

Effects of gamma irradiation on chickpea seeds vis-a-vis total seed storage proteins, antioxidant activity and protein profiling

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Abstract

The present work describes radiation-induced effects on seed composition vis-a-vis total seed proteins, antioxidant levels and protein profiling employing two dimensional gel electrophoresis (2D-GE) in kabuli and desi chickpea varieties. Seeds were exposed to the radiation doses of 1,2,3,4 and 5 kGy. The total protein concentrations decreased and antioxidant levels were increased with increasing dose compared to control seed samples. Radiation induced effects were dose dependent to these seed parameters while it showed tolerance to 1 kGy dose. Increase in the dose was complimented with increase in antioxidant levels, like 5 kGy enhanced % scavenging activities in all the seed extracts. Precisely, the investigations reflected that the dose range from 2 to 5 kGy was effective for total seed storage proteins, as depicted quantitatively and qualitative 2D-GE means enhance antioxidant activities in vitro.

Key words: Chickpea, 2D-GE, Gamma-Radiation.

Introduction

Genetic variability is the essence of crop improvement. The main objective of plant breeders is to develop of better quality genotypes producing high yield crops of nutritional value. Crop improvement can be achieved through hybridization and breeding programmes although are time consuming. Induced mutations are one of the promising approaches for broadening the genetic variation in crop plants. Mutation breeding techniques have been used to induce new genetic variations and improve agronomic traits. Mutational breeding is used to get new genetic combinations, without changing major total genetic setup (1). Combinations of physical and chemical mutagens are used for inducing mutation in crop plants. Gamma rays are one of the promising and extensively used combination treatments to activate biochemical parameters of the seed (2).

India is one of the leading nations doing mutational research and producing large number of mutant varieties (2,3). Bhabha Atomic Research Centre (BARC) Mumbai, Indian Agriculture Research Institute (IARI) New Delhi, Tamil Nadu Agricultural University (TNAU) in Coimbatore etc. are some of the pioneer research centres in India strongly committed to mutation breeding for several crops. A number of Indian researchers have contributed improving legumes like pigeon pea (4), winged bean (5), urdbean (6) which includes chickpea also (7).

Chickpea (*Cicer arietinum* L.), belongs to family *Fabaceae*, is an important grain legume crop of the world (8). A perusal of literature indicated that chickpea has received scant attention as regards its genetic improvement through mutation breeding approach. Mutation breeding techniques have made some contribution to chickpea improvement in India. Mutational breeding in chickpea is mainly carried out enhancing the high

yielding potential and nutritional components like total proteins, antioxidants and phytochemical compounds of pharmacological importance (9).

Madhya Pradesh (M.P.); a central part of India is one of the major producers of chickpea and wheat. Popular varieties of chickpea includes desi; BGD-112 (green colored seeds) and ICC-4812 black colored seeds) and a kabuli-Vijay (white colored seeds). Studies aiming to determine radiations effects on these varieties vis-a-vis antioxidant levels, total protein composition and qualitative analysis employing 2D-GE is lacking and predictable to generate some useful information. Therefore this study was performed to investigate the effects of gamma radiation on the seed proteins composition.

Materials and methods

Seed material

The study was carried out with 3 Indian chickpea varieties that are mostly cultivated in Madhya Pradesh region of India which includes two desi (BGD-112 and ICC-4812) and one kabuli (Vijay). BGD-112 is a desi green colored while ICC-4812 is a desi black colored seed genotype. Vijay is white bold seeded variety resistant to soil borne diseases having good cooking quality. BGD-112 is resistance to and drought *Fusarium* wilt. Seeds are dark green, uniform and excellent for cooking. The phenotypic seed qualities are represented in the Figure 1. To avoid heterogenicity, the seeds of chickpea varieties were collected from R. A. K. Collage of Agriculture, Sehore, M.P. India.

Irradiation of chickpea seeds

Collected seeds (250 g) were stored in polythene bags for irradiation following the method as described



Figure 1. Chickpea a) Vijay-Kabuli (White) b) ICC-4812 (Black) c) BGD-112 (Green).

(10). The seeds were exposed to a ^{60}Co gamma source for irradiation at 25°C at the cancer hospital and research institute, Gwalior (M. P.), India using exposure doses in the range of 1-5 kGy (0.12 kGy/h). The radiation doses were measured with a Fricke Dosimeter (11). All chemicals used for the experiments were procured of analytical grade and highest purity available.

DPPH radical scavenging assay

Scavenging activity on DPPH free radicals by the extracts was assessed according to the method reported by (12) with slight modifications (13). Briefly, a 2.0 ml solution of the extract, at different concentrations diluted twofold (2–125 µg/ml) in methanol, was mixed with 1.0 ml of 0.3 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 min. Blank solutions were prepared with each test sample solution (2.0 ml) and 1.0 ml of methanol while the negative control was 1.0 ml of 0.3 mM DPPH solution plus 2.0 ml of methanol. L-Ascorbic acid was used as the positive control. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with Systronics 2203 UV–vis spectrophotometer. Lower absorbance of the reaction mixture indicated higher radical-scavenging activity.

DPPH radical-scavenging activity was calculated using the equation; $\text{DPPH \%} = (\text{A blank} - \text{A sample}) / \text{A blank} \times 100$.

2D-GE analysis

Total protein extract preparation

Total seed storage proteins were extracted using the procedure (14). The defatted chickpea flour was extracted with a solution consisting of 7 M urea, 2M thiourea, 2% CHAPS, 65 mM 1, 4-dithiothreitol (DTT) in a ratio of 1/30 (w/v) under stirring at room temperature for 2 h. The slurry was centrifuged at 10,000 g for 30 min at 4°C and the extracted proteins in the supernatant were analyzed immediately.

The extracted proteins were subjected for 2D-GE analysis as per the standard procedure (15). The isoe-

lectric focusing (IEF) was performed using 7 cm; pH 3-10 gradient IPG strips (Bio-Rad, USA). The strips were rehydrated overnight in a solution containing of 7 M urea, 2% w/v CHAPS, 15 mM DTT and 0.5% v/v IPG buffer pH 3-10 (Bio-Rad, USA) containing the protein sample. For the first dimension, 300 µg of protein sample was loaded. These amounts were optimized for the best electrophoretic performance. After 16 h of passive rehydration at 20°C, isoelectric focusing was performed and strips were focused initially at 250 V for 3 h till 8000 volt hours under mineral oil. Strips of IPG were equilibrated for total 25 min prior to SDS-PAGE. After the first dimension, strips were equilibrated for 15 min in the equilibration buffer-I (50 mM Tris-HCl buffer, pH 8.8 containing 6 M urea, 30% w/v glycerol, 2% SDS and 1% DTT) and then for 10 min in the equilibration buffer-II (equilibration buffer I containing 4% w/v iodoacetamide instead of DTT). After equilibration, strips were transferred to 12% SDS-PAGE for second-dimension separation at a constant voltage of 200V for 3 h. Following electrophoresis, 2D-gels were visualized by staining with colloidal coomassie blue G-250. Protein spots were visualized under white light in a UV trans-illuminator at 280 nm.

Statistical Analysis

All work was done in triplicates and the data presented are means \pm S.D. of three independent determinations. Significance was accepted at $p \leq 0.05$.

Results

The effect of gamma radiations on chickpea seed types caused conspicuous decrease in total protein contents and increase in antioxidant activity. The total protein content was found depleted by gamma irradiation in all the irradiated chickpea seeds as shown in the Table 1. The pattern of change in protein content, as a function of ionizing radiation, is different for different seed types compared to their respective controls. Maximum loss (24%) of soluble protein in kabuli seeds was registered when irradiated at 5 kGy. The loss of soluble protein was observed to be dose-dependent and exhibited negative correlation. Black seed variety ICC-4812, showed maximum (0.2%) loss of protein when irradiated at 1 kGy and exhibited maximum loss (23%) at 5 kGy dose of gamma irradiation. BGD-112, a green genotype, exhibited maximum (17%) protein loss compared to control seeds at 5 kGy. Hence a dose assortment of 1 kGy to 5 kGy resulted loss of total protein

Table 1. Effects of gamma radiation on total seed Protein contents and free radical scavenging activities of chickpea seeds.

Variety	Vijay- Kabuli (White)		ICC-4812 desi (Black)		BGD-112 desi (Green)	
Dose (kGy)	Total seed protein	DPPH	Total seed protein	DPPH	Total seed protein	DPPH
Control	5.34 \pm 0.01	21.8 \pm 0.05	5.47 \pm 0.01	35.1 \pm 0.05	5.56 \pm 0.01	30.4 \pm 0.03
1kGy	5.10 \pm 0.01	21.2 \pm 0.01	5.45 \pm 0.02	35.1 \pm 0.03	5.52 \pm 0.01	30.5 \pm 0.03
2kGy	4.25 \pm 0.01*	21.2 \pm 0.03	4.70 \pm 0.02*	36.1 \pm 0.05	5.05 \pm 0.03	32.4 \pm 0.20*
3kGy	4.22 \pm 0.02*	20.7 \pm 0.02*	4.53 \pm 0.02*	38.1 \pm 0.03*	4.87 \pm 0.05	34.5 \pm 0.03*
4kGy	4.19 \pm 0.01*	19.8 \pm 0.05*	4.25 \pm 0.03*	39.2 \pm 0.05*	4.62 \pm 0.09*	35.8 \pm 0.10*
5kGy	4.10 \pm 0.01*	18.1 \pm 0.04*	4.21 \pm 0.01*	40.2 \pm 0.08*	4.60 \pm 0.02*	39.8 \pm 0.06*

Values are mean \pm SE; *P<0.05 compared to control.

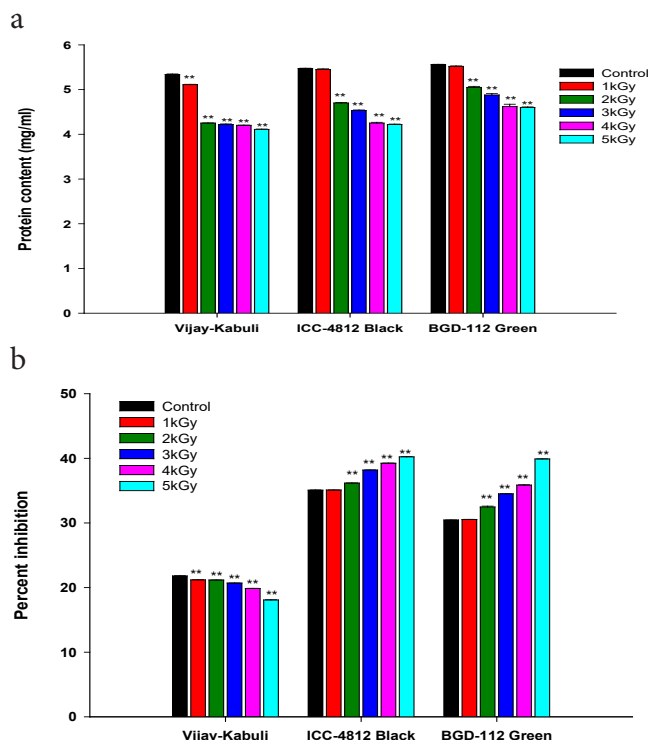


Figure 2. (a) Total protein content (mg/ml) expressed as mean \pm SE (n=3). (b) DPPH assay (% inhibition) expressed as mean \pm SE (n=3).

contents in a range of 0.2 % - 24 % for all the three seed types studied.

The DPPH radical scavenging assay was performed in normal and irradiated seed extracts. The radical-scavenging activities of all the seed extracts showed compound trend with increasing doses of gamma irradiation and revealed dose dependent inhibition (Table 1). The highest antioxidant capacity displayed by BGD-112 (39.8%) while lowest (18.1%) was observed in kabuli variety as compared to control. Interestingly dose of 5 kGy resulted maximum scavenging of the formed DPPH radicals and thereby decreased a color intensity showing antioxidant potentials of chickpea seed extracts.

Qualitative assays of the protein profiles employing 2D-GE was performed in normal and a gamma irradiated green seeds of BGD-112 (figure 3 and 4). The proteins were fractionated using 7 cm IEF strip focused in a range of 3-10 pH which were further separated based on mass using SDS-PAGE. Few of the spots depicted be differentially expressed as a result of $-NH_2$ sensitive

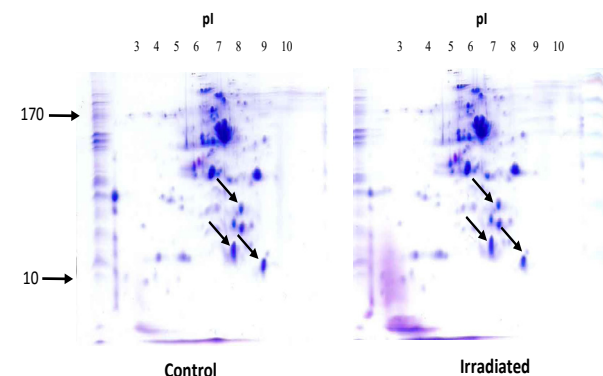


Figure 3. 2D-gel fingerprint of BGD-112 variety (Control and irradiated) using 3.0- 10.0 IPG strips.

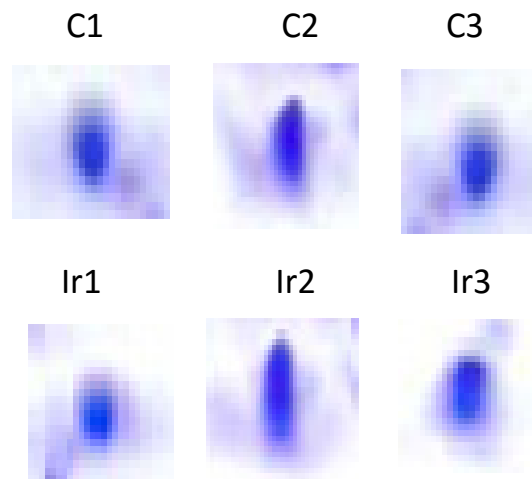


Figure 4. A close-up view showing differentially expressed protein spots in control (C) and irradiated (I) chickpea.

doses of irradiation. The closes up views of such spots are shown in the figure 4 to mark the effects of gamma irradiations on seed proteins.

Discussion

In India chickpea is consumed as a vegetarian pulse fulfilling a major requirements of protein. Crop improvement through breeding/induced mutagenesis is the major concern for the developing countries like India. However, the extent of production compounding nutritional evenness has failed to keep the pace with demands of ever-increasing populations (16). Madhya Pradesh (M.P.) is the centrally located of India, known for wheat and chickpea productions. The variety improvement through radiation needs basic studies to distinguish performance of affect variety for planning future breeding strategies. Therefore, the positive and negative consequences of gamma intensities on three popular varieties of M.P. were evaluated in this paper. Ionizing radiations have been proved effective improving in overall nutritional attributes (17). A joint expert committee convened by the FAO/IAEA/WHO sated that irradiation of any food commodity up to 10 kGy presents no toxicological hazard (18, 19). Hence a dose assortment of 1 kGy to 5 kGy was employed on these varieties to ascertain the effects of gamma radiations vis-à-vis total protein contents and antioxidant activities coupled to 2D-GE protein profiling.

Total protein constitutes the major part of the seeds. Gamma irradiation resulted decrease in total protein with increasing dose concentration. In present study irradiations altered the total seed proteins quantitatively at all the doses except for 1 kGy. The total protein composition on irradiation showed a dose-dependent decrease may be due to higher dose of irradiation and this was not in line with the studies in other legumes like pigeon pea (20). This may be due to depolymerization and denaturation of amino acids. Such decrease in protein levels may on the other hand, influence some of the physicochemical properties of the seed flour proteins like water absorption capacity, protein solubility and foaming properties which are of nutritional importance. Some researchers have shown that the higher exposures of gamma radiation were usually inhibitory by reduction of mitotic activity, whereas low doses could bring

variations of agronomic importance. Our results are in agreement with findings of previous researchers who reported that total protein contents and seed germination of different crops decreased by increasing irradiation doses (21).

The total protein content depleted by gamma irradiation in all the seeds revealed negative correlation. Irradiation at 1 kGy in desi seeds induced minimal 0.2% loss compared to kabuli seed types which showed 5% of loss. The chickpea seeds demonstrated more radio-sensitive nature in terms of degradation of protein contents at high doses of gamma irradiation. Such findings were also observed by Maity *et al.* (22). Dose dependent effects in cowpea flours were observed by Joseph *et al.* (9) who reported that protein-related functional properties are not affected at low dose irradiation (2 kGy) however, higher doses (10 and 50 kGy) affected significantly.

In present investigation, gamma radiation enhanced free radicals scavenging activities in the chickpea cultivars that are studied. Progressive increase and thus positive correlation was depicted in desi seed types as opposed to kabuli. Free radical scavenging in kabuli seed types exhibited lower activities to 5 kGy dose of irradiation as compared to desi varieties. Our results are in line to the results of Marathe *et al.* (23) for DPPH antioxidant activity of legumes where they observed low activities in control chickpea seeds. Thus we strongly suggest that the dose concentration of about 5 kGy is significant for enhancing the antioxidant activity potential of chickpea. However, this needs to be further investigated using large number of chickpea varieties. Irradiation induces the unfolding denaturation of proteins, thus exposing non-polar groups that were previously locked. Earlier studies reported that gamma irradiation induces oxidative stress with overproduction of free radicals such as superoxide radicals, hydroxyl radicals and hydrogen peroxides, which reacts rapidly with proteins, lipids and nucleic acids causing disturbance of cellular metabolism (24).

The consequence effects of gamma radiation were also reflected in the 2D-GE fingerprint. This needs further characterization using more sophisticated techniques of mass spectroscopy to identify the differentially expressed proteins of nutraceutical importance. Hence a dose concentration of 5 kGy produced better antioxidant potentials of chickpea in desi compared to kabuli seeds and correspondingly reduced the total protein compositions. Further, projections of this study is observe heritable nature of these biochemical parameters by developing mutant progenies. In conclusion, green BGD-112 variety exhibited to gamma radiation relatively high tolerance is possibly, linked to its ability to protect its biochemical machinery, and may be because of desi seed nature and can be used as one of the parents for chickpea mutational breeding programmes.

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