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RESEARCH ARTICLE

Effect of photon irradiation on seed propagation and seedling growth of Radish and Spinach

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Abstract

An investigation was conducted to assess the impact of different doses (Control, 01 Gy, 05 Gy, and 10 Gy) of photon radiation from a 6 MV Linear Accelerator (LINAC) on the germination of Radish and Spinach seeds and subsequent seedling development in controlled conditions. The results revealed that irradiated seeds displayed improved germination rates, accompanied by increased lengths of seedlings and leaves, as well as higher fresh and dry weights in the leaves of seedlings. However, elevated radiation doses induced stress, leading to significant reductions in all measured parameters.

The highest seed germination percentage, at 65.6%, was observed twenty days after the commencement of the study in seeds exposed to 01 Gy photon radiation, followed closely by those exposed to 05 Gy radiation. At forty days into the experiment, the highest initiation of shoot growth, at 75.9%, was again noted with 10 Gy photon radiation, closely followed by 05 Gy. Radiation doses exceeding 10 Gy resulted in marked declines across all parameters. By day sixty, the largest leaf sizes were recorded from seeds exposed to 10 Gy photon radiation, measuring 1.9 cm and 3.8 cm, respectively.

At the end of sixty days, the most favorable outcomes in terms of seedling and leaf lengths, fresh weight, and dry matter content were observed with 01 Gy photon radiation, with measurements of 9.6 cm, 6.7 cm, 0.39 g, 0.09 g, and 471.6 mg/g fresh tissue, respectively. This study highlights the importance of acknowledging that the stimulating effects of low doses of photon radiation on germination and seedling growth may not be consistent.

Keywords: Photon irradiation, Seed propagation, Seedling growth, Radish, Spinach

Introduction

Photon, acting as ionizing radiation, influences the growth and development of plants by initiating various changes at the cellular, biochemical, physiological, and morphological levels. These alterations occur through the creation of free radicals (Gunckel et al., 1967; Kim et al., 2004; Wi et al., 2005). Higher doses of photon radiation have been observed to have inhibitory effects, while lower doses may exhibit stimulatory effects. Research indicates that low doses of photon rays can boost cell proliferation, propagation, cell growth, enzyme activity, stress resistance, and crop yields.

Using aseptic seedlings as explant sources is strongly recommended in tissue culture investigations. The genus Lathyrus, which belongs to the Fabaceae family, encompasses 187 taxa and is distributed across the Mediterranean region, Asia Minor, East Africa, and North and South America. *Lathyrus chrysanthus* is being evaluated as an ornamental plant due to its large, attractive, colorful, and fragrant flowers. However, achieving a high frequency of healthy seedlings suitable for use as explant sources for further studies, such as shoot regeneration and transformation, is challenging due to low in vitro seed propagation rates caused by dormancy in Radish and Spinach.

Various methods, including scarification of the seed coat, temperature and light treatments, growth regulators, and chemicals, have been widely employed to overcome seed dormancy. Studies have demonstrated that sodium hypochlorite solutions can effectively break dormancy in Radish and Spinach seeds. This study aimed to evaluate the impact of different doses of photon radiation on seed propagation, seedling growth of Radish and Spinach seeds, and dormancy breaking under in vitro conditions.

Materials and Methods

Plant material, seed irradiation, and propagation

Seeds of Radish and Spinach sourced from Banaras Hindu University in India were utilized for the experiment. These seeds were exposed to varying doses (control, 01 Gy, 05 Gy, and 10 Gy) of Varian Unique 6MV radiation, with a source-to-surface distance of 100 cm, at different dose rates at Banaras Hindu University, Varanasi. Each photon dose was administered to two sets of 50 seeds independently to facilitate parallel experiments as depicted in fig. 1. Fricke and alanine dosimeters were employed to map the doses and determine the photon dose rates accurately. To ensure consistent ionization, seeds were irradiated alongside a dosimeter for each dose. Prior to propagation, seeds were surface-sterilized using a 3.75% sodium hypochlorite solution at 35°C for 15 minutes, following the method outlined in reference, to obtain healthy, uninfected seedlings under controlled conditions (Telci et al., 2011).

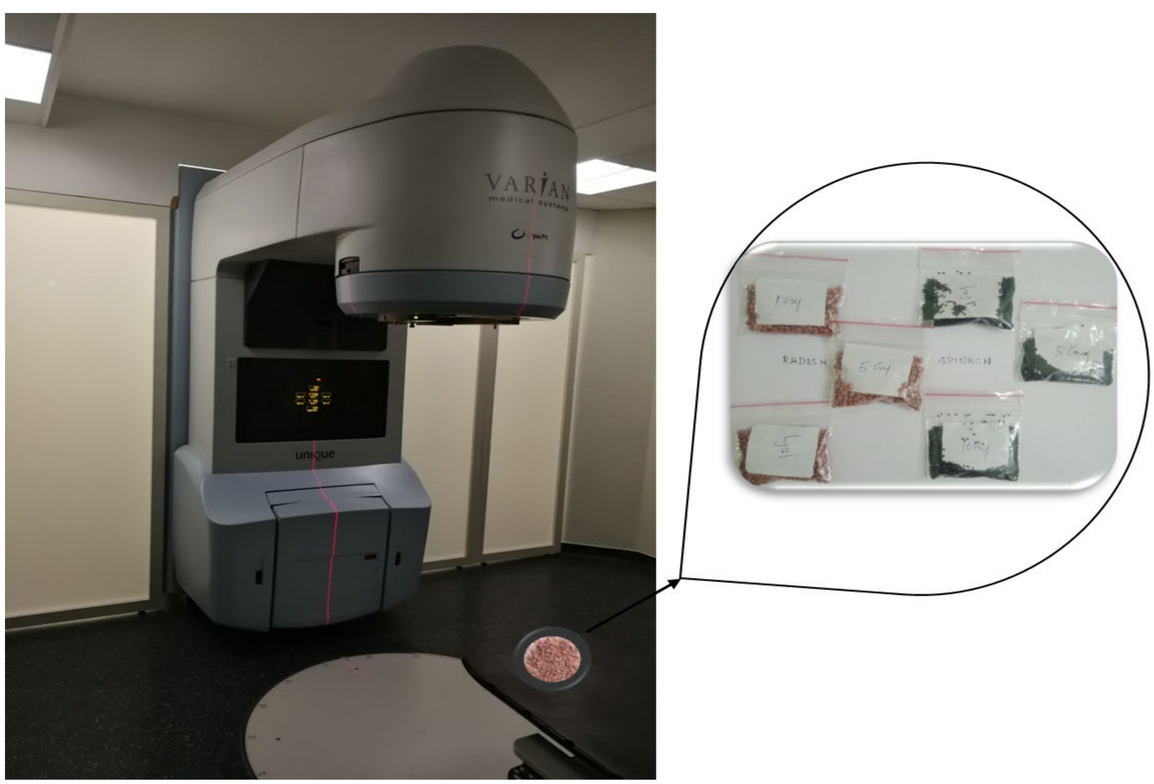

Figure 1. Shows that the experimental setup of seed irradiation

Thirty sterilized seeds were evenly distributed into three replicates, each placed between filter papers in Petri dishes containing 6 mL of distilled water. These Petri dishes were subsequently incubated in darkness at a temperature of 15°C ± 1°C for a period of 20 days to facilitate seed propagation. After 40 days from the commencement of the experiment, the germinated seeds were transferred to Magenta vessels filled with an autoclaved basal medium composed of Murashige and Skoog's (MS) mineral salts and vitamins, 3% sucrose, and 0.7% agar. The pH of the medium was adjusted to 5.8 prior to autoclaving. Subsequently, all cultures were relocated to a growth chamber set at 25°C ± 1°C, illuminated with cool white fluorescent light at an intensity of 27 µmol m^{-2} s^{-1}, and maintained under a photoperiod of 16 hours light and 8 hours dark.

Observations

Each photon dose was evaluated with three replicates, each containing 30 seeds. To ensure the accuracy of the study, all experiments were conducted in duplicate simultaneously, resulting in two parallel experiments. Each of these parallel experiments comprised 3 replicates of 30 seeds. Seed propagation percentages was determined at the conclusion of the 20th day, while the initiation of shoot growth, as well as shoot and leaf lengths, were measured 40 days after the commencement of the culture, adhering to the guidelines outlined in ISTA 2003. On the 60th day of the study, measurements were taken for seedling and leaf lengths, seedling fresh weight, seedling dry matter content, and total chlorophyll content in the seedling leaves. A seed was deemed germinated once the emerged radicle reached a length of 2 mm.

Results and Discussion

The impact of various photon doses on seed propagation, shoot growth initiation percentages, shoot and leaf lengths, seedling fresh weight, seedling dry matter content, and total chlorophyll content in the leaves of 60-day-old seedlings (Fig. 2).

(A)

(B)

Figure 2. **The seed propagation and seedling growth of A) Spinach and B) Radish at 20th day**

Our study corroborated the previously reported stimulatory effects of low doses of photon radiation, particularly evident at the 10 Gy photon irradiation level. At this dosage, optimal outcomes were observed for seed propagation percentage on the 20th day, as well as shoot growth initiation percentage, shoot, and leaf lengths on the 40th day. This is consistent with findings from previous studies (Charbaji and Nabulsi, 1999; Kim et al., 2000, 2005; Chakravarty and Sen, 2001; Baek et al., 2005). However, doses exceeding 10 Gy demonstrated inhibitory effects on seed propagation percentage, consistent with prior research (Abdel et al., 1999). Seed propagation percentage steadily increased up to 10 Gy by the end of the 20th day, with the highest recorded percentage (65.6%) observed at this dose. The stimulatory effect of low-dose photon irradiation on seed propagation may be attributed to the activation of RNA or protein synthesis (Fig. 3) (Chaomei et al., 1993).

Figure 3. **The seed propagation and seedling growth of radish at 40th day**

On the 40th day of the study, similar trends were observed for shoot growth initiation percentage, shoot, and leaf lengths, as depicted in fig. 4. Once again, the highest values were recorded at the 10 Gy photon dose, with shoot growth initiation percentage, shoot, and leaf lengths reaching 75.9%, 1.9 cm, and 3.8 cm, respectively. Notably, leaf length increased significantly from 1.9 cm in the control (0 Gy) to 3.1 cm at 10 Gy, consistent with previous findings. However, as photon doses exceeded 10 Gy, all parameters decreased significantly, in line with reports indicating that higher doses of photon irradiation reduce seed propagation and plant growth (Xiuzher et al., 1994).

Figure 4. The seed propagation and seedling growth of Radish at 60th day

By the 60th day of the study, the most favourable outcomes were observed from the 5 Gy treatment across parameters such as seedling and leaf lengths, seedling fresh weight, seedling dry matter content, and total chlorophyll content, as illustrated in Figure 4. Results from the control and doses exceeding 5 Gy were inferior to those at 5 Gy. Seedlings irradiated with 5 Gy exhibited accelerated growth compared to other doses. The highest recorded seedling and leaf lengths were 9.6 cm and 6.8 cm, respectively. Conversely, consistently lower results were observed with 01 Gy photon radiation, indicative of its inhibitory effects. These findings align with previous studies indicating that high doses of photon rays disrupt protein synthesis, water exchange, enzyme activity, production of growth hormones and Indole Acetic Acid (IAA), leaf gas exchange, and overall plant physiology (Rabie et al., 1996; Chandorkar et al., 1986; Stoeva et al., 2001; Kovacs et al., 2002).

Notably, the highest seedling fresh weight (0.39 g) and seedling dry matter content (0.09 g and 23.08% of seedling fresh weight) were recorded at 5 Gy photon irradiation. The gradual decrease in seedling dry matter content percentage with increasing photon irradiation dose suggests a relationship between tissue water content and photon dose. Seedling water content percentage increased with increasing photon doses, possibly due to reduced fresh weight and dry matter content with doses over 5 Gy. The favorable outcomes observed with 5 Gy irradiation could be attributed to increased water and hormone uptake facilitated by a softened seed coat, as reported (Dale et al., 1988). This increase in water and hormone uptake likely led to enhanced tissue metabolic activity, resulting in higher seedling fresh weight, seedling dry matter content, and total chlorophyll content.

In summary, the results demonstrate the dose-dependent effects of photon irradiation on seed propagation, seedling growth, and chlorophyll content in Radish and Spinach. Low doses, particularly 5 Gy, appear to have stimulatory effects on these parameters, while higher doses lead to inhibitory effects, likely due to disruptions in cellular processes and physiology. In our study, we observed that tissue water content increased with higher photon doses, reaching 78.96% of seedling fresh weight at 0.30 g (0.39-0.09). This phenomenon can be attributed to the concurrent decrease in seedling fresh weight and seedling dry matter content with photon doses exceeding 01 Gy. It has been previously noted that cell enlargement through water absorption, cell vacuolation, and turgor-driven wall expansion predominantly contribute to fresh weight increase (Melki et al., 2008). Additionally, dry weight increase has been linked to cell division and new material synthesis. Reports suggest that low doses of photon irradiation may soften the seed coat, potentially increasing permeability and facilitating higher tissue metabolic activity by enhancing water and hormone uptake from the medium (Dale et al., 1988). Consequently, 01 Gy irradiation resulted in higher seedling fresh weight, seedling dry matter content (both in grams and percentage), and total content, potentially due to increased absorption of water and other components from the basal medium, as suggested (Abdel et al., 2008).

Moreover, plants grown from seeds irradiated with 01 Gy exhibited enhanced leaf elongation, allowing them to maintain higher water levels and stable membrane structures compared to non-irradiated plants, as reported (Abdel et al., 2008). Conversely, lower levels of all parameters were observed at 10 Gy, possibly due to decreased water uptake from the environment, as reported, leading to reduced solute mobilization. Further note, Irradiation can induce genetic mutations in plants (Chandorkar et al., 1986). While this is sometimes desired to create new varieties with improved traits (like disease resistance or higher yield), it can also lead to unintended changes that may be undesirable or unpredictable.

Conclusions

In conclusion, our study suggests that low doses of photon radiation show promise in overcoming dormancy and promoting healthy seedling growth in Radishes and Spinach under in vitro conditions. While 10 Gy irradiation yielded optimal results for certain parameters like seed propagation and shoot growth initiation, our findings indicate that 5 Gy irradiation consistently produced the healthiest and best-grown seedlings overall. These results highlight the potential for photon radiation as a tool in optimizing seedling development, with further research warranted to explore its application in agricultural practices. Irradiation of seeds can still be a valuable tool in agriculture and research when used responsibly and within regulatory guidelines. It offers benefits such as pest control, disease prevention, and the development of new plant varieties with improved characteristics.

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