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Deciphering the Impact of Gamma Radiation on Photosynthesis and Cell Viability in *Chlamydomonas reinhardtii*

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Abstract

Chlamydomonas reinhardtii, a unicellular eukaryotic organism, has the potential to produce biomass in aquatic environments. Ionizing radiation, known for its genotoxic effects, can damage DNA and induce mutations in living organisms. This study aimed to understand the impact of γ-irradiation at varying doses (50 Gy to 400 Gy) on *C. reinhardtii*, focusing on survivability and photosynthetic activity. The results demonstrated that gamma irradiation caused alterations in cell number and photosynthetic activities in a dose-dependent manner under circadian conditions. Notably, a dose of 200 Gy resulted in enhanced photosynthetic activity. The observed reductions in cell number and photosynthetic efficiency at higher doses are likely due to random mutagenesis and cellular damage in the chloroplast. Understanding the effects of ionizing radiation and dosage levels provides insights into the epigenetic stress responses and priming effects of photosynthesis and carbon-concentrating mechanisms (CCM) in *C. reinhardtii*.

Keywords: Gamma-irradiation, toxicity, photosynthesis, CO_2 fixation.

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1. Introduction:

Since the dawn of time, aquatic environments have been immersed in ionizing radiation from natural sources and anthropogenic sources, such as radioactive and medical radionuclides wastage, leading to augmented environmental radiation levels UNSCEAR (2008). This exposure can significantly affect microalgae biomass production. Ionizing radiation, including α, β-particles and γ-rays (photons), is emitted by atomic nuclei during decay processes and induces adverse effects on living organisms. These effects include morphological alterations, growth reduction, and genomic-level reproductive impairments Reisz (2014). On the other hand, obtaining superior strains will produce huge biomass, mutant induction will be considered a key solution. Various mutagenesis can be applied to enhance the yield for biodiesel production from microalgae Baek (2016). Likewise, these ionized irradiations in algal species improve the tolerance to different abiotic stresses (heavy metals, cold, drought and salinity) Wang (2018); Qi (2015); Haleem (2012).

Numerous studies have confirmed that lower doses of non-ionizing radiation (γ-radiation) can prime physiological, biochemical, and molecular-level plant changes Daniel (2023). Contradictory trends have been observed in plants regarding chlorophyll content stability, with *Arabidopsis thaliana* showing stability at 60 Gy and cowpea exhibiting a significant increase at 50 Gy Vanhoudt (2013); Hallem (2012). The reticence of chlorophyll molecules *a* and *b* synthesis results in impaired photosystem functions that decelerate the oxygen-evolving complex (OEC) in algae following gamma irradiation. Kohn (1967); Rea (2008).

Despite some existing data on the effects of gamma irradiation, its impact on morphological alterations, photosynthesis, metabolite production, and CO_2 assimilation in *Chlamydomonas reinhardtii* remains inadequately understood. In this context, we hypothesize that random mutagenesis induced by low-dose gamma irradiation could enhance tolerance to abiotic stresses, such as light and CO_2 conditions. This study aims to investigate the survivability and photosynthetic activities of *C. reinhardtii* under circadian rhythmic conditions to elucidate the effects of gamma irradiation.

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2. Material and Methodology:

2.1 Culture and conditions:

In this study, we utilized *Chlamydomonas reinhardtii* (CC-124, mt^+), a wild-type strain obtained from the *Chlamydomonas* Resource Centre at the University of Minnesota. The culture was maintained in a TAP medium, agitated at 120 rpm, and kept at 25°C under circadian rhythmic light conditions (100 ± 5 µmol/m²/s).

2.2 Exposure to gamma-irradiation:

Gamma-irradiation (G-5000) exposure was carried out at the central lab experimental facility, NABTD, Baba Atomic Research Centre (BARC) in Mumbai. Logarithmic-phase C. reinhardtii cells were exposed to various doses of gamma radiation ranging from 50 Gy to 400 Gy. The exposure was completed in a short period to minimize fluorescence artifacts caused by significant microalgal growth. A total of ten different dosages were administered, with each dosage applied to wild-type cells (WT) in triplicate to ensure replicability.

2.3 Cell density, cell number & colony number:

Cell number and motility were monitored under a microscope to ensure cell health and the absence of contamination. Optical density (O.D.) was measured using UV-spectrophotometry (Agilent) at 730 nm. To determine cell count, 1 ml of culture was fixed with 0.025% iodine solution and counted using a hemocytometer. The effects of gamma irradiation on C. reinhardtii were assessed by counting colonies exposed to doses of 50 Gy, 80 Gy, 100 Gy, 125 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy, and 400 Gy with a colony counter (NTS Microprocessor, India). The average colony count was calculated from three biological replicates for each radiation dose.

2.4 Pigment estimation:

Total chlorophyll was extracted using 80% acetone from 1 ml of cell culture, and the mixture was centrifuged at 10,000 × g for 5 minutes at 25°C. The absorbance of the resulting solution was measured with a UV-visible spectrophotometer (Agilent) at wavelengths 645, 663, and 480 nm. The concentrations of chlorophyll a, chlorophyll b, and carotenoids were determined following the methods described by (Lichtenthaler 1987; Porra 1989 and Zivcak

2014). The ratio between chlorophyll and carotenoids was derived from the absorption spectrum of the individual carotenoid content.

3. Results:

3.1 Colony survivability and number:

Gamma-irradiated cells exhibited varying colony numbers and sizes depending on the dosage and survivability. In contrast, the control cells demonstrated higher colony numbers than the irradiated cells (Figure 1). Our observations indicated that both colony numbers and cell numbers decreased with increasing radiation dosage (Figure 2 B and C). Interestingly, there was a sudden drop in colony numbers at the 50 Gy dosage, while the cell number remained higher compared to the higher dosages.

3.2 Growth curve:

In this study, the growth curve data revealed the growth patterns of irradiated cultures under air-level CO_2 conditions and circadian rhythmic conditions. The growth curve was plotted by measuring the optical density at 750 nm at 24-hour intervals over 5 days. We observed that cultures exposed to 125 Gy and 150 Gy exhibited higher growth rates. Interestingly, the culture exposed to 200 Gy showed maximum growth compared to the wild type (WT). Conversely, cultures exposed to 50 Gy, 100 Gy, 250 Gy, 300 Gy, and 350 Gy exhibited lower growth rates (Figure 2 A).

3.3 Estimation of chlorophyll pigments:

Photosynthesis is essential for cell survival and growth in algae. This study investigated the impact of gamma irradiation on photosynthetic pigments in the *C. reinhardtii*. Total chlorophyll content, an alternative for photosynthetic activity, was measured in wild-type (WT) and irradiated cells. Our findings revealed no significant correlation between gamma irradiation dosage and overall photosynthetic activities. Interestingly, a non-monotonic response of chlorophyll content to irradiation was observed. Compared to WT, cells exposed to 50 Gy, 80 Gy, 125 Gy, and 150 Gy exhibited increased chlorophyll content. The maximum chlorophyll level was detected at 200 Gy. Conversely, chlorophyll content decreased significantly at higher doses (250 Gy, 300 Gy, and 350 Gy) compared to WT (Figure 3 A).

We further investigated the chlorophyll a/b ratio, an indicator of the relative abundance of light-harvesting complexes, in irradiated cells. Interestingly, cells exposed to 50 Gy, 80 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, and 350 Gy displayed moderate chlorophyll a/b ratios compared to the wild type (WT). However, the 125 Gy treatment resulted in a significantly higher a/b ratio than WT (Figure 3 B).

Additionally, the content of carotenoids, another pigment involved in stress response, was measured. Similar to chlorophyll content, a non-monotonic response was observed. Carotenoid content in 50 Gy and 100 Gy cells remained comparable to WT. Cells exposed to 80 Gy, 125 Gy, and 150 Gy exhibited increased carotenoid levels, with the highest content found in 200 Gy cells. As expected, a substantial decrease in carotenoid content was observed in cells treated with 250 Gy, 300 Gy, and 350 Gy (Figure 3 C).

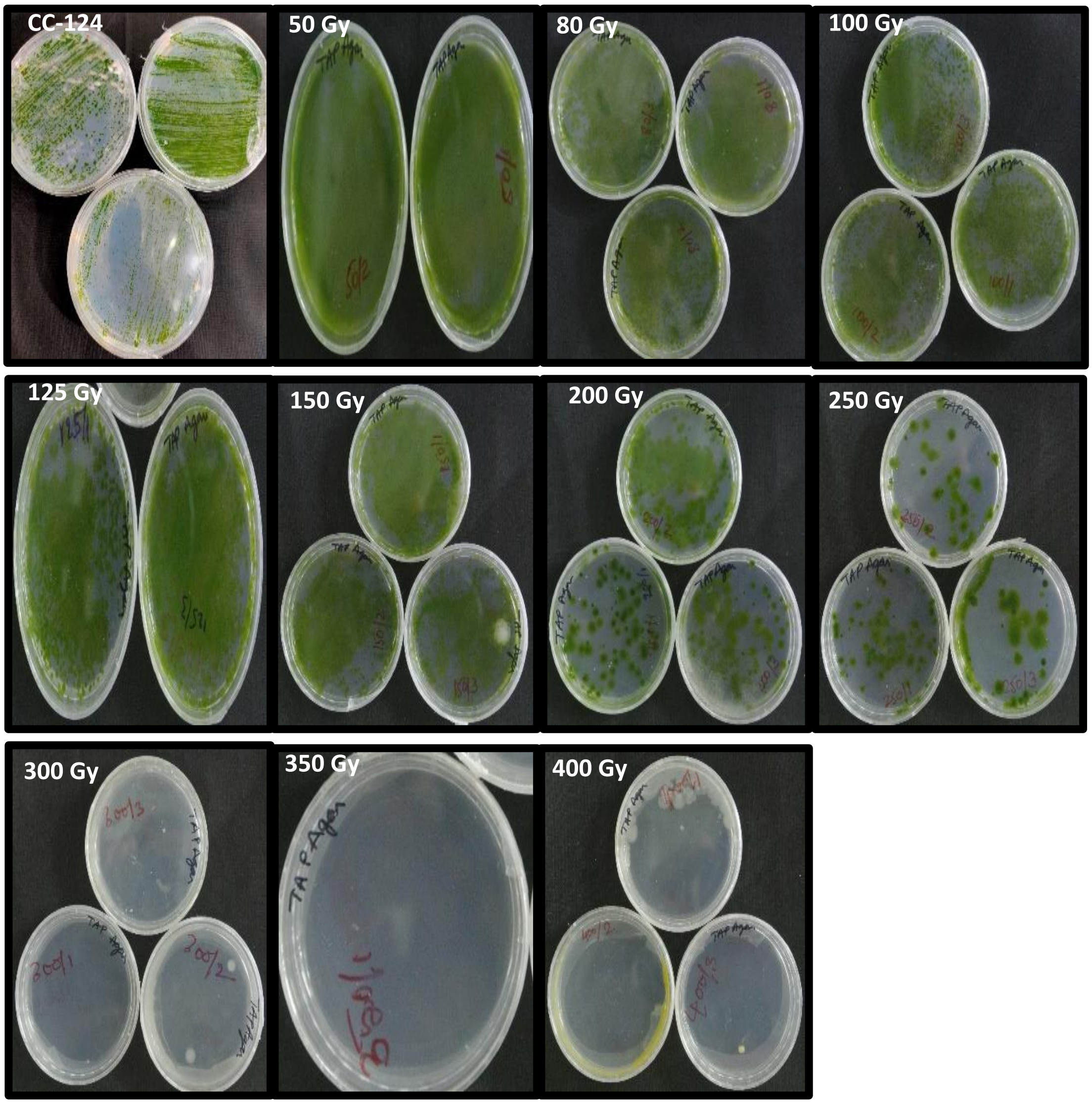


Figure 1: Gamma radiation reduced cell growth in *C.reinhardtii* in a dose-dependent manner.

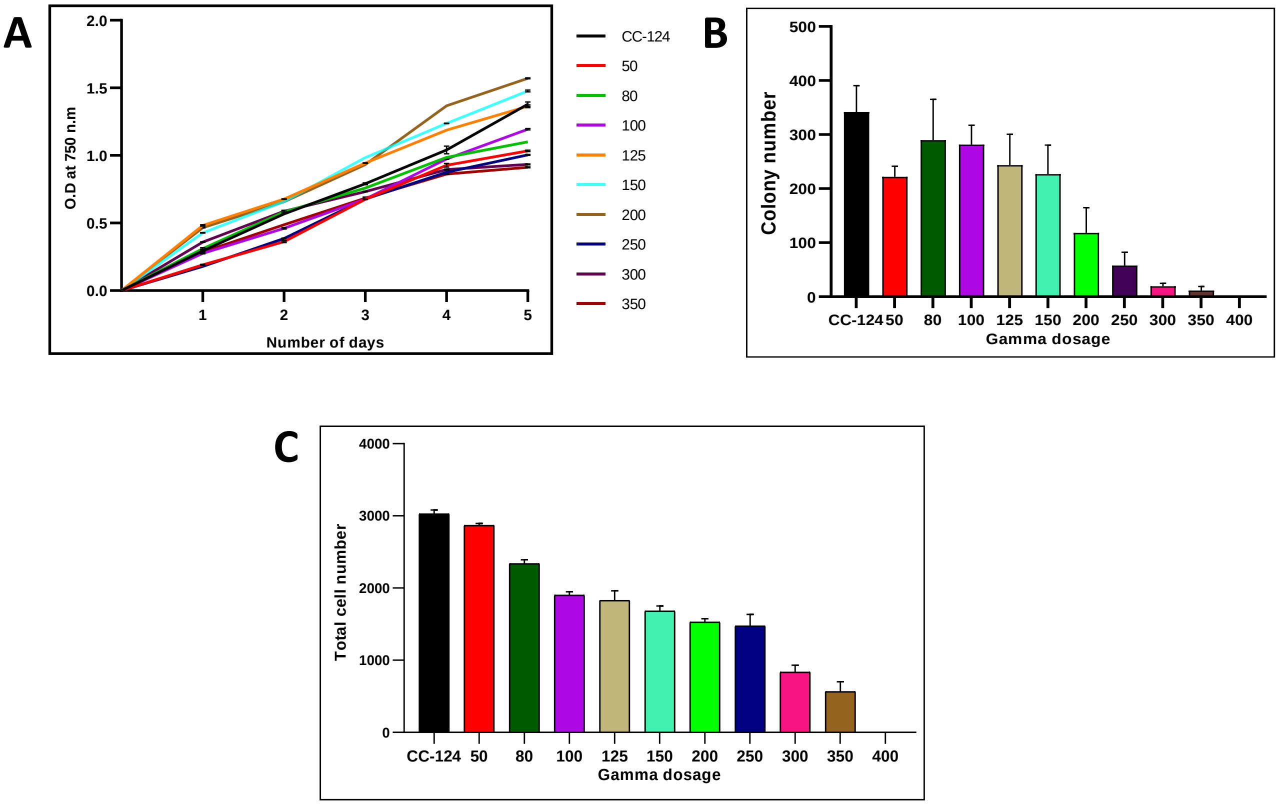
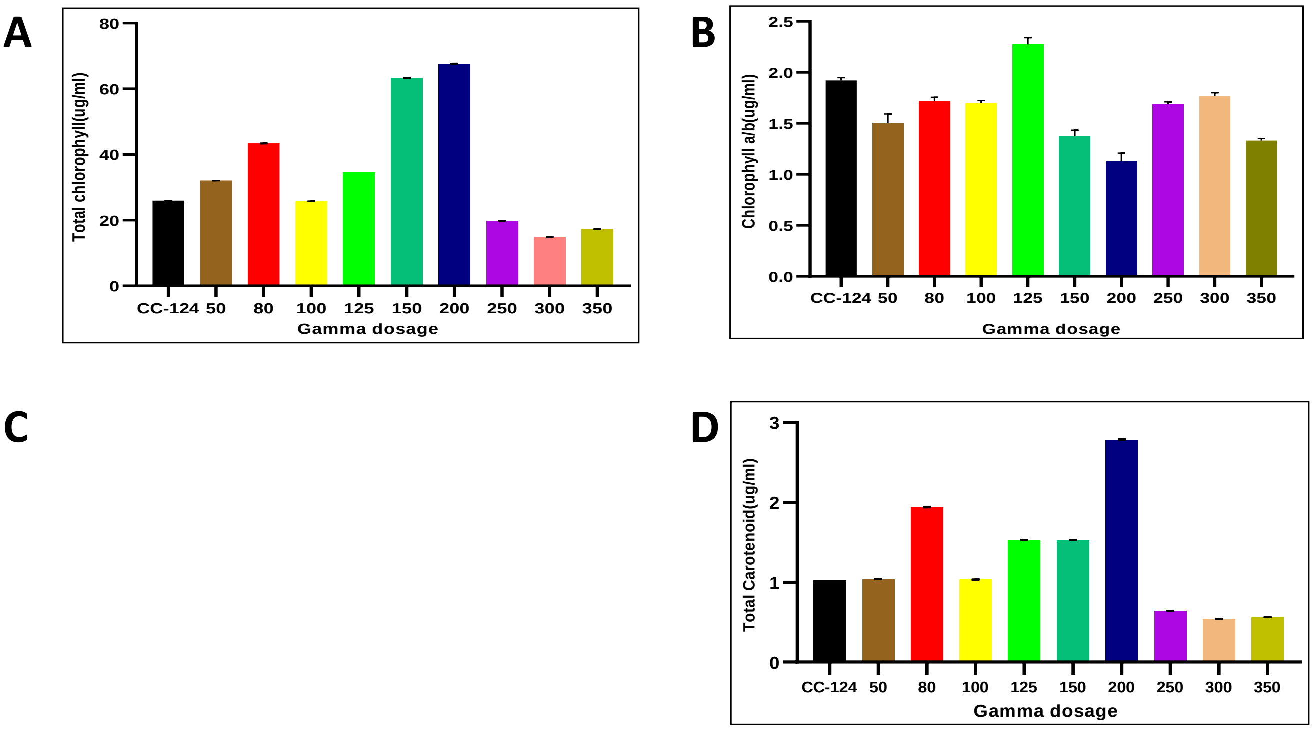


Figure 2: Gamma irradiation dosage negatively affects the survival of *Chlamydomonas reinhardtii*, i.e. (A) Growth curve, (B) Cell number, and potentially (C) Colony number.



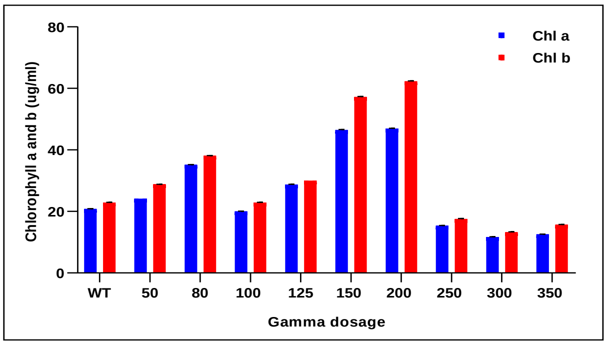


Figure 3: Gamma-irradiated dosage effect decelerated the photosynthetic pigments in *C.reinhardtii* i.e. (A) Chlorophyll content, (B) Chlorophyll a/b, (C) Chlorophyll *a* and *b* (D) carotenoid content.

4. Discussion:

Microalgae are crucial components of aquatic ecosystems, contributing significantly to biomass production and CO_2 fixation. However, ionizing radiation, like gamma rays, can pose a hazard to these organisms, potentially impacting their growth and photosynthetic activity. Limited research has explored the effects of gamma irradiation on microalgae, making this a relatively understudied area in environmental radioprotection.

This study aimed to elucidate the detrimental effects of gamma irradiation on *Chlamydomonas reinhardtii* by assessing its impact on cell survival and photosynthesis. Our findings revealed a dose-dependent response to gamma irradiation. While higher doses (300 Gy, 350 Gy, and 400 Gy) significantly inhibited growth, surprisingly, lower doses (50 Gy, 80 Gy, and 100 Gy) also exhibited reduced growth compared to the control—conversely, moderate doses (150 Gy and 200 Gy) enhanced growth. Furthermore, microscopic observations revealed significant decreases in colony and cell numbers at higher radiation doses, suggesting increased cell death. Damaged cell walls and degraded nuclei accompanied these observations. Interestingly, cells exposed to 50 Gy displayed a lower colony number than those at higher doses, potentially reflecting a slower death rate.

We further investigated the effects of gamma irradiation on photosynthetic parameters, aiming to establish a mechanistic understanding of growth inhibition and recovery. Total chlorophyll content exhibited fluctuations up to 250 Gy. However, at higher doses (300 Gy, 350 Gy, and 400 Gy), cell death and reduced cell numbers led to a decline in chlorophyll content. Conversely, low-dose priming, a phenomenon where exposure to a low dose enhances tolerance to a subsequent higher dose, increased the content of chlorophyll b content more than *Chl* a, suggesting adaption against stress conditions (Figure 3 C). Increased chlorophyll a/b ratio in WT and 125 Gy (Figure 3 B) may be a part of the adaptive stress response and optimize photosynthetic efficiency under stress conditions for ensuring maximum energy capture with reduced light-harvesting capacity.

Carotenoids are vital pigments that stabilize the plasma membrane and scavenge free radicals during abiotic stress (Tania Gomes 2017; Apel K 2004). Similar to chlorophyll, higher gamma irradiation doses reduced carotenoid content. However, the 200 Gy dose-primed strain displayed a remarkable recovery in carotenoid content, even at near-high doses. The observed correlation between physiological and photochemical parameters suggests that low-dose gamma irradiation can stimulate cell viability and photosynthetic activity. However, despite the stimulatory effect of low doses of radiation on Rubisco activity, based on our results complete inhibition of growth at higher doses might occur by low CO_2 assimilation rate and disintegration of chlorophyll activities. Furthermore, the study revealed a strong correlation between primary stress response parameters and the defense mechanisms employed by *C. reinhardtii* in response to a dose-dependent manner of gamma irradiation.

5. Conclusion:

The outcomes obtained in this work confirmed that long-term exposure to gamma-irradiation leads to an inhibition of cell survivability and photosynthetic performance in *C.reinhardtii*. In the present study, we have demonstrated that Gamma-irradiation reduces the colony and cell number, reduction of photosynthesis and accessory pigments content was also responsible for the higher dosage toxicity, causing stunted cell viability and induced cell death. Our findings deliver insights into the mechanism elaborate in gamma-irradiation toxic mechanism alteration cell number, survivability and photosynthetic activities in *C.reinhardtii*. Forthcoming studies should be directed towards a complete description of the biochemical and physiological data along with molecular mechanisms of gamma-irradiation-induced alterations in PSII and Carbon assimilation in CCM pathways.

Conflict of interests:

The authors declare that no conflict of interest exists.

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