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Morphological characters variation of Indonesian accession *Echinacea purpurea* in response to gamma-ray irradiation

AGUSTINA PUTRI CAHYANINGSIH[1], NITA ETIKAWATI[1,2], AHMAD YUNUS[3,4,♥]

[1]Graduate Program of Bioscience, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia

[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia

[3]Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret. Jl. Ir. Sutami 36 A, Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-637457, ♥email: yunus.uns7@yahoo.com

[4]Center of Biotechnology and Biodiversity, Research and Development, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia

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Abstract. *Cahyaningsih AP, Etikawati N, Yunus A. 2022. Morphological characters variation of Indonesian accession* Echinacea purpurea *in response to gamma-ray irradiation. Biodiversitas 23: 5351-5359.* Indonesia was one of the countries that introduced *E. purpurea* as a medicinal plant. Accessions of *E. purpurea* that have been successfully cultivated in Indonesia have narrow genetic diversity, lack accession variation, and have almost uniform tillers. This study was conducted to determine the effect of different doses of gamma-ray irradiation on morphological characteristics of *E. purpurea* accession B2P2TOOT. The experimental design was a Randomized Block Design with three replications, and six doses of gamma-ray irradiation (0, 15 Gy, 20 Gy, 25 Gy, 40 Gy, and 60 Gy) were used. The qualitative morphological data were presented descriptively; quantitative data were analyzed using ANOVA followed by a DMRT test at a 5% level with SPSS 16.0 application. The Dice similarity algorithm analyzed the similarity index, group analysis, and dendrogram construction using the UPGMA method with the NTSYS 2.02 application. Gamma-ray irradiation treatment increased the survival rate of *E. purpurea* plants grown in tropical lowlands. A dose of 15-60 Gy gamma irradiation did not affect the qualitative morphology of *E. purpurea* roots, stems, and leaves. Irradiation at doses of 40 Gy and 60 Gy resulted in flowers with more variation in color, overall flower shape, and arrangement of ray floret. Gamma irradiation significantly affected plant height, leaf length, leaf area, flower angle, and the first day of flowering. The 40 and 60 Gy doses resulted in longer leaves with wider leaf surfaces. The dendrogram revealed that *E. purpurea* irradiation resulted in two main groups, with doses of 40 and 60 Gy forming their groups and increasing morphological variation by 30% compared to controls.

Keywords: *Echinacea purpurea*, gamma irradiation, morphological variation

INTRODUCTION

Echinacea purpurea is one of nine perennial herbaceous Echinacea (Asteracea) species native to North America and has been used for centuries as a traditional medicinal plant (Kapteyn et al. 2002). *Echinacea purpurea* has numerous pharmacological benefits, including anti-inflammatory and immunostimulant properties. The plant *E. purpurea* is also useful as an antidepressant, antianxiety, cytotoxic, and antimutagen. Several biological activities, such as antiviral benefits, antibacterial, and antioxidant, have also been reported in *E. purpurea* plants (Manayi et al. 2015).

Indonesia was the first country to introduce *E. purpurea* as a medicinal herb. Rahardjo (2000) and Rahardjo et al. (2001) conducted preliminary research in Indonesia on the cultivation potential and adaptability of *E. purpurea* in tropical environments. *Echinacea purpurea* can thrive in both the highlands and the lowlands of the tropics (Rahardjo 2005). Gajalakshmi et al. (2012) reviewed that *E. purpurea* is the most adaptable species in the genus *Echinacea* and that cultivation development in Indonesia will provide good opportunities in the future. The Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) in Indonesia also cultivated *E. purpurea* (Fauzi et al. 2013) by obtaining ten accessions of *E. purpurea* (Subositi dan Fauzi 2011).

Research on developing *E. purpurea* accessions in Indonesia has been limited (Sidhiq et al. 2020). Developments in cultivation, production optimization, and variety formation are required to use *E. purpurea* as a medicinal plant to achieve optimal productivity in terms of quantity and quality. One approach to accession development is to create new superior varieties by leveraging the genetic diversity of existing accessions.

In this study, *E. purpurea* accession 3, one of the leading B2P2TOOT accessions, was utilized. Accession 3 was one of the *E. purpurea* accessions grown in the highlands that lacked accession variants with uniform tillers (Subositi and Fauzi 2016). Accession 3, with low growth yield and secondary metabolites, is grown in both highland and lowland areas (Sidhiq et al. 2020). Meanwhile, superior plant varieties can be obtained by increasing plant genetic variation.

Mutation induction is a widely used technique to increase genetic variation. Mutant plants produced through mutation induction can be used directly as new varieties or as parents to produce new varieties. The radiation-induced mutation is the most commonly used method for developing mutant varieties, accounting for up to 89% of all mutant varieties. Gamma-ray irradiation was used to create 64% of mutant varieties (Ahloowalia et al. 2004). Mutation induction with gamma-ray radiation effectively creates new and superior varieties in terms of increasing quantity, quality, and time efficiency. Morphological characteristics have been widely used to investigate irradiated mutant plants. Morphological characters are markers still used to differentiate and group mutant plants (Susila et al. 2019; Nurmansyah et al. 2020).

Based on this framework, this study was conducted to develop *E. purpurea* accessions successfully cultivated in Indonesia through mutation induction. The mutant *E. purpurea* lines obtained in this study can later be used to increase yields in terms of qualitative variation, growth, and quantifiable secondary metabolites and as a source for creating new varieties that excel as medicinal plant materials. Furthermore, the superior accessions to be developed through irradiation may also reduce imports of these plants to provide raw materials for the Indonesian pharmaceutical industry.

The main purpose of this study was to determine the effect of different gamma-ray irradiation doses on morphological characters of *E. purpurea* accession B2P2TOOT. Gamma-ray treatment in *E. purpurea* seeds is expected to increase the variety of *E. purpurea* plants and even provide a source for forming new varieties.

MATERIALS AND METHODS

Plant material

The seeds of *Echinacea purpurea* accession 3 were obtained from the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT), Tawangmangu, Central Java, Indonesia. Irradiation of *E. purpurea* seeds was conducted at the Isotope and Radiation Application Center - National Nuclear Energy Agency (PAIR-BATAN), Jakarta, on 29 April 2021. Seedlings of *E. purpurea* were grown in the greenhouse at the Integrated Laboratory, Sebelas Maret University, Surakarta. *Echinacea* seedlings are transferred to polybags before being transplanted to an experimental field at the Sebelas Maret University agricultural area in Jumantono, Central Java, Indonesia (293 m asl). The study was conducted between April to December 2021.

Procedure

This study was an experimental study that employed a Randomized Block Design (RBD) method with variations in gamma ray irradiation doses as a treatment factor. Treatments with six irradiation levels were planted in beds at random, with three groups serving as replicates.

Seed preparation

Flowers of *E. purpurea* accession 3 from B2P2TOOT were harvested and dried under the sun for two days. Then, the seeds attached to the dried flowers were separated and sorted by only taking the seeds containing the embryo.

Gamma-ray irradiation

A total of 900 *E. purpurea* seeds were used in this study, with 150 seeds for each treatment placed in plastic clips and labeled with the radiation dose. Seeds were irradiated with a Gamma Cell-220 upgraded at 0 Gy (control), 15 Gy, 20 Gy, 25 Gy, 40 Gy, and 60 Gy by a ^{60}Co source at a dose rate of 3789.4 Gy h^{-1}.

Seed seeding and polybags transplanting

The irradiated *E. purpurea* seeds were immediately planted in a plastic tray (34x27x5 cm) with a substrate mix comprised of soil, manure, and roasted husks (3:2:1) for recovery in a greenhouse. Sprouts were watered and maintained daily until 30 days after sowing (DAS).

E. purpurea sprouts were transplanted into polybags (15x15 cm) with a soil, manure, and roasted husks ratio of 3:2:1. *Echinacea purpurea* seedlings were kept in a greenhouse by watering every three days until they reached 90 days after planting polybags (DAPP).

Field planting

Echinacea seedlings were transplanted into the field using beds (300x100 cm) at 90 DAPP. The plants were planted 30 cm apart, with a 50 cm distance between beds; a 65% density paranet shaded the beds. A total of 360 *E. purpurea* plants were used in this study, with 60 plants for each treatment. In each bed, twenty *E. purpurea* seedlings were planted at random, with three groups of beds serving as replicates. In each treatment, 15 plants from 3 replicate groups were used as samples for 90 sample plants.

Plants were watered daily, weeds and pests were controlled mechanically, and manure fertilizer was applied to 20 kg for each bed. The *Echinacea* plants were kept alive until flowering, which occurred 120 days after field planting (DAFP).

Data collection

Observations were made on qualitative and quantitative morphological characteristics. The qualitative morphological characteristics observed were root system, root color, stem color, stem texture, leaf shape, leaf color, leaf edge, leaf tip, phyllotaxis, leaf surface, leaf venation, flower color, flower shape, the shape of the receptacle, the arrangement of ray floret. In addition, qualitative morphological characteristics were observed at the age of 120 DAFP. The quantitative morphological characteristics measured included the first day of flowering, plant height, stem diameter, leaf length, leaf width, leaf area (Due et al. 2019; Susila et al. 2019), number of flowers, flower diameter (Lin-na 2013), receptacle diameter, ray floret length, and the flower angle.

Data analyzes

The qualitative morphological data are presented descriptively. First, the description was completed by summarizing and comparing the outcomes of various treatments. Then, quantitative morphological data were analyzed using ANOVA, and the DMRT test was performed at the 5% level using the SPSS 16.0 application.

Qualitative and quantitative morphological data are scored based on the characteristics. The obtained values were then standardized into binary data (Elly et al. 2018). The Dice similarity algorithm was used to analyze the similarity index. The UPGMA (Unweighted Pair Group Method Using Arithmetic Method) on the NTSYS 2.02 (Numerical Taxonomy and Multivariate Analysis System) application was used for group analysis and dendrogram construction (Rohlf 2000).

RESULT AND DISCUSSION

Germination, seedling, and plant survival

The study used 900 *E. purpurea* seeds, but only 537 or (59.66%) germinated. While many seeds did not germinate, treatment at doses of 20 Gy and 40 Gy had the lowest germination percentage compared to the control and all treatments. Germination percentage increased at 15 Gy, 25 Gy, and 60 Gy doses. A total of 537 seedlings were transplanted into polybags, and 510 seedlings survived during the growth process in the greenhouse. Irradiation doses of 20 Gy and 40 Gy had a 100% seedling survival percentage. A total of 5% of seedlings died, especially in control and dose of 15 Gy, with the lowest percentage of seedling survival. It was revealed throughout the investigation that all plants planted in the field could live in the vegetative phase; however, some of the control plants died (3.3%) in the generative phase, but all irradiated plants survived until harvest time. The percentage of germination, seedling survival in the greenhouse, and plant survival in the field are shown in Figure 1.

The results showed variations in the effect of several doses of gamma radiation on the percentage of germination and seedling survival. These results align with the research of Amirikhah et al. (2021), gamma irradiation with low doses (up to 75 Gy) on *Festuca arundinacea* produced variations in germination and had a stimulating effect on seed germination. Low doses of radiation (25-75 Gy) cause an increase in the activity of seed germination enzymes, accelerate the decomposition of nutrients in seeds, and stimulate various biological processes in germination and sprout growth. In addition, the low concentration of H_2O_2 generated by irradiation acts as a key signal to regulate biological activity (Amirikhah et al. 2021).

The finding of plant survival in this study differs from previous studies on gamma irradiation in plants. Susila et al. (2019) reported that when irradiated at a dose of 10-20 Gy, *Chrysanthemum* plants died in huge numbers due to physiological abnormalities compared to the controls that survived better. In the study of Rifnas et al. (2020) on gamma irradiation of *Allamanda cathartica*, control plants had the highest survival rate, while increasing the irradiation dose decreased the survival rate.

Those showed that gamma-irradiated *E. purpurea* plants at a dose of 20-60 Gy increased plant survival in the field. Furthermore, *E. purpurea* plants from subtropical countries were grown in tropical lowlands, where physiological disturbances made it more difficult for control plants to survive. In contrast, irradiated plants experienced mutations (Hase et al. 2020) and improved biological activity by influencing antioxidant enzymes (Amirikhah et al. 2021). Increased proline and phenolic synthesis enhanced by gamma irradiation indicate protective mechanisms necessary to improve plant stress tolerance. A low radiation dosage can promote plant growth and development (Akshatha et al. 2013) and significantly improves physiological criteria (Hanafy and Akladious 2018), and affects increasing tolerance to abiotic stress and crop yields (El-Beltagi et al. 2011). The *E. purpurea* plants produced in this study can be seen in Figure 2.

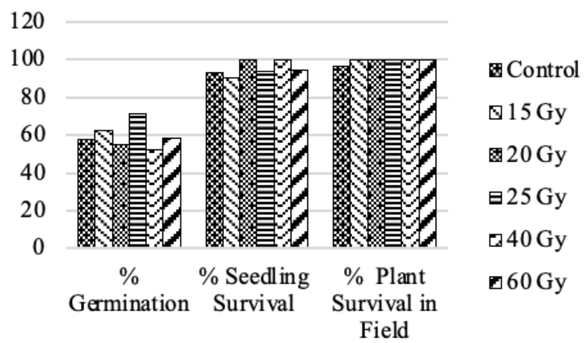

Figure 1. Percentage of germination, seedling survival in the greenhouse, and plant survival in the field of gamma-irradiated *Echinacea purpurea*

A B C D E F

Figure 2. Whole plant morphology of gamma-ray irradiated *Echinacea purpurea* at 120 DAFP. A. Control; B. 15 Gy; C. 20 Gy; D. 25 Gy; E. 40 Gy; F. 60 Gy. Scale bar: 10 cm

Qualitative morphological characters

Stem

Similar to the control plants, all treatments had light green stems with purplish stem bases and green or deep brown spots all over the main stem. Another aspect was the texture of the stems, with all *E. purpurea* treatments having the same texture, i.e., a rough surface due to the presence of trichomes. The same stem color and texture characters were observed in all treatments with gamma irradiation doses, indicating that the treatment in this study did not affect the qualitative morphology of *E. purpurea* stems. Figure 3 depicts the stem morphology of *E. purpurea*.

Research conducted by Choirunnisa et al. (2021) stated that stem color and texture observations could represent variations in the morphological characteristics of *E. purpurea* stems and that *E. purpurea* accession 3 stems had a dark green color with a rough surface and brownish-red spots. Meanwhile, Ferdyana et al. (2021) discovered that *E. purpurea* accession 3 has green stems with dark purple spots; which demonstrates that *E. purpurea* accession 3 from the tropical lowlands has variations in stem spot color, but irradiation treatment with a dose of 15-60 Gy was unable to add variations in stem color and texture. Therefore, variations in stem color in *E. purpurea* control plants in this and previous studies could be attributed to variations in environmental factors. The previous study of *E. purpurea* accession 3 used a plastic house with more homogeneous abiotic factors and a field with more heterogeneous environmental factors. Several environmental factors, including light intensity, solar radiation, wind speed, and soil criteria, play important roles in controlling and influencing plant morphological characteristics (Hassan et al. 2020).

Leaf

Gamma irradiation of *E. purpurea* did not affect the qualitative morphological characters of the leaves. In both the irradiation and control treatments, several qualitative leaf characteristics were observed, including leaf color, leaf shape, leaf venation, leaf surface, leaf edge and tip type, and phyllotaxis. The leaves of *E. purpurea* are dark green and lanceolate in shape, with curved veins (cervinervis) and a rough textured leaf surface. In addition, *E. purpurea* has two types of leaf edges, including serrated and flat, with a pointed leaf tip and rosette root phyllotaxis. At a dose of 40 Gy, gamma ray irradiation produced one variation of leaf morphology, namely blunt leaf tips. However, the presence of one such variation indicates that gamma irradiation at a dose of 15-60 Gy could not significantly increase the qualitative morphological variation of *E. purpurea*. Figure 4 depicts the leaf morphology of *E. purpurea*.

A B C D E F

Figure 3. Stem morphology of gamma-ray irradiated *Echinacea purpurea*. A. Control; B. 15 Gy; C. 20 Gy; D. 25 Gy; E. 40 Gy; F. 60 Gy. Scale bar: 2 cm

A B C D E F

Figure 4. Leaf morphology of gamma-ray irradiated *Echinacea purpurea*. A. Control; B. 15 Gy; C. 20 Gy; D. 25 Gy; E. 40 Gy; F. 60 Gy. Scale bar: 3 cm

Echinacea purpurea leaves range from lanceolate to oval, with rough or sharply serrated edges. The leaves are dark-green and have three major veins that branch out (Gajalakshmi et al. 2012). This study's leaf morphology corresponds to an accession of *E. purpurea* cultivated in the lowlands of Central Java, Indonesia, which has leaves with an elongated oval shape with a pointed tip, pointed leaf edges, dark green, and rough leaf surfaces (Sidhiq et al. 2020). Meanwhile, Choirunnisa et al. (2021) discovered that the leaves of *E. purpurea* accession 3 were elliptical, with blunt leaf tips and flat and serrated leaf edges. Some of the differences in characters observed in previous studies may be due to differences in the age of the observed leaves, whereas the leaves observed in this study were old leaves located on the main stem of the root rosette. Ferdyana et al. (2021) also reported that *E. purpurea*'s serrated leaf edge appears only on old leaves, while young leaves have flat leaf edges.

In this study, the control plant of *E. purpurea* accession 3 was found to have variations in the qualitative morphological characteristics of the leaves in the form of serrated and flat leaf edges. Those contrasts with the results of mass selection stage I of *E. purpurea* cultivated in the highlands of Central Java, Indonesia, where *E. purpurea* accession BHU5 has no variation (Subositi and Fauzi 2016). Morphological variations can occur as a result of both internal factors, such as cross-pollination (Azad et al. 2015; Liyanage et al. 2021) and external factors, such as differences in cultivation altitude and planting season (Kofidis et al. 2007). This one variation, however, is consistent with the findings of Subositi and Widiastuti (2013), who discovered that *E. purpurea* accession 3 has uniform tiller characteristics and only slightly different morphological characters.

Flower

The effect of gamma irradiation treatment on flower morphology was significant. Flowers from *E. purpurea* accession 3 had similar floral characteristics, whereas irradiated flowers had more diverse floral characteristics. Flowers in control have a downward-curving flower shape, a bright pink-purple color, round and triangular receptacles, and a tight arrangement of ray floret. Flowers at a dose of 15 Gy have characters that are still similar to the control in terms of flower color, receptacle shape, and ray floret arrangement, only that there is a slight change with the shape of the flowers getting lower down. The flower shape shifts to parallel to the receptacle at 20 Gy, with a paler purplish pink blossom color, the receptacle is round and triangular, and the ray florets are in a very tight arrangement until they are stacked. Flowers at a dose of 25 Gy have similar characteristics to doses of 15 Gy and 20 Gy; the color of the flowers is bright and pale purplish-pink with a flower shape from curved down to straight down; the shape of the receptacle is round and triangular, the ray floret has a loose and tight arrangement. At a dose of 40 Gy, the flower shape is flat and slightly curved upwards. Some flowers are pale pink, and others are darker purplish; the receptacle is round and triangular, and the ray floret is a tight and loose arrangement. At 60 Gy, the flower shape is flat and curved downwards, with more varied flower colors; the flower color characteristics that appear are bright purplish pink, darker purplish pink, purplish pink with a combination of white, round receptacles, as well as triangular, ray floret in a tight and loose arrangement. Figure 3 depicts the flower morphology of *E. purpurea.*

Figure 3. Flower morphology of gamma-ray irradiated *Echinacea purpurea* A. Control; B. 15 Gy; C. 20 Gy; D. 25 Gy; E. 40 Gy; F. 60 Gy. Scale bar: 1 cm

Flower morphological characteristics are significant for the characterization, identification, and grouping of *E. purpurea* accessions because they have more diversity than other plant parts. The leading accession, B2P2TOOT, was recognized as having accession variants predominantly related to flowering characteristics. As a result, accession 3 did not have accession variants due to floral differences. Figure 3A shows that the flowers in the control have similar flower characteristics, particularly in terms of color and shape.

According to the findings, gamma-ray irradiation at a dose of 15-60 Gy can increase the variety of flower morphology by causing changes in flower color and shape. That is consistent with several results of gamma irradiation studies on plants in the Asteraceae family, such as the induction of mutations in *Chrysanthemum morifolium* (Kumari et al. 2013), *Zinnia elegans* (Pallavi et al. 2017), and *Chrysanthemum* spp. (Susila et al. 2019). Flower colors are related to flavonoid levels, especially anthocyanins. Moreover, the alterations in irradiated flowers can be induced by variations in pigment quality and quantity caused by mutations in the anthocyanin biosynthetic pathway gene (Streisfeld et al. 2013). A 40-100 Gy gamma irradiation dose, increased the levels of anthocyanins and flavonoids in *Tulipa gesneriana* (Li et al. 2022), which may have led to darker flowers at a dose of 40-60 Gy. The change in the shape of the flower may be caused by changes in the placement of cells from meristem cells at the beginning of flower development as a result of mutations (Kumari and Kumar 2015; Li et al. 2022).

Quantitative morphological characters

Gamma-ray irradiation significantly affected quantitative morphological characters in *E. purpurea* (Table 1). The most affected characteristics are found in the leaves and flowers. Irradiation treatment, depending on doses, created significant variations in plant height, either increasing or decreasing it. While doses of 15 Gy, 20 Gy, and 25 Gy had the same effect on the control plants, a dose of 40 Gy increased plant height to 5 cm; and a dose of 60 Gy decreased plant height to 7 cm compared to the control. At a dose of 60 Gy, gamma ray irradiation resulted in larger stem diameter.

The findings of this study are consistent with those of Asare et al. (2017) and Hapsari et al. (2021), who found that the lowest dose stimulated plant height while increasing the dose caused a decrease in plant height. Gamma rays are electromagnetic rays that can pass through cells and chromosomes and cause changes in the plant genome, affecting cytology, biochemistry, and plant physiology processes (El-Beltagi et al. 2011). Irradiation dose correlates with genomic changes caused by random mutations and affects physiological aspects; higher doses negatively affect plant growth, while lower doses have a stimulatory effect (Majeed et al. 2017). Furthermore, irradiation can affect the mitotic division activity of meristem tissue (El-Sherif et al. 2011), resulting in a decrease in the frequency of mitosis in the tissue and a reduction in plant growth (Hong et al. 2022).

Leaf length and leaf area were two quantitative morphological characteristics of leaves that were affected by gamma ray treatment. Irradiating *E. purpurea* at doses of 40 and 60 Gy resulted in longer leaves and wider leaf surfaces. Meanwhile, at doses of 15 Gy, 20 Gy, and 25 Gy, leaf length and area increased slightly but not significantly compared to the control. This finding is consistent with the research from Mounir et al. (2022), who discovered that a low dose of gamma irradiation could increase the leaf area. The dose of 40 and 60 Gy used in this study was categorized as low irradiation doses, which could have a stimulatory effect in a positive direction (Majeed et al. 2017), resulting in longer leaves and wider areas than the control. Low doses of gamma rays can increase enzyme activity, accelerate cell division in meristematic tissues, expand cells, and stimulate vegetative characteristics (Ali et al. 2015).

Table 1. The effect of gamma-ray irradiation on morphological quantitive in *Echinacea purpurea*

Characters	Control	15 Gy	20 Gy	25 Gy	40 Gy	60 Gy
Plant height (cm)	$76.15^{b} \pm 0,48$	$75.1^{b} \pm 1.5$	$75^{b} \pm 1.27$	$75.2^{b} \pm 1.63$	$81.98^{a} \pm 0.61$	$71.4^{c} \pm 1.2$
Stem diameter (mm)	$8.64^{b} \pm 0.73$	$10.1^{a} \pm 1.04$	$8.40^{b} \pm 0.47$	$8.72^{b} \pm 0.7$	$8.82^{ab} \pm 0.5$	$8.32^{b} \pm 0.38$
Leaves length (cm)	$27.28^{b} \pm 0.33$	$27.61^{b} \pm 1.1$	$27.33^{b} \pm 0.33$	$28.47^{b} \pm 1.6$	$33.02^{a} \pm 0.69$	$32.12^{a} \pm 1.4$
Leaves width (cm)	$6.11^{a} \pm 0.37$	$6.01^{a} \pm 0.82$	$5.51^{a} \pm 0.43$	$6.02^{a} \pm 0.35$	$6.36^{a} \pm 0.65$	$6.57^{a} \pm 0.2$
Leaves area (cm^2)	$61.13^{b} \pm 3.95$	$67.18^{b} \pm 5.74$	$65.32^{b} \pm 7.84$	$66.82^{b} \pm 2.3$	$77.64^{a} \pm 3.6$	$79.02^{a} \pm 3.05$
Number of flowers	$13.53^{a} \pm 1.28$	$15.86^{a} \pm 1.47$	$13.66^{a} \pm 1.85$	$13.6^{a} \pm 0.91$	$16.46^{a} \pm 4.22$	$12.8^{a} \pm 2.94$
Flower diameter (cm)	$10.56^{ab} \pm 0.11$	$11.5^{a} \pm 0.39$	$10.62^{ab} \pm 0.58$	$10.68^{bc} \pm 0.42$	$11.26^{a} \pm 0.33$	$10.37^{c} \pm 0.65$
Receptacle diameter (mm)	$31.8^{a} \pm 1.42$	$32.61^{a} \pm 1.8$	$31.32^{a} \pm 1.11$	$31.64^{a} \pm 2.09$	$30.7^{a} \pm 0.57$	$31.36^{a} \pm 1.63$
Ray-floret length (cm)	$4.28^{a} \pm 0.12$	$4.61^{a} \pm 0.1$	$4.43^{a} \pm 0.26$	$4.52^{a} \pm 0.23$	$4.58^{a} \pm 0.14$	$4.22^{a} \pm 0.32$
Days to first flowering (DAP)	$61.33^{b} \pm 1.52$	$63.33^{b} \pm 1.52$	$63.33^{b} \pm 0.57$	$64^{b} \pm 1$	$68.73^{a} \pm 5.14$	$72.73^{a} \pm 0.64$
Flower angle (º)	$55.33^{a} \pm 3.93$	$41.4^{b} \pm 7.2$	$39.46^{b} \pm 2.23$	$40.26^{b} \pm 7.08$	$63.13^{a} \pm 0.72$	$63.4^{a} \pm 0.72$

Note: DAFP: Days after field planting. Numbers followed by the same letter do not show a significant difference according to the results of the DMRT test at a 5% level

The flower characteristics are the most changed among all parts of the plant, both qualitatively and quantitatively. The gamma-ray irradiation treatment strongly influences the angle of the flower and the first day of flowering. The number of flowers, the diameter of the flower, the diameter of the receptacle, and the length of the ray floret was not affected. Among all treatments, the dose of 15 Gy produced the largest flower and receptacle diameter. The dose of 20 Gy produced the lowest flower angle among all treatments and the controls but did not differ significantly with 15 Gy and 25 Gy. The 40 Gy dose treatment resulted in more flowers and required a longer time to first flower than the control and lower dose treatments. In addition, for flowers at doses of 40 Gy and 60 Gy, there was an increase in the flower angle compared to all treatments. The plants treated with 60 Gy produced the fewest flowers and took the longest to flower for the first time. These findings are consistent with those of Bharathi et al. (2013), Singh et al. (2017), and Kumar and Mishra (2021). They found that increasing the gamma irradiation dose causes plants to take longer to produce flower buds and enter a generative phase. According to the findings of this study, gamma ray irradiation can also delay plant maturity (Verma et al. 2017).

Dendrogram

A dendrogram was constructed using data from observed qualitative and quantitative morphological characters. The dendrogram results revealed that the gamma irradiation treatment of *E. purpurea* accession 3 resulted in two main groups (I and II) with a 70% similarity (Table 2). The control and irradiation treatment with a dose of 15 Gy, 20 Gy, and 25 Gy formed one group (I) with 77-100% similarity, while the gamma irradiation treatment with doses of 40 Gy and 60 Gy formed one group (II) with 70-88% similarity. The dendrogram also revealed that gamma-ray irradiation on *E. purpurea* increased morphological diversity by up to 30% compared to the control plant group. *Echinacea purpurea* accession 3 is one of the Indonesian *E. purpurea* accessions with slightly different morphological characteristics. The dendrogram also demonstrates that two of the three control sample plants had 100% similarity, indicating that the plants shared the same traits.

Gamma irradiation at doses of 15-25 Gy only increased the similarity distance by 4-23%, and those treatments remained in the same group as the control plants. Gamma irradiation of *E. purpurea* at the doses of 40 Gy and 60 Gy significantly increased the similarity distance by 12-30%. The obtained variations occurred in the morphological characteristics of the leaves and flowers. However, some morphological characters of *E. purpurea* at a dose of 15-25 Gy did not experience significant changes compared to control plants and caused the plants in the treatment to remain in the same group as the control. Those could be related to *E. purpurea*, which has a fairly high antioxidant content of various secondary metabolites found in all parts of the plant as a medicinal plant. When gamma rays are applied to plant parts as mutagens, they cause the formation of several types of free radicals, which then affect DNA structure, gene expression, cytology, biochemistry, and plant physiology (El-Beltagi et al. 2011; Majeed et al. 2014). Plant antioxidant content can reduce or eliminate free radicals in cells, allowing the effects of low-dose mutagens to be mitigated further (Hong et al. 2022). The plant defense mechanism is activated under a low dose of gamma rays to cope with the damage (Ali et al. 2015). The 40 Gy and 60 Gy of irradiation can be used as an effective irradiation dose for *E. purpurea*, as it does not cause too many mutations, leading to positive results, such as quality and quantity variations in plant morphology after irradiation (Majeed et al. 2017).

Table 2. Similarity coefficient of gamma ray-irradiated *Echinacea purpurea* based on morphological characters

	C_1	**C_2**	**C_3**	**15_1**	**15_2**	**15_3**	**20_1**	**20_2**	**20_3**	**25_1**	**25_2**	**25_3**	**40_1**	**40_2**	**40_3**	**60_1**	**60_2**	**60_3**
C_1	1.00																	
C_2	0.92	1.00																
C_3	1.00	0.92	1.00															
15_1	1.00	0.92	1.00	1.00														
15_2	0.88	0.88	0.88	0.88	1.00													
15_3	0.85	0.85	0.85	0.85	0.88	1.00												
20_1	0.92	1.00	0.92	0.92	0.88	0.85	1.00											
20_2	0.96	0.88	0.96	0.96	0.92	0.88	0.88	1.00										
20_3	0.85	0.77	0.85	0.85	0.88	0.92	0.77	0.88	1.00									
25_1	0.96	0.96	0.96	0.96	0.92	0.81	0.96	0.92	0.81	1.00								
25_2	0.88	0.88	0.88	0.88	0.85	0.88	0.88	0.92	0.88	0.85	1.00							
25_3	0.85	0.77	0.85	0.85	0.88	0.92	0.77	0.88	1.00	0.81	0.88	1.00						
40_1	0.88	0.81	0.88	0.88	0.77	0.74	0.81	0.85	0.74	0.85	0.77	0.74	1.00					
40_2	0.77	0.70	0.77	0.77	0.70	0.66	0.70	0.77	0.74	0.74	0.77	0.74	0.88	1.00				
40_3	0.70	0.70	0.70	0.70	0.66	0.70	0.70	0.70	0.66	0.66	0.74	0.66	0.81	0.81	1.00			
60_1	0.77	0.70	0.77	0.77	0.70	0.66	0.70	0.77	0.70	0.74	0.74	0.70	0.85	0.85	0.88	1.00		
60_2	0.74	0.74	0.74	0.74	0.74	0.66	0.74	0.74	0.66	0.77	0.66	0.66	0.81	0.77	0.77	0.88	1.00	
60_3	0.70	0.77	0.70	0.70	0.74	0.70	0.77	0.66	0.66	0.74	0.70	0.66	0.77	0.70	0.81	0.81	0.81	1.00

Note: C: Control; 15: 15 Gy; 20: 20 Gy; 25: 25 Gy; 40: 40 Gy; 60: 60 Gy

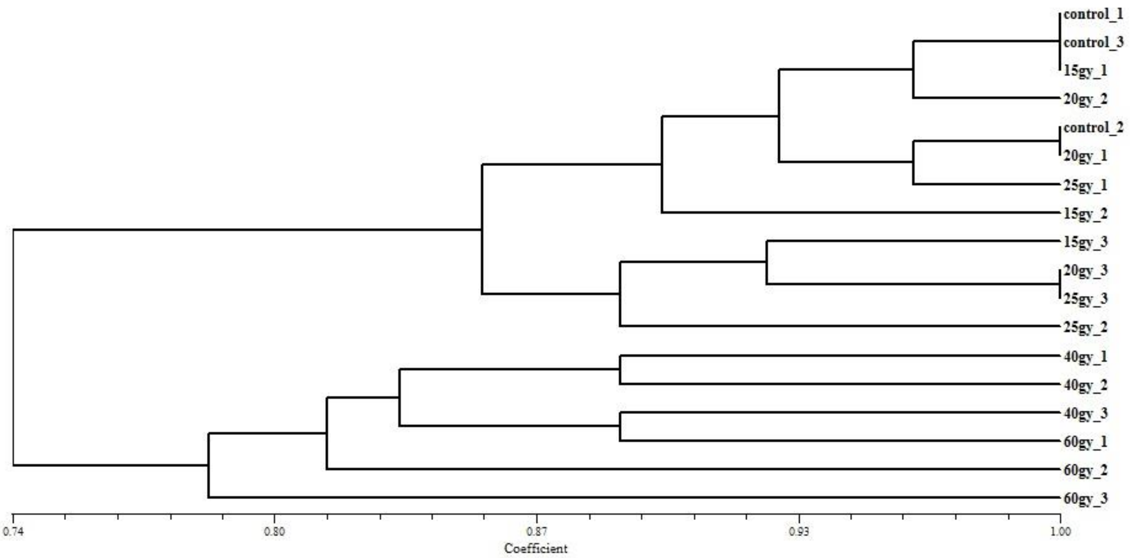

Figure 5. Dendrogram of gamma-ray irradiated *Echinacea purpurea*

In conclusion, gamma ray irradiation treatment increased the seedling and plant survival of *E. purpurea* accession 3 in the field under tropical lowlands conditions. A dose of 15-60 Gy gamma irradiation did not affect the qualitative morphology of *E. purpurea* roots, stems, and leaves. Irradiation at doses of 40 Gy and 60 Gy resulted in flowers with more variation in color, flower shape, and arrangement of ray floret. Gamma irradiation had a statistically significant effect on plant height, leaf length, leaf area, flower angle, and the first day of flowering. Doses of 40 and 60 Gy resulted in longer leaves with wider leaf surfaces and the highest flower angle and took longer to flower for the first flowering. The dendrogram revealed that *E. purpurea* irradiation resulted in two main groups, with doses of 40 and 60 Gy forming their groups and increasing morphological variation by 30% compared to controls. Mutant *E. purpurea* plants induced by gamma irradiation at a dose of 40-60 Gy in this study can be used for varietal assembly selection.

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