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Morphological, agronomic characteristics, and flavonoid content of *Echinacea purpurea* at various gamma ray doses

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Abstract

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Purple coneflower is an introduced herbal plant that has anti-inflammatory and antibiotic benefits. The pharmaceutical industry is currently starting to develop the use of herbal plants as medicinal raw materials, including this plants. Indonesia has started cultivating purple coneflower but it is only cultivated in the highlands which have low temperatures. The one method of plant breeders to expand plant adaptation in high temperature environments without reducing the content of secondary metabolites is by using gamma ray irradiation. This study aims to determine the effect of gamma ray irradiation on the diversity of growth, yield and flavonoid content of purple coneflower. Purple coneflower was irradiated with doses of 0 Gy (control), 5 Gy, 15 Gy, and 25 Gy. The research design used simple design experiment in plots without repetition. The results show that the diversity in plant height, number of leaves and flowers, and biomass weight of the mutant plants is higher than the control. The mutant plants in dosage 15 Gy are the highest in diversity. The flavonoid content of mutant plants is lower than the control, except in dosage 25 Gy.

Keywords: medicinal plant; mutation; purple coneflower; diversity; secondary metabolites

Introduction

Purple coneflower (*Echinacea purpurea* (L.) Moench) is a herbaceous plant of the Asteraceae family introduced from North America. This plant is famous for its various secondary metabolites, such as phenols, polyphenols, flavonoids, alkamides, caffeic acid, polyacetylene, phenylpropanoids, and p-coumaric (Subositi & Fauzi, 2016; Zayova et al., 2012). These compounds have benefits as antioxidants, anti-inflammatories and immunomodulators (Sidhiq et al., 2020). The plant is widely used as a treatment of upper respiratory tract infections, colds, coughs, bronchitis, urinary tract infections, and some inflammatory conditions (Kumar & Ramaiah, 2011). The use of herbal medicine as an alternative to modern allelopathic medicine has become known globally (Mukherjee, 2019). Purple coneflower extract is widely used as a raw material for the pharmaceutical industry and continues to grow rapidly in various countries, including Indonesia. *Echinacea purpurea* extract as a medicinal raw material is widely produced in America and some European countries (Kindscher, 2016). The pharmaceutical industry in Indonesia began to utilize purple coneflower extract as a medicinal raw material, although it was still sourced from othe country (import) (Rahardjo et al., 2001).

Echinacea purpurea plant was first introduced in Indonesia in 1998, but only can grow better in the highlands than lowlands (Rahardjo, 2005). Research of (Sidhiq, 2020) planted three accessions of *Echinacea purpurea* in the highland, middle, and lowland showed decreased growth, yield and content of secondary metabolites along with low altitude. Efforts to improve plant genetics can be made by using technology of gamma ray irradiation. Plants resulting from irradiation at certain doses will make the plant mutated so that changes occur in both the characteristics and physiology of the plant. Various studies of gamma-ray irradiation in plants showed changes in germination, plant growth and yield, the amount of chlorophyll, the level of oxidative stress, as well as the content of secondary metabolite compounds (Hong et al., 2022; Moghaddam et al., 2011). Only through small doses, gamma-ray irradiation becomes a large, cost-effective instrument to produce quality extracts of raw materials with high values (Pelcaru et al., 2021). Research on the right dose of gamma-ray irradiation in purple coneflower aims to obtain *Echinacea purpurea* plants that are able to increase production and content of secondary metabolite compounds in tropical climate conditions in the lowlands.

Materials and Methods

Materials needed for field research were manure, organic fertilizer, and seed plants. The purple coneflower seeds used was accession two obtained from the Medicinal Plants and Traditional Medicines Research and Development Centre (B2P2TOOT) Tawangmangu. The materials needed for laboratory research were samples of purple coneflower, aqueous, quercetin, methanol, ethanol, Folin-Ciocalteu reagan, acetic acid, Na_2CO_3, $AlCl_3$, and filter paper.

This field research was carried out on agricultural fields of the Faculty of Agriculture, Sebelas Maret University, Jumantono, Indonesia at an altitude of 173 m above sea level out from May 2021 to January 2022. The study design used a simple randomized experimental design by planting the population of each treatment into plots without repeatation.

- Seed Irradiation

The purple coneflower seeds was irradiated using cobalt-60 gamma-ray chambers at the National Nuclear Energy Agency of Indonesia. The dose rate used consists of 4 dosages, i.e 0 Gy (control), 5 Gy, 15 Gy, and 25 Gy.

- Qualitative Observations

The morphology of the purple coneflower plant was identified descriptively by observing the plant's characteristics. The observed morphological character variables include leaf color, leaf shape, leaf edge, leaf texture, flower color, and flower shape. Observations were carried out when the plant enters the generative phase.

- Quantitative Observations

Echinacea purpurea plants were harvested when 85% of the plant population had flowered. The harvest of the plant covers all parts of the plant; roots, stems, leaves and flowers. Quantitative observations include plant height, number of leaves, number of flowers, and plant biomass weight.

- Flavonoid Analysis

The extract was concentrated with a rotavator until it forms a viscous extract. 100 mg of viscous extract was weighed and then dissolved with 10 ml of 70% ethanol. The solution was personified for 15 min then precipitated overnight. There were 2 solutions made. The first solution contained 0.2 ml of the extract and 4.8 ml of aquabidest. The second solution contained 0.2 ml of the extract, 1 ml of $AlCl_3$, 3.8 ml of aquabidest. Both solutions were incubated for 15 min. The flavonoid test used a spectrophotometer with a wavelength of λ 370 – 450 nm three times. The calculation of the total flavonoids using the formula:

$$F = \frac{CV\,Fp\ 0{,}001}{m} \times 100\%,$$

where:

F = total flavonoids of the $AlCl_3$ method, %;
C = quercetin equality, μg/ml;
V = extract volume, ml;
Fp = dilution factor
m = sample weight, mg.

- Data Analysis

Qualitative data in the form of morphological characteristics of purple coneflower plants were analyzed descriptively. Quantitative data were displayed using RStudio in boxplot form to show the diversity of agronomic characteristics.

Results and Discussion

Morphological Characteristics

The leaves of the purple coneflower plant are non-irradiated and 5 Gy have a dark green color (Figure 1), while at doses of 15 Gy and 25 Gy have a bright green color. The purple coneflower leaf shape between the control plant and the mutant is both ovoid with a pointed tip but has differences in the edge of the leaf, where the leaves of the control plant have a smooth leaf edge, while the mutant plant has a jagged leaf edge. The texture of the leaves of both mutant plants and the controls on the upper part are smooth and on the underside of the leaves are rough.

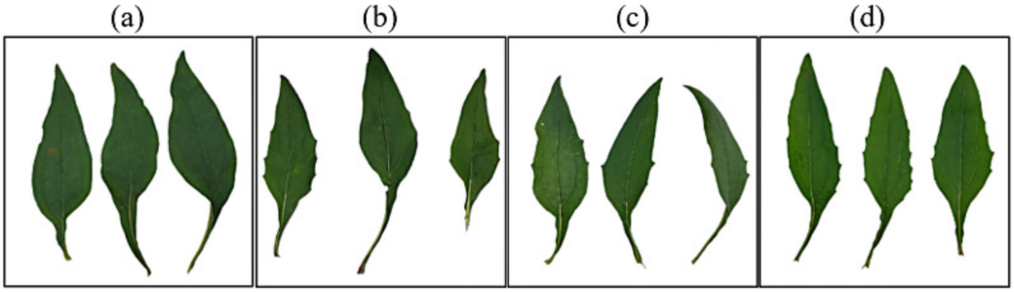

Fig. 1. Leaf color of *Echinacea purpurea* (L.) at doses of (a) 0 Gy / control, (b) 5 Gy, (c) 15 Gy, (d) 25

Fig. 2. Stem pattern color of *Echinacea purpurea* (L.). (a) green, (b) purple, (c) white Gy

The stems of the purple coneflower plant both irradiated and non-irradiated (control) show no morphological differences, both in stem color, stem texture, and stem pattern. The color of the stem is light green with the pattern colors are varying such as light green, purple, and white (Figure 2). The texture of the stem is coarse-hair. Differences in the stems of *Echinacea purpurea* can be caused as a form of plant tolerance to differences in environmental conditions (Sidhiq, 2020).

The most visible diversity is found in the flower part, especially in the color of the crown and the size of the flower. The un-irradiated purple coneflower flowers have a pale pink color, while the color of the mutant purple coneflower flowers is more pigmented and in the mutant plant 25 Gy tends to be magenta in color (Figure 3). Discoloration is caused by mutations in flower color control genes in somatic cells (Rizqiani et al., 2018). The difference in the color of *Echinacea purpurea* flowers is influenced by the level of accumulation of anthocyanin pigments which also affects the level of formation of flavonols and flavonols (Fedenko et al., 2020). Mutations in flower color occur inside somatic cells and are expressed in apical cells (Susila et al., 2019). The crown end of the purple coneflower flower is both mutant and the control is “W” shaped with a groove of 2 or 3. The crown shape of purple coneflower flowers at various doses of irradiation is directed downwards with different angles, while the mutant purple coneflower 25 Gy has a horizontal flower shape. Differences in the shape and color of flowers from their elders, in addition to being influenced by gamma rays, can occur due to the self-incompabilty nature of the purple coneflower plant or the mechanism to prevent self-pollination so as to trigger cross flowering (Choirunnisa et al., 2021; Sidhiq et al., 2020).

Fig. 3. Color of flowers *Echinacea purpurea* (L.) at doses of (a) 0 Gy / control, (b) 5 Gy, (c) 15 Gy, (d) 25 Gy

Electromagnetic waves from gamma-ray radiation generate radiant energy, by releasing electrons from the target cell, which are absorbed by the molecules in the cell thereby causing damage and producing free radicals and eventually altering the DNA molecule (Muhallilin et al., 2019). Long strands of DNA can split into short DNA at low-dose irradiation, however higher doses can damage DNA (Jan et al., 2012). The most prominent form of damage is the deletion of DNA segments, where the segments are lost when the single or double strands of DNA are cut off due to hydroxyl radicals (free radicals) will cause genetic changes (Ahmed et al., 2020; Susila et al., 2019).

Agronomic Characteristics

Agronomic characters can be indicators of the influence of irradiation on plant growth and yield. Measurements of plant height are commonly used to determine the influence of biological sides on physical (Silva et al., 2011). The results of the study found that the height of irradiated purple coneflower has a higher diversity than non-irradiated one. About 25% of the irradiated plant population has a much lower plant height than the non-irradiated plant population (Figure 4). The cessation of the cell cycle in the G2/M phase in somatic division and damage to the genome are the causes of stunted growth (Preuss & Britt, 2003). Gamma-ray irradiation exerts a random influence on each individual plant, can stimulate plant growth or damage plant cells. Disruption of the balance of hormones, enzymes, and protein synthesis in irradiated seeds can interfere plant growth (Khah et al., 2015), one of the conditions such as a decrease of the number or inhibition of signals in Giberalic Acid (GA) (Ahumada-Flores et al., 2021). The reaction between the water contained in plants and gamma rays forms hydroxyl radicals that can damage chromosomes (Abdullah et al., 2018).

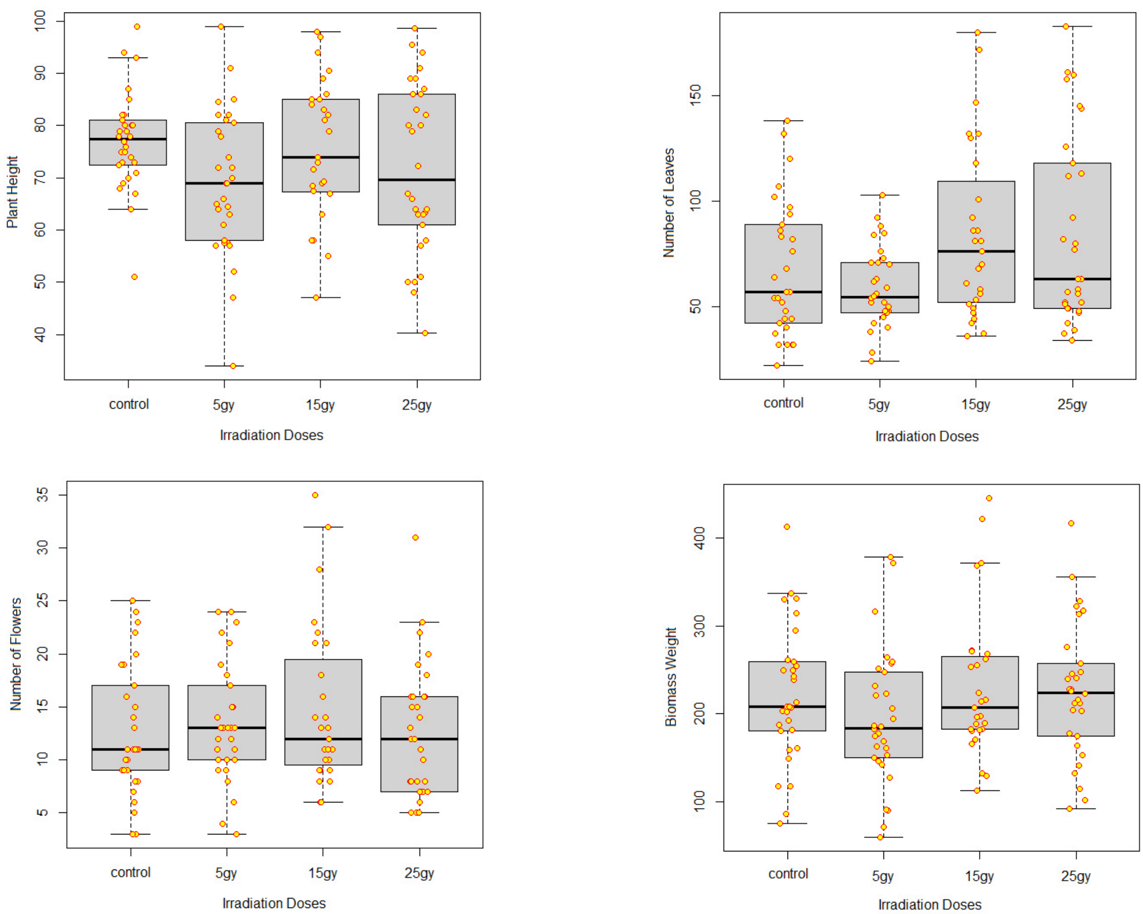

Fig. 4. The diversity of plant height, number of leaves and flowers, and biomass weights of *Echinacea purpurea* (L.) at various of gamma doses

The effectiveness of certain irradiation doses in high temperature environments, one of which can be seen from the number of leaves of the plant. The number of purple coneflower leaves which have been irradiated of gamma rays at high doses show its ability to adapt in better high-temperature environments. There are showed at Figure 4, around 50% of populations irradiated plants at dose 15 Gy and 25 Gy have higher median value than other two doses. Irradiation helps the adaptation of plants from environmental stress by increasing the number of antioxidative cells and altering hormonal signals (Abdullah et al., 2018). Suppression of leaf counts, as in irradiated plants at dose 5 Gy, is likely due to changes in energy production and distribution, accumulation of free amino acids, and chromosomal changes cause the leaves of mutant plants to differ (Kumari et al., 2013).

The highest diversity of flower numbers was shown in irradiated plants at dose 15 Gy. This dose is the optimal dose for increasing the number of flowers, then start to decreasing at dose 25 Gy. The decrease in the number of flowers at doses of 25 Gy is likely due to the presence of chromosomal damage in plants that produce flowers. There is no difference in harvesting time either at doses of 0 Gy, 5 Gy, 15 Gy, and 25 Gy. Research on daisy plants (Rizqiani et al., 2018) irradiated by gamma rays also showed no difference in the time of emergence of flowers with control plants. There was no difference in flowering time between control and irradiated plants, indicating the absence of the influence of gamma-ray irradiation on the flowering time of purple coneflower. The delay of the harvesting time of flowers can be caused by environmental influences especially related to temperature. Fluctuations in environmental temperature not only affect the timing of flowering, but also have a great effect on the physiological processes and development of plants (Capovilla et al., 2015).

The diversity of biomass shows no difference among all radiation doses (Figure 4), and the 5Gy dose has slightly higher in diversity than the other doses. Similar results occurred in gamma-ray irradiated white ginger resulting in rhizome weights that were not too different from control (Bermawie et al., 2015), where it can also be influenced by plant genotypes which can affect plant radiosensitivity. Gamma ray doses of 15 Gy are the optimal dose in both growth and yield, where the weight of linear biomass is large with a large number of leaves and flowers.

Total of Flavonoid Content

One of the content of secondary metabolite compounds in *Echinacea purpurea* is flavonoids (Figure 5). Flavonoids as secondary metabolites have no effect on plant growth and survival, but rather as a form of response to biotic and abiotic stress (García-Pérez et al., 2017).

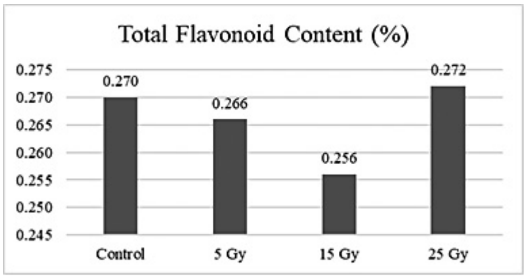

Fig. 5. Total of flavonoid content of *Echinacea purpurea* (L.) at various of gamma doses

Irradiated gamma-ray plants at low doses had lower total flavonoids than control plants. There are no significant different in the content of flavonoid total between irradiated and non-irradiated plants. The order of irradiated plants with the most flavonoid content is 25 Gy, 5 Gy, and 15 Gy. The low content of flavonoids in irradiated plants at dose 15 Gy was probably due to the better adaptability compared to the other two doses irradiated plants, which was indicated by the better growth rate and yield. According to Pelcaru et al. (2021) that gamma-ray alter the composition of antioxidants thereby affecting their properties.

Gamma ray energy can ionize water molecules in exposed plant parts, both through interactions in cell macromolecules and water radiolysis products, resulting in free radicals in the form of Reactive Oxygen Speciation (ROS) (Beyaz & Yildiz, 2017; Muhallilin et al., 2019). ROS triggers lipid peroxidation and forms melonaldehyde (MDA), causing membrane stability to decrease, membrane permeability to increase, and cell damage (Moghaddam et al., 2011). According to Jan et al. (2012) that lipid reactions quantitatively play only a small role, where electrons in these fats provide the main effect in the form of cation radicals that cause proton removal (deprotonation), dimerization, or disproportionation. The form of plant defense against oxidative stress due to ROS is in the form of antioxidants, where the form of protection mechanism is divided into two ways, namely enzymatic antioxidants (SOD, APX, CAT, MDHAR, DHAR, GR, GPX) and non-enzymatic antioxidants (ascorbic acid (AA), GSH, α-tocopherol, carotenoids, prolines, flavonoids) (Das & Roychoudhury, 2014).

Conclusions

Gamma-ray irradiation exerts an influence on the color of the leaves and flowers of the purple coneflower. Irradiated plants at dose 15 Gy shows the best growth, number of leaves and flowers, and yield rather than another irradiated plants. The highest flavonoid content in irradiated plants is dose 25 Gy, although there was no significant effect between irradiated and non-irradiated plants.

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