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Performance of M1 Generation of *Echinacea purpurea* Accession Two Resulting From Gamma (Ray) Irradiation

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ABSTRACT

Echinacea (*Echinacea purpurea*) is an herbaceous plant used as a medicinal raw material. Efforts required simplicia extract of Echinaceae is by increasing the diversity and content of secondary metabolites of Echinaceae through gamma ray irradiation. This study was conducted to determine the morphological characteristics as well as the diversity of growth and results of Echinaceae accession two from the results of gamma ray irradiation. The research was carried out by the Tawangmangu Center for Research and Development of Medicinal Plants and Traditional Medicine and in the Experimental Land of the Faculty of Agriculture, Universitas Sebelas Maret Surakarta, Jumantono Subdistrict, Karanganyar Regency in August 2022-February 2023 with a single plant method and without replication. Data analysis was carried out descriptively and boxplot analysis to see the distribution of data and outlier values. The results showed that the dose of gamma ray irradiation gave rise to new variations in the observed character. Irradiation dose increases diversity in plant growth character and yield. A dose of 40 Gy produced amendment extract yield of 12.32%. A dose of 20 Gy produced total flavonoid level of 0.048%.

Keywords: Flavonoid; Medicinal plant; Morphology; Mutants; Secondary metabolites

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INTRODUCTION

Echinacea purpurea (L.) Moench. or called purple coneflower belongs to Asteraceae originating from North America. Echinacea is one of medicinal herbs containing secondary metabolite compound (Fu R 2021). The content of secondary metabolites such as flavonoid, caffeine acid, polyacetylene, essential oil, polysaccharide and alkylamide have anti-inflammatory, antifungal, antioxidant, anti-immunosuppressant properties (Kumar & Ramaiah, 2011). The development in cultivation, the improvement of secondary metabolite content and the formation of variety are very desirable for the use of Echinacea as medicinal herb to obtain optimum productivity, either quantitatively or qualitatively. The attempt of developing accession can be taken, among others, through creating new variety by utilizing the diversity of accession genetics existing.

A plant breeding technique that can be used to improve genetic diversity of plant is mutation. Mutation induction using gamma irradiation is an evidently effective method of improving a species character and of spurring higher genetic diversity. Physical mutation leads to the change of genome level, chromosome, and DNA so that the physiological process of plant is abnormal and results in new genetic variation (Balai Penelitian Pertanian, 2011).

Considering the background, this research is conducted as an attempt of developing Echinaceae diversity as the source of medicinal herbs for Indonesians need. The choice of accession 2 was made based on a statement that accession A2 has highest flavonoid content at lowland (Sidhiq DF et al., 2020) and the Accession 2 response shows more tolerant characteristic to salt stress and produces the highest flavonoid content (Choirunnisa JP, 2021). The gamma irradiation treatment on Echinaceae is expected to improve the Echinaceae plant diversity. This research is aimed at finding out the morphological characteristic, the growth diversity, and the output of accession 2-Echinaceae resulting from gamma irradiation.

MATERIAL AND METHOD

This research was conducted in two different locations. The seeding activity up to 8 weeks after planting (wap) was conducted in Center for Research and Development of Medicinal Plants and Traditional Medicine (B2P2TOOT) and 12 (twelve) weeks after planting, the plant moving was conducted in Trial Land of the Faculty of Agriculture, Sebelas Maret University of Surakarta, Jumantono Sub District, Karanganyar Regency. This research was conducted from August 2022 to February 2023. The materials used were, among others, irradiated Echinaceae (0, 20, 30, 40 Gy), soil, fertilizer, and paddy husk in 2:1:1 ratio, and water. The tools used during the research activity were RHS Color Chart and camera.

This research employed a single plant method and without repetition. This research used one-factor treatment, the four levels of irradiation dosage: 0 Gy, 20 Gy, 30 Gy, and 40 Gy, with population number being the sample. The research procedure involved seed preparation, seed irradiation, seeding, land preparation and cultivation, planting, maintenance, observation, harvest, and laboratory analysis. Seed preparation was conducted by selecting ready-to-harvest seed conforming to the criteria and then delivered it to the National Nuclear Power Agency (BATAN) in South Jakarta. The seeding was conducted by sowing the irradiated seed on B2P2TOOT land in Tawangmangu. Land preparation was conducted on land compartment with 10 m x 8 m dimension. Land preparation and land cultivation involves weeds clearing, bed preparation, and paranet installation. Plant moving process was conducted after echinacea plant seed has been 3-4 months old and has fairly stroong root. This maintenance includes irrigation and weeds clearing weekly.

The obeservation of morphological characteristics included stem color, stem motif, stem surface texture, leaf color, leaf shape, leaf surface texture, edge flower color, middle flower shape and edge flower shape. The observation on growth and output involved plant height, number of leaf at flower appearance age, number of flower, harvest time, harvest time, wet weight, dry weight, extract yield and flavonoid level. Data resulting from the observation of morphological characteristics was analyzed descriptively by observing individual plants. Data of growth and output were analyzed in the form of boxplot chart.

RESULT AND DISCUSSION

Morphological Characteristics of Stem

The observation on stem color and texture can represent the varying morphological character of echinacea stem. Figure 1 shows dose of 30 Gy found in 1 (one) plant (A2.D3.T5) with strong yellow color. It is confirmed by a study on chrysant finding that irradiation treatment can generate variation in the stem color (Maharani S and Khumaida N 2013).

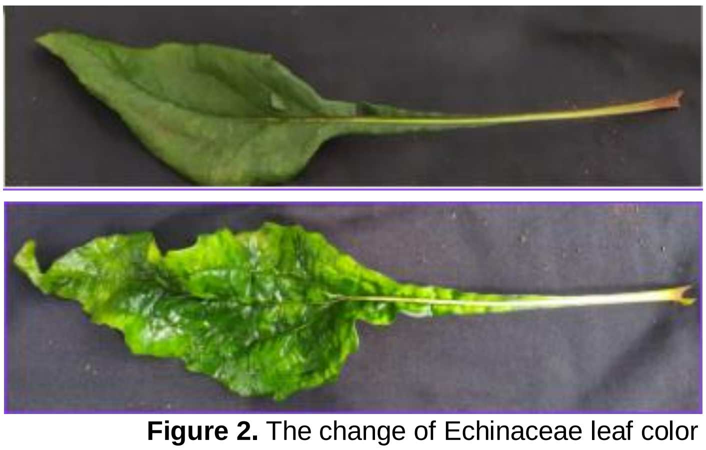
Strong Yellow Stem Color (30 Gy)   Dark Green Stem Pattern (30 Gy)

Figure 1. The change of Echinaceae stem color

Another character observed is stem texture. Considering the result of observation, all Echinaceae treatments have the same stem texture, coarse surface due to the presence of trichome. The appearance of mutant resembling the control plant occurs because the cell can survive so that the character of control plant will survive (Suwarno A et al., 2013).

Morphological Characteristic of Leaves

Figure 2 shows that the dose of 20 Gy results in 1 (one) plant (A2.D2.T3) generating different color variation, strong yellow green with real spot on its leaf color. The change of leaf color can be caused by the damage of chloroplast DNA (cpDNA). The mutation of chloroplast DNA (cpDNA) makes plastids in some tissue less capable or incapable of producing chlorophyll so that the deficiency of green color in the color (Anshori SR et al., 2014). Another study found that gamma irradiation can cause the change of leaf color from brighter into darker one (Yadav V, 2016).



Dark green (0 Gy)

Strong yellow green (20 Gy)

Figure 2. The change of Echinaceae leaf color

Echinaceae leaf has lanceolate leaf structure and the leaf has cervinervis with coarse texture of leaf surface. The edges of Echinaceae leaves are serrated with tapered tip. However, at the dose of 20 Gy there is 1 (one) plant (A2.D2.T3) with wavy edge-leaf shape. The change of plant elements, including the shape of leaves, can be an indicator of mutating plant (El OA et al., 2019). Gamma irradiation can cause the change of morphological character in Echinaceae, one of which is the shape of leaves (Fathin TS et al., 2021).

The Morphological Characteristics of Flower

At the dose of 0 Gy, the color of Echinaceae flower tends to be bright red to the combination of fading bright red and white (Figure 3). It is similar to a statement that at some altitudes, the accession 2 has bright red-to-pale bright red color (Kumar & Ramaiah, 2011). The doses of 20 Gy, 30 Gy, and 40 Gy generate strong reddish purple flower. At the dose of 40, Echinaceae generates two new colors: strong reddish purple (A2.D4.T10) and deep purplish red (A2.D4.T11 and A2.D4.T17). The irradiated plant flower has more pigmented color than the non-irradiated one. The dose of 40 Gy generates more new color than the non-irradiated Echinaceae

Strong reddish purple Deep purplish red

Figure 3. The change of flower color

Flower color pigment relates to the flavonoid level including anthocyanin and the change occurring in the irradiated flower can be caused by the change of pigment, either qualitatively or quantitatively (Zalewska M et al., 2011), due to the mutation occurring in anthocyanin-biosynthesis path (Streisfeld MA et al., 2013); Quantitatively, anthocyanine and flavonoid levels increase at the gamma irradiation doses of 40-100 Gy (Kurniasih D et al., 2016) it will likely make Echinaceae flower has more varied darker color at the doses of 20 Gy, 30 Gy and 40 Gy.

The irradiated flower results in more varied character. The Echinaceae flower is a single-shape flower on the top of stem stalk. Considering the result of research, the dose of 20 Gy produces 57.1% of downward-edge flower shape and 42.9% horizontal shape parallel with receptacle. 57.1% of middle flowers are round-shaped, the dose of 30 Gy results in 22.2% of downward edge flower and 44.4% horizontal or parallel with receptacle. 22.2% of middle flowers are round flat-shaped and 22.2% horizontal or parallel with receptacle. 11.1% of middle flowers are round-shaped (Figures 4 and 5). Irradiation can generate morphological variation in flower shape and density (Kurniasih D et al., 2016).

Flat Curving down Go downstairs

Figure 4. Changes in the character of ekinase edge flowers

Round Round flat Spherical buds

Figure 5. Changes in the character of the middle flower of Echinaceae

At the dose of 40 Gy, there is 1 (one) plant (A2.D4.T10) with up to 15 cm flower diameter; generally, the Echinaceae flower petal is 3-8 cm long with bright red, reddish purple, and purple colors like lavender (Gilman EF, 2014). It occurs because Echinaceae diversity can occur due to the uneven growth of mutant plant so that the plants responds varyingly as the consequence of gamma irradiation working randomly (Rahman & Aisyah, 2018).

The tip of Echinaceae edge flower is "W"-shaped with 2 or 3 curvatures. Based on the result of research, it can be seen that gamma irradiation at doses of 20 Gy, 30 Gy and 40 Gy can improve the morphological variation of flower by resulting in the change in color, shape of middle flower and shape of edge flower. The change of flower shape can be caused by the change of meristem cell positioning in the beginning of flower development due to mutation (Kumari & Kumar, 2015).

Analysis of Flavonoid Level

Figure 6 shows that the mean level of flavonoid produced by Echinaceae with irradiation treatment is higher than that by non-irradiated Echinaceae. The highest mean level of flavonoid is found at the doses of 20 Gy (0.048%), 40 Gy (0.047%), and 30 Gy (0.038), while the lowest flavonoid level is found is Echinaceae with non-irradiation treatment (0.037%). Gamma irradiation can change secondary metabolite content (Lee MB et al., 2013). The lowest total flavonoid content is 0.032% found in Echinaceae plant A2.D4.T9 and the highest one is 0.042% found in A2.D2.T11.

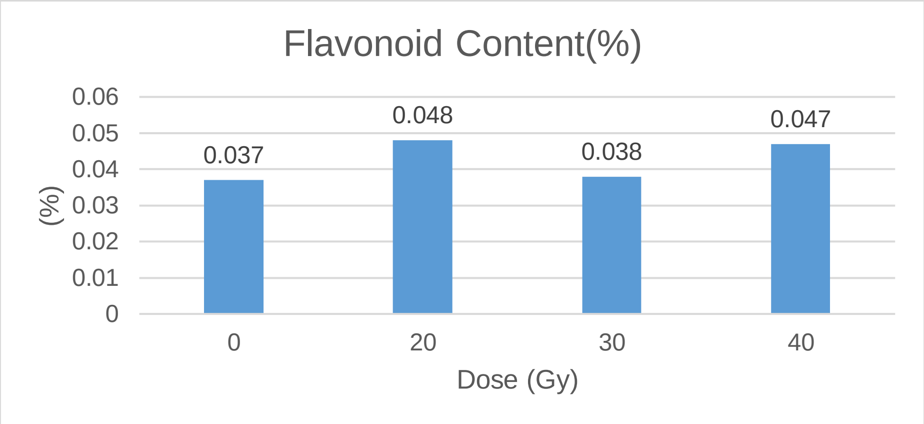


Figure 6. Total flavonoid levels resulting from gamma ray irradiation

The doses of 20 Gy and 40 Gy provide similar total mean level of flavonoid (Figure 6). It can be due to the fairly high anthocyanin content in the morphological character of flower color yielded at doses of 20 Gy and 40 Gy. Anthocyanin is one of flavonoid compounds involved as the pigment providing red, blue, and purple colors to the plant (Ramos P et al., 2014). Gamma irradiation can improve flavonoid content because gamma irradiation undermines the cellullar membran of plant's active compound. It is in line with a previous study finding that the flavonoid content of plant with gamma irradiation is significantly higher than that of the control (Moghaddam SS et al., 2011).

CONCLUSION

Irradiation treatment generates the varied morphological characteristic in the stem color (strong yellow), dark green motif of stem, leaf color (strong yellow green), wavy leaf edge shape, flower color, flower diameter, middle flower shape, and shape of Echinaceae edge flower. Gamma irradiation at doses of 20, 30, and 40 Gy can improve the variation in the characters of leaf number, flower number, age at which flower appears, wet weight and dry weight compared with non-irradiated plant and provides the mean highest extract yield of 12.32% at the doses of 40 Gy and the highest total mean content of flavonoid (0.048%) at the dose of 20 Gy.

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