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Original Article

Influence of gamma irradiation on the growth, photosynthesis and phenolic content of basil (ocimum sanctum L.)

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ABSTRACT

Basil is an aromatic herb commonly used as medicine by many. Its different parts like leaves, stem, flower, root, and seeds are used for different purposes. Phenols are secondary metabolites that play an important role as plant-derived medicinal compounds and these mostly have low quantity on plants, like in Basil. High-energy electromagnetic radiation is highly used and an effective method to induce mutation and enhance secondary metabolite production in plants. Basil seeds were treated with 0 kGy, 0.1 kGy, 0.2 kGy and 0.3 kGy gamma irradiation. Li-Cor P6800 portable photosynthesis system was used to gather photosynthesis data. The highest dosage, 0.3 kGy, yielded the shortest plants at an average of 17±0.4SE mm. Exposure to gamma radiation also lowers the plant's survival by 47%. On photosynthetic responses, transpiration rate decreased at -2.80 µmol H2O m^{-2} s^{-1} as the gamma dosage was increased. However, total leaf conductance to H_2O and CO_2 increased to 0.2 kGy and 0.3 kGy at 0.01 and 0.007 mmol m^{-2} s^{-1} as the gamma dosage was increased. Plants treated with 0.3 kGy gamma radiation also showed 86% higher amounts of intercellular CO_2 (80,438.16 umol mol-1) than those not exposed to gamma irradiation. On phenolic content, the highest was those exposed to 0.2 kGy and 0.3 kGy at 200.384±5.543 mg GAE/g and 210.574±12.003 mg GAE/g, respectively. These resultant characteristics can therefore be a source of parent material for breeding.

KEYWORDS: *Photosynthetic responses, Gamma radiation, Secondary Metabolites, Physical mutagen*

1 INTRODUCTION

Ocimum sanctum L., known as 'Basil' in English, is *Ocimum sanctum* L., known as 'Basil' in English, is an aromatic herb found throughout the Philippines. It is commonly used as medicine by many, using its different parts like leaves, stem, flower, root, and seeds (Prakash *et al*., 2005). Because of Basil's popularity, the plant is often referred to as the "king of the herbs." Aside from being used as a medicine, this is also used as a spice in various dishes, making it more significant for its many uses (Bragaa *et al*., 2006).

The plants' secondary metabolites are considered unique sources for pharmaceuticals, food additives, flavors, and other industrial materials (Tiwari and Rana, 2015). Metabolites are mainly described as small molecules with various functions that would include fuel, structure, signaling, stimulatory and inhibitory effects on enzymes, as defense, and interactions with the other organisms. However, their quantity is very low (≤1% dry weight) and depends significantly on the plant's physiological and developmental stages (Facchini 2001).

Plant phenolic compounds can act as antioxidants and are usually related to defense responses in the plant. These also play an essential role in other processes, like in incorporating attractive substances to accelerate pollination and defense against herbivores. These are also known to have antibacterial and antifungal activities (Lin et al., 2016). It was reported by Juliani and Simon, (2002) that the Holy Basil has a total phenolic content equal to 51.1 mg gallic acid equivalent per gram dry weight. In fact, in the report of Wangcharoen and Morasuk, (2007), the highest total phenolic content of white and red Holy Basil was 12.60±1.02 and 19.46±1.97 mg gallic acid equivalent per gram of dry weight, respectively where it was too low than the previous studies. Enhancing the phenolic content of basil would make the plant more useful and beneficial and survive more on different stresses.

The content of the phenolic compounds can be increased through mutagenesis using gamma irradiation. Gamma rays are reported to have high-energy electromagnetic radiation that can induce mutation and enhance secondary metabolite production in plants. Thus, the plant system produces higher amounts of

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antioxidant enzymes and would increase the secondary metabolites' content like phenolic compounds. However, higher doses could damage the tissue, thus it is important to know the right dosage for every plant and plant part (Vardhan and Shukla, 2017).

Biotechnological tools, like tissue culture and plant genetic transformation, are increasingly being employed to produce high-quality and rare Plant-Derived Medicinal Compounds (PDMC) under *in vitro* conditions. This is the main reason this study employed tissue culture and gamma radiation to enhance phenolic compounds in Basil

2 MATERIALS AND METHODS

Preparation, Irradiation and Inoculation

Seeds of Basil were obtained from Cebu Technological University – Barili Campus. Five thousand seeds were used. The seeds were washed thoroughly with tap water. Afterwards, the seeds were then surface sterilized with 0.1% sodium hypochlorite for 15 minutes, followed by their immersion to 75% ethyl alcohol for 10 minutes. Lastly, the seeds were washed with sterilized distilled water three times. Surface sterilized seeds were then allowed to dry, before packing and then shipped to the Philippine Nuclear Research Institute (PNRI) for irradiation. The seeds were irradiated at different doses between 0.1 kGy, 0.2 kGy, and 0.3 kGy. Three seeds were inoculated per culture bottle on MS medium supplemented with growth hormones.

Data Gathering

Plants were observed weekly to monitor their development. The following data were obtained; (a) percent survival - this accounted as the number of germinated seeds per treatment (b) plant height - three sample plants were randomly selected per treatment per replication from which the parameter will be measured. Plant height was measured from the plant's base to the tallest part in their natural stand (c) photosynthesis rate - samples were examined for carbon dioxide, water content, light and temperature using LiCor P6800 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE, USA). Two fully expanded leaves were measured on the top leaf of the plant after one month of inoculation.

Extraction and Quantitatively Determination of Phenolic Compound

Phenolic content from gamma-treated and untreated Basil plants was extracted through the infusion method. *In-vitro* samples of Basil were soaked in a beaker containing acetone (150 mL) to remove the chlorophyll. After 12 hours, acetone was decanted from the samples. The samples were then soaked with an equal volume of methanol and stored at room temperature for 24 hours. The obtained extracts were kept in a sterile beaker for total phenolic assay. Plant extracts were subjected to UV-Vis spectrophotometer to determine total phenolic compound using Folin-ciocalteu assay (Apostol, *et al.,* 2012). The standard gallic acid solution was prepared by dissolving 10 mg of it in 10 mL of methanol (1 mg/mL). Various concentrations of gallic acid solutions in methanol (25, 50, 75, and 100 µg/mL) were prepared from the standard solution.

Statistical Analysis

The study was laid out in Completely Randomized Design (CRD) with four treatments, including the control, and replicated three times. The data will statistically analyze through Analysis of Variance (ANOVA), and a further test was done using Tukey's test at $p<0.05$ to test for differences between treatment means.

3 RESULTS AND DISCUSSION

In Figure 1, it can be clearly seen that after exposure to gamma radiation, plant height and plant survival immensely decreased. The highest dosage which is 0.3 kGy produced the shortest plants (17.67±0.40SD cm) and lowest percentage survival (33%). This result also corresponds with the result of Songsri et al., (2011) wherein the highest dosage of gamma radiation resulted the shortest height, germination, and stem diameter of physic nut and also in the study of Alvarez-Holguin, (2019) which observed that seeds with high radiation doses cannot germinate, or their seedlings cannot survive beyond a few days after 4 kGy dose. As reported by Berry (2012), shorter plants have its advantages with seed establishment, which also prevents lodging and yield loss. Increased gamma radiation doses also increase free radicals' production, leading to diminished growth and sometimes to a shortened lifespan of plants (Marcu et al., 2013). Possible inhibition of cells during the germination process could have contributed to shorter growth as cellular mass may have also been affected.

It can clearly be seen in Figure 1A that exposure to 0.1 kGy has a comparable transpiration rate with those not irradiated. On the other hand, exposure to 0.3 kGy resulted in lowest transpiration rate at -2.8 µmol H2O m-2 s -1. A critical reason for decreased transpiration might have been due to some physiological and morphological changes arising from gamma-induced mutations such as smaller stomatal openings, decrease in stomatal opening periods, the formation of thicker cuticle layers, or through modification of the boundary layers in leaves (Sterling, 2004). The lowered transpiration rate found might also be due to an accumulation of respiratory carbon dioxide in the explants' intercellular spaces. Such

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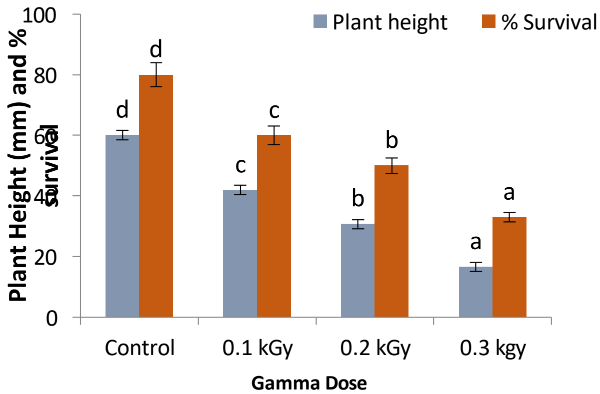
accumulation is known to lead to increased acidity, which favors starch formation from existing soluble sugars. This leads to a decrease in the osmotic pressure of the guard cells, causing a narrowing of the stomatal apertures (Santelia and Lunn, 2017).

A.

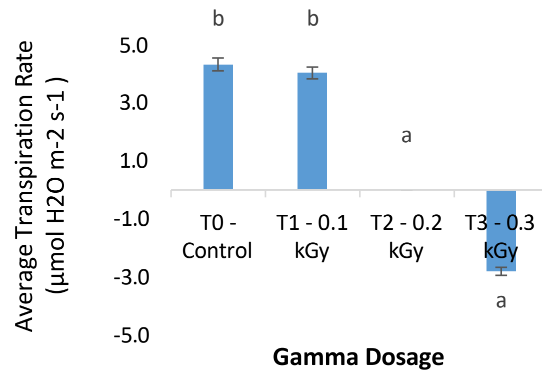


B.

Figure 1. Average plant height (mm) and percent survival of Basil after four weeks as affected by gamma radiation doses: (A) Graph of data gathered, (B) Photo of the plant hight difference between treatments. Different small superscript letters indicate significant differences, determined by tukey's test at p = 0.05

Intercellular rate however is not the reason for reduced transpiration since the highest gamma dosage of 0.3 kGy has produced the highest intercellular rate at 76159.93± 27689.99SD umol mol-1. Due to reduced CO_2 assimilation, CO_2 is not absorbed by the cell into the chloroplast. Rather, most of it remained outside of the cell at the intercellular matrix. In a review by Groszmann et al., (2017), water flux and cell permeability to CO_2 in plants can be affected by aquaporin's. According to them, it is estimated that there are 7 to 28 plasma membrane intrinsic proteins (PIPs), a subgroup of aquaporins, in flowering plants. These PIPs are of great interest as they can affect plant-water relations and photosynthesis. It might be possible that gamma irradiation of *O. sanctum* might have caused deleterious mutations on these PIPs resulting in reduced assimilation and increased intercellular CO_2. The carbon dioxide (CO_2) flows from the atmosphere to intercellular air spaces through the stomatal pore, diffuses across the wall, plasmalemma, cytosol and the chloroplast envelope, and the stroma.

A.



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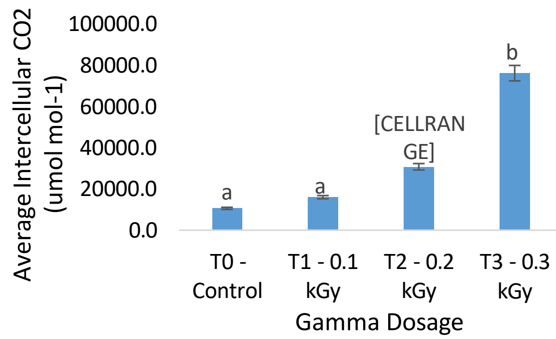

Figure 2. Photosynthetic reactions of Basil after exposure to gamma radiation: (A) Transpiration Rate (B) Intercellular CO2. Different small superscript letters indicate significant differences, determined by Tukey's test at p = 0.05.

It was exposure to 0.2 kGy followed by 0.3 kGy, that has the highest rate among the treatments in which stomatal conductance has reached 0.012± 0.003SD mmol m^{-2} s^{-1} while the total conductance is to CO_2 and H_2O is at 0.007±0.001SD mmol m^{-2} $s^{-1}$and 0.0115±0.003SD mmol m^{-2} s^{-1}, respectively. The study of Urban et al. (2017) pointed out that increased stomatal conductance at high temperatures can be advantageous to trees in increasing the photosynthesis rate. Overall, net CO2 assimilation is expected to increase to a certain extent due to increased atmospheric CO2 concentration. This increase occurs due to increased CO2 available for the Rubisco active site and decreased transpiration (Qaderi et al., 2019). Pascual et al. (2012) reported that on oil palm culture, an increased net photosynthetic rate (elevated CO and PPFD), dry weight and percent dry

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matter were 0.26 and 0.11 times higher, respectively, as compared to those cultured under ambient CO and PPFD. CO_2 and H_2O conductance are the leading indicators of the rate of gas exchange inside the leaf. It can also be used to determine the status of water vapor released and CO_2 absorption. The gas exchange rate is of vital importance to photosynthesis as it can account for photosynthetic activity and the amount of bioavailable CO_2. Increased conductance to CO_2 and H_2O can also be an underlying cause for increased stomatal conductance, or stomatal conductance may have caused an increase in the gas exchange rate. According to Xu et al. (2016), elevated CO_2 concentration in the leaf can cause an abrupt decrease in stomatal conductance in the case of trees. However, in small plants such as A. thaliana, it can still result in elevated stomatal conductance. Because of increased stomatal opening due to increased stomatal conductance, the rate of H_2O released is more likely to increase (Waring and Running, 2007).

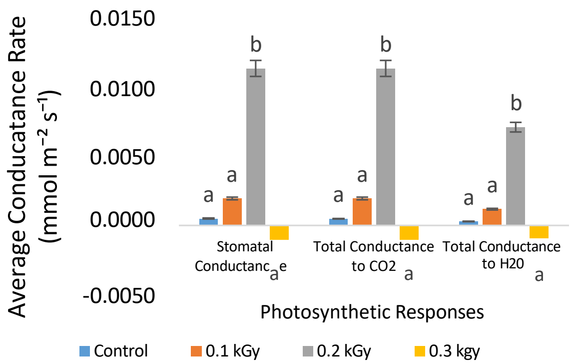

Figure 3. Photosynthetic reactions of basil after exposure to gamma radiation: Stomatal Conductance and Total Conductance to CO2 and H2O. Different small superscript letters indicate significant differences, determined by tukey's test at p = 0.05

In Figure 4, *O. sanctum* plantlets' phenolic content increased with gamma radiation exposure. In this case, 0.2 kGy and 0.3 kGy exposed plants demonstrated the highest phenolic content at 200.384±5.543 mg GAE/g and 210.574±12.003 mg GAE/g, respectively. The possibility for increased phenolic content in plants is the activation of stress pathways that can induce phenolic compounds' production. Stress in biological tissue has been known to elicit a biochemical response that results in phenolic compounds' production, resulting in increased phenolic content. This has been well documented in several plants (Boscaiu et al., 2010; Waskiewicz et al., 2013) under different stress conditions. Activation or deregulation of genes responsible to abiotic stress can also result to the induction of phenolic content production. This was supported by Madureira et al., (2020) that gamma radiation at 5 kGy can be a suitable technology for oil pomaces waste valorization, which contributes to enhancing the extraction of phenolic compounds bioactive properties.

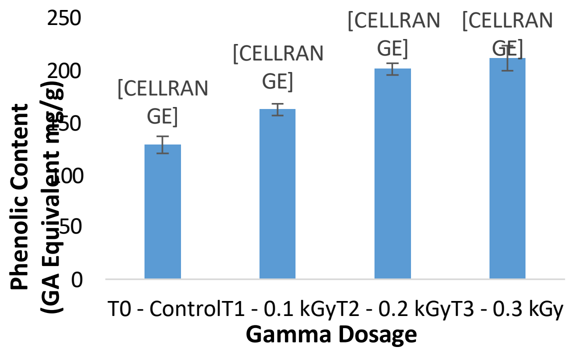

Figure 4. Phenolic content (mg GAE/g) of basil plantlets treated with different doses of gamma radiation.

Figure showed a highly significant correlation between phenolic content of *O. sanctum* and gamma irradiation dosage at (R=0.957**). Research by Harrison (2007) reported that on the effect of gamma irradiation on almond skin, this showed that the almond skin extract had increased phenolic content with an irradiation dose of 4 kGy and above. The high phenolic content in irradiation can be attributed to the release of the phenolic compound from a glycosidic component and degradation of larger phenolic compounds to smaller ones by gamma radiation (Gumus et al., 2011). This was also supported by the study of Ito et al., (2015) that the dose of 1 kGy at time 0 provides better antioxidant activity, total phenolic compounds, and some individual compounds.

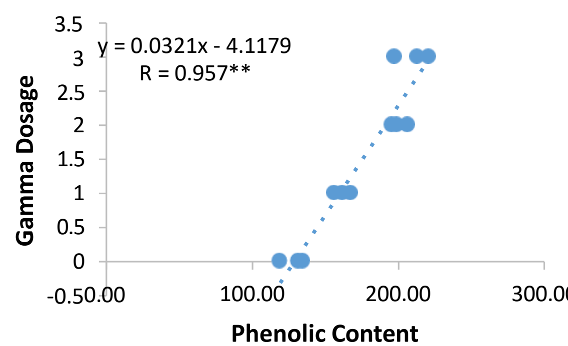

Figure 5. Correlation between Phenolic content (mg GAE/g) and gamma dosage of basil

Gamma radiation showed moderate and strong relationships with transpiration rate, intercellular CO2, and plant height at R=-0.885**, R=0.783** and R= -0.987**, respectively, while plant height and transpiration also showed strong relationship with each other at R=0.826**. Low transpiration rate resulted in shorter plant height. This decreased transpiration rate may also have contributed to the reduced growth of gamma-irradiated plants, as decreased osmotic pressure

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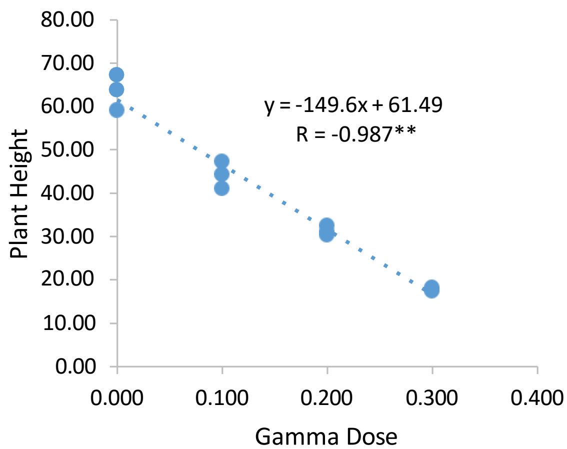
can slow down the uptake of nutrients and minerals from the growth medium to the various parts of the plant. The reduced nutrient movement hindered by reduced transpiration rate may have caused a slower replenishment of nutrients necessary for the plant's growth and development. This also aligns with lower plant height as water potential can limit the maximum height grown by plants.

It was reported by Singh et al., (2013) that at higher dose of 0.1 kGy, there was an immense reduction in flag leaf membrane stability index (MSI), photosynthesis, and nutrients like K, P, Mg, Fe, and Zn. It was then added that irradiation indicating that the negative effect of high dose (0.1 kGy) on the grain yield was caused by the adverse effect of radiation on the gas exchange, particularly photosynthesis, carbon, and nitrogen assimilation efficiency and the plant uptake of mineral nutrients thus the reduction of the plant height and survival.

A.



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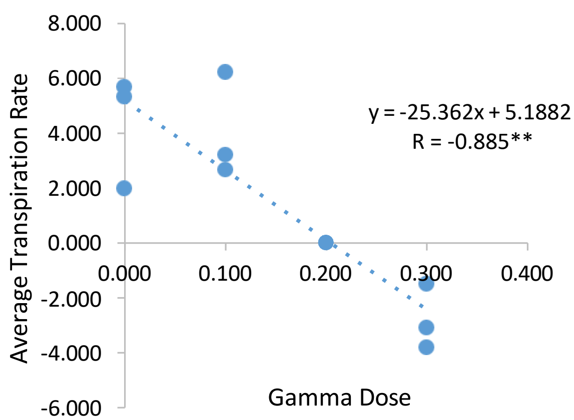

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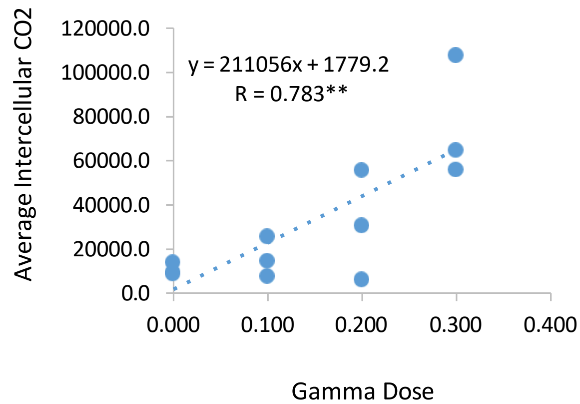

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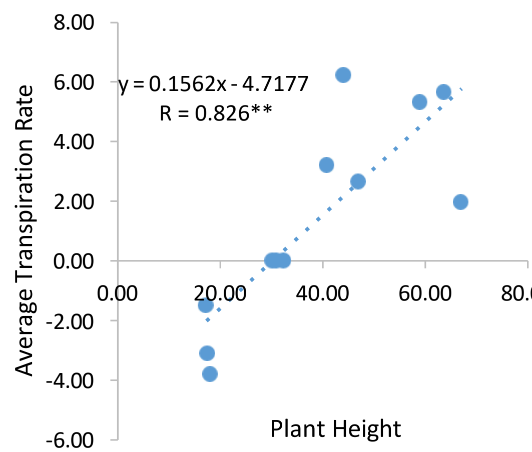

Figure 6. Correlation between gamma dosage of basil and; (A) Plant height, (B) Transpiration rate, (C) Intercellular CO2, and between (D) Plant height and Transpiration rate.

4 CONCLUSIONS

Gamma radiation is effective in enhancing the phenolic content of *Ocimum sanctum*. The treatment at 0.3 kgy demonstrated the highest phenolic content and is also the most effective among treatments used. Irradiation resulted in shorter plant height and a high mortality rate however, this still showed good characteristics, which is suitable to be a mutant variety or a parent material for a breeding program.

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realization of this study.

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