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# Gamma-ray irradiation induced polymorphism in *Echinacea purpurea* revealed by RAPD markers

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**Abstract.** *Cahyaningsih AP, Etikawati N, Yunus A. 2025. Gamma-ray irradiation induced polymorphism in* Echinacea purpurea *revealed by RAPD markers. Nusantara Bioscience 17: 289-297.* One of the major challenges in the development and cultivation of *Echinacea purpurea* in Indonesia is the narrow genetic diversity and the lack variation of local accessions, which restricts breeding potential to produce superior varieties with improved traits in terms of morphological characteristics and phytochemical content as medicinal ingredients. To overcome this challenge, gamma-ray irradiation methods can be used as an effective tool to induce mutations and increase genetic variability. This study investigated the impact of gamma irradiation on the genetic diversity of *E. purpurea* using Random Amplified Polymorphic DNA (RAPD) markers. The seeds of *E. purpurea* were irradiated using gamma-ray with irradiation doses of 0 Gy (control), 20 Gy, 40 Gy, and 60 Gy. Nine RAPD primers, including OPA-10, OPA-16, OPA-18, and OPA-19, were used to amplify DNA segments. The binary data obtained from the electrophoresis visualization for each treatment were analyzed using the Dice similarity index to calculate the similarity index. Dendrogram construction were performed using the NTSYS. The analysis revealed a significant increase in genetic diversity at doses of 40 Gy and 60 Gy, with 58.04% of the total bands showing polymorphism across all treatments. The 60 Gy treatment, in particular, resulted in the highest genetic dissimilarity compared to the control, indicating a dose-dependent response. The similarity coefficients between control and irradiated plants ranged from 63.7% (20 Gy) to 84.4% (0 Gy), with a noticeable trend toward greater genetic differentiation as the irradiation dose increased. These findings suggest that gamma irradiation effectively induces genetic variation in *E. purpurea*, which could be harnessed for mutation breeding programs aimed at improving desirable traits such as phytochemical production. However, further studies with larger sample sizes and long-term evaluation are needed to assess the stability of these mutations and their potential for incorporation into breeding programs. This study provides preliminary evidence supporting the use of gamma irradiation in enhancing genetic diversity and its potential application in breeding superior *E. purpurea* varieties with improved agronomic or medicinal traits.

**Keywords:** *Echinacea purpurea*, gamma irradiation, medicinal plant, molecular marker, mutation breeding

## INTRODUCTION

*Echinacea purpurea* (L.) Moench is a medicinal plant long recognized in traditional medicine, particularly as a powerful immunostimulant. It is widely used to boost the immune system and reduce symptoms of common colds and influenza. Extracts from the aerial parts of *E. purpurea*, including the stem, leaves, and roots, are commonly used in pharmaceutical preparations to accelerate the healing of upper respiratory tract infections (Burlou-Nagy et al. 2022). The use of *E. purpurea* extracts is not limited to immunomodulation therapy but is also known for its significant antioxidant and anti-inflammatory properties (Manayi et al. 2015; Miroshina and Poznyakovskiy 2023). These pharmacological qualities make it one of the key plants in the herbal medicine industry, especially in Europe and North America, where it was first widely cultivated. In Indonesia, the use of *E. purpurea* is relatively new, but its potential in the pharmaceutical industry is substantial, particularly in disease prevention and public health improvement. In Indonesia, *E. purpurea* has been used in health supplements to help maintain the immune system (Pramesty 2021).

One of the major challenges in the development and cultivation of *E. purpurea* in Indonesia is the narrow genetic diversity and the lack variation of local accessions, which restricts breeding potential to produce superior varieties with improved traits in terms of morphological characteristics and phytochemical content as medicinal ingredients. To overcome this challenge, gamma-ray irradiation methods can be used as an effective tool to induce mutations and increase genetic variability. This technique has proven successful in various plants, including *Musa paradisiaca* (Due et al. 2019) and *Oryza sativa* (Purwanto et al. 2019), as well as ornamental plants like *Chrysanthemum* (Susila et al. 2019) and medicinal plant such as *Celosia cristata* (Muhallilin et al. 2019). Gamma irradiation induces mutations that lead to increased phenotypic diversity and altered chemical content, potentially improving the medicinal properties of plants.

Although gamma-ray irradiation is a promising technique, there remains a gap in knowledge regarding the dose-

specific effects of gamma irradiation on *E. purpurea*'s genetic traits. While prior studies have used irradiation in other plants, the impact of different doses on the genetic diversity of *E. purpurea* has not been well-explored. Specifically, the effects of varying irradiation doses on the genetic markers of *E. purpurea* need further investigation to optimize breeding strategies. In this context, molecular markers such as Random Amplified Polymorphic DNA (RAPD) provide an efficient and cost-effective means of detecting genetic changes induced by irradiation. RAPD markers are ideal for *E. purpurea*, whose genome has not been fully mapped, and they are useful for assessing genetic variations in plants subjected to mutagenesis (Nasution et al. 2021).

While RAPD markers have been used in previous studies on *E. purpurea* to explore genetic diversity (Subositi and Widiastuti 2013), this study introduces a novel approach by combining gamma-ray irradiation with RAPD analysis to investigate the genetic variation induced by different irradiation doses in Indonesian accessions of *E. purpurea*. Unlike earlier research, which focused on natural genetic diversity without the use of mutagenesis, this study aims to fill the knowledge gap on the dose-specific effects of gamma-ray irradiation, providing insights into how different doses impact genetic variation in *E. purpurea*.

Gamma-ray irradiation in mutation breeding not only aims to increase genetic diversity but also to influence key agronomic traits that can improve plant production. Previous studies on horticultural crops have shown that the appropriate irradiation dose can accelerate flowering time, increase biomass yield, and shorten the harvest period (Holme et al. 2019), which is particularly beneficial for plants with long vegetative phase like *E. purpurea*. Moreover, inducing genetic diversity through irradiation can improve secondary metabolite content, enhancing the pharmacological value of *E. purpurea*, especially regarding flavonoid content, which is crucial for its medicinal properties (Hanifah et al. 2024).

This study specifically aimed to investigate the effects of different gamma-ray irradiation doses on the genetic diversity of Indonesian accessions of *E. purpurea* using RAPD markers. The findings of this study are expected to provide a foundation for developing superior varieties with higher pharmacological potential and improved agronomic traits. Additionally, the superior accessions developed through irradiation are anticipated to reduce the need for plant imports, thereby providing raw materials for the pharmaceutical industry in Indonesia.

## MATERIALS AND METHODS

### Plant material

The plant material used in this study was *Echinacea purpurea* Accession 3 (A3) seeds from Research and Development Center for Medicinal Plants and Traditional Medicine (B2P2TOOT), Tawangmangu, Central Java, Indonesia. *Echinacea purpurea* accession at B2P2TOOT is an *Echinacea* plant imported from Germany through PT. Deltomed Laboratories. Accession A3 is one of three leading accessions of B2P2TOOT planted in the highlands, classified based on minor morphological variations in flower shape and stem color. In the highland, this accession noted for lacking variants with uniform tillers (Subositi and Fauzi 2016). Both highland and lowland, this accession has low growth yield and secondary metabolite levels (Sidhiq et al. 2020). The irradiation of *E. purpurea* seeds was conducted at the PAIR-BATAN Laboratory (Center for Isotope and Radiation Applications - National Nuclear Energy Agency), Jakarta, Indonesia. The irradiated seeds were sown, and the seedlings were planted in the agricultural land of Universitas Sebelas Maret, Jumantono, Central Java, Indonesia. Molecular analysis was performed at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. The research was conducted from May 2021 to April 2022.

### Gamma-ray irradiation and seed sowing

The flowers of *E. purpurea* A3 from B2P2TOOT were collected and sun-dried for two days. Afterward, the seeds attached to the dried flowers were separated and sorted, selecting only those seeds that contained the embryo. A total of 120 *E. purpurea* seeds, with 30 seeds for each treatment, were placed in plastic clips and labeled according to each gamma radiation dose. The seeds were irradiated using a Gamma Chamber/Gamma Cell-220 upgrade with irradiation doses of 0 Gy (control), 20 Gy, 40 Gy, and 60 Gy by a $_{60}$Co source at a dose rate of 3789.4 Gy $h^{-1}$. The gamma irradiation time for a dose of 20 Gy was 19 seconds, a dose of 40 Gy was 38 seconds, and a dose of 60 Gy was 57 seconds. Irradiated *E. purpurea* seeds were sown in seed trays filled with a soil and manure mixture at a ratio of 3:2. The seeds were placed on the surface and gently pressed to ensure good contact with the soil. They were then covered with 3-5 mm of soil and watered gently but thoroughly to moisten the soil. The trays were placed in a bright and warm location, with the soil kept moist until germination. Seedlings aged 30 DAS (Days After Sowing) were transplanted into polybags (15x15 cm) filled with the same soil and manure mixture at a ratio of 3:2. Watering and seedling care were carried out daily until 120 DAS. Germination and seeding were carried out in a greenhouse.

### Field planting

*Echinacea purpurea* seedlings aged 120 DAS (Days After Sowing) were transplanted into beds (300x100 cm) for each treatment. *Echinacea purpurea* plants were planted with a spacing of 30 cm. The beds were shaded using a shade net with 65% density. In each bed, 10 *E. purpurea* seedlings were planted for each treatment. Two plants from each treatment were taken as samples. *Echinacea purpurea* plants were maintained until they were 120 Days After Field Planting (DAFT) by watering once a day. Control plants and gamma-ray irradiated plants are displayed in Figure 1.

**Figure 1.** *Echinacea purpurea* plant material irradiated with gamma-rays. A. Control, B. 20 Gy, C. 40 Gy, D. 60 Gy

**Molecular analysis using RAPD markers**

*DNA extraction*

DNA extraction from *E. purpurea* leaves for each treatment was performed according to Plant Genomic Mini Kit protocol provided by Geneaid, which consists of five stages of DNA isolation: tissue dissociation, lysis, DNA binding, washing, and DNA elution. The quality and quantity of extracted DNA were examined using an Eppendorf Biophotometer Plus. The DNA purity determined based on the absorbance measurement at a wavelength of λ 260/280. DNA isolates with a purity value of 1.8-2.0 were considered suitable for the next stage. This range indicates minimal contamination, which is crucial for efficient DNA amplification in PCR (Sophian et al. 2021).

*DNA amplification (PCR-RAPD)*

The DNA amplification of *E. purpurea* plants for each treatment, based on RAPD markers, was conducted using 9 primers (Table 1). The RAPD primers used were based on a literature review of several studies on the genetic variation of *E. purpurea* species, consisting of 3 accessions from the study by Subositi and Widiyastuti (2013) and 6 accessions from the study by Lema-Rumińska et al. (2019). PCR amplification preparation began by making a PCR mix with a total volume of 25 µL, consisting of 2 µL of DNA template, 1 µL of primer, 12.5 µL of MyTaq HS Red Mix, and 9.5 µL of $ddH_2O$. The amplification process was performed using a Veriti™ thermal cycler with the following stages: pre-denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing (depending on the primer, 36°C-38°C) for 1 minute, elongation at 72°C for 2 minutes, extension at 72°C for 8 minutes, and a final holding temperature of 4°C (Subositi and Widiyastuti 2013). The amplified products were stored at -20°C.

*Electrophoresis*

The PCR products were checked using 2% agarose gel electrophoresis. A total of 2 g of agarose powder was dissolved in 100 mL of 0.8X TAE buffer, and 10 µL of SYBR safe DNA gel stain was added. A total of 5 µL of PCR product DNA was mixed with 2 µL of loading dye on a piece of parafilm until homogeneous using a micropipette. The mixture was then loaded into the wells of the agarose gel. A 100 bp DNA ladder (3 µL) was loaded into the leftmost well. Electrophoresis was carried out by closing the electrophoresis tank and connecting it to the power supply at 100 volts for 30 minutes. Gel images were recorded using Bio-Rad Gel Doc XR+ System. The agarose gel was placed into the Gel documentation system to observe the polymorphism bands for each treatment of mutant *E. purpurea* plants.

**Table 1.** RAPD primers used for PCR

| Primer | Sequence (5'-3') | Tm (ºC) | Reference |
|---|---|---|---|
| OPA-10 | GTGATCGCAG | 38 | Subositi and Widiyastuti (2013) |
| OPA-16 | AGCCAGCGAA | 37 | Lema-Rumińska et al. (2019) |
| OPA-17 | GACCGCTTGT | 38 | Lema-Rumińska et al. (2019) |
| OPB-4 | GGACTGGAGT | 36 | Lema-Rumińska et al. (2019) |
| OPE-6 | AAGACCCCTC | 36 | Subositi and Widiyastuti (2013) |
| OPF-6 | GGGAATTCGG | 36 | Lema-Rumińska et al. (2019) |
| OPG-4 | AGCGTGTCTG | 38 | Lema-Rumińska et al. (2019) |
| OPH-13 | GACGCCACAC | 37 | Subositi and Widiyastuti (2013) |
| OPO-15 | TGG GTCCTT | 38 | Lema-Rumińska et al. (2019) |

Note: Tm: Melting temperature of primer

### Data analysis

Molecular data observation was conducted by examining the visualization of DNA fragments based on the RAPD primers used. In the visualization results, a score of 1 was assigned if a fragment was present, and a score of 0 if no fragment was present. The binary data representing the presence or absence of fragments were used to calculate the number and percentage of polymorphisms. The binary data obtained from the electrophoresis visualization for each treatment were analyzed using the Dice similarity index to calculate the similarity index. Dendrogram construction was performed using the NTSYS 2.02 software with the UPGMA method (Rohlf 2000).

## RESULTS AND DISCUSSION

### Amplification products using RAPD primers

In this study, RAPD markers were used to assess the increase in genetic variation due to the application of gamma-ray irradiation on *Echinacea purpurea* plants. A total of 9 primers were selected and optimized to identify polymorphisms in the DNA amplification products resulting from gamma irradiation. Two plant samples were used for each primer and irradiation dose. All samples irradiated with doses of 0, 20 Gy, 40 Gy, and 60 Gy successfully produced amplicons with the nine primers used, detecting both polymorphic and monomorphic bands. The amplified DNA bands can be seen in Figure 2.

In this study, a total of 135 DNA bands were generated using 9 primers, with an average polymorphism rate of 58.04%, and band sizes ranging from 200 to 2000 bp. Among these, 79 bands were polymorphic, while 56 were monomorphic. Five primers (OPA-16, OPA-17, OPB-04, OPE-06, and OPG-04) produced polymorphism rates greater than 50%, whereas the remaining four primers (OPA-10, OPF-06, OPH-13, and OPO-15) had polymorphism rates below 50%. Of the five primers with more than 50% polymorphism, only OPE-06 reached 100%. The number of amplified DNA bands is summarized in Table 2.

The use of RAPD markers to detect polymorphism in *E. purpurea* plants has been demonstrated in studies by Subositi and Widiastuti (2013) and Lema-Rumińska et al. (2019). Subositi and Widiastuti's study produced 48 fragments with an average polymorphism of 75%, while Lema-Rumińska et al. (2019) reported 110 fragments with a polymorphism percentage of 97.35%. In the current study, the total number of DNA fragments was higher, but the percentage of polymorphism was lower. The differences in polymorphism percentages are related to the type and number of primers used, as well as the *E. purpurea* accessions serving as DNA samples. The two previous studies used more than three accessions known to have morphological variation, whereas this study used a single accession irradiated at various doses, with the DNA samples coming from irradiated plants. This study combined primers from both previous studies. Amplified DNA bands come from DNA samples complementary to the primer, while polymorphic bands result from bands not amplified at a specific locus (Sulistyawati and Widyatmoko 2017), indicating one or more alternative phenotypes and suggesting genetic variation (Singh and Kulathinal 2013). Polymorphism in mutant plants can occur due to substitution, deletion, or insertion of nitrogenous bases at the primer binding sites, preventing amplification, or due to insertions or deletions that change the size of the amplified fragment (Riviello-Flores et al. 2022).

While compared to studies on other medicinal plant, the research by Magdy et al. (2020) found a polymorphism percentage of 74.14% in the RAPD analysis of gamma-ray irradiated ginger (*Zingiber officinale*), where they used 5 primers and obtained 58 bands (15 monomorphic and 43 polymorphic). The varying levels of polymorphism across each primer confirm genetic variation in the gamma-irradiated plants compared to the control (Riviello-Flores et al. 2022). RAPD molecular markers have been used in several studies to determine genetic variation in mutant plants induced by gamma irradiation. Previous studies on *Typhonium flagelliforme* (Sianipar et al. 2015), *Tectona grandis* (Parlaongan et al. 2022), *Coriandrum sativum* (Jabbar SM and Al-Tamimi 2022), and *Persea americana* (Ihsan et al. 2023) reported that RAPD markers are highly useful for identifying mutant plants based on detected polymorphisms, though not all primers consistently yield optimal polymorphic bands. However, each study identified some of the most effective primers. In this study, primers OPA-16 and OPB-04 showed strong polymorphism.

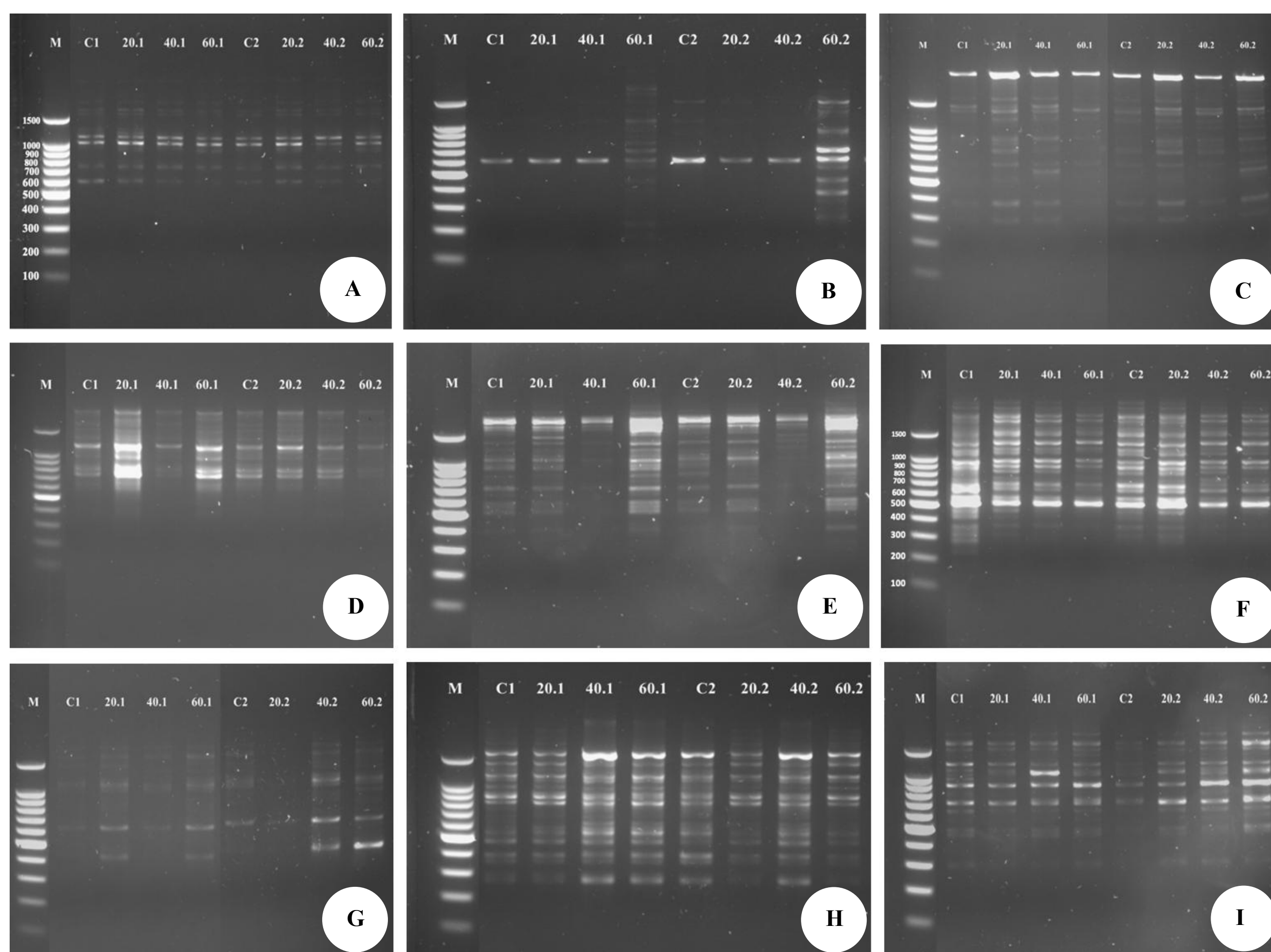


**Figure 2.** DNA bands resulting from amplification using RAPD primers. A. OPA-10, B. OPA-16, C. OPB-04, D. OPA-17. E. OPG-04. F. OPF-06, G. OPE-06, H. OPH-13. I. OPO-15. C: Control, 20: 20 Gy, 40: 40 Gy, 60: 60 Gy. M: Marker 100 bp

**Table 2.** Amplified DNA bands using RAPD primers

| Primer | Size of DNA band (bp) | Loci | | | | % polymorphism |
|---|---|---|---|---|---|---|
| | | Amount of DNA band | Polymorphic | Monomorphic | Unique | |
| OPA-10 | 600-2000 | 10 | 2 | 8 | - | 20 |
| OPA-16 | 200-1700 | 16 | 15 | 1 | 5 | 93.75 |
| OPA-17 | 300-1700 | 16 | 9 | 7 | 1 | 56.25 |
| OPB-04 | 300-1800 | 16 | 11 | 5 | 1 | 68.75 |
| OPE-06 | 400-1800 | 11 | 11 | 0 | - | 100 |
| OPF-06 | 275-2000 | 22 | 9 | 13 | - | 40.90 |
| OPG-04 | 400-2000 | 18 | 14 | 4 | - | 77.77 |
| OPH-13 | 600-1800 | 10 | 4 | 6 | - | 40 |
| OPO-15 | 275-1800 | 16 | 4 | 12 | - | 25 |
| Total | | 135 | 79 | 56 | 7 | |
| Average | | 15 | 8.7 | 6.2 | - | 58.04 |

*Unique DNA fragments*

Several unique or specific fragments were also obtained from amplification results using RAPD primers (Table 2). Unique or specific bands are those that appear in certain genotypes but are absent in others (Sharma et al. 2019). The 20 Gy treatment produced two specific fragments: a 500 bp fragment with primer OPB-04 and a 1400 bp fragment with primer OPA-17. Meanwhile, the 60 Gy treatment produced five specific fragments with primer OPA-16: fragments of 200 bp, 800 bp, and 1000 bp in the first sample, and fragments of 250 bp and 350 bp in the second sample. One of the advantages of using RAPD markers is the ability to obtain unique fragments from amplification results. These unique fragments can be used for the identification and characterization of accessions and as character materials for plant breeding purposes (Subositi

and Widiastuti 2013).

At the 20 Gy dose, although considered a relatively low irradiation dose, mutations were still induced; this effect was detectable through RAPD analysis as this molecular marker is sensitive to genetic changes such as small insertions or deletions (Riviello-Flores et al. 2022). These genetic changes led to the formation of unique fragments that could be distinguished from the control group, as the mutations altered the binding site locations of the RAPD primers. Studies have confirmed that RAPD markers are effective in identifying polymorphic and unique bands induced by gamma-ray irradiation (Roy et al. 2006; Jabbar and Al-Tamimi 2022). On the other hand, 60 Gy induces a broader range of genetic mutations, including large-scale structural changes such as deletions, duplications, or rearrangements of the DNA (Shu et al. 2012). Primers like OPA-16 can amplify these regions with larger structural variations, leading to the appearance of unique bands. The higher mutation rate associated with the 60 Gy dose increases the probability of generating genetic variation in loci that are targeted by these primers, resulting in more specific fragment patterns.

Data on the effect of gamma irradiation on the morphological diversity of *E. purpurea*, both qualitatively and quantitatively, has been published by Cahyaningsih et al. (2022). The unique fragments that appeared in *E. purpurea* plants irradiated at doses of 20 Gy and 60 Gy may be related to their morphological characteristics. At 20 Gy, the variation observed was a distinct flower shape compared to all other treatments. At 60 Gy, more variations were noted, corresponding to the unique fragments, such as the shortest plant height, darker flower color, and the longest time to first flowering compared to all other treatments (Cahyaningsih et al. 2022). Primers OPB-04, OPA-17, and OPA-16 can be used to detect mutant plant characteristics and may later be used in marker-assisted selection, particularly for flower traits in *E. purpurea*, however, further validation studies are required.

Marker-assisted selection can be performed by analyzing the relationship between DNA markers and specific genes or phenotypes (Ben-Ari and Lavi 2012). RAPD markers can be used to evaluate diversity and identify potential sources of unique genetic material by indicating the presence of specific fragments (Nadeem et al. 2018). Several studies have evaluated the use of RAPD as markers that can link specific fragments to desired phenotypes (Ntuli et al. 2015; Vishalakshi et al. 2012; Miladinović et al. 2018; Subositi et al. 2021).

**Dendrogram**

Table 3 shows the similarity coefficient values analyzed based on Dice similarity. Coefficient values closer to 1 indicate a high degree of similarity between samples, while values closer to 0 indicate increasing dissimilarity (Due et al. 2019). Gamma-ray irradiation treatments at doses of 0, 20 Gy, 40 Gy, and 60 Gy resulted in similarity coefficients ranging from 0.637 (63.7%) between the control and 60 Gy, to 0.844 (84.4%) between the control and 20 Gy. Based on these coefficient values, it is evident that the control plants and those treated with 20 Gy still have a close similarity, while the control plants compared with those treated with 40 Gy and 60 Gy show increasing dissimilarity.

The study by Subositi and Widiastuti (2013) reported that RAPD analysis of 3 accessions and 8 variants of *E. purpurea* showed narrow genetic diversity, with similarity indices ranging from 75.49% to 84.20%. This aligns with the results of this study, where the similarity index for *E. purpurea* control plants and those treated with 20 Gy ranged from 81.4% to 84.4%. This indicates that gamma-ray irradiation at 20 Gy was not able to significantly widen the similarity distance, as the similarity index remained high compared to the control plants.

For the 40 Gy treatment, the similarity distance from the control plants began to widen, with a similarity index ranging from 66.6% to 74.8%, indicating a 14.8% increase in dissimilarity. Meanwhile, for the 60 Gy treatment, the similarity distance widened further, with a similarity index of 63.7% to 70.3% compared to the control, showing a 17.7% increase in dissimilarity. These results demonstrate that gamma-ray irradiation at doses of 40 Gy and 60 Gy can induce mutations, leading to the formation of new alleles and thus increasing dissimilarity. The use of gamma-ray irradiation, which also depends on the dose, can cause larger chromosomal deletions through the occurrence of deletions or insertions of various sizes, as well as translocations (Sikora et al. 2011). Mutations in genes can result in the creation of new alleles (Ulukapi and Nasircilar 2019). The formation of new alleles through mutations induced by gamma-rays contributes to the overall genetic diversity of a species (Beyaz and Yildiz 2017).

**Table 3.** The similarity coefficient value of the gamma-ray irradiation results on *Echinacea purpurea* based on the RAPD marker

| | C_1 | 20gy_1 | 40gy_1 | 60gy_1 | K_2 | 20gy_2 | 40gy_2 | 60gy_2 |
|---|---|---|---|---|---|---|---|---|
| **C_1** | 1.000 | | | | | | | |
| **20gy_1** | **0.844** | 1.000 | | | | | | |
| **40gy_1** | 0.733 | 0.740 | 1.000 | | | | | |
| **60gy_1** | 0.696 | 0.748 | 0.740 | 1.000 | | | | |
| **K_2** | 0.814 | 0.792 | 0.740 | 0.703 | 1.000 | | | |
| **20gy_2** | 0.814 | 0.792 | 0.711 | 0.644 | 0.837 | 1.000 | | |
| **40gy_2** | 0.666 | 0.718 | 0.814 | 0.674 | 0.748 | 0.733 | 1.000 | |
| **60gy_2** | 0.644 | 0.651 | 0.688 | 0.725 | **0.637** | 0.681 | 0.740 | 1.000 |

Note: C: Control

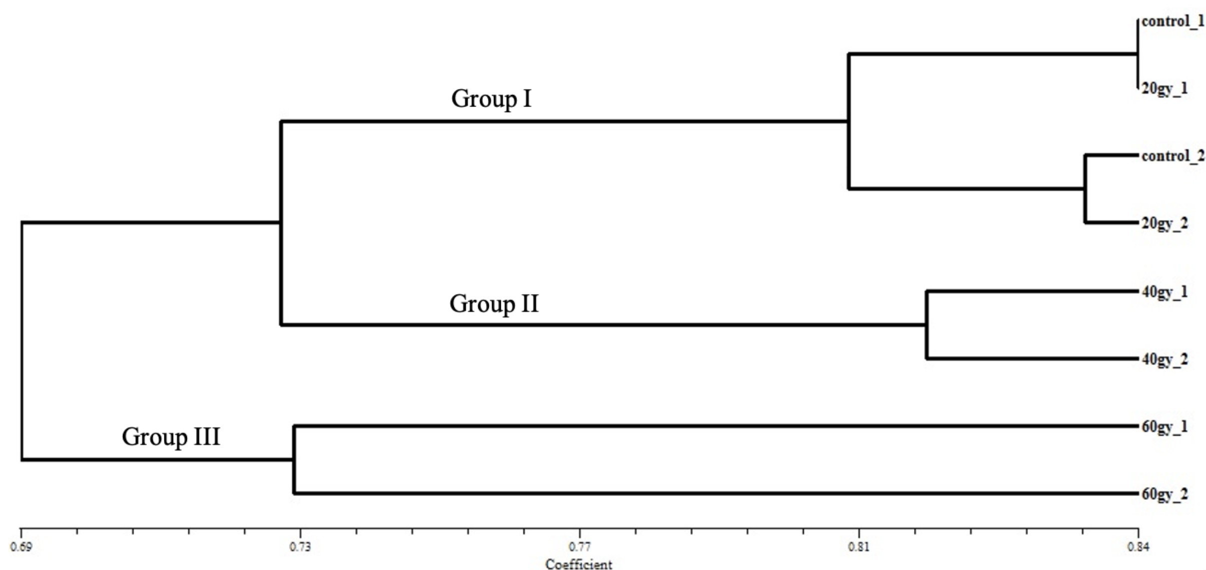


**Figure 3.** Dendrograms based on the estimation of genetic distance coefficient and UPGMA clustering of gamma-ray irradiation treatment on *Echinacea purpurea* based on RAPD markers

The dendrogram was constructed based on the appearance of DNA bands resulting from the amplification of 9 RAPD primers. The dendrogram construction (Figure 3) shows that gamma-ray irradiation of *E. purpurea* resulted in 3 main groups (I, II, III): the first group consists of the control and the 20 Gy treatment, the second group corresponds to the 40 Gy treatment, and the third group corresponds to the 60 Gy treatment. The dendrogram also indicates that the 60 Gy treatment has the greatest distance from the control and other irradiation treatments, while the 20 Gy treatment has the closest distance to the control.

Gamma-rays are physical mutagens that, when applied to plant organs, induce oxidative stress by generating excessive reactive oxygen species (ROS). The ROS produced can include superoxide radicals ($O^{2-}$), hydroxyl radicals ($OH^-$), and hydrogen peroxide ($H_2O_2$) (Yin et al. 2024). High concentrations of free radicals can affect cells, including chromosomes, leading to instability in DNA structure and causing DNA damage. However, exposure to low doses of gamma-ray irradiation, combined with DNA repair mechanisms and the role of non-enzymatic antioxidants, allows mutations to occur without resulting in significant damage (Riviello-Flores et al. 2022). Non-enzymatic antioxidants, such as phenols, have protective properties and reduce free radicals by donating hydrogen atoms or electrons, stabilizing the radicals (Hanafy and Akladious 2018). At low doses of gamma-ray irradiation, cells rely on a combination of direct and indirect DNA damage repair mechanisms to mitigate the impact of reactive oxygen species (ROS) and the resulting damage. While ROS can contribute to indirect DNA damage, various DNA repair pathways, including antioxidant defenses, play a crucial role in reducing ROS-induced damage by scavenging ROS and preventing further oxidative stress (Alanazi et al. 2024).

*Echinacea purpurea* is a medicinal plant with high secondary metabolite content, including flavonoids or phenols (Sidhiq et al. 2020; Ferdyana et al. 2021), which can act as antioxidants (Banica et al. 2020). Gamma-ray irradiation at doses of 20-60 Gy is still relatively low, meaning that the resulting free radicals can be reduced, leading to a low possibility of nitrogen base mutations. The changes occurring in the DNA may also relate to functional alterations; the mutations produced can lead to the formation of new alleles and increase the dissimilarity between mutant plants and control plants. Changes in DNA can manifest as base breaks, translocations, or nitrogen base deletions (Shu et al. 2012), resulting in the emergence of new DNA structures or the loss of DNA bands, which are detected as polymorphisms in molecular markers. This study's results demonstrate that RAPD markers can detect changes occurring in *E. purpurea* plants subjected to gamma-ray irradiation, also resulting in groupings of plants based on their similarity distances, which can be used as a basis for breeding and developing new varieties.

In conclusion, gamma irradiation at doses of 20 Gy, 40 Gy, and 60 Gy induces significant genetic variation in *E. purpurea* as revealed by RAPD markers. A total of nine primers, including OPA-10, OPA-16, and OPA-18, generated 58.04% polymorphism, with Dice similarity coefficients ranging from 63.7% to 84.4%. The highest genetic dissimilarity was observed at the 60 Gy dose, indicating a dose-dependent increase in genetic divergence. UPGMA clustering analysis successfully separated plants into distinct groups based on irradiation dose, further supporting the dose-dependent nature of the genetic variation induced. These results suggest that gamma irradiation can enhance the genetic diversity of *E. purpurea*, providing valuable genetic resources for breeding programs aimed at improving agronomic traits and phytochemical content. The findings also provide promising preliminary evidence for the potential use of gamma-induced genetic variation in *E. purpurea*, further research is required with larger sample

sizes, multiple accessions, and multi-generational analysis to confirm the stability of the mutations and assess the practical utility of these mutants for breeding. Future studies should also integrate agronomic and phytochemical evaluations to determine the full potential of gamma irradiation in enhancing desirable traits for crop improvement. Additionally, primers OPA-16 and OPB-04, which showed strong polymorphism in this study, are recommended for future marker-assisted selection in *E. purpurea* breeding programs.

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