

## Radiation induced enhancement of protein content in Soybean (*Glycine max L. Merr*)



## Biotechnology

**KEYWORDS :** Electron irradiation, F1 generation, SDS-PAGE, Soybean

**Deepthi Somasekharan**

Research Centre in Botany, Sir Syed College, Taliparamba 670142, India

**Dr. K. M. Khaleel**

Research Centre in Botany, Sir Syed College, Taliparamba 670142, India

### ABSTRACT

*Soybean samples were exposed to various doses of electron beam irradiation (50, 100, 150, 200 and 250Gy). The irradiated and non-irradiated soybean seeds were grown in greenhouse condition. Proteins were extracted from the seeds of F1 generation soybean plants and its protein content was estimated using Lowry's method. Protein profiling of the F1 generation soybean plants of irradiated and non-irradiated seeds was done using SDS-PAGE. The results demonstrated that the total protein in the F1 soybean plants of irradiated seeds increased with the increase in the radiation dosage and was found to be higher than the seeds of non-irradiated F1 soybean plant. Also, the protein profile in F1 seeds of irradiated sample showed significant difference in the band patterns, compared to the non-irradiated. Electron irradiation seems to be a good procedure for improving the nutritional quality of soybean.*

### INTRODUCTION

Soybean have been recognized as a plant food that, when compared with other plants, is relatively high in protein. The seeds are a major source of high- quality protein and oil for human consumption.<sup>3</sup> Protein is the reason that soybeans have historically been called "meat of the field" or "meat without bones". Soybean and its processed products have been acclaimed as health foods due to their high content of protein and essential amino acids, omega-3-fatty acids, fat soluble vitamins, polysaccharides and insoluble fibres.<sup>11</sup> A principal factor that limits the cultivation or increase of yield of soybean is the attack by fungus, bacteria or virus.<sup>6</sup> Irradiation, carried out under conditions of Good Manufacturing Practice, is commended as an effective, widely applicable food processing method judged to be safe on extensive available evidence that can reduce the risk of food poisoning, control food spoilage and extend the shelf-life of foods without detriment to health and with minimal effect on nutritional or sensory quality.<sup>2</sup> Physiological and biochemical processes in plants are significantly affected by radiation stress.<sup>10</sup> High doses of radiations disturb the synthesis of DNA, RNA, protein<sup>8</sup> and enzyme activity.<sup>1</sup> Relatively low doses usually alter growth characteristics whereas, very low doses have been shown to stimulate plant growth.<sup>5</sup> In this context the present study aims to detect the Soybean's behaviour, when treated by electron beam in different radiation doses and the effect of radiation on the protein profile of soybean.

### MATERIALS AND METHODS

#### Sample Collection

Soybean seed samples were collected from Department of pulses, Tamilnadu Agricultural University, Coimbatore.

#### Irradiation Treatments

Electron beam irradiation was carried out at Microtron Centre, Mangalore University (Microtron accelerator designed by Centre for Advanced Technology, India) to absorbed doses of 50, 100, 150, 200 and 250Gy at room temperature. Irradiation was carried out by exposing both sides of the packets for dose uniformity.

#### Germination Studies

Irradiated and non-irradiated soybean seeds were sown separately in petridishes lined with filter paper, soaked in distilled water. Healthy seedlings were selected from petridishes for transplantation and grown in green house condition.

#### Protein Extraction

Protein was isolated from the seeds of F1 generation soybean plants using the following procedure.

The seeds were grinded in the mortar and pestle till they were powdered finely. 0.5 g seed flour and 1.5ml of buffer [0.05 M Tris base 6.5 g/L; 0.007 M citric acid (monohydrate) 1.5 g/L; 0.1% cysteine hydrochloride 1 g/L; 0.1% ascorbic acid 1 g/L; 1.0%

polyethylene glycol (3500) 10.0 g/L; 1 mM 2-mercaptoethanol 0.08 mL/L, the final pH 8.0] were mixed and homogenized for 1 min. Homogenates in the tubes were transferred into eppendorf tubes and centrifuged at room temperature at 18000 rpm for 20 min. Supernatants were transferred into the new eppendorf tubes and used for estimation studies.<sup>9</sup>

#### Estimation

The proteins were determined by Lowry's method.<sup>4</sup>

Suitable aliquots were taken in triplicates from each preparation. Volume was made up to 1ml with double distilled water. Then 5ml of reagent 1 [48ml of reagent A (2.0% Na<sub>2</sub>CO<sub>3</sub> + 0.1N NaOH) +1ml of reagent B (1% KNaC<sub>4</sub>H<sub>4</sub>O<sub>6</sub>·4H<sub>2</sub>O) + 1ml of reagent C (0.5% CuSO<sub>4</sub>·5H<sub>2</sub>O)] was added to each tube, mixed well and kept at room temperature for 10 minutes. After incubation 0.5ml of reagent 2 (1 part 1N Folin- Ciocalteu reagent : 1 part water) was added with immediate mixing. The tubes were incubated for 30 minutes, for colour development. Absorbance was read at 660nm using a Shimadzu UV Visible spectrophotometer and the standard graph was plotted.

#### Extraction of Protein for Protein profiling

Protein profile in the seeds of the F1 generation soybean plants of irradiated and non-irradiated samples was determined by SDS-PAGE (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis).

Protein was isolated from the seeds of F1 generation soybean plants using the following procedure.

The soybean seeds (1g) was homogenised thoroughly in 10ml of 100mM Tris Hcl (pH 7.2) and centrifuged at 10000rpm at 4°C for 15 minutes. The supernatant was collected and to one volume of supernatant nine volume of ice cold ethanol was added. It was then mixed and kept for 30 minutes at -20°C. The supernatant along with ice cold ethanol was then centrifuged for 15 minutes at 4°C at 15000rpm. The pellet was then dried in vacuum to eliminate the ethanol residues. The pellet was then resuspended in minimum volume of sample loading buffer and was used for PAGE studies.

The sample for SDS-PAGE was prepared by mixing the protein samples with sample loading buffer at 1:3 ratios. After mixing, the samples were heated in a boiling water bath for 2 minutes at 90°C. A 30µl of each sample and 10µl of molecular weight marker were loaded in each sample well. Electrophoresis of protein was carried out on 12% gel and the gel ran about 1.5 h with the power supplier set at 100V. The gel was stained by Coomassie brilliant blue (R-250) for 4 h and gel destaining was accomplished by using 150ml/l ethanol and 100ml/l acetic acid solution. The bands from the samples were compared with medium range protein molecular weight marker (Genei) of 97.4, 66, 43, 29, 20.1, and 14.3 KDa.

**RESULTS**

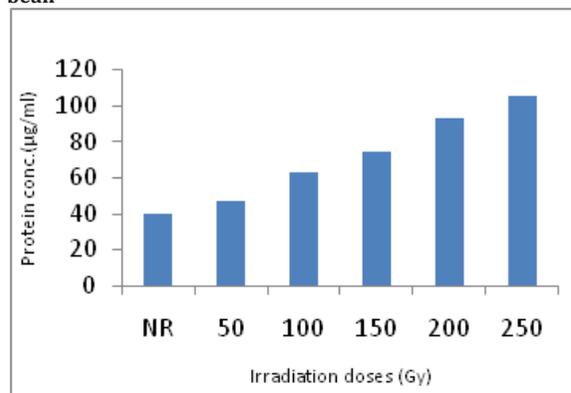
Irradiation with electron beams could produce specific biochemical changes in the soybean. The amount of protein in the F1 seeds of irradiated sample was found to be higher than the non-irradiated sample (Table 1 and Figure 1). The protein concentration increased with the increase in the radiation dosage with a maximum of 106µg/ml in F1 seeds of irradiated soybean sample with 250Gy dose, whereas the non-irradiated samples had only 40µg/ml.

The protein profile in the seeds of F1 generation soybean plants of the irradiated sample showed difference in the band patterns compared to the F1 seeds of non-irradiated sample (Figure 2). Protein in the F1 seeds of irradiated sample showed occurrence of comparatively thick bands when compared to the control and it also exhibited new bands which were absent in the F1 seeds of non-irradiated sample.

**Table 1: Changes in total protein (µg/ml) of soybean after electron irradiation**

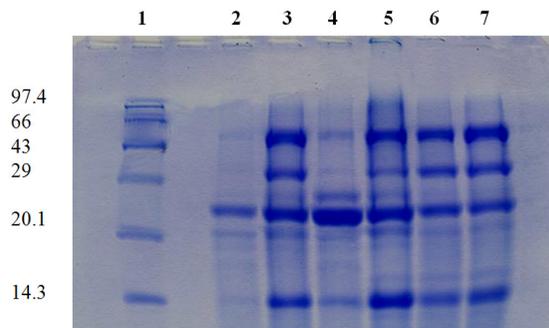
Radiation Dosage(Gray)	Protein Concentration(µg/ml)
Non-irradiated	40
50	47
100	63
150	75
200	93
250	106

**Figure 1: Effect of electron beams on protein content of soybean**



\*NR-Non-irradiated

**Figure 2: Protein profile of F1 soybean seed. Lane 1- Protein marker (14.3-97.4KDa), Lane 2- Protein sample of F1 plant from non-irradiated soybean seeds, Lane 3, 4, 5, 6, 7- Protein samples of F1 plants from irradiated soybean seeds in the order 50, 100,150,200,250Gy.**

**CONCLUSION**

Radiation processing of foods by ionizing radiation such as  $\gamma$  rays, X-rays and electron beams has in recent years assumed considerable importance as a technology to reduce postharvest food losses by increasing shelf life and to eliminate food poisoning microorganisms.<sup>7</sup> The overall goal of this study was to determine the effect of electron irradiation in the protein profile of soybean and our result shows that the irradiation with electron beams could enhance the protein content in Soybean. These findings suggest that electron irradiation had a potential for improving the nutritional quality of Soybean.

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