Gamma Ray Application for Increasing Kemenyan Toba (*Styrax sumatrana*) Seed Viability

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Abstract: Kemenyan toba (*Styrax sumatrana*) is the identity trees for Tapanuli community in North Sumatra, Indonesia. These non-timber forest products have been cultivated for generations. Over the past decade, the incense productivity have been decreasing due to the lower interest of farmer to cultivate kemenyan. The another reason also causing by longer germinate time so that the farmers difficulty obtaining high quality seedlings for replanting the old unproductive trees. Accelerate germination time through improving seed viability and genetic engineering were prospective ways for increasing kemenyan productivity. The objective of this research was to determine the effectiveness of gamma irradiation techniques application for increasing the viability of Kemenyan toba seeds. Randomized completely design, with 5 levels of radiation dose those were, control 0 Gy, 10 Gy, 20 Gy, 30 Gy, and 40 Gy with 4 replications was used in this research. The results showed that increasing the intensity of irradiation shortens the germination time. The highest germination rate occurred on day 61 on the intensity of irradiation 40 Gy whereas the control was day 197. The treatment of irradiation affected the germination. However, increasing irradiation intensity decreasing the sprouting ability. On seeds without irradiation, sprouting percentage reach an average of 83.8%. The germination rate was not different compared to the seeds that received irradiation treatment with intensities of 10 and 20 Gy with sprout power of 75.0% and 57.5%. It means that the low dose of gamma ray irradiation can be used to increase the viability and vigor of Kemenyan seeds.

1 INTRODUCTION

One of valuable non-timber forest product that has a long history and become the main community livelihood in Tapanuli region, North Sumatra is Kemenyan rosin. Historically and economically this commodity has been cultivated from *Styrax* spp trees for a long time and is a major source of regional income (Kholibrina *et al.*, 2018). BPS Sumut (2018) reported that incense production reaches 5,661.39 tons every year. It mean that if the price of incense at the farmer level reaches Rp 200 thousand/kg, the farmer will resulting around Rp1.2 trillion of income every year from kemenyan forest management.

Although valuable comodity, the sustainability of kemenyan production were constrain by some problems. In the last decade, the population of incense trees has declined due to logging and forest conversion (Susilowati *et al.*, 2018). The low willingnes of farmer to replant their unproductive trees also contribute the lower rosin production. Furthermore, *Styrax* seed also takes a long time for germinate. BPS North Sumatra (2018) states that kemenyan rosin productivity has been decline from 6,060.89 tons/ha in 2008 to 5,661.39 tons/ha in 2017. Therefore, it is necessary rapid effort to increase the rosin productivity in North Sumatra, one of which by improving seed quality.

The utilization of high quality seeds is the starting point of a stand development and improving kemenyan rosin quality. These efforts can be started from the seed stage, through increasing seed viability and vigor. Seed viability and vigor determine seed quality both physically and physiologically. Seed quality improvement can be conducted by gamma ray irradiation techniques (Piri *et al*., 2011). Aplication the irradiation techniques to improve vigor and seed quality has been carried out on agricultural crops, but in forest plant seeds are still limited (Iglesias-Andreu *et al*., 2012). In trees species, the application of gamma ray radiation at low doses can improve seed germination and seedling growth (Iglesias-Andreu.,

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2012; Akshatha *et al.*, 2013). Chan & Lam (2002) also reported that irradiation of papaya seeds with a dose of 10 Gy increased the germination percentage to 50%. Zanzibar & Witjaksono (2011) reported that irradiation of Suren seeds (*Toona sureni*) using low doses (5 Gy) able to increase the seedling growth rate. The plant sensitivity to radiation treatment depends on many factors, such as species or varieties, plant parts and the radiation doses (Esnault 2010; de Micco *et al*., 2011). The objective of this study was to determine the effectiveness of gamma ray irradiation techniques to increase the seed viability of Kemenyan toba (*Styrax sumatrana*).

2 METHODS

The material of this research was kemenyan seeds, and germination media. Kemenyan toba seeds for the research was obtained from Humbang Hasundutan district, North Sumatra Province. The gamma ray irradiation dose used were 0 Gy (control), 10, 20, 30, and 40 Gy. The Gamma irradiation was conducted at the National Atomic Energy Agency (BATAN), while seed viability and vigor testing were conducted in greenhouse in Environment and Forestry Research and Development Institute of Aek Nauli in Lake Toba region, North Sumatra.

Randomized completely design with irradiation dose treatments was used in this research. The treated seeds was germinated in polybags 12x17cm in size using sterilized mixture of soil sand media (1:1/v:v). The number of seeds sown for five treatments with 4 replications and 20 observation units reached 400 seeds. Observation of germination is carried out every day since being planted until no seeds germinate. The observation variables include days of germination which ae marked by the appearance of the radicles, germination rate, seedling height, diameter, branches and number of leaves. Data were analysed using analysis of variance to determine variation between treatments. If there were variations, the analysis was continued with Duncan's Multiple Distance Test (Duncan 's Multiple Range Test - DMRT).

3 RESULT AND DISCUSSION

The results of variance analysis for germination day, germination rate, diameter and height's seedlings, branching and number of leaves on irradiated seeds are shown in Appendix 1, Table 1 and Table 2.

Table 1: Analysis of variance for the observation variables of duration and germination rate.

Treatments	Germination days	Germination rate
Control	197^{a}	83.75^{a}
10 Gy	102^{b} (-48%)	75.00^{a} (-10%)
20 Gy	68^{b} (-65%)	57.50^{ab} (-31%)
30 Gy	66^{b} (-67%)	25.63^{bc} (-69%)
40 Gy	61^{b} (-69%)	4.15^{c} (-96%)

The irradiation treatment affects the rate of germination. Increasing the intensity of irradiation shortens the germination time. Based on Table 1, the normal seed (non treatment) starts the germination on 197th day. The shortest germination time occurred on day 61 at the intensity of irradiation of 40 Gy, thus reducing the germination time of 69% from normal time. The 40 Gy dose also gives the highest seedling high variable of 14.45 cm (Table 2). However, this was not followed by an increase in germination rate which decreased to 96%.

Table 2: Variance analysis for the seedling diameter, height, branches and number of leaves.

	Diameter (mm)	Height*	Branches	Number of leaves*
Control	34^{a}	157^{a}	2.3^{a}	10^{ab}
10 Gy	30^{a}	103^{b} (-35%)	2.6^{a}	8^{abc} (-26%)
20 Gy	29^{a}	105^{b} (-33%)	2.2^{a}	6^{c} (-38%)
30 Gy	29^{a}	116^{ab} (-25%)	1.4^{a}	7^{bc} (-34%)
40 Gy	35^{a}	145^{ab} (-7.7%)	2.5^{a}	11^{a} (5.33%)

Generally, application of gamma irradiation on Kemenyan seeds provides two response those were: supporting and inhibiting germination. The irradiation treatment affects the speed of germination. Increasing the intensity of irradiation will shortens germination time. According to Zanzibar (2015), the irradiation technique is an ionic process, when ionizing radiation is absorbed into biological material, the radiation will act directly on the critical target cells or indirectly through the generation of metabolites which can modify important cell components. According to Luckey (1980) irradiation at low doses can stimulate the physiological process (radiostimulation) of plants through excitation or known as hormesis. The influence of hormesis on various agricultural crops species can provide a positive or beneficial response (Luckey, 2003; Piri *et al*., 2011).

The germination rate decreases with increasing irradiation intensity. Based on Tukey HSD and Duncan test, there were three groups of responses (Table 1). On seeds without irradiation, percent germinate reached 83.8%. The germination rate was not different with another treatment (intensities of 10

and 20 Gy with germination rate of 75.0% and 57.5%).

Increased doses of up to 40 Gy reducing sprouting ability to 4.1% or decrease to 95.5% (Table 2). Therefore, the dose for kemenyan seeds should be lower than 40 Gy. Zanzibar (2015) states that the dose level application and its effect on seed germination varies for each species and genotype. But in general, higher irradiation doses tend to inhibit germination. Habba (1989) reported that an increase in irradiation doses of up to 100 Gy increased seed germination gradually, but then seed germination decreased with increasing irradiation doses. A high irradiation dose causes higher cell damage because the energy released by gamma rays is quite large and penetrates deeply. The amount of damage to cells cause's lower opportunities for survive (Hapsari, 2004). Wulandari (2003) on Chrysanthemum found that increasing gamma ray irradiation doses of 10, 15, 20 to 25 Gy decreased the percentage of plant life.

The germination phase of all seeds shows a natural response as well as germination of kemenyan without irradiation (control). The new response can be seen in the seedling phase which is marked by changing the shape of the leaves to wavy to curly and the large number of branches (Figure 1). Changes in plant morphology are common and are most easily seen from the irradiation of plants. The diversity due to gamma ray irradiation is most commonly found in leaf pinnate, both in terms of colour and shape. The diversity of seedling morphology due to irradiation is characterized by the occurrence of abnormalities or malformations of plant organs. Hartati (2000) states that irradiation treatment will cause cell damage or inhibition of cell metabolism due to interference with RNA synthesis so that the synthesis of enzymes needed for growth is inhibited. This phenomenon might be resulting enzyme to lose its function. The irradiation treatment can cause enzymes that stimulate growth to become inactive. Soeranto in Herison (2008) states that the occurrence of abnormalities in irradiated populations shows that there have been changes at genomes level, chromosomes, and DNA or genes that are very large so that genetically controlled physiological processes in plants become abnormal and cause variations in new genetic variations. Abnormalities to plant irradiation deaths are caused by the formation of free radicals such as Ho, which are highly labile ions in the reaction process due to irradiation. It resulting in many collisions in various directions, which consequently will make changes or mutations at the DNA, cell and tissue levels and organs, even causing death in plants. Abnormalities began to appear since the leaves of the irradiated plants began to develop, at 47 HST. Abnormalities occur in the shape of leaves in plants for all irradiation doses

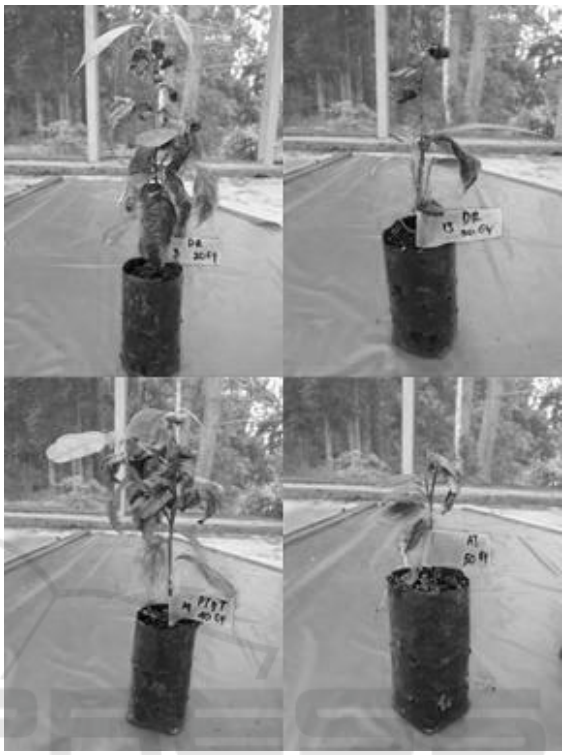

Figure 1: The changes of the leaves shape and number of branches.

Furthermore, irradiation affects the number of leaves. The number of leaves in normal conditions reaches an average of 10 sheets. The number of leaves decreased after the seed was irradiated at an intensity of 10 Gy to 8 sheets. But statistically the response of the two treatments is the same. In seeds with irradiation intensity of 20 and 30 Gy the number of leaves decreases to 6 and 7 consecutive leaves. But the increase in intensity to 40%, the number of leaves increased to 11 sheets. Marcu et al (2012) on *Lactuca sativa* plants, showed that the effective dosage for increasing germination was not more than 30 Gy. While at doses above 70 Gy, the vegetative part of plant growth began decrease.

The irradiation treatment affects plant height. In seeds without irradiation treatment, the response of height reached 15.6 cm. At dose of 40 Gy the response of the plants average height decreases to 14.45 cm. This high response is not statistically different with the seeds with irradiation intensity 30 Gy. Conversely, irradiation treatment does not affect stem diameter and number of branches.

4 CONCLUSIONS

Our research point out that, increasing the intensity of irradiation reducing the germination time from 197 days (without treatment) become 61 days (dose 40 Gy). Increasing irradiation intensity also decreasing the sprouting ability from 83.8% (without treatment) to 75.0% and 57.5% (dose 10 and 20 Gy). The irradiation also affects the number of leaves. The number of leaves was 10 sheets (without treatment) but decreases after irradiated at an intensity of 10 Gy to 8 sheets. The irradiation treatment also affects plant height. But statistically the response of the two treatments is the same. Conversely, irradiation treatment does not affect stem diameter and number of branches.

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APPENDIX

ANOVA Germination days

Source	Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	52483.5[a]	4	13120.9	11.69	.000
Intercept	195881.4	1	195881.4	174.59	.000
Perlakuan	52483.5	4	13120.9	11.69	.000
Error	16829.0	15	1121.9		
Total	265193.9	20			
Corrected Total	69312.6	19			

a. R Squared = .757 (Adjusted R Squared = .692)

Post Hoc Tests of Germination days

	Treatment	N	Subset	
			1	2
Tukey HSD[a,b]	5	4	61.2250	
	4	4	65.9250	
	3	4	68.4500	
	2	4	101.9500	
	1	4		197.2750
	Sig.		.452	1.000
Duncan[a,b]	5	4	61.2250	
	4	4	65.9250	
	3	4	68.4500	
	2	4	101.9500	
	1	4		197.2750
	Sig.		.133	1.000

The error term is Mean Square(Error) = 1121.936.
a. Uses Harmonic Mean Sample Size = 4.000.
b. Alpha = 0.05.

ANOVA of Germination rate

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	21137.6[a]	4	5284.4	7.7	.001
Intercept	52439.0	1	52439.0	76.4	.000
Perlakuan	21137.6	4	5284.4	7.7	.001
Error	10288.5	15	685.9		
Total	83865.2	20			
Corrected Total	31426.2	19			

a. R Squared = .673 (Adjusted R Squared = .585)

Post Hoc Tests of Germination rate

	T	N	Subset		
			1	2	3
Tukey HSD[a,b]	5	4	4.1500		
	4	4	25.6250	25.6250	
	3	4	57.5000	57.5000	57.5000
	2	4		75.0000	75.0000
	1	4			83.7500
	Sig.		.073	.107	.331
Duncan[a,b]	5	4	4.1500		
	4	4	25.6250	25.6250	
	3	4		57.5000	57.5000
	2	4			75.0000
	1	4			83.7500
	Sig.		.264	.106	.082

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 685.900.
a. Uses Harmonic Mean Sample Size = 4.000.
b. Alpha = 0.05.

ANOVA of Diameter

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.228[a]	4	.307	1.398	.282
Intercept	198.671	1	198.671	905.224	.000
Perlakuan	1.228	4	.307	1.398	.282
Error	3.292	15	.219		
Total	203.190	20			
Corrected Total	4.520	19			

a. R Squared = .272 (Adjusted R Squared = .077)

ANOVA of Height

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	93.973[a]	4	23.493	3.220	.043
Intercept	3125.625	1	3125.625	428.430	.000
Perlakuan	93.973	4	23.493	3.220	.043
Error	109.433	15	7.296		
Total	3329.031	20			
Corrected Total	203.406	19			

a. R Squared = .462 (Adjusted R Squared = .319)

Post Hoc Tests of Height

	T	N	Subset	
			1	2
Tukey HSD[a,b]	2	4	10.2500	
	3	4	10.5250	
	4	4	11.6250	
	5	4	14.4500	
	1	4	15.6562	
	Sig.		.080	
Duncan[a,b]	2	4	10.2500	
	3	4	10.5250	
	4	4	11.6250	11.6250
	5	4	14.4500	14.4500
	1	4		15.6562
	Sig.		.060	.063

The error term is Mean Square(Error) = 7.296.
a. Uses Harmonic Mean Sample Size = 4.000.
b. Alpha = 0.05.

Table ANOVA of Number of Leaves

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	67.022[a]	4	16.755	3.271	.041
Intercept	1409.521	1	1409.521	275.127	.000
Perlakuan	67.022	4	16.756	3.271	.041
Error	76.848	15	5.123		
Total	1553.390	20			
Corrected Total	143.869	19			

a. R Squared = .466 (Adjusted R Squared = .323)

Table Post Hoc Tests of Number of Leaves

	T	N	Subset		
			1	2	3
Tukey HSD[a,b]	3	4	6.3750		
	4	4	6.8500		
	2	4	7.6000		
	1	4	10.3000		
	5	4	10.8500		
	Sig.		.085		
Duncan[a,b]	3	4	6.3750		
	4	4	6.8500	6.8500	
	2	4	7.6000	7.6000	7.6000
	1	4		10.3000	10.3000
	5	4			10.8500
	Sig.		.480	.058	.072

The error term is Mean Square(Error) = 5.123.
a. Uses Harmonic Mean Sample Size = 4.000.
b. Alpha = 0.05.

ANOVA of Number of Branch

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.282[a]	4	.821	1.485	.256
Intercept	96.141	1	96.141	173.972	.000
Perlakuan	3.282	4	.821	1.485	.256
Error	8.289	15	.553		
Total	107.713	20			
Corrected Total	11.571	19			

a. R Squared = .284 (Adjusted R Squared = .093)