conditions, several types of beans irradiated to insect disinfestation dose of 0.5 kGy or higher can be detected for radiation treatment. The DNA comet assay is a relatively simple and rapid screening test that makes use of low-cost equipments. However, the method is not radiation specific and suspicious samples may need to be confirmed by an officially validated method.

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