

Low-dose gamma radiation varied growth and potential of paclitaxel biosynthesis in callus culture of hazel (*Corylus avellana* L.)

Razieh Adabi

Shahed University

Ayatollah Rezaei (✉ arezaei@shahed.ac.ir)

Shahed University <https://orcid.org/0000-0003-4472-8282>

Research Article

Keywords: *Corylus avellana* L., In vitro culture, Elicitation, Variation, Biomass, Paclitaxel

Posted Date: March 23rd, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2655677/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Plant Cell, Tissue and Organ Culture (PCTOC) on December 10th, 2023. See the published version at <https://doi.org/10.1007/s11240-023-02618-z>.

Abstract

Using the *in vitro* culture system along with related strategies is one of the most promising sources to improve the production of antitumorous agent paclitaxel and other valuable bioactive metabolites. To the best of our knowledge, hazel is the only angiosperm plant that has the capacity to produce paclitaxel and other taxanes. In this study, stable 14-day-old calli initiated from fresh hazel cotyledons were exposed to low-dose γ -radiation for 3, 9, and 15 seconds, which produced 10, 20, and 30 Gy of radiation dose, respectively. Non-irradiated cultures were considered as control. The cultures were picked up after 2 weeks of exposure to radiation and the parameters measured. The results showed that radiation changed callus growth and cells viability. Instead, by increasing radiation dose, antioxidant potential, activity of phenylalanine ammonia-lyase enzyme, and biosynthesis of biochemicals such as paclitaxel significantly increased. A negative correlation was observed between studied biochemical traits including paclitaxel and callus biomass production. The highest paclitaxel specific yield was measured in 30 Gy irradiated calli which was $241.77 \mu\text{g g}^{-1} \text{DW}$, 21.17 times higher than the control culture. Exposure to 30 Gy radiation resulted in the development of a new callus line with a stable high content of paclitaxel. It seems that by eliciting and varying cells, γ -radiation improved the metabolism in callus culture of hazel and could be used to produce such valuable metabolites on a commercial scale.

Introduction

Paclitaxel is a natural antitumor agent that was first extracted from the bark of the yew tree (*Taxus brevifolia*) (Wani et al. 1971). This compound is effective in treating many types of tumors such as breast, stomach, head and neck cancers as well as AIDS-related Kaposi's sarcoma (Agarwal et al. 2020). Although a part of the commercial production of paclitaxel is done using cell culture, the current production of this compound can't meet the high demand for it, and therefore alternative sources for its production are considered. Some studies have shown that the hazel plant also has the capacity to produce paclitaxel (Hoffman, 1998). Later, other studies showed that hazel tissue and cell culture could also produce this valuable compound and other related taxanes (Bestoso et al. 2006; Rezaei et al. 2011; Salehi et al. 2020). In addition, it was revealed that hazel cell extract inhibited the growth of cancer cells, which was attributed to paclitaxel and similar compounds (Bemani et al. 2013). Recently, the biosynthetic pathway of this important molecule in hazel has been taken into consideration as a step towards its development and engineering (Raeispour Shirazi et al. 2021).

In biotechnology, alternative methods are often considered to produce natural compounds. Today, plant cell and tissue culture is more important than any other subject in the study of plant metabolites. High growth rate and isolation from environmental factors and geographical changes are the advantages of plant *in vitro* culture. In addition, in cell and tissue culture, it is possible to produce compounds that are not naturally present in the mother plant (Kartal et al. 2004). Due to the high economic importance of secondary metabolites and the fact that plant species are limited in natural habitats, tissue culture is a suitable alternative to the production of valuable chemical compounds (Farkya et al. 2004). The plant cell cultures have a high potential for large scale production of plant-derived metabolites (Komaraiah et al.

2005). In addition, in this method, it is possible to use various strategies, including elicitation, to change cell metabolism and increase metabolite biosynthesis.

Stress-induced defense responses, lead to increased production of secondary metabolites in plant cell cultures (Akula and Ravishankar 2011). Ionizing radiation such that of γ -rays is harmful in high doses due to the production of free radicals that leading to DNA, protein, and lipid membrane damage. Conversely, it has been shown that low-dose γ -radiation improved biomass and production of valuable metabolites in plant cell culture (Fulzele et al. 2015; Kim et al. 2013). Irradiation of plants can be used to improve the secondary metabolites production (Vardhan and Shukla 2017). The use of biotechnological approaches with low-dose radiation treatment to increase the production of bioactive plant metabolites in medicinal plants has been documented (Aly 2010). The study of the applying γ -radiation as a mutagenic agent in combination with plant tissue culture technique can increase the knowledge of using this technology to produce secondary metabolites and important pharmaceutical products (Kovacs and Keresztes 2002). Compared to the aspects of biological effects of γ -radiation, there is limited information about its effect on the biosynthesis of bioactive compounds, especially paclitaxel. Currently, hazel has been considered as the only angiosperm plant that has the ability to biosynthesize paclitaxel and other taxanes. The numerous advantages of hazel, such as more variation and distribution, faster growth, and also less information than the other source of paclitaxel, i.e., gymnosperm yew, deserve more attention and study. Therefore, the aim of the present work was to study the effect of various levels of low-dose γ -irradiation on the growth, antioxidant activity, some biochemical parameters and paclitaxel production in hazel callus culture.

Material And Methods

Culture conditions and callus induction

Fresh cotyledons of hazelnut (Gerd Eshkevar cultivar) which were harvested in September 2021, surface sterilized, used as explant source and cultured on solidified MS (Murashige and Skoog 1962) according to the method of Rezaei et al. (2011). The developed calli were kept in darkness at 25°C and subcultured every 2 weeks for one year.

Calli irradiation

Low-dose γ -radiation for 3, 9, and 15 seconds, which produced 10, 20, and 30 Gy of radiation, respectively, was applied to stable 14-day-old calli on MS medium. Non-irradiated cultures were considered as control. Radiation was produced by a ^{60}Co source (γ -cell 220 irradiator, Nordion, Ottawa, Canada) with a dose intensity of 1.62 Gy S^{-1} . Optimal dose is important to maximize metabolite production while having minimal effect on growth and viability in plant tissue culture. To achieve this, a preliminary test was performed in which the effect of radiation on callus culture was investigated. It was observed that calli growth severely inhibited at doses above 30 Gy, so lower doses were used for further experiments. In both

experiments, after two weeks, the treated calli were collected, frozen in liquid nitrogen and then transferred to -80°C until used.

Growth assessment

To measure the growth, calli were removed from the culture medium and weighed immediately to determine fresh weight. To measure the dry weight, fresh calli were placed in a fanned oven at 35°C for 72 hours, dried and then weighed.

Biochemical analysis

Total anthocyanin content was measured using the method described by Jeong et al. (2010). The results were expressed as mg g^{-1} FW. The amount of total phenolics was determined based on Folin–Ciocalteu reagent method (Shen et al. 2010). Gallic acid was used as the calculation standard ($R^2 = 0.985$) of phenolics contents and the results were expressed as the equivalent of gallic acid (GAE) per gram of sample. To measure total flavonoids, Djerdane et al. (2006) method was used. An extinction coefficient of $33,000 \text{ cm M}^{-1}$ was used to calculate the compounds concentration.

To determine the antioxidant capacity of the samples, DPPH (2, 2-diphenyl-1-picrylhydrazyl) test was used according to the method described by Brand-Williams et al. (1995). Finally, the radical scavenging activity (%) was calculated using the following equation: Radical scavenging activity (%) = $[(A_o - A_s)/A_o] \times 100$, where A_o is the absorbance of control blank, and A_s is the absorbance of sample extract.

The damage to the membrane lipids was determined by measuring the amount of malondialdehyde (MDA) as the final product of lipid peroxidation (Heath and Packer 1968). The H_2O_2 content of calli was measured using titanium tetrachloride assay as designed previously by Brennan and Frenkel (1977). H_2O_2 concentration was estimated using a standard curve drawn from different concentrations of H_2O_2 . The amount of total protein was evaluated by Bradford (1976) assay using bovine serum albumin as standard. Catalase activity was measured by following the reduction of H_2O_2 according to the method of Cakmak and Horst (1991). Catalase activity was measured by decreasing the absorbance at 240 nm due to H_2O_2 consumption, against mg of protein. Phenylalanine ammonia-lyase (PAL) enzyme activity was determined by converting L-Phenylalanine to trans-cinnamic acid as described method by Ochoa-Alejo and Gómez-Peralta (1993). Using the standard curve of trans-cinnamic acid, the PAL activity of the samples was quantified.

Paclitaxel extraction and measurement

The method proposed by Wu and Lin (2003) was used to extract and measure the paclitaxel content. The HPLC system (Agilent Technologies, 1260 Infinity II) was equipped with a C-18 column (Perfectsil Target ODS3, $5\mu\text{m}$, $250 \times 4.6 \text{ mm}$, MZ-Analysentechnik, Mainz, Germany). Paclitaxel was eluted at a flow rate of 1 ml min^{-1} methanol and water (45:55, V/V)

and was detected at 227 nm. The amount of paclitaxel was determined by comparing the retention time and peak area with the standard sample (Sigma).

Statistical analysis

A completely randomized design with 4 replications was conducted for this experiment. SAS statistical software (version 9.2.) was used for analysis of variance and comparison of means (Duncan's test).

Results

Callus growth

Hazel callus cultures were irradiated with different doses (0, 10, 20 and 30 Gy) and kept under normal conditions. Analysis of variance showed that all studied traits were significantly affected by γ -radiation (Table 1.). Callus growth was reduced in both fresh and dry biomass under the influence of radiation. It was observed that the fresh weight of irradiated calli decreased by increasing radiation dose, which was 64, 71 and 42% of the control culture at 10, 20 and 30 Gy after 14 days of incubation, respectively (Fig. 1A). Regarding the dry biomass of irradiated calli, the trend of change was somewhat different. The dry biomass showed a significant decrease only under 30 Gy and no significant differences were observed at lower doses compared to the control culture (Fig. 1B). Of course, observing the calli cells under the microscope showed that the radiation reduced their survival rate, which could be a reason for the decrease in the growth of treated calli (Fig. 2).

Table 1
Variance analysis of γ -radiation effects on some traits of hazel plant callus

Mean squares						
Source of variations	DF	Fresh weight	Dry weight	Viability	Phenolics	Flavonoids
Irradiation	3	139.86**	0.18*	10.77**	0.009*	0.03**
Error	12	8.67	0.042	0.66	0.002	0.0002
CV (%)		15.39	17.11	24.87	21.69	16.71
*, **: significant at 5% and 1% probability levels, respectively						

Table 1
continued

Mean squares						
Source of variations	DF	Anthocyanins	Paclitaxel	Antioxidant activity	Catalase	PAL
Irradiation	3	0.012**	1271.73**	970.84**	129.76*	0.14**
Error	12	0.00015	99.41	182.47	0.2	0.027
CV (%)		15.66	29.30	20.57	14.93	11.03
*, **: significant at 5% and 1% probability levels, respectively						

MDA and H₂O₂ content

MDA is an important sign of cell state such as lipid peroxidation, extent of membrane damage, amount of electrolyte leakage, oxidative stress and membrane integrity, which is produced under the effect of several abiotic stresses. As shown, exposure to 20 and 30 Gy doses significantly increased the cells MDA content compared to control (Fig. 3A). Nevertheless, no significant difference was observed in the 10 Gy-treated culture with the control culture. The production of H₂O₂ free radical in callus cells was also affected by radiation (Fig. 3B). As can be seen, with the exception of 10 Gy, two higher doses significantly increased H₂O₂ production compared to control culture. The highest H₂O₂ free radical and MDA content in cells was measured at 30 Gy dose, which was 3.2 and 14 times higher than that of the control culture, respectively.

Metabolite production and antioxidant activity

The accumulation of phenolic compounds, flavonoids and anthocyanins in the irradiated cultures are shown in Fig. 4. The compounds were significantly increased in the treated cultures compared to the control. Increasing the radiation dose from 10 to 30 Gy resulted in phenolics production about 1.05, 1.35, and 1.82 times higher than that of the control culture (Fig. 4A). Also, in the case of flavonoid compounds, when the radiation dose increased from 10 to 30 Gy, their production increased by 1.3, 8.3, and 14.0 times compared to the control culture (Fig. 4B). There was a sharp increase in anthocyanins accumulation in cultures when doses of radiation increased from 10 to 30 Gy (Fig. 4C). The anthocyanins production at 10, 20 and 30 Gy was almost 6.1, 12 and 15.1-fold amounts to that of control cultures, respectively.

Exposure to all doses of radiation significantly increased the antioxidant activity (radical scavenging potential) of calli compared to the control culture. According to the obtained results, the maximum significant increase in antioxidant activity was observed with 78% in the dose of 30 Gy, which was 1.81 times more than the control culture. The lowest amount (43%) of radical inhibition was also recorded for control culture (Fig. 4D).

Hazel calli extracts were analyzed by HPLC and their paclitaxel content also measured. Retention time matches were observed for paclitaxel in calli extract and standard, which are displayed in Fig. 5. A significant increase in paclitaxel content and paclitaxel specific yield (PSY) of calli was found along with the increase in radiation dose (Fig. 6A and B). The highest paclitaxel content and PSY were obtained under the effect of 30 Gy dose, which were 8.75 mg L^{-1} and $241.77 \mu\text{g g}^{-1} \text{ DW}$, respectively. Productions of only 2.68 and 6.88 mg L^{-1} were achieved at 10 and 20 Gy, respectively. The paclitaxel content of control cultures was only 0.63 mg L^{-1} . Paclitaxel content at 30 Gy was 13.88 times that of control cultures, with 10.92 times at 20 Gy and 4.25 times at 10 Gy. The PSY of calli at 10, 20 and 30 g were 55.83, 132.3 and $241.77 \mu\text{g g}^{-1} \text{ DW}$, which were 4.88, 11.58 and 21.17 times higher than the control culture, respectively. It should be noted that 30 Gy irradiated calli with highest paclitaxel content were subcultured for 8 generations and their biomass production and biosynthesis potential measured each time. The observations showed that the increase in paclitaxel biosynthesis was stabled in them and therefore they were selected, preserved and named as a new callus line (CHPCA) (Fig. 6C). In the case of biomass production, an average value of 1.04 g was obtained during 8 generations, which indicates the stability in CHPCA growth (Fig. 6D). It appears that the increase in callus paclitaxel production was done at the cost of growth reduction.

Catalase and PAL activity

Catalase and PAL enzymes activity was estimated as important enzymes in antioxidant defense system and biosynthesis pathway of phenolic compounds. It was revealed that the activity of catalase and PAL enzymes changed significantly under the effect of radiation. The activity of the enzymes significantly enhanced with increasing radiation dose and implied a dose dependent response. The activity of the enzymes was higher under 30 Gy radiation dose than other doses. The catalase activity at 30 Gy was 15 times higher than the control culture, with 7-fold at 20 Gy and 4-fold at 10 Gy (Fig. 7A). The PAL activity of calli significantly increased as 1.6, 2.1 and $2.9 \mu\text{g cinnamic acid mg}^{-1} \text{ protein min}^{-1}$ after treated by 10, 20 and 30 Gy doses, respectively, compared to the control culture as $1.1 \mu\text{G cinnamic acid mg}^{-1} \text{ protein min}^{-1}$ (Fig. 7B).

Table 2
Analysis of correlation between growth and biochemical traits of hazel calli affected by γ -radiation

	1	2	3	4	5	6	7	8	9	10	11
1	1										
2	0.95	1									
3	-0.89	-0.75	1								
4	-0.74	-0.78	0.80	1							
5	-0.77	-0.70	0.76	0.74	1						
6	-0.74	-0.69	0.71	0.83	0.86	1					
7	-0.78	-0.71	0.73	0.77	0.88	0.92	1				
8	-0.82	-0.81	0.71	0.76	0.85	0.81	0.68	1			
9	-0.72	-0.79	0.78	0.82	0.79	0.84	0.88	0.74	1		
10	-0.87	-0.84	0.79	0.72	0.74	0.78	0.73	0.71	0.87	1	
11	-0.81	-0.78	0.74	0.75	0.94	0.91	0.89	0.86	0.78	0.89	1
1: Fresh weight; 2: Dry weight; 3: MDA content; 4: H ₂ O ₂ content; 5: Phenolics content; 6: Flavonoids content; 7: Anthocyanins content; 8: Paclitaxel content; 9: Antioxidant activity; 10: Catalase activity; 11: PAL activity											

Discussion

There is a high attention to the use of *in vitro* systems such as cell and tissue culture to produce valuable compounds. In order to increase the production of valuable compounds in plant *in vitro* culture systems, some strategies are used like the applying elicitation, precursor feeding, optimization of culture conditions, mutation and selecting high yielding cell lines. The role of physical or mechanical stimuli in increasing the production of substances in *in vitro* plant culture systems has been documented. Gamma radiation is one of these factors that has many biological effects on plant cells and tissues, including changes in growth and development and stimulating the production of valuable metabolites (Fulzele et al. 2015; Kim et al. 2013; Kovacs and Keresztes 2002). In addition, γ -radiation has been pointed out as a fast and reliable tool for inducing metabolic and physiological changes in plants (Parchin et al. 2019). This radiation in biological systems causes the release of free radicals after collision with atoms or molecules. It has been revealed that molecules, processes and membranes of plant cells, can be damaged or changed by these free radicals, depending on the radiation energy level (Ashraf et al. 2004).

By this research, it was shown that the effect of radiation on fresh and dry biomass was different. As the radiation dose increased, the calli fresh biomass decreased significantly compared to the control culture. However, in the case of dry biomass, a significant decrease was measured only at the 30 Gy dose.

Consistent with this research, increasing the γ -radiation up to 20 Gy significantly reduced the fresh biomass of callus in *Stevia rebaudiana* and increased doses prevented the proliferation of callus (Khalil et al. 2015). Similarly, the accumulation of fresh callus biomass of *Gerbera jamesonii* (Hasbullah et al. 2012) and hairy roots growth of ginseng (*Panax ginseng*) (Zhang et al. 2011) was inhibited by increasing the dose of γ -irradiation. Arrest of cell divisions and/or damage to plant genetic materials is thought to account for reduced growth at higher radiation doses (Preuss and Britt 2003). The viability of cells decreased under radiation treatment. In dead cells, complete plasmolysis was observed, which was clearly shown by Evans blue staining. Induction of cell death and inhibition of DNA synthesis were shown under the influence of γ -radiation (Vucić et al. 2006). Meanwhile, the stimulating effects of γ -radiation at low doses have been demonstrated by some studies. For example, the *Arthrospira platensis* alga showed resistance to growth reduction at lower applied γ -doses (Abomohra et al. 2016). The growth of turmeric seedlings was slightly stimulated at the dose of 20 Gy, while the highest dose (80 Gy) significantly reduced the parameter compared to the control seedlings (Chusreeaeom and Khamsuk 2019). In addition, higher doses of γ -radiation significantly decreased callus growth in *Cucumis melo* while lower doses stimulated it (Venkateshwarlu 2008). Studies revealed that the applied dose and the exposure time to γ -radiation were decisive in the biological effects on plant cells and tissues. There is evidence that the change in the cross-talk of hormone signaling pathways and the increasing the antioxidant capacity of cells are the reasons for the stimulation of low doses of γ -radiation on plant cells and tissues (Wi et al. 2007). Some studies have also shown that the resistance of living organisms to γ -radiation is related to the change in the concentration of metabolites (Abomohra et al. 2016; Choi et al. 2015; Farhi et al. 2008). It was thought that the increase in the amount of free amino acids under low doses of radiation and the subsequent increase in the pool of heat shock proteins were involved in the DNA repair process and the strengthening of coping with stress in cells (Farhi et al. 2008; Yu et al. 2016). Similarly, in this study, it was found that with the increase of radiation dose, the antioxidant activity and the pool of related metabolites increased, and a positive correlation was also shown between them (Table 2). This may explain the resistance of cells in terms of dry biomass production, especially at low doses of radiation.

According to the results, increased tissue MDA content was determined after exposure to irradiation, which represents the oxidation process. A similar increase in MDA content was also shown after 200 Gy irradiated soybean (Štajner et al. 2009), 50 and 100 Gy irradiated *Zizania latifolia* seedlings (Fan et al. 2014) and *in vitro* cultured turmeric plantlets after exposed to 40 Gy (Chusreeaeom and Khamsuk 2019). The relationship between free radicals and membrane lipids peroxidation as well as phenylpropanoids induction has been shown (Tang and Smith 2001). However, a decrease in MDA amount was shown in some plants such as chickpea irradiated with 700–1000 Gy (Hameed et al. 2008) and turmeric seedlings after 40 Gy irradiation (Chusreeaeom and Khamsuk 2019). The amount of MDA is a measure of oxidative stress in the organism. This compound, which is produced by the peroxidation of polyunsaturated fatty acids in cell membranes, leads to cell damage (Del Rio et al. 2005). The observed decrease in viability in irradiated cells may be due to this process. In addition, MDA production process along with the free radical chain-reaction leads to mass production of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radicals and hydroxyl radicals. Various protective mechanisms have been proposed

to reduce MDA content at high doses of radiation, including antioxidant activity, DNA repair, and cellular osmotic regulation (Kim et al. 2012).

Calli exposed to higher radiation doses shown a significant increase in H₂O₂ content compared to 10 Gy-treated and control cultures. In accordance with our findings, there are reports that γ -radiation at the applied dose increased the production of free radicals in some plants (Calucci et al. 2003; Štajner et al. 2009). In addition, γ -irradiation significantly increased the H₂O₂ content at 20.0 kGy to its maximum in broad bean plants compared to the control (Aly and El-Beltagi 2010). Some studies have suggested that the increase in H₂O₂ content is related to the increase in paclitaxel production (Wu and Ge 2004), which was also observed in this research. On the contrary, a decrease in H₂O₂ amount was observed in broad bean plants under γ -radiation at 20 Gy compared to control plants (Moussa 2009). It has been shown that exposure to γ -radiation causes oxidative damage associated with excessive production of free radicals in cells and tissues and damages or changes almost all structural and functional aspects of the cell depending on the applied dose (Apel and Hirt 2004; Ashraf et al. 2004; Salter and Hewitt 1992).

In this research, it was shown that when the radiation dose increased, the production of important metabolites such as phenolic, flavonoid, anthocyanin and paclitaxel increased. Consistent with this, some studies also showed that tissue and cell cultures treated with γ -rays increased the production of secondary metabolites. For example, by the increasing radiation doses saponins and ginsenosides content were increased in ginseng cell lines (Zhang et al. 2011), phenolic and flavonoid compounds in rosemary callus cultures (El-Beltagi et al. 2011), stevioside, phenolic and flavonoid compounds in stevia callus cultures (Khalil et al. 2015), camptotecin production in callus culture of ghanera (*Nothapodytes foetida*) (Fulzele et al. 2015), phenolic contents in the *Gnetum gnemon* seeds (Syahdi et al. 2019) and total phenol and flavonoid contents in mugwort extracts (Hwang et al. 2021). However, γ -radiation enhanced shikonins content in cell suspension culture of gromwell (*Lithospermum erythrorhizon*) up to 32 Gy and after that content wasn't significantly affected (Chung et al. 2006). Gamma radiation did not have a significant effect on the content of flavonoids, flavonols, and total polyphenols of *Polygoni Multiflora* Radix (Chiang et al. 2011) or phenolic content of peppermint and thyme irradiated at 2, 5 and 10 kGy (Pereira et al. 2016). Furthermore, doses up to 15 kGy had no significant effect on the amount of phenolic content in *Glycyrrhiza glabra* root (Khattak and Simpson 2008). It was even observed that, a dose of 5 kGy reduced the flavonoids and phenolics content in Chinese dodder extract (Alijaniha et al. 2021). In this regard, some researches also showed the inductive effect of low energy ultrasound radiation on Chinese yew (*Taxus chinensis*) and hazel cell culture in paclitaxel production (Abomohra et al. 2016; Rezaei et al. 2011; Wu et al. 2003). According to the available evidence, these different observations are suggested to be due to the radiation dose, exposure time, plant species, as well as the type of compounds studied.

The recent findings shown that irradiation (10 to 30 Gy) increased the calli antioxidant capacity with respect to the control samples. Our result agreed with other findings that showed free radical scavenging capacity increased under the effect of γ -radiation. For example, antioxidant capacity of black pepper increased under the influence of γ -radiation (Polovka et al. 2006). Stevia plant calli treated with γ -

radiation (20 Gy) showed higher antioxidant capacity (Khalil et al. 2015). In addition, with increasing γ -radiation dose (2 to 10 kGy), the antioxidant activity of mugwort extracts increased (Hwang et al. 2021). However, some conflicting reports have been presented about the effect of γ -rays on antioxidant capacity (Chandrashekar et al. 2013; Gumus et al. 2011). The plant's antioxidant system uses enzymatic and non-enzymatic mechanisms to regulate the level of free radicals in a non-toxic range in the cell. Based on this, the increase in the production of some compounds such as phenolics and flavonoids can be related to the increase in the antioxidant capacity of cells and tissues. In this study, in addition to antioxidant capacity, such compounds were also increased under the influence of radiation. It should be noted that a positive correlation was observed between such metabolites and the antioxidant capacity of cells. The increase in antioxidant capacity in *Pulchea indica* leaves was attributed to the increase in phenolic compounds under γ -ray effect (Suryadi and Mun'im 2021). Also, the antioxidant capacity and the production of phenolic compounds in some studied medicinal plants increased under the effect of γ -radiation. (Khawory et al. 2020). Antioxidant capacity by suppressing free radicals plays a central role in protecting plants against damaging environmental factors (Gudkov et al. 2019). It seems that the effect of γ -radiation on antioxidant capacity is also dependent on various factors, particularly the species and its dose.

It was revealed that the exposure of calli to radiation led to the induction of catalase and PAL enzymes activity. In this regard, it was shown that different doses of γ -rays affected the activity of antioxidant enzymes, including catalase, and their activity gradually increased with the increase of the dose (El-Beltagi et al. 2011). The same results was obtained by another study that showed that the activity of the catalase increased under the influence of γ -radiation at 10 and 30 Gy doses in potato tuber (Afify et al. 2012), below the 100 Gy in tall fescue (Amirikhah et al. 2021) and at 0.1 kGy in *Arabidopsis* wild type and mutants (Sultana et al. 2013). As in our experiment, when *Rosmarinus Officinalis* L. calli were exposed to γ -radiation, the PAL activity and the phenolics content increased (El-Beltagi et al. 2011). It was also showed the increase in the PAL and chalcone syntase activity and the amount of flavonoid compounds in *Centella asiatica* seedlings under the influence of 20 and 30 Gy γ -radiation (Moghaddam et al. 2011). The observed improve in the content of phenylpropanoids under irradiation can be correlated to variations in the enzyme activity of the relevant biosynthetic pathway.

Correlation analysis of traits was performed to better explain the findings. One of the important goals was to investigate the relationship between biomass production and metabolite biosynthesis. In this study, a high negative correlation between these two processes, which is very important in the field of *in vitro* culture, was shown. It is thought that under the effect of γ -radiation, metabolites such as amino acids and monosaccharides, which act as major building blocks for cellular components and growth, mainly shift towards the production of secondary metabolites, and therefore the growth rate is reduced. Such a correlation between biomass production and metabolite accumulation has already been concluded for *Arabidopsis thaliana* and tomato plants (Schauer et al. 2006; Meyer et al. 2007).

Conclusions

According to the results obtained in this study, under the effect of low-dose γ -radiation, while affecting the growth and viability of hazel cells, the production potential of bioactive compounds and antioxidant capacity significantly increased. In sum, the ability to use γ -irradiation method as an effective alternative tool in the field of *in vitro* cultures of medicinal plants to elicit the biosynthesis of valuable effective ingredients and also develop new cell lines, offers researchers an interesting study window. Of course, more research is needed to use this useful and powerful technology to achieve the desired goals in different fields of plant production in sustainable agriculture.

Declarations

Acknowledgments The authors express special gratitude to Shahed university for supporting this research.

Author contributions RA methodology, investigation, writing, formal analysis. AR supervision, conceptualization, methodology, writing.

Conflict of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Afify AE, El-Beltagi HS, Aly AA, El-Ansary AE (2012) Antioxidant enzyme activities and lipid peroxidation as biomarker compounds for potato tuber stored by iodine vapor. *Adv Food Sci* 34:93-99.
2. Agarwal G, Carcache PJB, Addo EM, Kinghorn AD (2020) Current status and contemporary approaches to the discovery of antitumor agents from higher plants. *Biotechnol Adv* 38:107337.
3. Akula R, Ravishankar GA (2011) Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal Behav* 6:1720-1731.
4. Alijaniha F, Emadi F, Naseri M, Kamalinejad M, Motevaseli E, Karimi R (2021) Effect of gamma irradiation on cytotoxicity, phenolics content and acute toxicity of *Cuscuta chinensis* L. extract. *Radiat Phys Chem* 185:109508.
5. Aly A (2010) Biosynthesis of phenolic compounds and water soluble vitamins in Culantro (*Eryngium foetidum* L.) plantlets as affected by low doses of gamma irradiation. *Analele Universitatii Din Oradea-Fascicula Biologie* 17:356-361.
6. Aly AA, El-Beltagi HE (2010) Influence of ionizing irradiation on the antioxidant enzymes of *Vicia faba* L. *Grasas Y aceites* 61:288-294.
7. Amirikhah R, Etemadi N, Sabzalian MR, Nikbakht A, Eskandari A (2021) Gamma radiation negatively impacted seed germination, seedling growth and antioxidant enzymes activities in tall fescue infected with *Epichloë* endophyte. *Ecotoxicol Environ Saf* 216:112169.

8. Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signaling transduction. *Annu Rev Plant Biol* 55:373.
9. Ashraf M, Cheema A, Rashid M, Qamar Z (2004) Effect of gamma rays on M1 generation in basmati rice. *Pak J Bot* 35:791-796.
10. Bemani E, Ghanati F, Rezaei A, Jamshidi M (2013) Effect of phenylalanine on taxol production and antioxidant activity of extracts of suspension-cultured hazel (*Corylus avellana* L.) cells. *J Nat Med* 67:446-451.
11. Bestoso F, Ottaggio L, Armirotti A, Balbi A, Damonte G, Degan P, Mazzei M, Cavalli F, Ledda B, Miele M (2006) *In vitro* cell cultures obtained from different explants of *Corylus avellana* produce taxol and taxanes. *BMC biotechnol* 6:1-11.
12. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248-254.
13. Brand-Williams W, Cuvelier M-E, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci Technol* 28:25-30.
14. Brennan T, Frenkel, C (1977) Involvement of hydrogen peroxide in the regulation of senescence in pear. *Plant Physiol* 411-416.
15. Cakmak I, Horst WJ (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol Plant* 83:463-468.
16. Calucci L, Pinzino C, Zandomenoghi M, Capocchi A, Ghiringhelli S, Saviozzi F, Tozzi S, Galleschi, L (2003) Effects of γ -irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. *J Agric Food Chem* 51:927-934.
17. Chandrashekar K, Somashekarappa H, Souframanien J (2013) Effect of gamma irradiation on germination, growth, and biochemical parameters of *Terminalia arjuna* Roxb. *Radiat Prot Environ* 36:38.
18. Chiang Y-C, Huang G-J, Ho Y-L, Hsieh P-C, Chung H-P, Chou F-I, Chang Y-S (2011) Influence of gamma irradiation on microbial load and antioxidative characteristics of *Polygoni Multiflori* Radix. *Process Biochem* 46:777-782.
19. Chung B, Lee Y-B, Baek M-H, Kim J-H, Wi S, Kim J-S (2006) Effects of low-dose gamma irradiation on production of shikonin derivatives in callus cultures of *Lithospermum erythrorhizon* S. *Radiat Phys Chem* 75:1018-1023.
20. Chusreeaeom, K., Khamsuk, O., 2019. Effects of gamma irradiation on lipid peroxidation, survival and growth of turmeric *in vitro* culture. *J. Phys.: Conference Series*. IOP Publishing, p. 012003.
21. Del Rio D, Stewart AJ, Pellegrini N (2005) A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr Metab Cardiovasc Dis* 15:316-328.
22. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N (2006) Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food chem* 97:654-66.

23. El-Beltagi HS, Ahmed OK, El-Desouky W (2011) Effect of low doses γ -irradiation on oxidative stress and secondary metabolites production of rosemary (*Rosmarinus officinalis* L.) callus culture. *Radiat Phys Chem* 80:968-976.
24. Fan J, Shi M, Huang J-Z, Xu J, Wang Z-D, Guo D-P (2014) Regulation of photosynthetic performance and antioxidant capacity by ^{60}Co γ -irradiation in *Zizania latifolia* plants. *J Environ Radioact* 129:33-42.
25. Farhi E, Rivasseau C, Gromova M, Compagnon E, Marzloff V, Ollivier J, Boisson A-M, Bligny R, Natali F, Russo D (2008) Spectroscopic investigation of ionizing-radiation tolerance of a Chlorophyceae green micro-alga. *J Phys Condens Matter* 20:104216.
26. Farkya S, Bisaria V, Srivastava A (2004) Biotechnological aspects of the production of the anticancer drug podophyllotoxin. *Appl Microbiol Biotechnol* 65:504-519.
27. Fulzele DP, Satdive R, Kamble S, Singh S, Singh S (2015) Improvement of anticancer drug camptothecin production by gamma irradiation on callus cultures of *Nothapodytes foetida*. *Intl J Pharma Res Allied Sci* 4(1):19-27.
28. Gudkov SV, Grinberg MA, Sukhov V, Vodeneev V (2019) Effect of ionizing radiation on physiological and molecular processes in plants. *J Environ Radioact* 202:8-24.
29. Gumus T, Albayrak S, Sagdic O, Arici M (2011) Effect of gamma irradiation on total phenolic contents and antioxidant activities of *Satureja hortensis*, *Thymus vulgaris*, and *Thymbra spicata* from Turkey. *Int J Food Prop* 14:830-839.
30. Hameed A, Shah TM, Atta BM, Haq MA, Sayed H (2008) Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in desi and kabuli chickpea. *Pak J Bot* 40:1033-1041.
31. Hasbullah NA, Taha RM, Saleh A, Mahmad N (2012) Irradiation effect on *in vitro* organogenesis, callus growth and plantlet development of *Gerbera jamesonii*. *Hortic Bras* 30:252-257.
32. Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts I Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125:189-198.
33. Hoffman A (1998) Bioprospecting for taxol in angiosperm plant extracts-using high performance liquid chromatography thermospray mass spectroscopy to detect the anticancer agent and its related metabolites in filbert trees. *Spectroscopy* 13:22-32.
34. Hwang K-E, Ham Y-K, Song D-H, Kim H-W, Lee M-A, Jeong J-Y, Choi Y-S (2021) Effect of gamma-ray, electron-beam, and X-ray irradiation on antioxidant activity of mugwort extracts. *Radiat Phys Chem* 186:109476.
35. Jeong J-H, Jung H, Lee S-R, Lee H-J, Hwang KT, Kim T-Y (2010) Anti-oxidant, anti-proliferative and anti-inflammatory activities of the extracts from black raspberry fruits and wine. *Food Chem* 123:338-344.
36. Kartal M, Konuklugil B, Indrayanto G, Alfermann A (2004) Comparison of different extraction methods for the determination of podophyllotoxin and 6-methoxypodophyllotoxin in *Linum species*. *J Pharm Biomed Anal* 35:441-447.

37. Khalil SA, Ahmad N, Zamir R (2015) Gamma radiation induced variation in growth characteristics and production of bioactive compounds during callogenesis in *Stevia rebaudiana* (Bert). New Negat Plant Sci 1:1-5.
38. Khattak KF, Simpson TJ (2008) Effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of *Nigella staiva* seed. Food Chem 110:967-972.
39. Khawory MH, Sain AA, Rosli MAA, Ishak MS, Noordin MI, Wahab HA (2020) Effects of gamma radiation treatment on three different medicinal plants: Microbial limit test, total phenolic content, in vitro cytotoxicity effect and antioxidant assay. Appl Radiat Isot 157:109013.
40. Kim DS, Song M, Kim S-H, Jang D-S, Kim J-B, Ha B-K, Kim SH, Lee KJ, Kang S-Y, Jeong IY (2013) The improvement of ginsenoside accumulation in *Panax ginseng* as a result of γ -irradiation. J Ginseng Res 37:332.
41. Kim S-H, Song M, Lee KJ, Hwang S-G, Jang CS, Kim J-B, Kim SH, Ha B-K, Kang S-Y, Kim DS (2012) Genome-wide transcriptome profiling of ROS scavenging and signal transduction pathways in rice (*Oryza sativa* L.) in response to different types of ionizing radiation. Mol Biol Rep 39:11231-11248.
42. Komaraiah P, Kishor PK, Carlsson M, Magnusson K-E, Mandenius C-F (2005) Enhancement of anthraquinone accumulation in *Morinda citrifolia* suspension cultures. Plant Sci 168:1337-1344.
43. Kovacs E, Keresztes A (2002) Effect of gamma and UV-B/C radiation on plant cells. Micron 33:199-210.
44. Meyer RC, Steinfath M, Lisec J, Becher M, Witucka-Wall H, Törjék O, Fiehn O, Eckardt Ä, Willmitzer L, Selbig J, Altmann T (2007) The metabolic signature related to high plant growth rate in *Arabidopsis thaliana*. Proc Natl Acad Sci 104(11):4759-4764.
45. Moghaddam SS, Jaafar H, Ibrahim R, Rahmat A, Aziz MA, Philip E (2011) Effects of acute gamma irradiation on physiological traits and flavonoid accumulation of *Centella asiatica*. Molecules 16:4994-5007.
46. Moussa H (2009) Effect of gamma radiation on antioxidant enzymes and G 6 PDH activities in *Vicia faba* plants. Acta agron Hung 57:79-86.
47. Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473-497.
48. Ochoa-Alejo N, Gómez-Peralta JE (1993) Activity of enzymes involved in capsaicin biosynthesis in callus tissue and fruits of chili pepper (*Capsicum annuum* L.). J Plant Physiol 141:147-152.
49. Parchin RA, Ghomi AA, Badi HN, Eskandari A, Navabpour S, Mehrafarin A (2019) Growth characteristics and phytochemical responses of Iranian fenugreek (*Trigonella foenum-graecum* L.) exposed to gamma irradiation. Ind Crops Prod 139(36):111593.
50. Pereira E, Pimenta AI, Calhelha RC, Antonio AL, Verde SC, Barros L, Santos-Buelga C, Ferreira IC (2016) Effects of gamma irradiation on cytotoxicity and phenolic compounds of *Thymus vulgaris* L. and *Mentha x piperita* L. LWT - Food Sci Technol 71:370-377.
51. Polovka M, Brezová V, Staško A, Mazúr M, Suhaj M, Šimko P (2006) EPR investigations of gamma-irradiated ground black pepper. Radiat Phys Chem 75:309-321.

52. Preuss S, Britt A (2003) A DNA-damage-induced cell cycle checkpoint in *Arabidopsis*. *Genetics* 164:323-334.
53. Raeispour Shirazi M, Rahpeyma SA, Rashidi Monfared S, Zolala J, Lohrasbi-Nejad A (2021) Identification and in-silico characterization of taxadien-5 α -ol-O-acetyltransferase (TDAT) gene in *Corylus avellana* L. *Plos one* 16:e0256704.
54. Rezaei A, Ghanati F, Behmanesh M, Mokhtari-Dizaji M (2011) Ultrasound-potentiated salicylic acid-induced physiological effects and production of taxol in hazelnut (*Corylus avellana* L) cell culture. *Ultrasound Med Biol* 37:1938-1947.
55. Salehi M, Moieni A, Safaie N, Farhadi S (2020) Whole fungal elicitors boost paclitaxel biosynthesis induction in *Corylus avellana* cell culture .*Plos one* 15(7):pe0236191.
56. Salter L, Hewitt CN (1992) Ozone-hydrocarbon interactions in plants. *Phytochemistry* 31:4045-4050.
57. Schauer N, Semel Y, Roessner U, Gur A, Balbo I, Carrari F, Pleban T, Perez-Melis A, Bruedigam C, Kopka J, Willmitzer L (2006) Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. *Nat Biotechnol* 24(4):447-454.
58. Shen X, Li X, Li Z, Li J, Duan L, Eneji A (2010) Growth physiological attributes and antioxidant enzyme activities in soybean seedlings treated with or without silicon under UV-B radiation stress. *J Agron Crop Sci* 196:431-439.
59. Štajner D, Popović BM, Taški K (2009) Effects of γ -irradiation on antioxidant activity in soybean seeds. *Cent Eur J Biol* 4:381-386.
60. Suryadi H, Mun'im A (2021) Effect of gamma irradiation on the caffeoylquinic acid derivatives content antioxidant activity and microbial contamination of *Pluchea indica* leaves. *Heliyon* 7(8):e07825.
61. Syahdi RR, Sakti AS, Kristiyanto A, Redmawati R, Mun'im A (2019) Effect of gamma irradiation on some pharmacological properties and microbial activities of Melinjo (*Gnetum gnemon* Linn) seeds. *Pharmacogn J* 11:(1).
62. Tang M, Smith C (2001) Elicitor induced defence responses in *Medicago sativa*. *New Phytol* 149:401-418.
63. Vardhan PV, Shukla LI (2017) Gamma irradiation of medicinally important plants and the enhancement of secondary metabolite production. *Int J Radiat Biol* 93:967-979.
64. Venkateshwarlu M (2008) Effect of gamma rays on different explants of callus treatment of multiple shoots in *Cucumis melo* cv Bathasa. *J Environ Biol* 29:789-792.
65. Vucić V, Isenović ER, Adžić M, Ruzdijić S, Radojčić MB (2006) Effects of gamma-radiation on cell growth cycle arrest death and superoxide dismutase expression by DU 145 human prostate cancer cells. *Braz J Med Biol Res* 39:227-236.
66. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT (1971) Plant antitumor agents VI The isolation and structure of taxol a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J Am Chem Soc* 93:2325-2327.

67. Wi SG, Chung BY, Kim JS, Kim JH, Baek MH, Lee JW, Kim YS (2007) Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron* 38:553-564.
68. Wu J, Ge X (2004) Oxidative burst jasmonic acid biosynthesis and taxol production induced by low-energy ultrasound in *Taxus chinensis* cell suspension cultures. *Biotechnol Bioeng* 85:714-721.
69. Wu J, Lin L (2003) Enhancement of taxol production and release in *Taxus chinensis* cell cultures by ultrasound methyl jasmonate and in situ solvent extraction. *Appl Microbiol Biotechnol* 62:151-155.
70. Yu Y, Cui Y, Niedernhofer LJ, Wang Y (2016) Occurrence biological consequences and human health relevance of oxidative stress-induced DNA damage. *Chem Res Toxicol* 29:2008-2039.
71. Zhang J-Y, Bae T-W, Boo K-H, Sun H-J, Song I-J, Pham C-H, Ganesan M, Yang D-H, Kang H-G, Ko S-M (2011) Ginsenoside production and morphological characterization of wild ginseng (*Panax ginseng* Meyer) mutant lines induced by γ -irradiation (^{60}Co) of adventitious roots. *J Ginseng Res* 35:283.

Figures

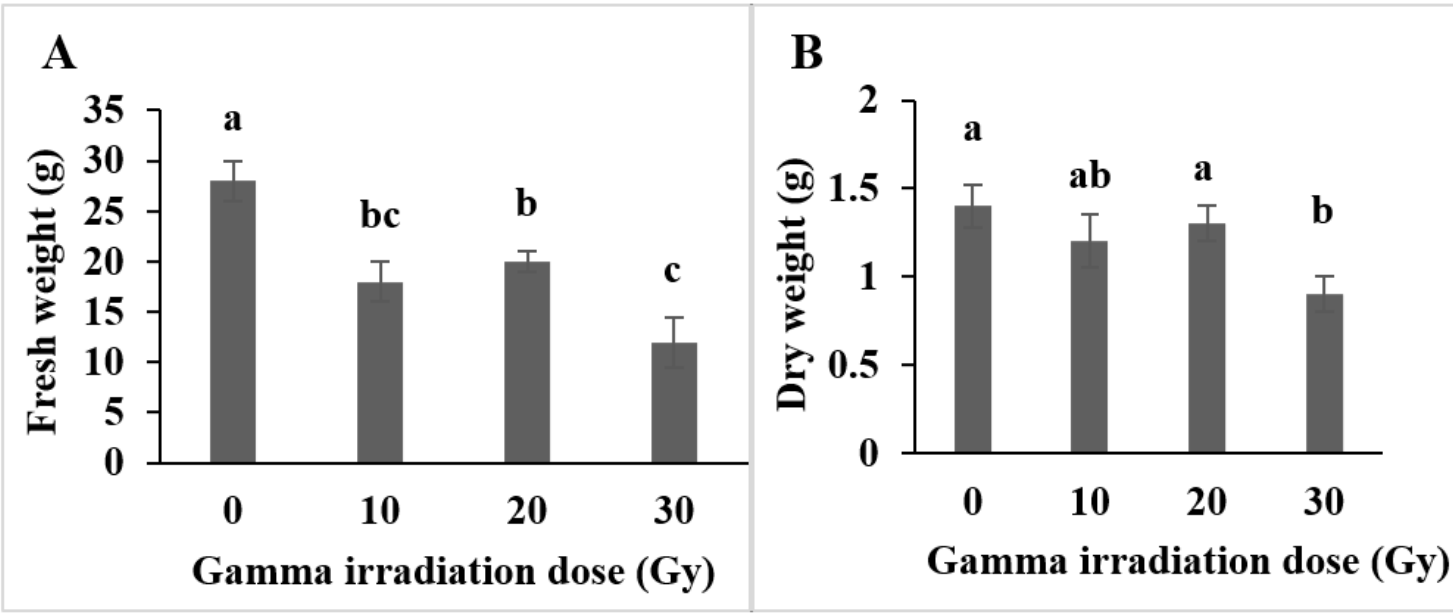


Figure 1

Fresh (A) and dry weight (B) of hazel callus as affected by γ -radiation. Data are means of 4 replicates. Means with the same letter are not significantly different according to Duncan's test ($P \leq 0.05$)

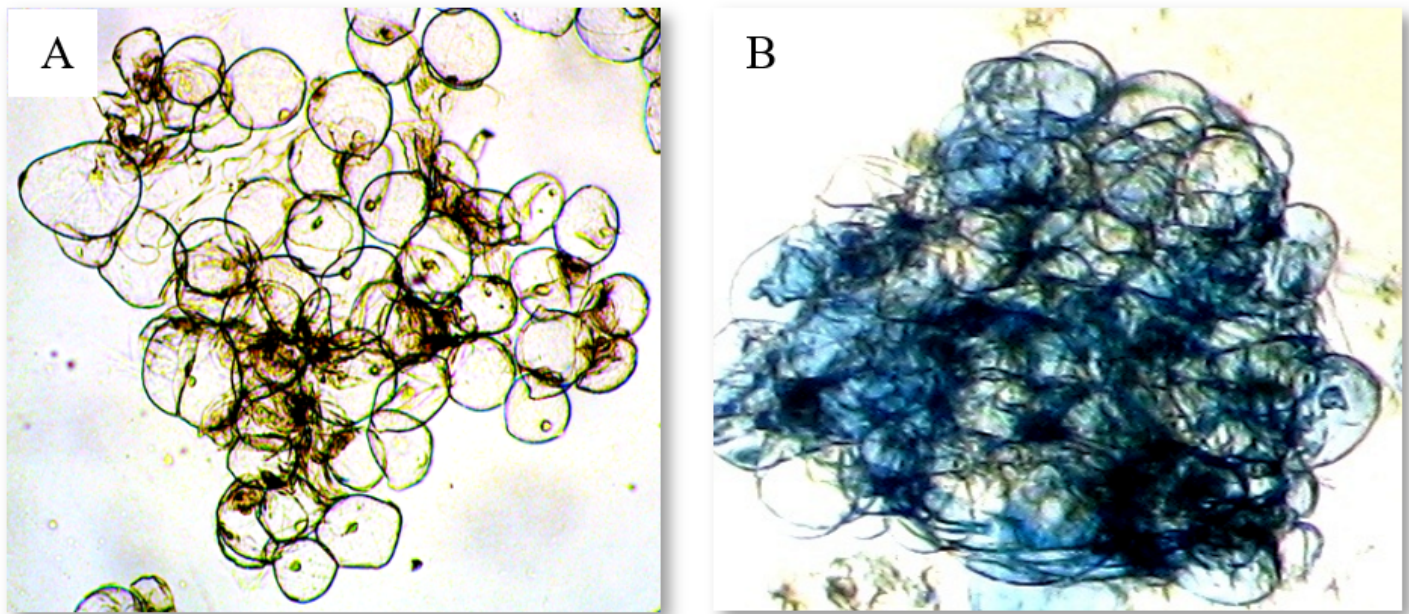


Figure 2

Comparison of the viability of hazel cells treated with γ -radiation. Control cells (A) and treated with 30 Gy (B), cells were stained with Evans blue and visualized by light microscope with magnification (20x). Blue colored cells are dead

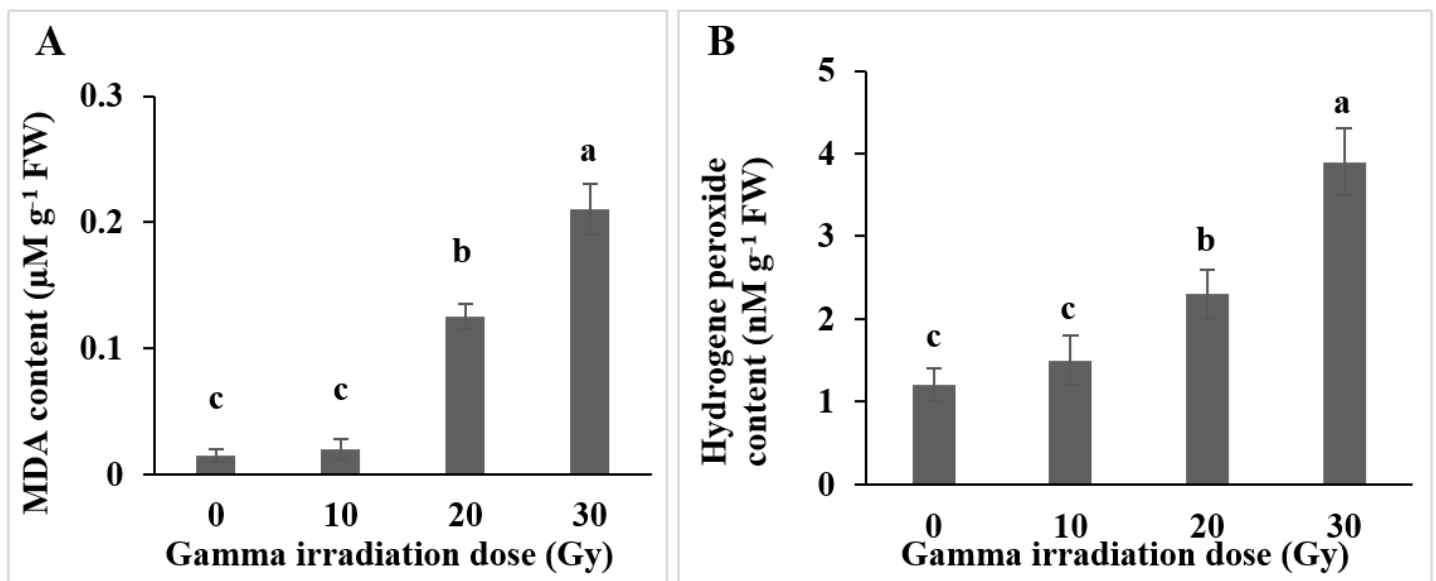


Figure 3

MDA (A) and H₂O₂ (B) content of hazel callus as affected by γ -radiation. Data are means of 4 replicates. Means with the same letter are not significantly different according to Duncan's test ($P \leq 0.05$)

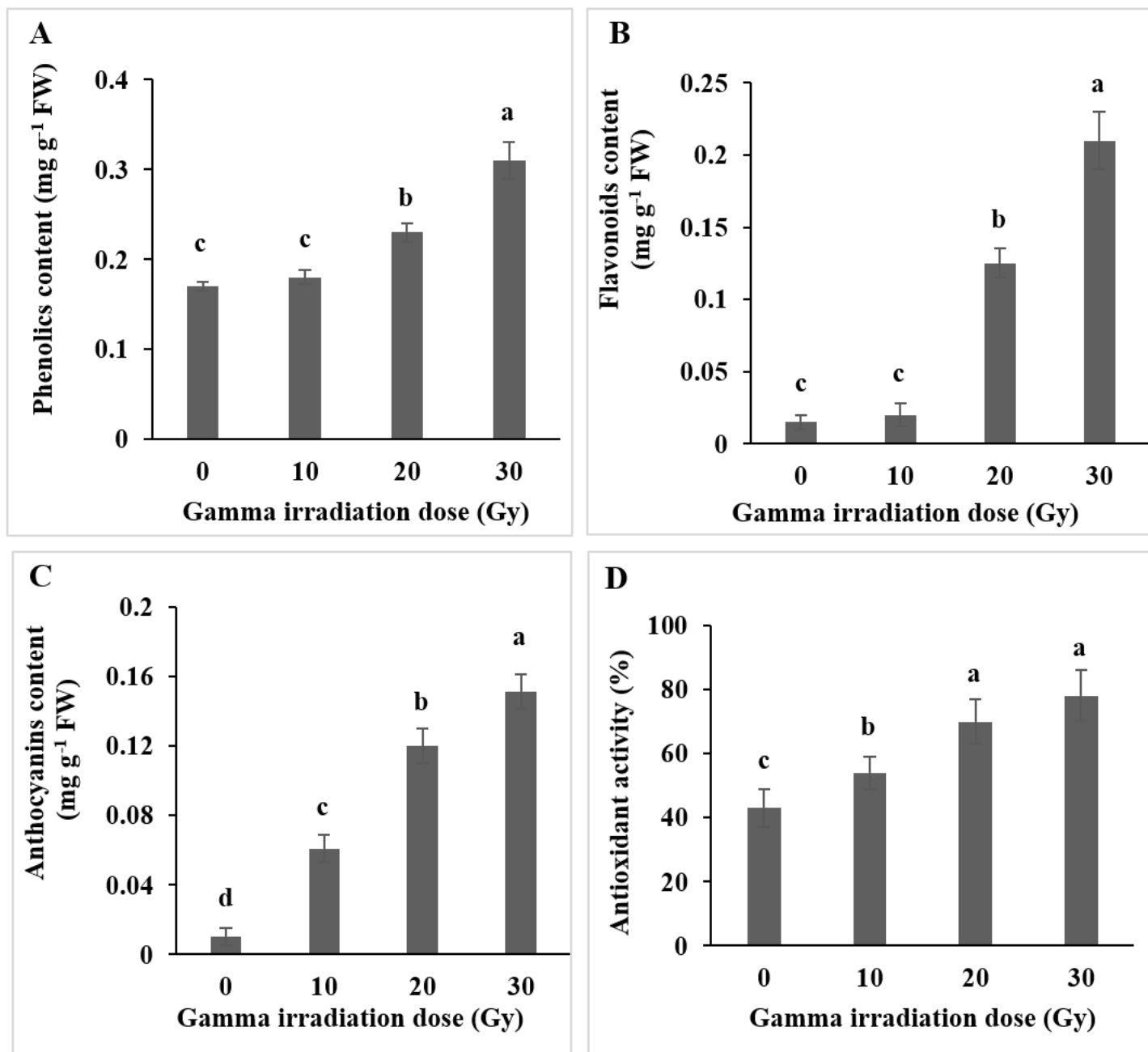


Figure 4

Content of total phenolics (A), flavonoids (B), anthocyanins (C), and antioxidant activity (D) of hazel callus as affected by γ -radiation. Data are means of 4 replicates. Means with the same letter are not significantly different according to Duncan's test ($P \leq 0.05$)

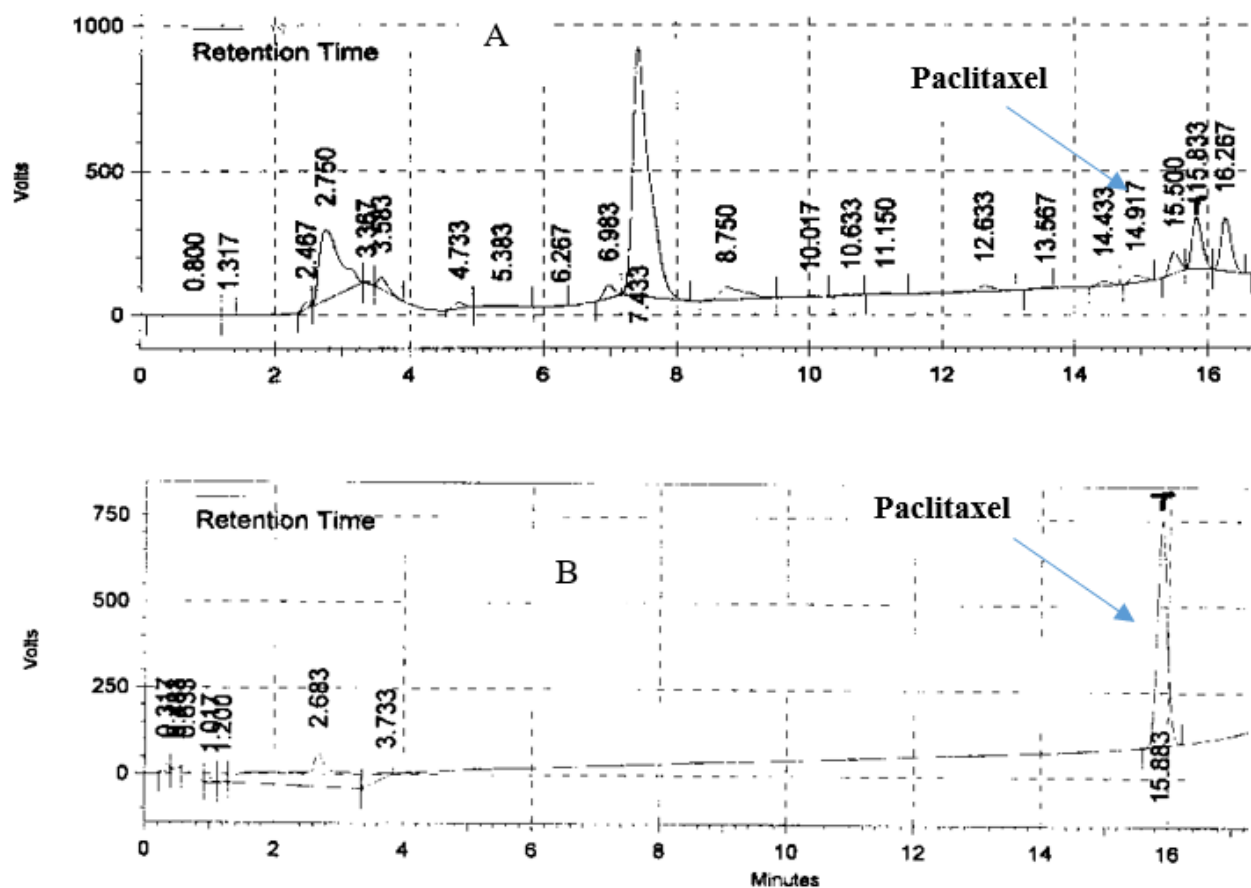


Figure 5

HPLC chromatograms of (A) hazel callus extract and (B) paclitaxel (T: taxol) standard

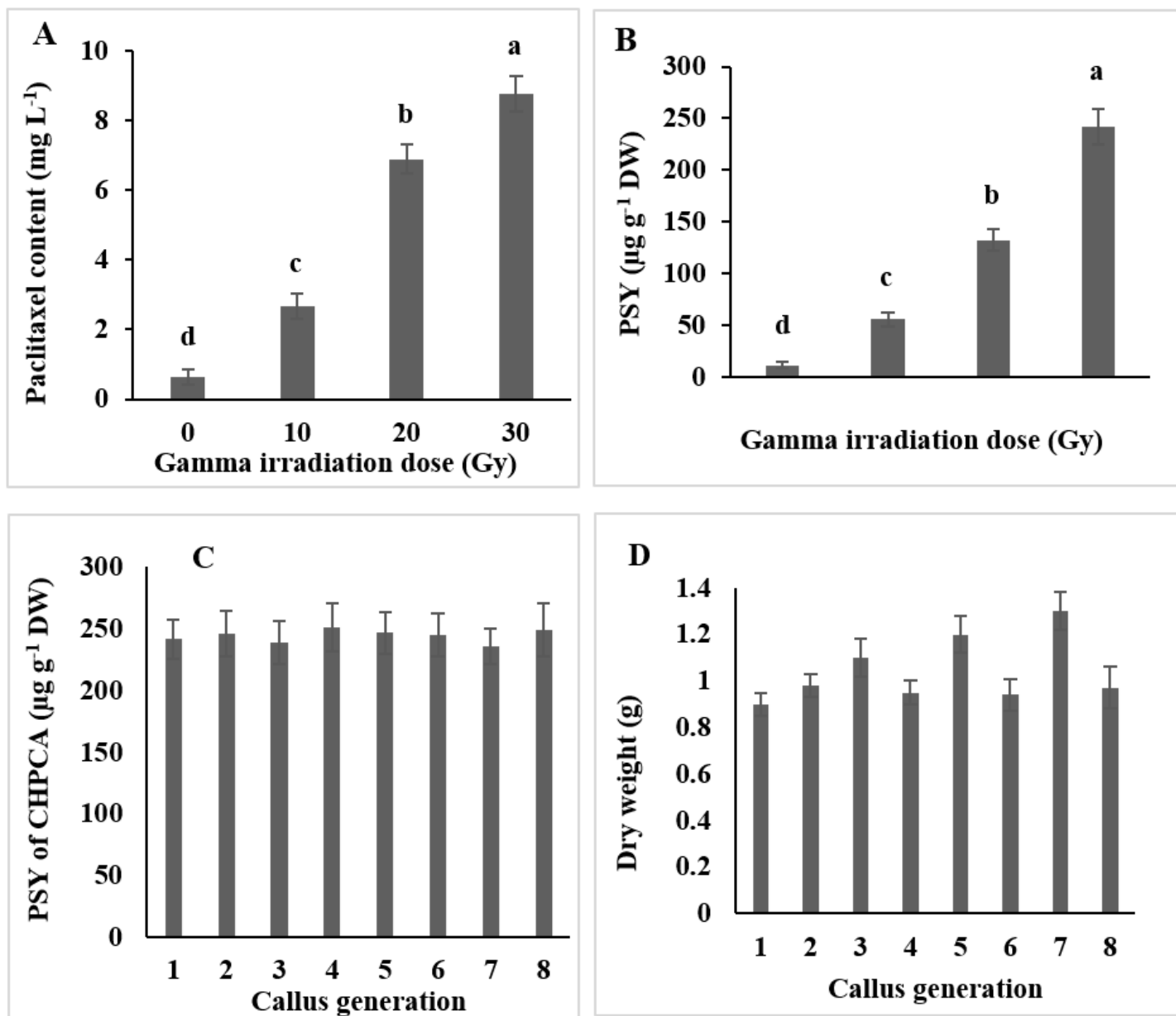


Figure 6

Paclitaxel content (A), PSY (B), new callus line (CHPCA) PSY (C) and dry biomass (D) over 8 generations of hazel callus as affected by γ -radiation. Data are means of 4 replicates

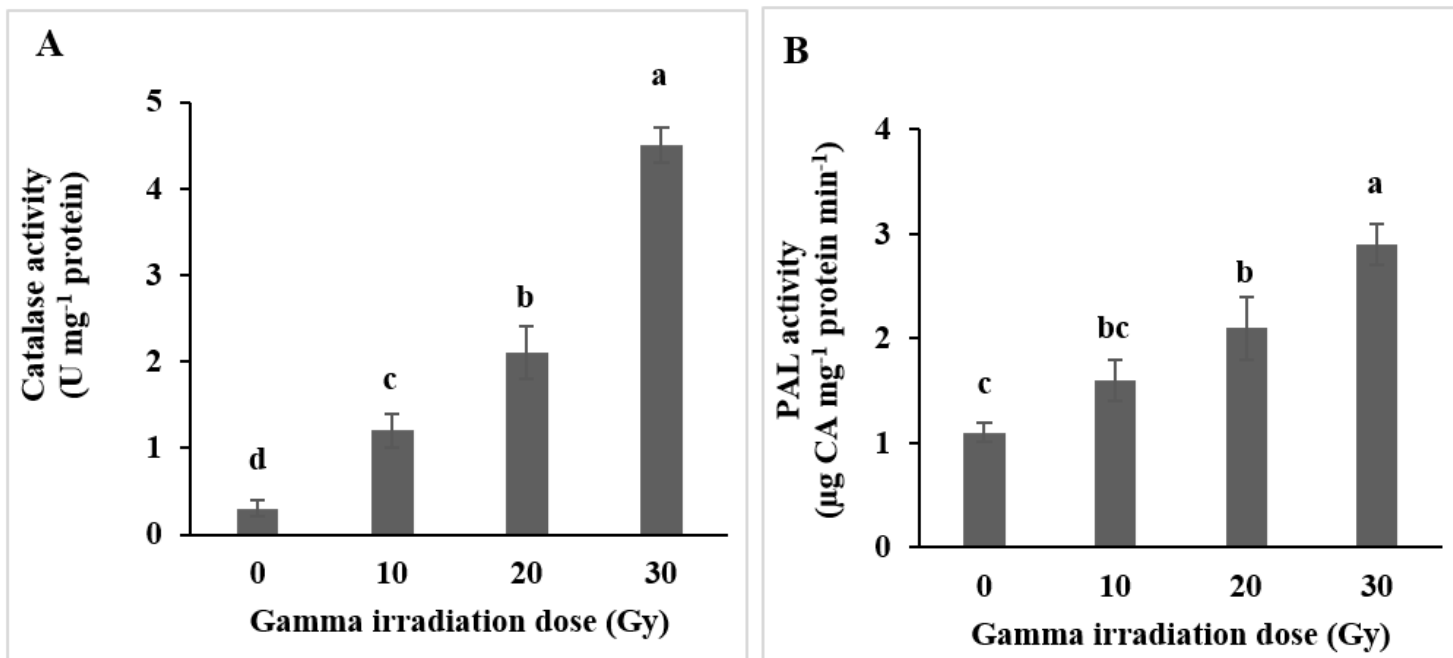


Figure 7

Catalase (A) and PAL (B) enzymes activity of hazel callus as affected by γ -radiation. Data are means of 4 replicates. Means with the same letter are not significantly different according to Duncan's test ($P \leq 0.05$)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Graphicalabstract.pdf](#)
- [Highlights.docx](#)