# Gamma Rays Radiation on Germination and Growth of *Sandoricum koetjape* Seedlings

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**Abstract.** Kecapi or santol fruit (*Sandoricum koetjape*) is currently very rare. Many factors are suspected of causing this crop scarcity, i.e, low economic value, less attractive because it quickly turns brown and land conversion. Kecapi contains antioxidants, beta carotene, polyphenols, sentulic acids and high pectin. This study aims to determine the effects of gamma radiation on the germination and growth of two kecapi seedling accessions. The experiment was conducted using a Split-plot Randomized Block Design with three replications. The treatments consisted of two kecapi accessions (Yellow and Green) and seven doses of gamma ray irradiation (0, 10, 20, 30, 40, 50, and 60 Gy). The results showed that the interaction between kecapi accession and radiation dose significantly affected the percentage of viability and seedling height in the nursery. Kecapi accession did not significantly affect viability or growth rate. However, the radiation dose significantly affected the percentage of viability, and the dose of 60 Gy produced the highest percentage. Radiation significantly suppressed seedling growth; higher radiation doses resulted in shorter seedling growth. The leaf colour was generally categorised as scale 5G (green-yellow) based on the colour chart observation. The radiation resulted in shorter stomata than the control. The stomata density was 476.4 to 985.8 stomata $mm^{-2}$.

## 1 Introduction

Indonesia is a country with high biodiversity, including a wide variety of fruit species. Research on Indonesian indigenous fruits conducted by the Horticultural Research and Development Center in the context of fruit domestication and commercialization recorded approximately 253 fruit species with the potential to be developed in Indonesia, one of which is kecapi [8]. Kecapi (*Sandoricum koetjape*), also known as santol fruit, is estimated to have originated from Indochina and the Malay Peninsula centuries ago [3]. Kecapi has considerable potential for further development due to its various functions and health benefits. Kecapi contains antioxidants such as beta-carotene and flavonoid bioactive compounds in relatively high amounts, reaching 6.5 millimhos per 100 g of

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fresh fruit [5]. Kecapi belongs to the Meliaceae family and is generally consumed fresh. The fruit has a sweet-to-slightly-sour taste. In several regions, kecapi is processed into jam, candy, and syrup. Another form of utilization is the production of fermented alcoholic beverages using rice. In Malaysia, young kecapi fruits are used as raw materials for candy production because they are rich in pectin [3]. According to a survey of fruit sellers in Jakarta, kecapi is rarely sold because it is less popular than imported fruits, which often have higher market value. The availability of kecapi at fruit stalls is generally limited and depends on the presence of local trees in the surrounding area. The existence of kecapi, a local fruit species, is increasingly rare; therefore, conservation and preservation efforts are necessary. Exploration and conservation are efforts to collect genetic resources by providing genetic material [6 & 23]. Furthermore, plant improvement programs can be carried out by evaluating and utilizing the existing characteristics of these genetic resources. The development of superior varieties requires access to diverse genetic resources. Germplasm collections must be maintained and enhanced in line with the need for variety development to enrich genetic reserves, and should be conserved ex situ to facilitate maintenance, evaluation, protection, and utilization [12].

One approach to providing genetic material is through seed propagation by improving seed viability and vigor. Seed viability and vigor determine seed quality, including both physical and physiological characteristics. Radiation treatment affects plant growth and development by increasing enzyme activity and inducing genetic, biochemical, cytological, physiological, and morphological changes in cells and tissues [21]. Gamma-ray radiation can also induce mutations, which play an important role in increasing genetic diversity and improving plant characteristics and productivity [18]. Biochemical changes induced by radiation affect cellular metabolic processes, which at certain levels can break down germination-inhibiting compounds and stimulate cell division. Consequently, radiation treatment not only influences germination but also affects seedling growth [21]. Sensitivity to radiation treatment depends on several factors, including plant species or variety, plant part, and radiation dose [9]. Therefore, testing different accessions, plant parts, and radiation doses is necessary to obtain optimal results. The aim of this study was to determine the germination and growth characteristics of two kecapi accessions (*Sandoricum koetjape*) treated with gamma-ray radiation. The data obtained from germination and growth observations are expected to provide useful information for breeding programs and the development of superior seeds.

## 2 Materials and Methods

This research was conducted at the Green House Bambu Kuning, Faculty of Agriculture, Universitas Nasional, Jl. Bambu Kuning, Jati Padang, Pasar Minggu, Jakarta Selatan. Implementation of seed radiation was carried out at the Research and Development Center for Isotope and Radiation Technology, National Nuclear Energy Agency (BRIN/P3TIR BATAN). Meanwhile, the stomatal anatomy observations were carried out at the Agronomy Laboratory for Evaluation of Biotechnology Products, Center for Biotechnology BRIN Cibinong, Bogor. The materials used in this study were seeds from two accessions of kecapi, which are kecapi kuning (yellow color) and kecapi hijau (green color) (**Fig. 1**), and planting media (sand, soil, and compost). Meanwhile, the tools used in this research were plastic for polybags, pots, treatment labels, measuring cups, a digital thermo-hygrometer, the Munsell Plant Tissue Color Book, and Nikon microscopes.


**Fig 1.** Yellow and green kecapi (moss green) fruits

This study was arranged in a Split-plot Randomized Block Design with three replications. The first factor was the accession of kecapi, which were yellow and green. The second factor was the dose of gamma rays radiation in units of Gray (Gy), which consisted of 7 levels, namely 0,10, 20, 30, 40, 50, and 60 Gy. The kecapi seeds were obtained from kecapi fruit from the area of Kebagusan, South Jakarta (yellow accession) and Nambo Village, Bogor Regency (green accession). Both types of kecapi accessions were extracted by separating the pulp from the seeds. After separation from the flesh, the seeds were washed with running water and then air-dried for 5-7 days. The dried seeds were then selected based on their size, which ranged from 1 to 1.5 cm. After that, the seeds were ready to be irradiated with gamma rays from the GammaCell-220 irradiator.

The germination medium used in this study was pasir malang (lava rock) at 150 g per plastic container. The media were sterilized by steaming for 1 h and subsequently air-dried. Irradiated kecapi seeds were then germinated on the sterilized media. The seedbeds were watered daily, and observations were conducted daily from planting until 50 DAP (Days After Planting). Parameters observed during the germination phase included seed viability, germination speed, sprout/seedling morphology, and leaf color. Seed viability was determined based on the percentage of normal germinated seeds using the following equation:

$$Seed\ Viability\ (\%) = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ planted}\ x\ 100$$

The germination speed was calculated according to the following formula:

$$\text{Germination Speed} = \sum \frac{Di}{Ni}$$

where N$i$ is the number of germinated seeds on day $i$, and D$i$ is the observation day after planting.

At 50 DAP, the seedlings were transplanted into pots containing a planting medium composed of soil, sand, and compost at a ratio of 2:1:1 (300 g per pot). Seedlings were maintained for 15 WAP (Weeks After Planting) through daily watering and manual weed removal. Biofarm liquid fertilizer was applied at a dose of 250 mL per plant. Observations during the seedling phase were conducted every week until 15 WAP and included seedling height, stem diameter, number of leaves, leaf color, and stomatal anatomy. Seedling height was measured using a ruler from the stem base to the shoot tip, stem diameter was measured using a caliper, and the number of leaves was determined by counting fully opened leaves.

# 3 Results and Discussion

## 3.1 Kecapi seed germination

Seed germination aims to determine the effectiveness of gamma-ray radiation in increasing seed viability in two accessions. Based on the position of the cotyledons to the surface of the germination media, kecapi seeds have an epigeal germination type, which is the cotyledons that are lifted above the surface of the media. Analysis showed that kecapi accession treatment had no significant effect on seed viability or growth rate (Table 1). It was suspected that radiation treatment could stimulate germination, so the accession of the yellow kecapi, in general, resulted in seeds with viability and growth rates that were not significantly different.

**Table 1.** Effect of kecapi accession on the viability and growth rate of the seed

| Kecapi Accession | Seed Viability (%) | Growth Rate KN/etmal |
|---|---|---|
| Yellow | 59.87a | 0.25a |
| Green | 56.12a | 0.20a |

*Note: The average number followed by the same letter in the same column indicates no significant difference based on the 5% BNT test.*

The low germination of kecapi in this study was presumably due to the seeds being classified as recalcitrant, with high water content; they could not survive a relatively long storage period. Kecapi seeds are also difficult to store because their high water content can lead to contamination by microorganisms and, ultimately, cause damage and deterioration. [25] stated that kecapi seeds can be classified as intermediate or recalcitrant seeds because the viability of the seeds can be maintained up to >30% moisture content. The gamma-ray radiation dose treatment had a significant effect on seed viability, but not on seed growth rate (Table 2). This result indicates that gamma-ray irradiation affected the ability of seeds to germinate. However, the seedling growth rate remained relatively similar across treatments.

**Table 2.** Effect of radiation dose on the viability and growth rate of the seed

| Radiation Dose (Gy) | Seed Viability (%) | Growth Rate (Kn/Etmal) |
|---|---|---|
| Control | 46.43c | 0.19a |
| 10 | 64.29ab | 0.24a |
| 20 | 54.76bc | 0.27a |
| 30 | 59.52b | 0.25a |
| 40 | 50.00bc | 0.18a |
| 50 | 57.14bc | 0.20a |
| 60 | 73.81a | 0.25a |

*Note: Numbers followed by the same letter in the same column indicate no significant difference based on the 5% LSD test*

The viability of irradiated seeds was significantly lower than that of untreated (control) seeds. Radiation doses above 30 Gy showed higher viability than the control (46.43%), and a dose of 60 Gy produced the highest viability (73.81%). This is in line with the research by Bramasto et al. [7], which found that gamma-ray irradiation of forest plant seeds at 60 Gy resulted in the greatest increase in viability, ranging from 13.5% to 50.9% compared to the control. The interaction treatment between kecapi accession and radiation dose significantly affected germination power (Table 3). In contrast, the interaction effect on seed germination speed was not significant (Figure 2). This finding suggests that the response of seed germination characteristics to gamma irradiation may vary across accessions, especially for seed viability parameters.

**Table 3.** Interaction of kecapi accession with radiation dose on seed germination power

| Radiation Dose (Gy) | Germinating Power of Seeds | |
|---|---|---|
| | Yellow | Green |
| Control | 57.14ba | 35.71db |
| 10 | 66.67aa | 61.90bb |
| 20 | 61.90ba | 47.62cb |
| 30 | 61.90ba | 57.14cb |
| 40 | 33.33cb | 66.67aa |
| 50 | 61.90ba | 52.38cb |
| 60 | 76.19aa | 71.43ab |

*Note: The average number followed by the same capital letter in the same column and the same lowercase letters in the same row showed no significant difference according to 5% LSD test*

Based on Table 3, the yellow accession shows that gamma-ray radiation at 60 Gy has a higher germination rate than the other doses and the control treatment. Similarly, the green accession showed the highest germination at 60 Gy compared to the other treatments. The interaction between radiation dose and kecapi accession showed a significant difference between the yellow and green accessions at all radiation doses. The yellow accession exhibited higher average germination values than the green accession under most irradiation treatments, although the green accession showed better performance at 40 Gy. This difference in germination indicates that different genotypes exhibit distinct germination rates. However, the interaction between the yellow accession and gamma irradiation doses of 40–60 Gy resulted in higher seed germination compared to other treatment combinations. This phenomenon may be associated with the stimulatory effect of low to moderate gamma irradiation doses on seed physiological activity, including enhanced enzymatic activity, cell metabolism, and membrane permeability during germination. Previous studies have demonstrated that appropriate gamma irradiation doses may induce hormetic responses that improve seed germination and vigour [4, 14].

The interaction between yellow accession seeds and radiation doses of 0–30 Gy resulted in a higher germination rate than for green accession seeds. In contrast, no consistent increase in germination rate was observed at radiation doses of 40–60 Gy (**Fig. 2**). The 20 Gy dose in the yellow accession tended to produce the highest germination rate (0.38 KN/day). This result indicated that germination speed was more strongly influenced by accession differences than by irradiation treatment. Differences in germination responses between yellow and green accessions may be associated with genetic variation among genotypes, which can affect seed vigour, metabolic activity, and tolerance to irradiation stress. Previous studies reported that genetic background plays an important role in determining seed germination performance and plant response to gamma irradiation treatments [4, 14].

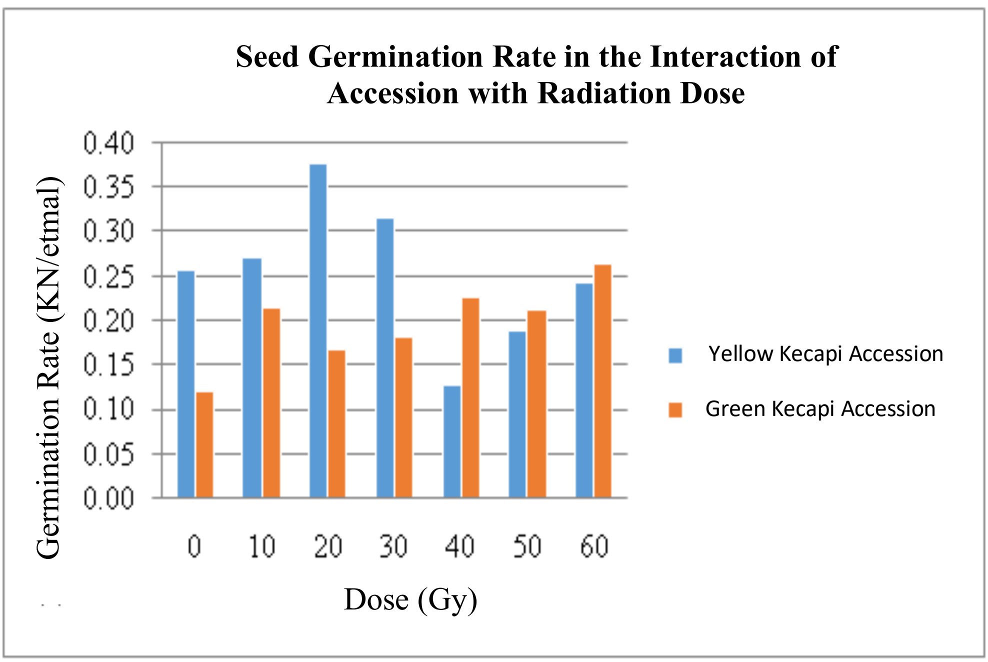



**Fig 2**. Interaction of Kecapi Accession with Radiation Dose on The Speed of Germination

### 3.2 The structure of the kecapi seedling

Observation of the growth structure of kecapi seedlings was carried out at the 50th day after transplanting or before transplanting, by taking one seedling per treatment.

Observations of the growth structure of kecapi seedlings included the number of leaves, the number of leaf tillers, the length of the hypocotyl, the length of the epicotyl, the number of branches, root length, and seedling height, which are presented in **Table 4.**

**Table 4.** The development structure of kecapi seeds

| Accession, Dosage (Gy) | Number of Leaves | Number of Baby Leaves | Hypocotyl Length (cm) | Epicotyl Length (cm) | Number of Branches | Root Length (cm) | Seedling Height (cm) |
|---|---|---|---|---|---|---|---|
| Yellow, Control | 4 | 3 | 2.0 | 3.50 | 0 | 7.3 | 16.2 |
| Yellow, 10 | 3 | 3 | 3.5 | 5.00 | 0 | 10.0 | 21.4 |
| Yellow, 20 | 3 | 3 | 5.0 | 4.00 | 1 | 12.0 | 21.5 |
| Yellow, 30 | 4 | 3 | 3.0 | 2.20 | 1 | 8.5 | 15.8 |
| Yellow, 40 | 3 | 3 | 2.0 | 1.00 | 1 | 7.2 | 13.0 |
| Yellow, 50 | 5 | 3 | 1.5 | 1.50 | 1 | 9.5 | 12.5 |
| Yellow, 60 | 3 | 3 | 2.5 | 1.00 | 1 | 6.0 | 6.2 |
| Green, Control | 4 | 3 | 4.0 | 2.40 | 0 | 8.0 | 14.5 |
| Green, 10 | 4 | 3 | 4.7 | 4.20 | 0 | 8.2 | 22.6 |
| Green, 20 | 4 | 3 | 4.0 | 6.00 | 1 | 8.2 | 19.0 |
| Green, 30 | 5 | 3 | 3.5 | 4.50 | 1 | 8.8 | 21.0 |
| Green, 40 | 5 | 3 | 3.3 | 3.00 | 1 | 8.8 | 15.0 |
| Green, 50 | 3 | 1 | 2.0 | 2.00 | 1 | 9.0 | 11.0 |
| Green, 60 | 5 | 3 | 2.0 | 2.90 | 1 | 7.0 | 17.3 |

In the table above, the root length and seedling height of yellow and green accessions treated with 60 Gy were the shortest, compared with the control and other radiation doses. This shows that high doses of radiation can suppress kecapi growth, as indicated by both root and seed length. However, a dose of 10-30 Gy resulted in good effects (**Fig. 3**). These results are in line with [31] research, which explains how gamma ray radiation with a dose of 5-30 Gy can have a hormonal effect that can stimulate a plant's root growth. Root length is the ability of plants to obtain water supplies and nutrients from deeper soil layers [20].

In the picture above, the yellow accession with a radiation dose of 60 Gy produced the lowest seedling, but the radiation dose of 10-30 Gy (I1–I3) produced better seedlings than the control. At a radiation dose of 10-60 Gy, both yellow and green accessions were seen to have branches; meanwhile, in the control treatment, there was no branching (**Fig. 4**).

In **Fig. 4** above, the 30 Gy radiation treatment showed that the seedlings began to form branches, whereas the control treatment showed only elongated stems; however, at low doses, branching did not fully form. It is suspected that radiation treatment significantly affects branch formation in the seedling structure. States that too low radiation doses are not sufficient to mutate plants because too low a frequency of mutation results in only a few mutated sectors [26]. At high doses (above 50 Gy), the growth of seedlings showed abnormal growth (K2I5) and symptoms of rosette growth (K1I5). Rosette is the growth of the intermedia stem that is hampered until the nodes are attached to each other; the leaves form a rosette/bouquet (**Fig. 5**).


**Fig. 3.** The structure of kecapi seedlings, 50th day after planting in various treatments


(a) (b) (c) (d)

**Fig. 4.** Comparison of Seedling Structure: (a) Yellow Accession, Control (K1I0), (b) Yellow Accession, Radiation 30 Gy (K1I3), (c) Green Accession, Control (K2I0), (d) Green Accession, Radiation 30 Gy (K2I3)


**Fig. 5**. Kecapi Seedlings with Rosette Growth Symptoms (K1I5) and Abnormal (K2I5)

### 3.3 Seedling Height

Seedling height is the easiest growth measure to be observed [30]. At 7 and 9 WAP, the height of the yellow accession seeds was significantly different from the green accession; however, at 15 WAP, there was no significant difference between the height of the yellow and green accession seedlings. This indicates that seedling height is more influenced by plant genotype. In radiation treatment, there was a very significant difference in seedling height (control) (Table 5). In **Table 5,** it can be seen that the higher the radiation dose given to the seed, the greater the suppression of seedling growth, resulting in shorter seedling height, which is very significantly different from lower doses and the control.

**Table 5.** Effect of kecapi accession and radiation dosage on seedling height at 7-15 weeks after planting (WAP)

| **Kecapi Accession** | **Seedling Height (cm)** | | | | |
|---|---|---|---|---|---|
| | **7 WAP** | **9 WAP** | **11 WAP** | **13 WAP** | **15 WAP** |
| Yellow | 16.94b | 17.29b | 18.68a | 19.86a | 20.82a |
| Green | 19.92a | 20.57a | 21.28a | 22.89a | 24.29a |
| **Radiation Dose (Gy)** | **Seedling Height (cm)** | | | | |
| | **7 WAP** | **9 WAP** | **11 WAP** | **13 WAP** | **15 WAP** |
| Control | 21.25ab | 21.90b | 22.74ab | 24.43ab | 24.85ab |
| 10 | 22.95a | 23.20a | 24.68a | 25.85a | 27.40a |
| 20 | 21.7ab | 21.90b | 22.42ab | 22.70b | 23.38bc |
| 30 | 19.35b | 19.44c | 20.52b | 22.30b | 23.77b |
| 40 | 16.55c | 17.42cd | 18.17c | 19.14c | 20.20c |
| 50 | 15.20c | 15.95d | 16.42cd | 17.07d | 18.50d |
| 60 | 12.02d | 12.68e | 14.90d | 18.15cd | 19.80d |

*Note: The numbers followed by the same letter in the same column indicate no significant difference based on the 5% LSD test.*

Plant stunting occurs due to sprout dormancy, which delays plant growth [22]. This is also followed by direct radiation that causes plant growth to stop at a certain time, resulting in stunting. Seedling height is influenced by internal and external factors. Based on the results, the diversity of attachment between internal factors (accessions) and external factors (radiation dose) showed that external factors (radiation dose) had a very significant effect on seedling height compared to internal factors (accessions).

### 3.4 Stem Diameter and Number of Leaves

The treatment of kecapi accession and radiation had no significant effect on stem diameter (Table 6). The results showed that all treatments produced relatively similar stem growth responses. This condition may indicate that the radiation doses used were still within the plants' tolerance range.

**Table 6.** Effect of kecapi accession and radiation dose on stem diameter at 7-15 weeks after planting (WAP)

| Kecapi Accession | Stem Diameter (mm) | | | | |
|---|---|---|---|---|---|
| | **7 WAP** | **9 WAP** | **11 WAP** | **13 WAP** | **15 WAP** |
| Yellow | 3.37a | 3.37a | 3.59a | 4.07a | 4.10a |
| Hijau | 3.68a | 3.68a | 3.87a | 3.73a | 4.36a |
| **Radiation Dose (Gy)** | **Stem Diameter (mm)** | | | | |
| | **7 WAP** | **9 WAP** | **11 WAP** | **13 WAP** | **15 WAP** |
| Control | 3.48a | 3.48a | 3.68a | 3.95a | 4.25a |
| 10 | 3.76a | 3.76a | 4.25a | 4.38a | 4.65a |
| 20 | 3.54a | 3.54a | 3.68a | 3.84a | 4.15a |
| 30 | 3.46a | 3.46a | 3.59a | 3.74a | 3.92a |
| 40 | 3.46a | 3.46a | 3.57a | 3.71a | 4.10a |
| 50 | 3.30a | 3.30a | 3.50a | 3.68a | 4.05a |
| 60 | 3.75a | 3.75a | 3.88a | 4.03a | 4.22a |

*Note: The numbers followed by the same letter indicate no significant difference based on the 5% LSD test.*

Table 6 shows that stem diameter was not significantly affected by different kecapi accessions or gamma irradiation doses applied to the seeds. This result indicates that stem growth in kecapi seedlings remained relatively stable under the applied irradiation treatments. Previous studies reported that low to moderate doses of gamma irradiation may not significantly affect vegetative growth, as plants can still maintain normal physiological activity and cell division [29]. Leaf number is an important indicator of vegetative growth and photosynthetic capacity in plants. In the present study, neither kecapi accession nor irradiation treatment significantly affected the number of leaves at the end of observation (15 WAP), although significant differences were observed during the early growth stages (Table 7). This condition suggests that the seedlings were able to adapt and recover from irradiation-induced stress during subsequent growth stages. Similar findings were reported by [13], who stated that irradiated plants may recover physiologically after exposure to low irradiation doses, resulting in relatively normal growth performance at later stages.

**Table 7.** Effect of kecapi accession on number of leaves at 7-15 weeks after planting (WAP)

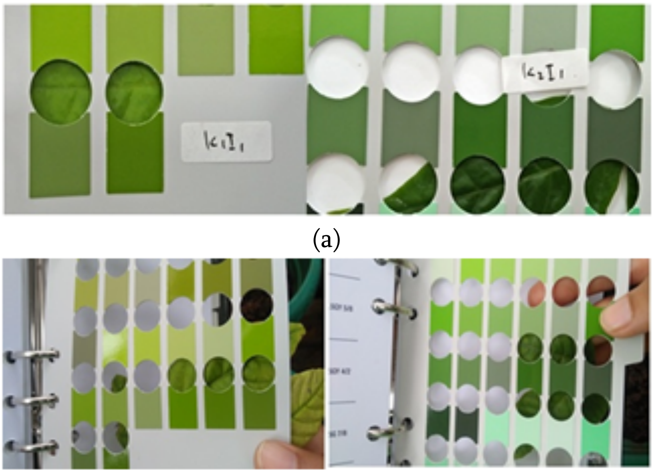
| **Kecapi Accession** | **Number of Leaves** | | | | |
|---|---|---|---|---|---|
| | **7 WAP** | **9 WAP** | **11 WAP** | **13 WAP** | **15 WAP** |
| Yellow | 3,53ab | 3,62ab | 4,67a | 5,19a | 5,95a |
| Green | 4,38a | 4,38a | 4,81a | 5,43a | 6,19a |
| **Dosis Radiasi (Gy)** | **Number of Leaves** | | | | |
| | **7 WAP** | **9 WAP** | **11 WAP** | **13 WAP** | **15 WAP** |
| Control | 3,16a | 3,16b | 3,50a | 4,16a | 5,50a |
| 10 | 3,34a | 3,34b | 4,00a | 4,83a | 5,00a |
| 20 | 3,67a | 3,67b | 5,34a | 5,00a | 5,50a |
| 30 | 3,84a | 3,84b | 4,83a | 5,50a | 6,17a |
| 40 | 5,50a | 5,84a | 6,50a | 7,00a | 8,67a |
| 50 | 4,34a | 4,34ab | 4,66a | 5,16a | 5,67a |
| 60 | 3,84a | 3,84b | 4,33a | 4,83a | 6,00a |

Note: The numbers followed by the same letter indicate no significant difference based on the 5% BNT test.

Based on **Table 7**, the number of leaves tended to increase with plant age in both kecapi accessions. The green accession generally produced a higher number of leaves compared to the yellow accession, although the differences were not statistically significant at several observation times. In terms of gamma irradiation treatment, the 40 Gy dose showed the highest number of leaves at 7–15 WAP compared to other treatments. This result indicates that an appropriate dose of gamma irradiation may stimulate vegetative growth, particularly leaf formation. According to previous studies, low to moderate doses of gamma rays can induce physiological and biochemical changes that stimulate cell division and plant growth, whereas excessively high doses may inhibit growth due to cellular damage and metabolic disruption [14, 29]. However, higher radiation doses generally tend to reduce leaf production because radiation stress can negatively affect photosynthetic activity and meristematic tissue development.

### 3.5 Leaf characteristics

Leaf characteristics were observed qualitatively based on leaf colour using the Munsell Tissue Colour Book. Observations were conducted at 50 days after planting (DAP) and at the end of the seedling stage, 15 weeks after planting (WAP). The results showed that kecapi seedlings generally exhibited green leaf colouration, with slight differences in colour intensity among treatments. At 50 DAP, the leaves tended to display lighter green colouration, whereas at 15 WAP the leaves became darker green, indicating further leaf development and chlorophyll accumulation during seedling growth. Figure 6(a) presents the qualitative morphological characteristics of kecapi leaves at 50 DAP, while Figure 6(b) shows leaf characteristics at 15 WAP.



(a)

(b)

**Fig 6.** Observation of leaf colour of kecapi seedlings at 50 DAP (a) and 15 WAP (b)

The leaf colour data showed several variations among the observed kecapi accessions (**Table 8**). Nevertheless, most samples were categorised as 5 GY (Green Yellow), indicating a relatively similar pigmentation. Leaf colour is considered an important morphological trait associated with chlorophyll content and plant physiological conditions. Environmental factors, nutrient availability, and genetic characteristics may influence leaf colour variation in plants [27].

**Table 8.** Qualitative Morphological Characteristics of Kecapi Seedling Leaves

| Accession, Radiation | Leaf Color | |
|---|---|---|
| | Germination | Nurseries |
| Yellow, Control | 5 gy 4/8 | 2,5 gy7/8 |
| Yellow, 10 | 5 gy 4/8 | 5 gy 5/8 |
| Yellow, 20 | 5 gy 5/8 | 7,5 gy |
| Yellow, 30 | 7,5 gy 4/6 - 7,5 gy 4/4 | 5 gy 4/4 |
| Yellow, 40 | 5 gy 4/4 | 5 gy ¾ |
| Yellow, 50 | 5 gy ¾ | 5 gy 4/6 |
| Yellow, 60 | 5 gy 5/4 | 5 gy 4/6 |
| Green, Control | 5 gy ¾ | 5 gy 5/8 |
| Green, 10 | 7,5 gy 4/4 | 5 gy 6/8 |
| Green, 20 | 5 gy 4/6 | 5 gy 5/8 |
| Green, 30 | 5 gy 5/8 - 5 gy 5/6 | 2,5 gy 7/8 |
| Green, 40 | 7,5 gy ¾ | 5 gy 4/8 |
| Green, 50 | 7,5 gy ¾ | 2,5 gy 7/8 |
| Green, 60 | 5 gy 5/8 | 5 gy 5/6 |

**Table 8** shows that leaf colour is not affected by kecapi accession or the radiation exposure to seeds. Neither at 60 Gy nor at lower doses showed any significant difference in leaf colour. In this study, leaf colour did not show chlorosis or albinism. Some leaves showed only abnormal growth, such as leaf unions with rosette symptoms. This result

aligns with [11], which reports that radiation can cause different leaf shapes, including stunted growth, leaf union, and discolouration. Aisha et al. (2019) also reported colour changes due to DNA mutations; in this case, chlorophyll mutations result in partial colour changes (chimaeras) and albino mutants. Albino mutants induced by gamma-ray irradiation result from impaired chlorophyll synthesis, leading to a green colour deficiency [10]. Furthermore, [28] stated that mutations result in the exchange of DNA base pairs, thereby causing changes in phenotypic or genotypic properties. In this study, it is suspected that the applied radiation dose is still low enough not to have caused DNA mutations that would alter chlorophyll.

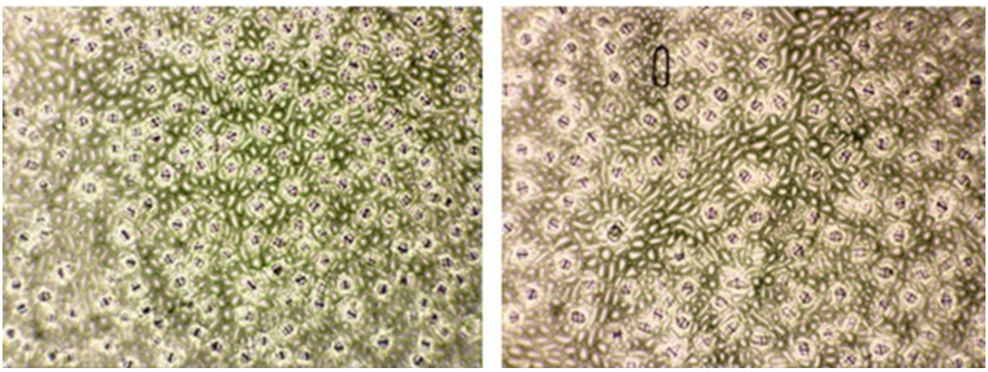
### 3.6 Stomata anatomy

Anatomical observations of stomata were conducted on kecapi seedlings at 15 WAP during the daytime. The observed leaves were the second to third leaves from the shoot. Observations were carried out using a Nikon light microscope at 10×10 magnification with a field of view of 0.212 mm² (**Fig. 7**). Based on **Table 9**, the stomatal length and width varied among treatments. In both yellow and green accessions, gamma-irradiated plants generally showed shorter stomata than the control treatment. The 60 Gy irradiation treatment produced the shortest stomata, but the stomatal width tended to be higher than in other treatments. These results indicate that gamma irradiation affected stomatal morphology in kecapi seedlings. The reduction in stomatal length may be associated with the inhibitory effect of irradiation on cell growth and development. In contrast, the increase in stomatal width at higher irradiation doses may indicate a morphological adaptation response of epidermal cells to radiation stress. Stomatal number and size are influenced by both genetic and environmental factors, which may explain the variation observed among treatments [2]. Stomata play an important role in regulating water loss and gas exchange as well as transporting water and minerals throughout the plant [16].

**Table 9.** Characteristics of the stomata of kecapi seeds

| Accession, Radiation | Number of Stomata | Stomata Length (μm) | Stomata Width (μm) | Stomata Density (mm²) | Leaf Size (mm²) |
|---|---|---|---|---|---|
| Yellow, Control | 167 | 22.1 | 4.8 | 787.7 | 67.83 |
| Yellow, 10 | 177 | 18.3 | 3.3 | 834.9 | 65.98 |
| Yellow, 20 | 187 | 18.3 | 6.7 | 882.0 | 58.24 |
| Yellow, 30 | 190 | 13.9 | 5.1 | 896.3 | 44.38 |
| Yellow, 40 | 168 | 24.7 | 5.2 | 792.5 | 169.07 |
| Yellow, 50 | 182 | 16.6 | 4.4 | 858.5 | 84.42 |
| Yellow, 60 | 185 | 20.0 | 6.4 | 872.6 | 54.49 |
| Green, Control | 208 | 21.2 | 5.6 | 981.2 | 80.12 |
| Green, 10 | 146 | 13.4 | 4.8 | 688.7 | 128.80 |
| Green, 20 | 150 | 14.9 | 5.8 | 707.5 | 62.34 |
| Green, 30 | 209 | 20.8 | 6.4 | 985.8 | 106.09 |
| Green, 40 | 120 | 15.5 | 4.6 | 566.0 | 117.13 |
| Green, 50 | 121 | 18.0 | 4.8 | 570.7 | 103.65 |
| Green, 60 | 101 | 19.0 | 6.9 | 476.4 | 80.81 |

Based on observations, the stomata density of yellow and green accessions at various radiation doses varied from 476.4 to 985.8 mm$^2$. The level of stomatal density is influenced by environmental factors such as water availability, light intensity, temperature, and $CO_2$ concentration, so the higher the intensity of the light, the more the stomata frequency on both leaf surfaces would increase, even though the increase in frequency is not significant [17]. An experiment on lily sprout radiation (*Lilium longiflorum*) in vitro culture with doses of 5, 7.5, and 10 krad produced differences in leaf length, width, density, y and number of leaf stomata at 12 WAP [15].



(a) (b)

**Fig. 7.** Stomata Magnification 40x10 (a) Yellow Accession, (b) Green Accession

## 4 Conclusion

There was no significant difference in germination percentage and germination rate between the yellow and green accessions, although the green accession tended to produce better seed performance. Gamma-ray irradiation at 60 Gy had the greatest effect on seed germination; however, higher doses significantly inhibited seedling growth, resulting in shorter seedlings. Furthermore, the interaction between the yellow accession and 60 Gy irradiation produced the highest germination percentage but also the lowest seedling height. Overall, gamma-ray irradiation influenced both seed viability and early seedling growth, indicating its potential to induce physiological responses and variability in the studied accessions.

## References

1. S.I. Aisyah, H. Aswidinnoor, A. Saefuddina and B. Marwoto, Induced mutations in cutting of carnation (*Dianthus caryophyllus* Linn.) through gamma rays irradiation. J. Agron. Indones. **37**, 1 (2009).
2. N.A. Andini, Anatomi jaringan daun dan pertumbuhan tanaman *Celosia cristata*, *Catharanthus roseus*, dan *Gomphrena globosa* pada lingkungan udara tercemar, Master thesis, IPB University (2011).
3. P. Aprilianti and W.U. Putri, Study sifat fisik biji kecapi (*Sandoricum koetjape* Burm.f. Merr) dan penyimpanannya dalam suhu kamar. Bul. Kebun Raya Indones. **12**, 2 (2009).

4. M. Ashraf, A.A. Cheema, M. Rashid and Z. Qamar, Effect of gamma rays on M1 generation in basmati rice. Pak. J. Bot. **35**, 5 (2003).
5. C. Benjawan and P. Chutichudet, Control of skin colour and polyphenol oxidase activity in santol fruit by dipping in organic acid solution. Pak. J. Biol. Sci. **12**, 11 (2009).
6. J. Berthaud, Strategies for conservation of genetic resources in relation with their utilization. Euphytica**. 96**(1997).
7. Y. Bramasto, P.K. Putri, M. Zanzibar and Danu, Pemanfaatan teknik irradiasi sinar gamma untuk meningkatkan viabilitas benih sengon. J. Hutan Trop. **4**, 1 (2016).
8. W. Broto, Teknologi penanganan pascapanen buah untuk pasar (2010).
9. M.A. Esnault, F. Legue and C. Chenal, Ionizing radiation: advances in plant response. Environ. Exp. Bot. **68** (2010). https://doi.org/10.1016/j.envexpbot.2010.01.007
10. M. Girija and D. Dhanavel, Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combined treatments in cowpea (*Vigna unguiculata* L. Walp). Glob. J. Mol. Sci. **4**, 2 (2009).
11. D. Grosch and E. Larry, Biological effects of radiations. (Academic Press, 1979).
12. Karsinah, F.H. Silalahi and A. Manshur, Eksplorasi dan karakterisasi plasma nutfah tanaman markisa. J. Hort. **17**, 4 (2007).
13. A.L.P. Kiong, A.G. Lai, S. Hussein and A.R. Harun, Physiological responses of Orthosiphon stamineus plantlets to gamma irradiation. Am.-Eurasian J. Sustain. Agric. **2**, 2 (2008).
14. E. Kovács and Á. Keresztes, Effect of gamma and UV-B/C radiation on plant cells. Micron **33**, 2 (2002). https://doi.org/10.1016/S0968-4328(01)00012-9
15. N.K.D. Lestari, I.A. Astarini and I.G.M.O. Nurjaya, Perubahan anatomi stomata daun lili trumpet (Lilium longiflorum) setelah pemaparan radiasi sinar X. J. Metamorfosa. **1**, 1 (2013).
16. K.H. McKown and D.C. Bergmann, Grass stomata. Curr. Biol. **28**, R803–R825 (2018).
17. L. Meriko and Abizar, Struktur stomata daun beberapa tumbuhan kantong semar (*Nepenthes* spp.). Berita Biol. **16,** 3 (2017).
18. H.R. Moussa, Low dose of gamma irradiation enhanced drought tolerance in soybean. Acta Agron. Hung. **59** (2011).
19. D. Mudiana, Germination of *Syzygium cumini* (L.) Skeels. Biodiversitas. **8**, 1 (2007).
20. Y.P. Munarso, Keragaan padi hibrida pada sistem pengairan intermittent dan tergenang. Penelit. Pertan. Tanam. Pangan. **30**, 3 (2015).
21. I. Piri, M. Babayan, A. Tavassoli and M. Javaheri, The use of gamma irradiation in agriculture. Afr. J. Microbiol. Res. **5**, 32 (2011).
22. S. Pramono, Studi irradiasi sinar gamma pada tanaman iles-iles (*Amorphophallus muelleri*), Master thesis, IPB University (2011).
23. T.S. Silitonga, Pengelolaan dan pemanfaatan plasma nutfah padi di Indonesia. Bul. Plasma Nutfah. **10**, 2(2004).
24. S. Soedjono, Aplikasi mutasi induksi dan variasi somaklonal dalam pemuliaan tanaman. J. Penelit. Pengemb. Pertan. **22**, 2 (2003).
25. U. Soetisna, D. Priadi, S. Hartati and E. Sudarmonowati, Storage and the use of peroxydase enzyme to detect germination capability of *Sandoricum koetjape* Merr. seeds. Biodiversitas. **6**, 1 (2005).

26. G.N. Sutapa and I.G.A. Kasmawan, Efek induksi mutasi radiasi gamma 60Co pada pertumbuhan fisiologis tanaman tomat (*Lycopersicon esculentum* L.). J. Keselam. Radiasi Lingkung. **1**, 2 (2016).
27. L. Taiz, E. Zeiger, I.M. Møller, and A. Murphy, Plant physiology and development. (Sinauer Associates, 2015).
28. T. Tounekti, M. Mahdhi, T.A. Al-Turki and H. Khemira, Genetic diversity analysis of coffee (Coffea arabica L.) germplasm accessions growing in southwestern Saudi Arabia using quantitative traits. Nat. Resour. **8**, 5 (2017). https://doi.org/10.4236/nr.2017.85020
29. S.G. Wi, B.Y. Chung, J.S. Kim, J.H. Kim, M.H. Baek, J.W. Lee and Y.S. Kim, Effects of gamma irradiation on morphological changes and biological responses in plants. Micron. **38**, 6 (2007). https://doi.org/10.1016/j.micron.2006.11.002
30. E. Yuniastuti, H. Sri and R.W. Slamet Karakteristik morfologi tanaman durian sukun (*Durio zibethinus* Murr), in Preosiding Semin. Nas. Pendidik. Biol. FKIP UNS. Universitas Negeri Semarang, Semarang (2010).
31. M. Zanzibar, Megawati, E.P. Dede and J.S. Dede, Iradiasi sinar gamma (60Co) untuk meningkatkan perkecambahan dan pertumbuhan bibit tembesu (*Fagraea fragrans*). J. Penelit. Hutan Tanam. **12**, 3 (2015).