EFFECT OF X-RAY IRRADIATION ON SEED GERMINATION AND SEEDLING GROWTH OF INDIAN GOOSEBERRY (*Phyllanthus emblica* L.)

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INTRODUCTION

The cost and value of medicines and nutrient substances have risen due to the prevailing crisis in the world. Affordability has been reduced due to higher prices and the unavailability of specific medicines. Micronutrient deficiency is another global issue existing due to the prevailing condition of the world (Ritchie & Roser, 2020). It is manifested as a hidden symptom but identified as a prevailing public health issue in the world. Sri Lanka also experiences these impacts to a certain extent. Being a tropical country, Sri Lanka has a great opportunity and potential to overcome these effects by consuming nutrient-rich underutilized fruits. Indian gooseberry (*Phyllanthus emblica* L.) is one such nutrient-rich medicinally important underutilized fruit crop found in Sri Lanka. Lack of quality planting materials is one of the main causes to remain the crop as underutilized state (Mawalagedera et al., 2014). Most of the underutilized fruit seeds exhibit long-term dormancy. Seed dormancy causes reduced planting materials and seed propagation potential. Most of the available seed treatment methods (Sharma et al., 2021) are very expensive and highly technical (Nayaka, 2006). Some other treatment methods cause damage to existing plant populations (Dahanayake, 2015). To increase the fruit from its underutilized state, there should be a low-cost, easy-access, and feasible method to overcome seed dormancy to create a viable plant population. Therefore, this research aims to investigate whether radiation technology can be applied as a low-cost, easy-access, and feasible seed treatment method to break up the seed dormancy and increase the propagation potential of *Phyllanthus emblica* L. seeds. To find out the optimum radiation dose which breaks seed dormancy and the X-ray doses, which cause detrimental and stimulatory effects on seed germination, and parameters of seedling growth of *Phyllanthus emblica* L. were assessed during this study.

METHODOLOGY

Time and location: A seed sample was collected from an open market in Nawala, Colombo District, Sri Lanka from February to April 2021. The study was performed as an in-vitro study. The treated seeds were stored in a mini-greenhouse (size: 1 m x 1.5 m x 1.5 m), Colombo district and the irradiation treatment was performed using a diagnostic Computed Tomography (CT) machine, Asiri Hospitals, Colombo-05.

Experimental design: The experiment was randomly designed as a single variable factor (X-ray dose). Randomized Complete Block Design was done with 6 replications for each dose level. 16 X-ray treatment doses ranging from 0-7.5 Gy with 0.5 Gy intervals (0- control, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 Gy) were used to treat the seed samples.

Method: In order to break up seed dormancy artificially, a method was developed using 960 apparently quality seeds. The seeds were sorted using a floating test and surface sterilized using 1% sodium hypochlorite (Al-Enezi et al., 2012). The sorted seeds were arranged in labelled Petri dishes containing wet paper towels. 10 seeds were placed per Petri dish in a sterilized environment. A CT X-ray exposure device was used to irradiate the samples. Finally, the irradiated seed-containing plates and non-irradiated control samples were stored inside a mini greenhouse for germination.

Data collection and calculation: Readings and measurements were collected every week after the treatment. The readings: germinated seeds count at each dose level was observed by the naked eye and hand lens, root length, shoot length, and leaf length of the seedling were measured using a calibrated vernier caliper (accuracy 0.1 mm). Shoot length was taken from the root-shoot junction (collar region) to the cotyledon of the emerging seedling in millimeters. Root length was taken from the shoot-root junction to the tip of the longest root in a millimeter (Nayaka, 2006). Finally, germination percentage, mean germination time, and vigour index were assessed to identify the outcome of the results using the equations.

Final Germination Percentage:
FGP= (No. of seeds germinated / Total no. of seeds) x 100%

Mean Germination Time: $MGT=\sum (n*d)/ N$
(n= number of seeds germinated on each day, d= number of days from the beginning of the test, N= total number of seeds germinated in 8weeks after tretment)

Vigour Index:
VI= (average root length + average shoot length) * Germination percentage

Statistical analysis: The study was performed as a single variable factor and one-way ANOVA was carried out to find out the significant difference of the findings. N=96 data were analyzed for variance and the least significant difference at 5% standard deviation was used to calculate in the analysis process. Microsoft Office Excel 2010 was used for descriptive statistical analysis and graphical data representation. Graphical data were represented as bar charts and 2-D line diagrams. Bar charts were used to represent the final percentage germination 6 weeks after treatment. An error bar with a 5% value was used to represent errors in bar charts. Line charts were used to give informative data on the percentage of germination in each week and average seedling growth.

RESULTS AND DISCUSSION

A significant enhancement in seed germination and seedling growth was observed in response to X-ray as compared to the control (non-irradiated) sample. Percentage germination is an estimation of the viability of the population in the plant community. The percentage of seed germination is shown in the graph presented in Figure 1. Based on the analysed results, *Phyllanthus emblica* L. seeds exhibited a significant highest percentage of seed germination (56.7%) in the 2.0 Gy dose exposed seed sample (Figure 3). A significant stimulation in seed germination and seedling growth parameters were obtained within the range of 0.5-4.0 Gy dose range expose samples. A significant inhibition started from the 4.5Gy and persisted until the 7.5 Gy dose exposed plate. The highest vigour index and seedling growth parameters were observed in the 1.5 Gy dose exposed sample (Figure 2). When considering the germination time, the fastest seed germination has been shown in the 1.0- 2.5 Gy exposure dose range. Seed germination has been noted during the 1 week after treatment (WAT) while the control (non-irradiated) starts germination during the 4WAT (Figure 1). Irradiated doses above 4.0 Gy did not induce germination in *Phyllanthus emblica* L. seeds during the testing period. However, negligible single seed germination appeared in the 5.5Gy irradiated plates with an elongation. More than 50 % germination had been achieved at three dose levels at the end of the experiment. The above results obtained at doses were 1.5, 2.0, and 2.5 Gy. Germination speed T50 is the time taken to obtain 50% of germination by the total population. T50 of 1.5 Gy and 2.0 Gy dose-exposed plates were obtained in 4 WAT and 2.5 Gy plates were obtained in 5 WAT (Figure 1).

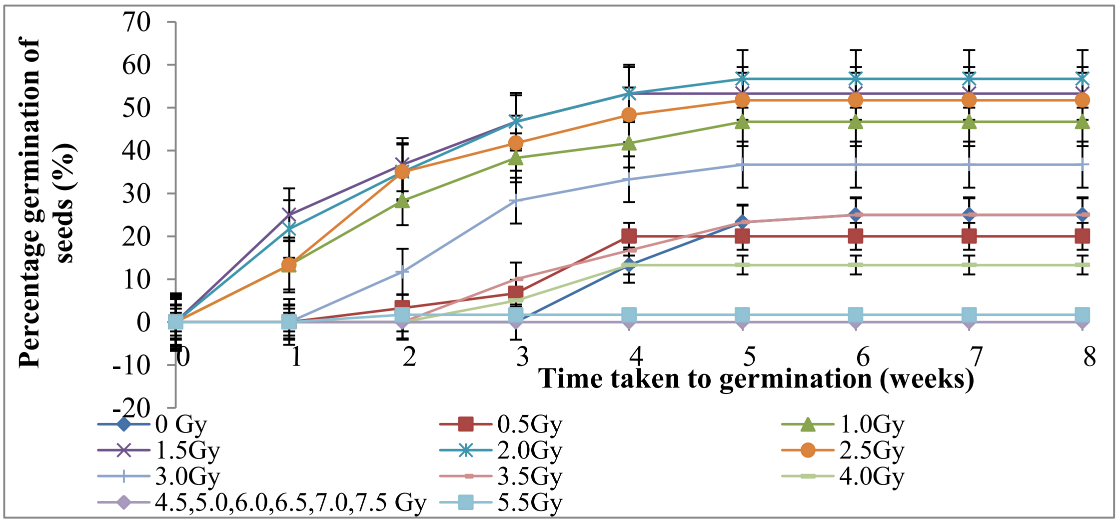

Figure 1. Effect of X-rays irradiation on seed germination percentage of P.*emblica* L. seeds.

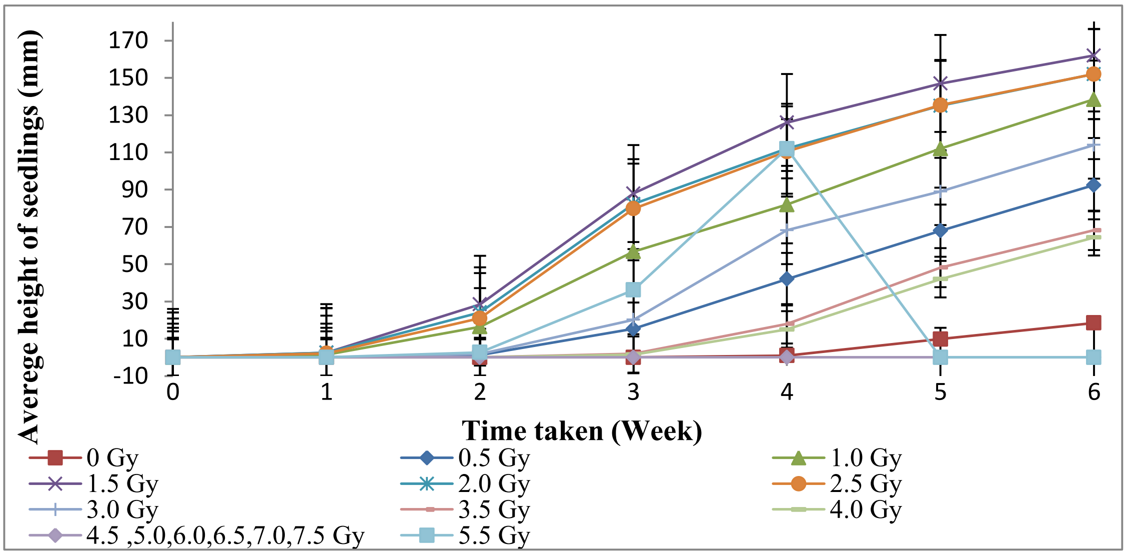

Figure 2. Effect of X-ray irradiation on seedling growth of P.*emblica* L.

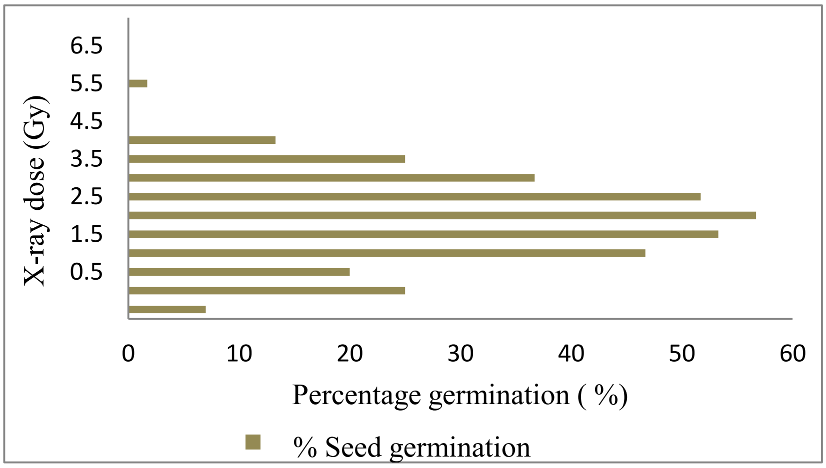

Figure 3.Final percentage germination variant (6WAT) with x-ray irradiated dose (Gy).

CONCLUSIONS/RECOMMENDATIONS

X-radiation dose from 0.5 to 4.0 Gy gives a stimulatory effect on seed germination and seedling growth parameters of P.*emblica* L.in tested conditions. The 2.0 Gy dose has been identified as the optimum radiation dose, which effectively breaks seed dormancy of the study crop. The dose above 4.0 Gy has been identified as an inhibition dose in seed germination and seedling growth of *Phyllanthus emblica* L. This observation can be suggested as irradiation stress due to radiation exposure. The selected radiation dose range of the study was not great enough to completely kill or damage the plant materials. The ionizing radiation causes vast damage and detrimental effects at higher doses and stimulatory and inducing effects on physiological processes at lower dose levels. According to the calculation of the benefit-cost ratio, the radiation treatment method was achieved the highest ratio (2.07) while comparing the existing treatment methods for *Phyllanthu emblica* L.: Gibberellic acid with seed scarification treatment, Sulphuric acid (H_2SO_4) scarification method, and tissue culture technique.

The information gained from this study may open up a pathway to develop X-irradiation treatments to break up seed dormancy and increase seed propagation of P.*emblica* L. According to the experiment, the use of X-radiation can be suggested as a low-cost and feasible pre-treatment method to obtain quality plant populations. This treatment can be applied to large-scale commercial propagation of Indian gooseberry to cater to the demand within the country.

Recommendations: This study has opened the pathway for further studies to apply this seed treatment method to overcome seed dormancy challenges in different underutilized fruit plant species. Further studies with various cultivars are essential to enhance our knowledge to find out different dose levels which stimulate germination percentage, breakup seed dormancy, and produce quality planting materials. Further study could be designed to identify the effect of X-radiation on underutilized fruit development and yield of the study crop. The study was performed specifically to find out the use of X-rays in seed germination in *Phyllanthus emblica* L. seeds. Similar treatment dose values will not be suitable for different plant species. Before applying a similar treatment method for different plant varieties, it is necessary to follow a pilot study to identify the optimum dose range which is suitable for each plant species. The reaction of different plant species to X-ray exposure can vary according to their genetic diversity.

REFERENCES

Al-Enezi, N. A., Al-Bahrany, A. M., & Al-Khayri, J. M. (2012). Effect of X-irradiation on date palm seed germination and seedling growth. *Emirates Journal of Food and Agriculture*, 24(5), 415–424.

Dahanayake, N. (2015). Some neglected and underutilized fruit crops in Sri Lanka. *International Journal of Science and Research Publications*, 5(2), 1–7.

Mawalagedera, S. M. U. P., Perera, G. A. D. and Sooriyapathirana, S. D. S. S. (2014) 'Prolonged Seed Dormancy in *Phyllanthus emblica* L. Can Be Overturned by Seed Scarification and Gibberellin Pre Treatment', *Open Journal of Forestry*, 04(01), pp. 38–41. doi: 10.4236/ojf.2014.41007.

Nayaka, D.R.G. (2006). PROPAGATION STUDIES IN AONLA (Phyllanthus emblica L.).*Indian National Agricultural Research System*

Ritchie, H., & Roser, M. (2020). Micronutrient Deficiency. In OurWorldInData.org.

Sharma, K., Singh, S. K., Mangal, S., & Sahu, K. (2021). Effect of different treatments on breaking seed dormancy in aonla (Phyllanthus emblica L .).The pharma innovation journal 10(1), 705–708.