Mutagenic efficiency of gamma rays on *in-vitro* regeneration in Indian mustard (*Brassica juncea*)

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ABSTRACT

In the study Indian mustard [Brassica juncea (L.) Czern. & Coss.] was treated with different doses (0, 2.5, 10, 20 and 100 Gy) of gamma rays (radioactive cobalt, 60Co) and the effects were investigated on callus initiation, seed germination, seedling growth, total chlorophyll, protein and proline contents of seedling leaves, which were grown from hypocotyl explant of non-irradiated and irradiated seeds. Experiment was conducted at School of Biotechnology, Gautam Buddha University during 2017–18. The results showed that irradiated seeds had increased seed germination per cent, seedling length, fresh and dry weight, total chlorophyll, protein and proline content in low dose exposure (2.5Gy). Callus cultures were established from hypocotyl explants of 15 days old seedlings obtained from irradiated and non-irradiated seeds of B. juncea on MS1 [MS+BAP (2 mg/ml) + 2,4 D (1 mg/ml)] and MS2 [MS+BAP (2 mg/ml) + 2,4 D (0.5 mg/ml)] medium. Cultured hypocotyls formed callus at their cut ends within a week and simultaneously differentiation started into nodular structures from their base. MS1 callus induction medium gave good callus induction percentage (58%) as compared to MS2. It was observed that the callus was green, compact and showed 83% induction frequency in 100Gy gamma dose in MS1, whereas in different dose treatment (2.5–20Gy), callus was white and friable. It turned light brown in 100Gy treatment in MS2 medium. Thus, results of the present study showed pre-sowing seed irradiation is an effective method of improving seed germination at the early developmental stage of the plant.

Keywords: Brassica juncea, Callus initiation, Gamma radiations, Hypocotyl explants, Seed germination

Brassica is oilseed crops sown over 11 million hectares of the world's agricultural land, under a variety of climatic conditions, include species *Brassica napus*, *B. rapa* and *B. juncea*. *Brassica juncea* (L.) Czern. & Coss. is widely grown in Indian subcontinent, and is used as a condiment, edible oil, lubricant, and its defatted cakes are used as a cattle feed and fertilizer. *Brassica juncea* breeders are indulged for improvement of agronomic performance, disease resistance and quality traits. For plant improvement, either its conventional or nonconventional breeding is done. Nonconventional genetic improvement includes mutation induction tissue culture and molecular genetics. Mutations have been accepted as a useful tool in plant-breeding programs in various crop plants (Tiliouine *et al.* 2018, Gupta 2019, Lal *et al.* 2020).

In nonconventional breeding, Gamma rays as an ionizing radiation affect plant growth and development by inducing cytological, biochemical, physiological and morphological changes in cells and tissues. The higher doses of gamma radiation were reported to be inhibitory whereas lower

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doses may be stimulatory. Breeding programme generated better outcome when both tissue culture and mutagenesis treatment are combined, to produce economically beneficial traits (Turkan *et al.* 2006). The study depicts the mutagenic effectiveness and efficiency of different doses of gamma rays on *in vitro* biotechnology technique (tissue culture) mainly used in improving *Brassica juncea*.

MATERIALS AND METHODS

Sample collection and in vitro gamma radiation: The healthy, dry and uniform sized seeds of *Brassica juncea* (var. Varuna) were obtained from Department of Genetics and Plant Breeding, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttrakhand. For in vitro studies, seeds were irradiated with gamma radiation of absorbed doses 0Gy (control), 2.5Gy, 10Gy, 20Gy, and 100Gy. The source of gamma radiation was Cobalt-60 gamma chamber; with a dose rate 2.08 Kilo Gray per hour (2.08 KGy/hr) at Radiological Safety office, Nuclear Research Laboratory (NRL), Indian Agriculture Research Institute, New Delhi. Experiment was conducted in the plant tissue culture laboratory, School of Biotechnology, Gautam Buddha University in the year 2017–18. The seeds (non-irradiated and irradiated with all doses) were surface sterilized for 5 min with aqueous solution of 0.1% HgCl₂ and few drops of Tween-20 for 10 min and rinsed repeatedly

(3-4 times) with sterile distilled water. Seeds were dried on blotting paper and cultured for germination. For *in vitro* germination, about 30 sterilized seeds were transferred aseptically to phyta jars/ Magenta vessels (12 cm \times 12 cm) contained an autoclaved 50 ml basal medium of Murashige and Skoog's (MS) mineral salts and vitamins (Murashige and Skoog 1962), 3% sucrose, and 0.7% agar. The *p*H of the medium was adjusted to 5.8 prior to autoclaving. Vessels were incubated at 26 ± 2 °C under photoperiod of 16 h light and 8 h dark and after 5 days, calculated the germination percentage of both non-irradiated and irradiated with all doses. Germination percentage (GP) was calculated by using the following formula: GP = Number of seeds germinated \times 100/Total number of seeds inoculated on MS medium.

After 15 days of seed germination in *in vitro* condition, seedling length was measured in centimeters. For biomass of the seedling, the samples were oven dried separately at 80°C for 48 h and dry weight was determined on a digital balance. Percent dry weight of samples was calculated by using following formula: Dry weight = Dry weight ×100/Fresh weight

Callus formation: For callus induction, hypocotyls were used as an explant from the in vitro grown seedlings source. About 1–2 cm long pieces of hypocotyls of 15 days old mustard (B. juncea) seedlings were cut with the help of sterile blades. Hypocotyls (6-7 pieces) were transferred to each of the petri plate containing MS medium supplemented with different combinations of hormones 2, 4 D (1 mg/ml and 0.5 mg/ml) and BAP (2 mg/ml) for 30 days (Table 1). Effects of different doses of gamma irradiation on callus induction percentage (%) and morphogenic response of callus were studied after 30 days of inoculation. For biochemical analysis, we selected 15 days old seedling leaves. Total amount of chlorophyll in non-irradiated and irradiated treatments were determined as suggested by Arnon (1949). The total chlorophyll content was calculated by following formulae:

Total Chlorophyll (mg/ g fwt.) = $[20.2 (A645) + 8.02 (A663)] \times V/1000 \times W$

The total soluble protein and proline contents were estimated as suggested by Bradford (1996) and Bates et al. (1973) methods, respectively. The soluble protein concentrations were quantified with the help of standard curve prepared from the standard of bovine albumin serum (BSA) and its content was expressed in mg/g fresh weight (fwt.) The corresponding proline concentration was

determined against the standard curve processed in the same manner using L-proline (sigma). The proline amount was expressed as mg/g fwt.

RESULTS AND DISCUSSION

The results for radiation treatment are based on germination percentage carried out under in vitro conditions. It demonstrated significant reduction in the germination percentage with increased absorption of the gamma radiation doses (Fig 1A). On an average, the mean germination percentage was greater in 2.5Gy (83%) when compared to control sample and lowest in 100Gy (45%). The study was conducted on the seedling length, fresh and dry weight of seedling increased in 2.5Gy treated dose when compared to control sample and thereafter showed a significant decrease with increase in absorbed 100 Gy doses. In order to evaluate callus induction ability, hypocotyl segments were cultured on MS medium supplemented with different plant growth regulator combinations. The cultured hypocotyls formed callus at the cut ends within a week and simultaneously started differentiating into nodular structures. We observed that MS1 callus induction medium [MS+BAP (2 mg/ml) + 2, 4 D (1 mg/ml)] gave good callus induction percentage (58%) in control when compared to MS2 callus induction medium [MS+BAP (2 mg/ml) + 2, 4 D (0.5 mg/ml)] (Munir et al. 2008, Ramakrishna et al. 2018) (Table 1). Results of the study showed that frequency and growth of callus initiation increased with the increasing doses of gamma radiation when compared to control. Callus was green, compact and showed 83% induction frequency in 100Gy treatment in MS1 callus induction medium. In different treatment (2.5-20Gy), callus showed white and friable morphological character, which turned light brown in 100Gy treatment in MS2 medium (Fig 1B-C). Total chlorophyll and protein contents of the seedling leaves enhanced significantly (P<0.05) in 2.5Gy doses of gamma radiation if compared with control under in vitro, after that they decline steadily with the increasing doses of gamma radiations. Proline content in in vitro conditions showed a significant (P<0.05) increase with increasing doses of gamma radiation when compared to control (non-irradiated) (Fig 2).

The radiation sensitivity test is a prerequisite step before the starting of the research on mutagenic treatments. The purpose of radiation sensitivity test is to investigate the most effective dosage of irradiation to be used and to estimate the frequency and mutation spectrum using gamma irradiation. The results in the present study for radiation

Table 1 Effect of different doses of gamma radiation on callus initiation of hypocotyl explants of B. juncea after 30 days of inoculation

MS media supplemented with BAP and 2, 4D	Callus Induction (Hypocotyl) and morphological features	0 (control)	2.5 Gy	10 Gy	20 Gy	100 Gy
MS1	Callus fresh wt. (%)	58	55	60	66	83
[MS+BAP (2 mg/l)+2, 4D (1 mg/l)]	Color	white	green	white	white	Whitish green
MS2 [MS+BAP (2 mg/l)+2, 4D	Callus fresh wt. (%)	40	38	46	57	63
(0.5 mg/l)	Color	white	white	Off white	Off white	Light brown

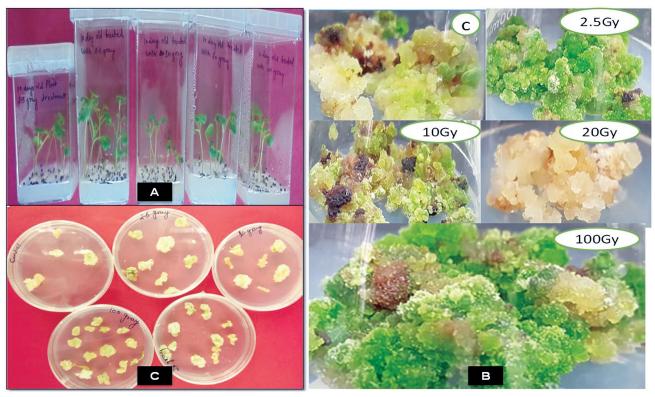


Fig. 1 Effect of different doses of gamma radiation: (A) *In vitro* seed germination and seedling growth of *B. juncea* (L.) seeds at the 10th day; (B) Callus initiation of hypocotyl explants after 30 days of inoculation in MS1 medium [MS+BAP (2 mg/l)+2, 4D (1mg/l)]; (C) MS2 medium [MS+BAP (2mg/l)+2, 4D (0.5mg/l)].

sensitivity test based on germination percentage of irradiated and non-irradiated seeds demonstrated that significant reduction in germination percentage was observed with increasing gamma dosage under *in vitro* conditions. These results are in accordance with the radiation sensitivity test done by (Norfadzrin *et al.* 2007) whereby increasing gamma dosages also decrease the germination percentage of tomato and okra. The seed germination inhibit at higher doses of radiation may have resulted from damage to chromosomes

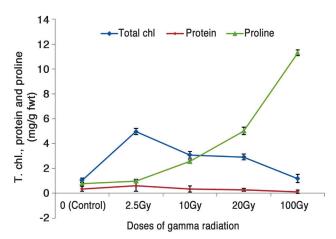


Fig 2 Variation in total chlorophyll (mg/g fwt), soluble protein (mg/g fwt) and proline content (mg/g fwt) of 15 days old seedling leaves of *B. juncea* under *in vitro* conditions treated with different doses of Gamma radiation.

and subsequent mitotic retardation (Kiong *et al.* 2008). A decreasing trend in plant biomass with increasing gamma dose exposure suggests that there was radiation impact on the physiological symptoms and carbon reduction, supported by earlier work (Jia and Li 2008).-

Gamma irradiation may prohibit the auxin and cytokinin activities that may change the morphogenetic responses (Hasbullah et al. 2012, Taheri et al. 2014). It was observed that there is a significant increase in the fresh weight of irradiated callus when compared to the control and inferences that callus growth responses were strongly influenced by the radiation dose. The fresh weight of callus had increased up to 83% compared to 58% of control in MS1 (MS+BAP [2mg/ml] + 2, 4 D [1 mg/ml]) (Table 1) when callus tissues were exposed upto 100 Gy gamma irradiation. When gamma irradiation doses increased, the color of the callus became dark greenish increase proliferation of callus tissues and their structure was more fragile in comparison to the control. Though, effect on the callus tissue varies when exposed to the irradiation doses. Good growth of callus tissue on high irradiation dose presumed that it may also influence the synthesis of endogenous hormones auxin, which altered the intake capacity of water by plant cell, and responsible for change in the cell size. Chlorophyll content in the present study showed a dosage dependent significant increases under in vitro conditions which are in accordance with the results of earlier work (Alikamanoglu et al. 2007).

In this study, it was found that there was an irregular distribution of total soluble protein content in irradiated plantlets. According to the results obtained in the present study, it was observed that it increases up to absorbed doses 2.5 Gy after that it showed a linear decrease with increasing doses of gamma radiation. These results are in accordance with Cho and Song (2000) who observed that gamma irradiation induce significant loss in total soluble proteins. During gamma irradiation of tomatoes, protein synthesis was not stopped but produced a form different set of proteins called as gamma induced proteins. The function of these is not yet known, but they may be involved in physiological disorders triggered by irradiation during the repair process. Present study showed that, proline content significantly increases with increasing doses of gamma radiation which are in accordance with the findings of Esfandiari et al. (2007). Gamma irradiation leads to modulation of certain metabolic, defensive pathways and promotes the level of antioxidants. One of the protective mechanisms is the synthesis of osmolytes which is essential to plant growth in proline synthesis. Gamma radiation at higher doses induce oxidative stress with overproduction of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide, which react rapidly with almost all structural and functional organic molecules, including proteins, lipids and nucleic acids causing disturbance of cellular metabolism.

Productivity of the crop plant and their economic gains could be enhanced through adoption of suitable cultivar. Findings of the present study provide sufficient evidence to the effect that Γ -irradiation activates a biochemical system, which alter the metabolic and defensive pathways of plant under stress condition. An optimum dose of ionizing radiations encompasses modulator position in the growth and developmental processes. In our study, 2.5Gy gamma radiation gave best germination and also facilitate raise of healthiest and well grown seedlings. The absorbed doses of 100Gy can prove detrimental for early development stage of plant growth, however under in vitro conditions; results hold 100Gy as threshold dose for increasing callusing. We finally concluded that gamma rays prove to be an important tool in increasing the breeding efficiency, and regeneration frequency in Brassica juncea crop plants.

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